ABSTRACT BOOK I

Oral Presentations

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CONTENTS

PUBLIC LECTURES

Ginkgo: An evolutionary and cultural biography

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Perhaps the world's most distinctive tree, living *Ginkgo biloba* is a botanical oddity that has remained little changed for at least 55 million years, and is also very similar to plants that flourished in the late Triassic and Jurassic over 200 million years ago. First brought to the attention of western science by Kaempfer, and named by Linnaeus only shortly before his death, *Ginkgo* was initially regarded as a conifer. It was later recognized as distinct in plant classifications of the early nineteenth century, a conclusion underlined subsequently by the remarkable discovery in *Ginkgo* (and also in cycads) of motile sperm cells. The precise phylogenetic position of *Ginkgo* among living and fossil seed plants remains uncertain, although most analyses using molecular data, as well as many morphological analyses that include fossils, associate *Ginkgo* with conifers. Classic work by Zhou Zhiyan has demonstrated that diverse *Ginkgo*-like plants existed during the Jurassic in China, and recent paleobotanical discoveries hint even greater diversity through the Jurassic and Early Cretaceous, as well as intriguing similarities to certain groups of Mesozoic "seed ferns". For much of the Mesozoic *Ginkgo*-like plants were widespread in both hemispheres, but their diversity declined during the mid-Cretaceous. They disappeared from the Southern Hemisphere early in the Cenozoic. In the Northern Hemisphere *Ginkgo* remained widely distributed through the Late Cretaceous and early Cenozoic, including at high paleolatitudes. Ultimately, however, *Ginkgo* barely survived the marked climatic changes of the later Cenozoic. It is last recorded in western North America about 15 million years before present. It disappeared still more recently from Europe, parts of Asia and Japan, and apparently survived the Pleistocene Ice Ages only in China. The only remaining putatively wild populations exist as relics in central-western and eastern China and recent research suggests that all living *Ginkgo* trees were ultimately derived from these two relictual populations. Despite its near extinction *Ginkgo* began its remarkable contemporary renewal and resurgence when people in China first found it useful as a "nut tree" about a thousand years ago. Subsequently *Ginkgo* was grown widely in China and appears to have been introduced into Korea and Japan in medieval times. In the mid-seventeenth century *Ginkgo* was introduced into Europe and by 1784 it was being grown outside of Philadelphia in North America. Today *Ginkgo* is an increasingly familiar living link to landscapes of the distant past. Its "nuts" are still enjoyed in the cuisines of eastern Asia, but it is also revered for its longevity, beloved for the elegance of its leaves, valued as a medicinal, and widely cultivated as one of the world's most popular street trees. In both the East and the West *Ginkgo* is also a source of artistic and spiritual inspiration. *Ginkgo* remains of great scientific importance and may provide our best window into the lives of diverse ancient seed plants that flourished between about 200 and 100 million years ago. The recent sequencing of the *Ginkgo* genome, and discoveries of new and exceptionally well-preserved fossil material, both offer exciting possibilities for a deeper insights into the life story of this remarkable tree, as well as its broader significance for understanding plant evolution.

People and plants – the unbreakable bond

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Plants form the scaffold for Earth's green ecosystems, but they are also essential for human survival. Plants provide most of the food we eat (directly or indirectly), our medicines, clothes, buildings, and even the air we breathe; they also beautify our daily lives. So we need plants, but do they need us? In this time of increasing human impact on plants, animals and natural habitats, our actions can make a big difference in whether plants are a part of an ecological civilisation for the future. Plants do in fact need us - they need us to study and use them responsibly, both as scientists and as members of human societies. So what can plant science contribute to the survival of these species on which we depend? I will draw on my own experiences as a scientist working on a flowering plant family of enormous economic importance, the Solanaceae, to explore how basic science can become part of many different aspects of people's use of plants. Members of the Solanaceae have uses in horticulture (petunias), medicines (mandrakes, tobacco, and henbane), and they are among our most important foods (potatoes, tomatoes, and eggplants). I will look at how people have changed and modified these species, and explore the changes that have driven the use of some species and not others. The journeys taken by plants in the company of people involve many changes, on both sides. It could be said that rather than domesticating plants for our own use, plants are in fact domesticating people! So our relationship with plants is deep and complex – but what should it be in the future? Should we confine plant species to wild areas where we can see them, but not interact or use them? Or should we manage plant diversity strictly for human use and enjoyment by bringing more plants into cultivation and into close relationships with us? Or perhaps something in between? I will argue that to really develop the bonds that link plants to people and create a society in harmony with nature all of use, not just scientists, must work together in order to maintain these relationships now and for future generations.

Saving plants to save ourselves: The Shenzhen Declaration

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Our planet consolidated into its present form about 4.5 billion years ago, with life appearing at least 3.9 billion years ago. The photosynthetic group of bacteria known as Cyanobacteria began producing oxygen about 2.7 billion years ago, with oxygen reaching present or even higher levels around the start of the Cambrian Period, 541 million years ago, about the same time that multicellular life first appeared. Animals, plants, and fungi colonized the land and began to diversify about 450 million years ago, with seed plants appearing about 370 million years ago and angiosperms some 135 million years ago. The ancestors of both dinosaurs and mammals appeared about 200 million years ago. The fourth great extinction occurred at about the time dinosaurs and mammals first appeared, with the last dinosaurs disappearing at the time of the fifth and last great extinction, at the end of the Cretaceous Period about 66 million years ago. Mammals then began to diversity, with primates, the mammalian order of which we are members, appearing about 55 million years ago. It is estimated that about 99% of all kinds of organisms that ever existed on earth are extinct, but when our ancestors diverged from the African apes about 6-8 million years ago, they did so in a world of biological richness, with perhaps 12 million species of eukaryotes and countless millions of species of prokaryotes supporting the functioning of the global ecosystem. The oldest fossils we know of our genus, *Homo*, date from about 2.8 million years ago, the oldest of our species, *Homo sapiens*, from about 200,000 years ago; these are both from Africa, with *Homo sapiens* migrating to Eurasia about 60,000 years ago. Therefore we are very new on Earth, but our activities have become devastating to our continued existence and that of other organisms as well.

The human development of agriculture as a main source of food about 10,000 years ago led to an explosion of our numbers from about 1 million people then to 7.4 billion today, projected to grow to 9.9 billion by mid-century, 33 years from now. This has made us an ecological force so great that we can be said to have entered the Anthropocene Period, one in which our actions will determined the fate of everything on Earth. For example, our bodies and those of our domestic animals now constitute 95% of all mammalian biomass on our planet. We are estimated to be consuming on a continuing basis about 164% of the planet's sustainable productivity, with some of us rich and many poor (www.footprintnetwork.org) . Like all organisms, we depend on the functioning of the global ecosystem for every aspect of our lives, and yet we are currently tearing it apart. Extinction is currently taking place at a rate 1,000 times or more greater than it has since the end of the Cretaceous Period 66 million years ago, so that as many as half of the estimated 12 million species of eukaryotes existing now are likely to have disappeared from nature by the end of the 21st Century, and we shall not even have recorded most of them.

Plants, and photosynthesis in general, do not receive the attention they deserve, and if we want to attain global sustainability, we shall have to work much harder to gain the knowledge that we need to save ourselves and to apply it to the maintenance of a sustainable world. We shall need to emphasize field studies in order to understand better the functioning of our globe. We have little or no knowledge about the majority of species of plants or other organisms on earth, despite our living during the last decades when many of them will exist. Nations and peoples have become starkly unequal, women and children often neglected or suppressed, and yet we are in danger of perishing together unless we do much better in these respects. We need to learn to cooperate with and love one another while there is still time to do so. We must attain a level population, equality between peoples, and better technologies while we still can do so. Plants, the subject of this Congress, and agriculture must receive greater attention since we depend so clearly and completely on them. Let us make this Congress a time of commitment to do better and resolutely seek a sound and sustainable future for all people.

Plants and people: History and future

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The relationship between humans and plants extends far back into hominid history. Plants have shaped our evolutionary past, adapting humans to a series of diet changes by alterations in morphological traits such as the structure of teeth, biochemical adaptations to novel foods and plant secondary compounds, and even alterations in our taste receptors. Humans early on have used plants as part of their culture, their medicine and their religion, forming a deep bond with native species. In turn, humans, with the advent of plant domestication and agriculture, have changed plants, altering them to increase yield, to simplify cultivation, and to modify their basic biochemistry for enhanced taste, and reduction in noxious compounds. This evolutionary dance between humans and plants has been central for the development and flourishing of agriculture. Agriculture, in turn, allowed humans to settle in towns and fostered the subsequent development of more complex civilizations. This legacy has persisted for at least 10,000 years with plants providing fiber and food for both people and animals. The complexity of our modern cities and cultures rests in part on the reliable production and transportation of food.

But, what is the future? How do we provide food security and proper nutrition to a growing global population? How do we grow more food on less land and reduce the environmental footprint of agriculture? Can plants be part of the new bio-economy, providing income to farmers in both developed and developing countries? And how do we affect all of these advances in the face of a rapidly changing climate? These are monumental changes facing the entire globe and they present both a challenge and an opportunity for plant science.

Green life, beautiful genes

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BGI, Shenzhen, China

We have all the reasons to sing praises for plants: We survive by take-in plants everyday, and we, together with all other animals, breath with the oxygen made by plants every minute. We live in a world to which plants contribute so much to its sustainability and balanced ecological environment.

Plants do not only teach us the sense of harmony and colorfulness, but also help us open the doors to understanding the secrets of all life. It is the plant that provided the tools to discover the genetic rules of genes; it is the plant that helped us find out the genes are even able to "jump"; it is the plant, listed as one of the model organisms of the International Human Genome Project (HGP), helped understand ourselves, as well as providing the new strategy to understand plants.

The discovery of DNA double helix structure opened a new epoch of life sciences and biotechnologies, which revolutionized our understanding of the life and diversity of plants. The HGP, with its belief that "*Life is of Sequences, Life is Digital*", provides a now concept of genomics and all other omics, a new technology of sequencing, and a new culture of globalized collaboration under the HGP Spirit, "*needed by all, owned by all, done by all, and shared by all*".

The Earth BioGenome Project (EBP), which was announced in February this year, has the ambitious missions to sequence "all life on Earth", and plants accounting for a major part. We call again for international collaboration, especially all those working on plant sciences and omics-based breeding.

PLENARY LECTURES

Evolution of plant sexual diversity

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Flowering plants possess striking sexual diversity despite the hermaphroditic condition of most species. Male and female gametes are deployed in a variety of spatial and temporal combinations at the flower, inflorescence and population level resulting in diverse sexual systems. As Darwin recognized, species with polymorphic sexual systems are especially valuable for evolutionary analysis, and studies of heterostyly and dioecy continue to provide key insights into evolutionary processes. Here, I provide an overview of our current work on the causes and genetic consequences of two of the most important evolutionary transitions in angiosperms: the pathway from outcrossing to predominant self-fertilization, and the origin of separate sexes (females and males) from hermaphroditism. The approaches used to address these problems include field studies of the ecology, demography and genetics of populations; comparative methods; genetic mapping of floral traits; and genomic and molecular evolutionary analyses.

The evolutionary breakdown of heterostyly to homostyly represents a classic system for investigating the shift from outcrossing to selfing. In *Eichhornia* this transition has occurred multiple times both within and among species. Evidence from *E. paniculata* indicates that the joint evolution of genetic drift and selection for reproductive assurance explains repeated transitions to selfing. Genotyping-by-sequencing and composite interval mapping of independently derived selfing forms that have colonized the Caribbean and Central America from Brazil, indicate that stamen modifications governing selfing occur on different linkage groups from the loci controlling tristyly. Each selfer has modifiers on a different linkage group consistent with their separate origins. This demonstrates that selfing can evolve via different genetic pathways within the same species. Transitions to selfing have resulted in a reduced efficacy of selection, the purging of deleterious mutations and changes in gene expression across the genome.

The transition from hermaphroditism to dioecy has occurred on multiple occasions in angiosperms. Although theory predicts 1:1 sex ratios in dioecious populations, biased ratios are common but the causes are not well understood. Our comparative analyses of angiosperms have revealed an association between heteromorphic sex chromosomes and female-biased sex ratios. In *Rumex,* a genus with heteromorphic sex chromosomes, natural populations often exhibit female-biased sex ratios and experimental studies have demonstrated that increased pollination intensity causes female bias. Moreover, theoretical work indicates that a balance between Fisherian sex-ratio selection and selection against deleterious mutations during the haploid phase can explain stable female biased sex ratios. These findings motivated us to investigate if the observed bias in populations is associated with Y-chromosome degeneration. Studies of sex-linked SNPs and transcriptome data have provided evidence of different degrees of Y-chromosome degeneration, depending on the time since recombination suppression, and a loss of neutral diversity consistent with selective interference among linked sites. Future comparative work on the genus will further investigate biased sex ratios, haploid selection and sex chromosome evolution across the phylogeny. Collectively, these studies of two polymorphic sexual systems both highlight the important genomic consequences of recombination suppression and also the insights that can be gained from intraspecific studies or natural variation.

Applications of genomics to crop plant improvement

Michael Bevan
John Innes Centre, Norwich, UK

Recent advances in genome sequencing technologies, coupled to radically improved assembly and annotation methods, have revolutionized genomics. These approaches are now being used to tackle crop plants with large and complex polyploid genomes. New methods for sequencing populations of crop plants and their wild relatives are also providing unprecedented access to a wide range of genetic variation. In my presentation I will use the wheat genome as an example of such progress. The question now is how can genomics guide and accelerate the breeding and engineering of crops with improved characteristics. I will provide examples of how our research teams at the John Innes Centre and the Earlham Institute in the UK are going about delivering the great promise of genomics.

On the exceptional value of model lineages in systematics and comparative biology

Michael J. Donoghue
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With reference to a model portraying the scope of systematics and comparative biology, I will argue that comprehensive analyses of model lineages have a critical role to play in advancing our understanding of plants and their evolution. Such studies provide new avenues for analyzing how organisms are integrated and the evolutionary causes and consequences of this integration. They also provide a sound basis for the comparative analyses that are most likely to yield insights of lasting significance. As a concrete example I will highlight our ongoing studies of the angiosperm clade *Viburnum* (Adoxaceae). Specifically, I will develop connections between our analyses of leaf structure, function and evolution in *Viburnum* and global patterns in leaf form and leafing habit. These studies identify new traits of potential interest in studies of global change.

Fertilization mechanisms: A comparison of eudicots and grasses

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After leaving their aquatic environment land plants adapted to various environmental challenges and evolved specialized reproductive structures. Originally, their sperm cells were motile and capable to swim to egg cells. This mode of fertilization is still preserved in most mosses and ferns preferring moist environments. However, flowering plants or angiosperms, which dominate in colder and dryer habitats, evolved embedded gametophytes to protect egg and immobile sperm cells. Male gametophytes or pollen grains develop in anthers, while female gametophytes or embryo sacs are produced deeply inside the maternal tissues of ovule and ovary, respectively. After release pollen grains of angiosperms are transported by wind or animals to female flower organs of the same species. They germinate, penetrate stigmatic tissue and grow through the style towards the ovule to deliver their passive sperm cell cargo. Inside the female gametophyte double fertilization is executed resulting in embryo and endosperm formation, respectively. To avoid fertilization failures and to promote outcrossing, angiosperms have developed various mechanisms to distinguish between own and alien pollen as well as pollen of the own species. Using *Arabidopsis* as a eudicot and maize as a grass model, I will compare morphological differences and focus on similar and distinct molecular mechanisms associated to fertilization. These include various hurdles along the pollen tube pathway, during sperm cell release and gamete activation. The analysis of genes involved in pollen adhesion, hydration and penetration, pollen tube growth and guidance as well as double fertilization and onset of embryo development will be presented. Some genes with key functions for fertilization and egg activation in maize lack homologs in *Arabidopsis*, while other genes are highly polymorphic. Molecular systems to discriminate own and foreign tubes will be presented and possibilities discussed to generate a new species.

Deconstructing the plant regulatory genome: Enabling technologies

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Genetic and epigenetic variation can impact gene transcription and phenotypic diversity and may thus contribute to adaptation. We have produced and characterized > 1000 high quality single-base resolution genomes, methylomes, and transcriptomes from a global collection of *Arabidopsis thaliana* ecotypes/accessions. Although the genetic basis of methylation variation is highly complex, geographic origin is a major predictor of global DNA methylation levels and altered gene expression caused by epialleles. Analysis of the cistromes and epicistromes of these 1001 accessions revealed significant association between transcription factor binding (TF) sites, methylation and nucleotide variation and co-expression modules. To more directly interrogate the effects of genetic and epigenetic variation on TF binding, we developed a high-throughput TF binding site discovery method and defined the *Arabidopsis* cistrome by resolving motifs and peaks for a large number of TFs. We have also developed a massively multiple yeast 2-hybrid based method for studies of protein:protein interactions and applied this approach to study of *Arabidopsis* TF:TF interactions. These approaches are advancing our understanding of how both genetic and epigenetic variation contribute to phenotype in natural populations.

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Structural characteristics of forests and diversity of woody plants in China

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China is rich in forest communities and woody plants. To systematically investigate species composition and structure of China's forest communities, we launched a long-term project consisting forest vegetation surveys across China's mountains in the mid 1990s. Over the study period, we have conducted vegetation surveys for more than 70 mountains and collected vegetation data from about 2000 forest plots, using consistent sampling protocols. Meanwhile, to explore geographic patterns and their environmental controls of woody species diversity, we established a huge database of distribution of China's woody plants that contains all 11405 woody species in the country. Our results indicated that as latitude increased, diameter at breast height (DBH) and height of trees increased, while individual density of trees and woody species richness decreased. Total basal area (TBA) of trees and species richness of herbs did not vary with latitude. Contemporary climate seems to drive these patterns: temperature was the leading factor for DBH, precipitation was most important for tree height and individual density, actual evapotranspiration (a surrogate of productivity) determined woody (trees and shrubs) species richness, and rainfall was the major controller of the herb species richness. The species–abundance relationship showed that species dominance (measured by the number of individuals per species) declined significantly from boreal forests to evergreen broadleaf forests from north to south. Further, using the database of distribution of woody plants, we compared the effects of habitat heterogeneity, human activities and climatic variables (particularly environmental energy, water–energy dynamics and winter frost) on the woody species diversity, and found that the contemporary climate explained the spatial variation of more than 80%, of which winter mean temperature of the coldest quarter was the strongest predictor, thus supporting the freezing-tolerance hypothesis.

Understanding the molecular basis of rice plant architecture

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Rice is the most important staple crop in China and an ideal model plant. How to increase yield and improve quality has been the major challenge in rice research and breeding. However, to meet the security of food supply, the crop yield per unit area has to be increased by 50% before 2030. My lab has been focused on systematical dissection of key genes regulating rice plant architecture, elucidation of their molecular mechanisms and the genetic regulatory network, and application of this fundamental knowledge to breed new high-yield elite varieties.

Plant architecture is a primary determinant of crop yield and has profound adaptability in response to internal and external factors. Research on rice plant architecture and understanding of the molecular basis and regulatory mechanism are of great importance to both basic science and practical application. Plant architecture is mainly determined by plant height, tiller number, tiller angle and panicle morphology. My lab has molecularly characterized a series of rice mutants that show various altered plant tillering or panicle phenotypes, including *monoclum 1* (*moc1*), *tillering and dwarf 1* (*tad1*), *ideal plant architecture 1* (*ipa1*), *lazy1* (*la1*), s*mall panicle 1* (*sp1*), *dwarf 27* (*d27*), and *dwarf 53* (*d53*). We cloned these corresponding genes through map-based cloning approaches and in-depth characterization of their functions, demonstrating that the tillering phenotypes mainly result from either the deficiency in the branching plant hormone strigolactone biosynthetic and signaling pathways or the transcription factors that play key roles in plant architecture formation. The understanding of these gene functions enable us to elucidate a comprehensive regulatory network underlying the formation of plant architecture and greatly expanded our knowledge of plant development.

Based on the knowledge from the study on plant architecture of rice, our lab and collaborators have made enormous efforts in breeding new elite varieties through developing a molecular breeding system. These results demonstrate that this system could truly speed up the rice breeding course and generate new super rice with multiple advantages. However, to fully meet the challenge of food supply, more efforts need to be made by providing more genetic resources and designing different allele combinations to meet the unique demands in different regions in China.

Computational morphodynamic approaches to pattern formation in the shoot apical meristem of *Arabidopsis*

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The shoot apical meristem is the stem cell niche responsible for generating the above-ground parts of flowering plants, including stems, leaves and flowers. Though only a few hundred cells, it has a large number of overlapping but different domains of gene expression. These gene expression domains are dynamic – some genes change their expression relative to appearance of new leaf or flower primordia. Others are expressed in what appear to be stable domains in the meristem – but are not stable, as the cells that express the genes are dividing, and some progeny cells enter these domains as others depart, making them dynamic at the cellular level, even though stable in comparison to the meristem boundaries. How are these gene expression patterns controlled? By iteration between computational models of cell-cell interactions, and experiments to test the models, we have developed robust hypotheses for flower primordium-influenced gene expression that involve interactions between chemical (auxin) and mechanical signals in the meristem, and for meristem region-specific expression we have models where cell-cell communication is mediated by a combination of small-molecule (cytokinin) and peptide signals.

The origin of economically important species of *Cucumis*, *Citrullus*, and *Momordica* – combining Chinese genomics know-how and old European herbaria to find wild relatives

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China is World-leading in Cucurbitaceae genomics, having produced the draft genomes of cucumber, *Cucumis sativus* (Huang *et al.,* Nature Genetics 2009; also Qi *et al.,* Nature Genetics, 2013; Shang *et al.,* Science 2014; Zhou *et al.,* Nature Plants 2016) and watermelon, Citrullus 'lanatus' (Guo *et al.,* Nature Genetics 2013). Old-fashioned Sanger sequencing over the past 10 years, however, has revealed numerous misclassifications of material of Cucurbitaceae, from simple misidentification of germplasm to serious misplacements of species in unnatural genera, meaning that names do not reflect evolutionary relationships and cannot be trusted to guide sampling for genomic projects. The family Cucurbitaceae is underrepresented in herbaria because wild cucurbits often are climbers in disturbed habitats or desert plants that are hard to find at the right stage. Fruits of the family are difficult to preserve. Only with DNA barcoding has it become possible to figure out what is what (simply put). In the talk I will illustrate how molecular data are revolutionizing our knowledge about the wild relatives of *Cucumis sativus* (in India, Thailand, and tropical China), *Cucumis melo* (in India and Australia), *Citrullus lanatus* (in West or Northeast Africa), *Momordica charantia* (in Africa) and *Luffa* (in India and Australia), using examples of recent and ongoing work in my lab, in collaboration with genomics experts from China.

EXAMPLES:

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Plant evolution in the Anthropocene

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Humans have emerged as the dominant selective force on our planet. This provides unparalleled opportunities for evolutionary biologists to study evolution in action. However, it also leads to the question of whether plants (and other organisms) can evolve fast enough to keep up with the rapid pace of human-caused environmental change. I will discuss what we have learned about plant adaptation and speciation from studies of contemporary evolution. Plant evolution in the Anthropocene is pervasive and can be rapid, but in many species it lags behind environmental change due to long generation times and limited dispersal. In the absence of intervention, extinction of such taxa seems likely. To maintain productive communities and ecosystems, plant biologists must develop strategies to aid adaptation when desired (e.g., evolving climate-adapted crops or forests) and hinder it when necessary (e.g., in weeds). More generally, our focus going forward should be on adaptation rather than preservation of the *status quo*.

Linking heterogeneous data in botanical research

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Plant diversity is the product of complex interrelationships among genetics, ecology, and evolution, yet integration of these disciplines of botanical research remains elusive.

Emerging cyberinfrastructure and new data sources provide unparalleled opportunities for mobilizing and integrating massive amounts of information from organismal biology, ecology, genetics, phylogenetics, climatology, and other disciplines. Key among these data sources is the rapidly growing volume of digitized specimen records from natural history collections. With nearly 100 million specimen records currently publically available online, these data provide excellent information on species distributions and changes in phenology and distributions over time. Particularly powerful is the integration of phylogenies with specimen data, enabling analyses of phylogenetic diversity in a spatio-temporal context, the evolution of niche space, and more, while phylogenomic analyses that synthesize phylogenies and genome-scale data shed new light on the role of genomic change in plant diversification. The burgeoning field of phenomics offers still further data for integration with phylogenetic, genomic, and ecological data. However, a major challenge to synthetic research is the heterogeneous nature of complex data, and new methods are needed to link these divergent data types. Ongoing efforts to link and analyze diverse data are yielding new perspectives on a range of evolutionary and ecological problems. We will present multiple case studies that attempt to integrate heterogeneous data sources in analyses of plant diversity. Although many specific hypotheses may be addressed through integrated analyses of linked biodiversity and environmental data, additional value of such data-enabled science lies in the unanticipated patterns that emerge.

Decoding the epigenetic language of life

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Epigenetics refers to the study of heritable information that is not contained in DNA sequence. An important epigenetic mark conserved in mammals and plants is DNA methylation, a chemical modification of DNA that controls gene function. Proper DNA methylation patterns are critical for development, diseases and stress responses in humans as well as in plants. Plants are excellent biological systems to study how DNA methylation patterns are generated. DNA methyltransferase enzymes that deposit the DNA methylation mark are guided to specific DNA sequences, and DNA demethylase enzymes that remove the DNA methylation mark are also guided to distinctive sequences to erase unwanted DNA methylation. I will describe work in my lab that has shed light on how DNA methyltransferases and demethylases are guided to specific sequences, and how the antagonistic actions of the enzymes are coordinated to generate proper DNA methylation patterns. I will also describe some of our recent work on how DNA methylation influences transgenerational inheritance.

KEYNOTE LECTURES

Plant speciation across environmental gradients and the occurrence of hybrid zones

Richard Abbott
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The success of individual plant species to spread across environmental gradients is dependent on their ability to adapt to changed local conditions via adaptive phenotypic plasticity and/or adaptive genetic divergence. There are many examples of heritable adaptive changes in phenotype across environmental gradients and the underlying molecular genetic bases for some of these changes are beginning to be resolved. Such heritable change can occur in the presence or absence of gene flow and may be manifested as continuous change in phenotype (clinal variation) or the establishment of discrete ecotypes. In both cases, reproductive barriers may evolve, often gradually, between locally adapted forms and ultimately result in the formation of new species.

At an intermediate stage in this process, hybrid zones may originate as a result of primary intergradation (with continuous gene flow) or through secondary contact between locally adapted forms previously isolated by means of an eco-geographical barrier. The study of both types of hybrid zone can be highly informative of reproductive barriers that evolve between incipient species across an environmental gradient. Whereas in animal groups hybrid zones are recognised as useful systems for studying the nature and sequence of reproductive barriers that evolve across the speciation continuum, rather surprisingly, they have been little used for this purpose in plants, where they remain a relatively underutilised resource in studies of plant speciation. Nonetheless, a few recent studies of plant hybrid zones involving detailed genomic analysis have been conducted and begun to shed light on the nature of breeding barriers that may evolve during divergence across environmental gradients. These studies provide direction for the future use of hybrid zones in plant speciation research.

One possible reason for why studies of plant hybrid zones are relatively rare in the current literature is that though hybridization occurs frequently among plant species, the establishment of hybrid zones may be uncommon, particularly under relatively stable conditions, i.e. low levels of disturbance. Rarity of hybrid zones, if true, would be an interesting phenomenon in itself and raise questions as to why it is the case. However, more information is required on the occurrence of hybrid zones between plant species before reaching conclusions on their frequency.

Saving plants

Stephen Blackmore

Queens Botanist and Honorary Fellow, Royal Botanic Garden Edinburgh, UK

The international botanical community, meeting here in Shenzhen, is far more aware than most people in wider society of the fundamental role played by plants in shaping and maintaining the biosphere. However, a significant proportion of plant species are now threatened with extinction and plant biodiversity is being lost in other ways, so that even species that were once common are becoming rare. As botanists we also recognise that our current knowledge of plant diversity is far from complete: many plant species remain to be discovered and we are still in the early stages of exploring the genetic diversity. Maintaining plant diversity, at ecosystem, species and genetic levels, Is especially important in an era of global change and has direct relevance to future resilience and adaptation. The continuing erosion of plant diversity has major consequences for our planet and all of its inhabitants: threatening biodiversity in general and making the internationally agreed Sustainable Development Goals even more difficult to achieve.

I will explore some of the key actions humanity needs to undertake in order to conserve global plant diversity, considering conservation in the broadest sense to include any and all interventions that help to secure pant diversity and the ecosystem services delivered by plants. I will emphasise that many different sectors and stakeholders will need to work closely together if we are to succeed in passing on the Earth's rich, green inheritance to future generations. Direct action to conserve plants is carried out by agencies and disciplines as diverse as forestry, agriculture, conservation biology, urban and rural development planning, protected area management and other fields. An even wider set of institutions and organisations are involved in education and outreach, enabling people in wider society to understand the issues and to engage in addressing them.

My focus will be on the potential contribution of botanic gardens, arboreta, seed banks and other collections of living and preserved plants and on the strategic challenges they and other players need to overcome for global efforts in plant conservation to accelerate and expand to an appropriate degree.

Genes gone wild: Experimental evolution meets synthetic biology

Ralph Bock

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In my talk, I will describe experimental approaches to study genome evolution in real time. I will discuss three fundamental processes in eukaryotic genome evolution and show how they can be reconstructed in laboratory experiments: (i) the transfer of organellar (plastid and mitochondrial) genes to the nuclear genome, (ii) the horizontal movement of organellar DNA between plants, and (iii) the movement of nuclear genetic material between plants by horizontal genome transfer. Finally, I will show how horizontal gene transfer can be employed as a versatile tool in plant biotechnology and synthetic biology.

Molecular mechanisms of histone demethylases in genome-wide targeting in *Arabidopsis*

Xiaofeng Cao

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Transcription activity of chromatin is regulated by covalent modifications on nucleosomes and DNA. Recent efforts have identified a lot of chromatin modifiers, which play important roles in various aspects of biological processes. These chromatin modifiers normally bind a subset of specific genomic loci. Discovering the mechanisms of recruiting these modifiers is essential for understanding the biological function of them. We previously identified *Arabidopsis* JMJ14 and JMJ12/REF6 as H3K4 and H3K27-specific histone demethylases, which use two distinct mechanisms to their genome-wide targets. The C-terminal FYR domain of JMJ14 interacts with a pair of NAC domain containing transcription factors, which bring JMJ14 to their common target genes; whereas REF6 recognizes its target loci by direct recognizing specific DNA sequence through its tandem C2H2-Zinc finger domains. In this talk, I will illustrate how other factors affect REF6 genome-wide targeting in *Arabidopsis*.

Pant sesquiterpenes: Biosynthesis and bio-interactions

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Terpenoids constitute the largest group of plant secondary (or specialized) metabolites, and they play important roles in plant adaptation to environments. Sesquiterpenes are derived from the 15-carbon farnesyl diphosphate. We are interested in sesquiterpene biosynthesis in plant and the role of sesquiterpene phytoalexins in plant defense against insect pests.

The plant defense hormone jasmonate (JA) plays an important role in regulation of secondary metabolism pathways. The *Arabidopsis* MYC2, a bHLH transcription factor, acts as a key regulator of JA response. We found that in *Arabidopsis* inflorescence MYC2 integrates both gibberellin (GA) and JA signals in regulating the expression of terpene synthase genes. In addition, transcription factors of other families, such as WRKY and AP2/ERF, are also involved in induced formation of sesquiterpenes.

The level and accumulation of secondary metabolites change during plant development and growth. The miR156-targeted SPLs function as an age cue in plant phase transition and maturation, our recent study demonstrated that the SPL factors directly regulate sesquiterpene synthase genes in *Arabidopsis* and of *Pogostemon cablin*, linking the plant age cue to elevated accumulation. The SPLs also attenuate JA signaling through direct interaction with JAZ proteins, leading to age-dependent JA response decay. Conversely, phytoalexins may accumulate constitutively to a higher level in later stages, providing a strong insect resistance to old plants.

*I thank Ying-Bo Mao, Zong-Xia Yu, Gao-Jie Hong and others who contributed to this research.

Mechanisms of microRNA degradation

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The abundance of miRNAs is determined by both biogenesis and degradation. While the mechanisms of miRNA biogenesis are fairly well understood, how miRNAs are degraded in vivo, especially when miRNAs are associated with their effector protein ARGONAUTE 1 (AGO1), is poorly understood. In 2005, my group showed that miRNA degradation entails 3' truncation and 3' uridylation, but the enzymes responsible for these processes were unknown. In recent years, we identified the exonucleases and nucleotidyl transferases that are responsible for miRNA 3' truncation and 3' uridylation, respectively. We showed that these enzymes not only act on free miRNAs but also AGO1-bound miRNAs. While these enzymes are largely non-sequence-specific, the degradation of specific miRNAs in vivo can be achieved. One example of specificity determination is the promotion of miR165/6 degradation by AGO10. My talk will be focused on the molecular framework of miRNA degradation.

O-GlcNAc signaling mediates vernalization sensing in wheat

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Wheat and rice show different preference to low temperature since their various origin and evolution. Cold can damage for living of rice, but be required for flowering of winter wheat. Sensitivity of rice for cold limits its planting only in certain climate-zones. Our previous study suggests COLD1 with a localization on plasmamembrane and ER, interacts with rice G-protein α subunit 1 (RGA1) to activate the Ca^{2+} channel for sensing chilling and to accelerate G-protein GTPase activity. Based on the characters of temperature sensor in mammalians and its Calcium signaling and electriphysiological response to temperature changes, COLD1 is a cold sensor in plants. Vernalization is the acquisition of a plant's ability to flower which requires a prolonged cold temperature. It is controlled by a genetic network such as *VRNs*. However, it's less known that sensing mechanism for prolonged cold, vernalization. Here reports that the *O*-GlcNAcylation and phosphorylation signaling were involved in sensing vernalization in wheat, and *O*-GlcNAc signaling mediated flowering through effecting epigenetic process in *Arabidopsis*.

The post-translational *O*-linked N-acetylglucosamine (GlcNAc) modification of proteins, catalyzed by *O*-GlcNAC transferase (OGT), is involved in multiple important biological processes in animals. However, whether protein *O*-GlcNAcylation affects plant development is largely unknown. Here, we show that loss of function of SECRET AGENT (SEC), an OGT in *Arabidopsis*, leads to weak early flowering time phenotype, which results from decreased transcription of FLC. And ChIP analysis indicates that H3K4me3 level is significantly reduced in sec-5 mutant comparing with that of wild type. We also found that SEC can interact with a protein and catalyzed *O*-GlcNAcylation in vivo to regulate H3K4 methylation impacting the transcription of *FLC*. So the *O*-GlcNAc signaling plays an important role in flowering by affecting the activity of specific protein and the epigenetic process in *Arabidopsis*. Meanwhile we studied the possible functions of *O*-GlcNAc signaling in wheat vernalization. The approaches of LWAC and iTRAQ-TiO2 were used to enrich *O*-GlcNAcylated peptides and phosphopeptides of the plumules from vernalization and non-vernalization wheat, respectively. As a result, about 200 *O*-GlcNAcylated proteins and 164 alternatively changed phosphoproteins were identified. Functional analysis showed that these proteins are mainly involved in metabolic processing, cellular processing and response to stimulus. The inhibitors, such as alloxan for OGT and PUGNAc for OGA were used to treat wheat plants with nonvernalization and vernalization. And the data showed that *O*-GlcNAc signaling in vernalization accelerated flowering transition in winter wheat. During vernalization, gradually increased *O*-GlcNAc modification was detected for TaGRP2, the RNA-binding protein directly binding to *TaVRN1* pre-mRNA to repress its activation. Phosphorylated VER2, a jacalin lectin, recognizes the *O*-GlcNAcylated TaGRP2 to repress its accumulation in the nucleus and attenuate its binding to *TaVRN1*, thereby releasing the repression of *TaVRN1*, ultimately promoting flowering. We speculated that *O*-GlcNAcylation and phosphorylation modification may act as signals to sense vernalization and regulate the network of *VRNs* for flowering in wheat.

Fitter with litter: Plant traits and their legacy for ecosystem services

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Litter, i.e. the finer fraction of dead plant material, provides a wide variety of important ecosystem services. However, the role of litter in regulating ecosystem processes and services has mostly been studied in terms of litter presence or amount. Besides in biogeochemistry, we still do not know how litters from distinct plant species differ in their effects on other ecosystem processes and services including biodiversity support. I will propose a new conceptual model that will help us to investigate these differences based on species traits, by explicit reference to two highly distinct and possibly interacting plant 'trait spectra': the widely known (Plant or) Resource Economics Spectrum (RES), and the newly defined Size and Shape Spectrum (SSS). Different ecosystem services provided by plant litter are driven by either one of the trait spectra or by both. I will introduce this concept by drawing from examples from a range of contrasting ecosystem services, including regulatory, recreational, provisioning and biodiversity support services. I will encourage the audience to start empirical work to quantify the linkages between the multi-species RES-SSS trait space and these various services, thereby enhancing our understanding about how exactly dead plant matter matters not only to ecosystems but also to people in different parts of the world.

Seed germination and seedling emerging out of soil: Life and death decisions

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In natural and agriculture systems, seeds often germinate and emerge from the soil to start new life cycles. How plants regulate seed germination and seedling development to emerge from soil is crucial for plants' survival and growth. In the past few years, our series of research greatly advance the knowledge of seed germination and seedling emergence out of soil. We identify the novel positive transcription factor and central repressor in mediating light-induced seed germination. Combining the genetics, molecular biology and mathematical modeling methods, we revealed that seeds use a multilevel regulatory circuit of triple feed-forward loops to sensitively and precisely control seed germination in various light conditions (Shi *et al.,* 2013; Shi *et al.,* 2015). In order to study the molecular mechanism underlying seedling emergence from the soil, we design a convenient and highly reproducible soil assay and show that the transcription factor in ethylene signaling pathway EIN3 is essential for the seedling emergence (Zhong *et al.,* 2014). We further demonstrate that the COP1-EBF1/2-EIN3 tandem E3 ligase module channels the light and mechanical pressure signals to elaborately adjust EIN3 protein levels to ensure seedling emergence out of soil (Shi *et al.,* 2016a). After growing out of the soil to reach the sunlight, dark-grown seedlings face dramatic environmental changes. Our recent studies show that photoreceptor phyB manipulates substrate-E3 ligase interactions in a light-dependent manner, thus directly controlling the stability of EIN3. Therefore, the plants adopt the tandem E3 ligase module to gradually adjust EIN3 protein accumulation in the soil, and elicit a rapid degradation of EIN3 to shut down the ethylene responses by a light-dependent "molecular glue" phyB upon emergence (Shi *et al.,* 2016b). Taken together, our findings demonstrate the molecular actions of key factors and regulatory mechanism during seed germination and seedling emergence out of soil, which helps us to understand the natural phenomena and provide the mechanistic basis for crop genetic improvement in future.

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Distinct evolutionary dynamics of C4 and CAM photosynthesis

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CAM and C4 photosynthesis are two carbon-concentrating mechanisms (CCMs) that increase photosynthetic efficiency, and together have evolved hundreds of times in flowering plants over the past 30 million years. Although C4 and CAM share a primary biochemical module, their anatomical specializations are quite distinct, and carry significant ecological repercussions. I'll present the latest ideas on the evolutionary trajectories of both C4 and CAM syndromes, highlighting areas of convergence and divergence. An emerging hypothesis is that anatomical modifications preceeded the emergence of CCM biochemistry during C4 evolution, while the reverse may be true for CAM. I'll discuss the evolutionary implications of this order of events, and suggest that structural innovations may act more generally as key evolutionary "bottlenecks": events that happen relatively rarely, and dramatically alter the adaptive landscape and subsequent evolutionary accessibility of novel phenotypes.

Early angiosperms: Key fossils and extinct diversity

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Fossil floral structures and other remains of angiosperms recovered from Cretaceous sediments have yielded a wealth of data on the organization and structure of early angiosperms. These discoveries are important in the search for character evolution and systematic diversification in early angiosperms. Results obtained so far from the study of Cretaceous angiosperms corroborates to a large extent patterns of diversification obtained from analyses of extant angiosperms using molecular data. However, the fossils also witness of extensive extinction among early angiosperms that cannot be detected studying extant plants alone. In several cases the fossils possess a suite of distinctive characters that are unknown among extant angiosperms, but that may help bridge morphological splits between extant lineages. In other cases the fossils cannot be place in any existing group. We illustrate extinctions and missing links among Cretaceous angiosperms using a number of exquisite preserved key fossils. Focus will be on fossil members of Chloranthaceae and Laurales, but other groups will also be discussed with examples from early diverging eudicots and core eudicots. It has long been suggested that the Chloranthaceae have an extensive geological history extending back to at least the mid-Early Cretaceous. Fossil flowers, fruits, seeds and stamens with pollen in situ particularly from the Early Cretaceous of Portugal have confirmed this suggestion and show considerable diversification very early in angiosperm history with several extinct forms, but also forms that are closely similar to extant taxa. A key fossil among extinct chloranthoids is *Canrightiopsis* that includes several different species. The affinity to extant Chloranthaceae is documented by unique seed coat features as well as details of the tiny embryo and nutritive tissue surrounding the embryo. *Canrightiopsis* is important as a key for understanding character evolution in the Chloranthaceae suggesting an evolutionary link between extinct *Canrightia* and extant *Chloranthus* and *Sarcandra* and a model for the derivation of the tripartite androecium of *Chloranthus*. *Canrightiopsis* co-occur with several other chloranthoid taxa, several of them not described earlier. A new fossil genus with three species under description from the Early Cretaceous is significant for understanding structural and systematic diversity among early members of the Laurales. The fossils show a combination of characters that is unknown among extant or fossil members of the group, but phylogenetic analyses clearly places the fossils in the Laurales. Several features are shared with extant core Laurales such as the presence of a single, pendulous seed and valvate anther dehiscence. In gynoecium features it is particularly similar to extant Gomortegaceae. However, other characters such as dicolpate pollen and continuous tectum are shared with extant Calycanthaceae, the earliest branching lineage of Laurales and the new genus thus provide a link between Calycanthaceae and core Laurales.

Another abominable mystery: Gene-body methylation

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Darwin thought the abrupt origin and rapid diversification of angiosperms to be an abominable mystery. Here I discuss another ongoing mystery within the genomes of land plants: the evolution and function of DNA methylation in coding genes. Plants methylate both genes and transposable elements (TEs). For the latter, the function is clear: DNA methylation suppresses TE activity. The methylation of TEs has corollary effects on the expression of nearby genes, and thus TE methylation has major consequences for the function and evolution of plant genomes. Just as TEs are methylated in plants, so is a subset of genes. Genic methylation was initially thought to be sporadic, but it is now clear that methylation is a conserved evolutionary property of individual genes. These genes tend to evolve slowly, they are expressed broadly, and their methylation is not a property of their GC content. The cause (s) of conservation remain unclear, as does the function of genic methylation, if indeed it has a function. I will present current information about evolutionary patterns of genic methylation, a recent idea about the mechanism of genic methylation, and varied evidence that genic DNA methylation correlates with gene expression. The function and evolution of genic methylation remains mysterious, however, because it has been difficult to separate cause from effect.

Genetic basis of complex traits and heterosis in rice

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Most of agronomically important traits are called complex traits, which are usually controlled by multiple genes and affected by various environmental conditions. Although a lot of QTLs and genes related to rice complex traits have been cloned and functionally characterized, genetic basis and regulatory mechanisms underlying these complex traits are still unclear. We have implemented an integrated approach of genome-wide association study (GWAS) with functional analysis on agronomic traits in a diverse cultivated rice population, which is so called as a CAT-Gs (Catching Agronomic Trait Genes) approach. The associated loci with the agronomic traits such as panicle length, grain sizes, grain weight and grain filling rate can be further characterized through expressional profiling, in-depth genome analysis, transgenic study, genome editing, and population genetic analysis. We believe that allelic genetic variations responsible for the panicle and grain size complex traits can be effectively explored. The genetic basis of these complex traits will be characterized.

Exploitation of heterosis is one of the most important applications of genetics in agriculture. However, the genetic mechanisms of heterosis are only partly understood, and a global view of heterosis from a representative number of hybrid combinations is lacking. We have developed an integrated genomic approach to construct a genome map for elite hybrid rice varieties and their inbred parental lines. We therefore identified that the accumulation of numerous rare superior alleles with positive dominance is an important contributor to the heterotic phenomena. We have further done large-scale genomic mapping for yield related traits and heterotic effects by analyzing over 10,000 rice lines produced from 17 elite rice lines. The large data of genomics and phenomics from the well-designed populations enabled us, for the first time, to identify the genetic contributors comprehensively and find out the exact causes of heterosis. We found that modern rice varieties can be classified into three major types, reflecting the major breeding systems. Within each group a few genomic regions from female parents linked to heterosis effects for improved yields were identified, but these loci varied across the three groups. The key heterosis-related genes often controlled several yield-related components simultaneously, severing as the major contributors of heterosis. For the individual yield components, the heterozygous state of the heterosis-related genes generally acted through the way of dominance complementation. Taking all the components into account, the hybrids with yield heterosis resulted from an optimal combination of multiple yield-related components, meaning better performance of overall yield in crop productions. These results inform on the genomic architecture of heterosis for yield traits in rice, which will be useful information for crop improvement program.

Long-term field experiments and observation data in combination in Inner Mongolia grasslands of China successfully addressed many fundamental questions in ecology

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The typical steppe ecosystem of China is an important part of the vast Eurasian grasslands in the world. With an area of 0.4 $*10^6$ km^2 in China, these ecosystems not only harbor rich biodiversity resources, but also serve as important bases of livestock production and ecological shelters for northern China. However, Inner Mongolia grassland is experiencing serious degradation resulting from overgrazing and climate change. During the last decades, we have conducted intensive studies focusing on the fundamental questions in ecology with collaborators from home and abroad. Through long-term field monitoring, transect surveys and controlled field experiments, we are trying to address the nature of fundamental concepts and principles in ecology includes 1) formation mechanisms of primary and secondary production, 2) biogeochemistry of major elements (C, N, S, P), 3) Coupled cycling of Carbon, nitrogen and H_2O, 4) stoichiometry of C: N: P ratio and its application. We seek to understand how organisms interact with each other and the abiotic environment, and also apply this knowledge to the management of grassland ecosystem, and the services it provides. Both the science of ecology and researchers on the Inner Mongolia grassland have come a long way over the last decades. Today, it is a dynamic international base for scientists with a focus on studying ecology in natural environments.

Ex situ cultivated flora and germplasm discovery for novel crop plants

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There are c. 3,000 botanical gardens in the world, distributed widely across different climatic zones and floristic regions. Most importantly they provide habitats for >120,000 vascular plants in *ex situ* cultivation, accounting 1/3 of known plant species in the world, including many economically important taxa or groups, such Aceraceae, Ericaceae, Fagaceae, Leguminosae, Magnoliaceae, as well as a large number of crop wild relatives, medicinal and aromatic, and horticultural plants. This *ex situ* cultivated flora contains an enormous amount of plant diversity in many well documented living collections in different botanical gardens and plays a critical role in maintaining the security of plant diversity, and in conservation, sustainable agriculture and other related bio-industries.

China has approximately 33,000 higher plants, of which more than half are endemic to China and encompasses a vast number of species of bryophytes (c. 2,200 species), pteridophytes (c. 2600 species), gymnosperms (c. 250 species), and angiosperms (>30,000 species), accounting for 9.1%, 22%, 26.7% and 10% of the world total, respectively. The Chinese flora also represents the living remnants of the early Miocene floras of the whole North Temperate regions and is the source of numerous crops and of medicinal and horticultural plants. There are c. 190 Chinese botanical gardens, harboring 22,375 species belonging to 3,927 genera and 382 families, of which 20,000 species, 2911 genera and 288 families are native to China, accounting for 91%, 86% and 60% of native families, genera and species, respectively. As an example of initiatives to utilize the ex situ cultivated flora to address plant diversity conservation and germplasm discovery for sustainable agriculture and the bio-industries, the *Ex situ Cultivated Flora of China* project aims to catalogue and document this mega diversity of plants that are cultivated in the Chinese botanical gardens.

The initiative has three main goals: 1) To enhance taxonomic research on common garden-based living collections: the morphological and biological data collected from these collections should provide adequate and accurate descriptions for the delimitation of difficult taxa in cases when traditional taxonomic revision has been based only on herbarium specimens; 2) To support comparative biology and frontier plant science research: with increasing awareness of environmental and habitat changes in the overall background of climate change on plant distributions *in situ*, the *Ex situ* cultivated flora program should provide detailed plant biological information from different gardens across a wide spectrum of different latitudes, climates and habitats for research on adaptive evolution of species, plant migration and distribution shifts and physiological or/and biochemical changes, *etc.*; 3) To strengthen germlasm discovery and sustainable uses of plant resources, which should enhance our current ongoing efforts in medicinal plants, industrial bio-energy plants, landscaping and ornamental plants, new functional fruits and vegetables, environment-ameliorating plants, *etc.*

The presentation will provide a comprehensive introduction and well documented case studies. The project of *Ex situ* Cultivated Flora is planned to be one of the most important initiatives of the plant diversity research platform for sustainable economic and social development in China.

Plant growth in a high CO_2 world

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Plant growth needs resources and physical environmental conditions that permit acquiring these resources. CO_2 is a central resource (close to 50% of plant biomass is carbon), hence, elevated CO_2 supply is widely held to enhance plant growth and mitigate growth constraints set by resources other than carbon and by environmental stress. I will present theory and evidence that questions such a simple rationale. It is a trivial fact that carbon can only become incorporated into new biomass to the extent soil nutrients permit, because plants are not made of carbon only. Hence, whenever soil nutrients are short, particularly when neighbouring plants compete for the same resources, there is little leeway for more carbon incorporation. Further, when abiotic limitations such as low temperature or low soil moisture cause soil nutrient shortage and a slowing of meristematic activity (tissue formation), it becomes a question of whether carbon uptake limitations or carbon investment limitations weigh more heavily. The two effects (source limitation *versus* sink limitation) are very hard to separate. At high CO_2, water shortage exerts less of a constraint, for stomatal CO_2 uptake, while at the same time, elevated CO_2 reduces stomatal conductance, thus, causing soil moisture to decline more slowly, also causing nutrients to remain available for a little longer. This non-photosynthetic effect of drought mitigation on growth can be simulated by similarly dosed water addition without any change in CO_2-supply. The effect of such water savings on future plant growth depends on future climate driven moisture regimes. Constraints of growth by low temperature do not seem to get less severe in a high CO_2 world. Alpine and arctic plant growth has been shown to be unresponsive to elevated CO_2. Similar constraints to CO_2 ‘fertilization’ effects apply to ecosystems that have arrived at a natural steady state of the nutrient cycle and canopy density. In summary, the carbon limitation paradigm of plant growth is ill founded when long-term nutrient shortage, low temperature or drought come into play. The simple reason is that under those three conditions, it is not the photosynthetic machinery that sets the long-term limit to plant growth but sink activity (tissue stoichiometry, meristem functioning). Constraints acting upon sink activity are far more challenging to model than those on source activity. In most cases we simply lack appropriate algorithms and parameters to represent sink activity in a mechanistic way. The problem cannot be solved by tuning the output of source activity driven models, because the actual causality is the reverse: the ability to grow new tissue defines the demand for fresh carbon, a demand set by meristems and their direct stoichiometric and developmental controls, and these demands are communicated to foliage through the phloem pipelines. In other words, in all those cases, photosynthesis (source activity) is driven by the rate of growth (sink activity). Sink control over source activity (CO_2 uptake) can only be eliminated by ample nutrient and water supply, as happens under horticultural growth conditions, which do not represent appropriate test conditions for the likely responses of wild plants to elevated CO_2 concentrations in the atmosphere.

Tropical Plant-Animal Interactions: Coevolution in the Anthropocene

W. John Kress

National Museum of Natural History, Smithsonian Institution, USA

The complexity of life on earth is a product of the diversity of species multiplied by the diversity of their interactions. An understanding of species diversity, which requires correct species identification, is a prerequisite to accurately interpreting and understanding their interactions. Investigations of plant-herbivore and plant-pollinator interactions have provided classic examples of the interdependent relationships that exist among species within and across ecosystems. The charismatic tropical Zingiberales, including bananas, birds-of-paradise, heliconias, gingers, and prayer plants, offer illustrative and often surprising insights into the ecology of the animals with which they have coevolved. Specifically, the ecological interactions between colleopteran herbivores and hummingbird pollinators with their Zingiberalean hosts reveal intricate patterns of their evolutionary history and how they may respond to today’s rapidly changing planet. The geographic mosaic of these relationships across tropical islands, fragmented landscapes, and elevational gradients suggests that human-caused habitat alterations, biological invasions, and climate change may significantly modify and disrupt through time and space the historical patterns of ecological interactions. The future of today’s biological complexity in the Age of Humans, in the Anthropocene, remains to be determined.

A Ca^{2+} sensor switch for tolerance to elevated salt stress in *Arabidopsis*

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Excessive sodium (Na^+) in soils inhibits plant growth and development. The Ca^{2+}-activated salt overly sensitive (SOS) pathway formed by the Ca^{2+} sensor SOS3/CBL4 interacting with the kinase SOS2/CIPK24 to activate the Na^+/H^+ antiporter SOS1, represents a core module for regulating Na^+ homeostasis and establishing salt tolerance in plants. In natural conditions, the actual soil Na^+ concentration can vary over a wide range. If and how the intensity of Na^+ stress is reflected by quantitative parameters of downstream signaling components and adjustment of the SOS remains to be investigated. Here, we identify a Ca^{2+} sensor switch that is specifically activated under highly elevated Na^+ stress conditions. We report that Na^+ triggers a primary Ca^{2+} signal specifically localized in root hair differentiation zone of the root from where it expands as a wave. The amplitude of this primary signal rises and the speed of wave propagation accelerates reflecting increasing external Na^+ concentration, thereby providing quantitative information about stress intensity to the plant. Moreover, by analyzing mutant and over-expression plants, we identified the Ca^{2+} sensor CBL8 as crucial for enhanced salt tolerance in *Arabidopsis*. Like SOS3/CBL4 also CBL8 interacted with SOS2/CIPK24 and activated SOS1. While the phosphorylation and conformation of SOS3/CBL4 remained largely unaffected by Ca^{2+}, CBL8 exhibited conformational changes and enhanced phosphorylation with increasing Ca^{2+} concentrations. Together our data identify a Ca^{2+} sensor switch to optimize plant salt tolerance over a range of stress conditions in which the SOS3-SOS2-SOS1 pathway conveys basal and essential salt tolerance, while in response to intensified Ca^{2+} signals, elicited by highly elevated Na^+, in addition CBL8-SOS2 complexes are activated to further enhance SOS1 activity for salt extrusion.

Understanding Kranz anatomy in maize with a view to achieving C_4 rice

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A multi-national effort aimed at introducing components of C_4 photosynthesis into the C_3 plant rice is underway. A fundamental requirement of the 'C_4 Rice Project' is the need to understand how Kranz anatomy develops in the leaves of C_4 plants so that leaf anatomy can be manipulated in rice. I will provide an overview of our understanding of Kranz development, discuss recent research aimed at identifying genetic regulators of Kranz anatomy and illustrate how this research fits into the wider C_4 Rice Project.

The quest for the origin and evolution of flowering plants using time-calibrated plastid phylogenomics

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The angiosperms, i.e., flowering plants, are the most species-rich plant lineage on Earth. Because angiosperms are critical components of nearly all terrestrial habitats, knowledge on their origin and evolution can provide the framework for understanding the history and composition of major terrestrial ecosystems and general patterns of biodiversity. Over the last two decades, molecular phylogenetic studies have greatly advanced our understanding of angiosperm relationships and evolution, as reflected in the latest update to the Angiosperm Phylogeny Group (APG) classification system (2016). However, the composition and relationships of some clades recognized as orders and families have remained weakly supported, with recent studies often based on either sparse gene sampling or low taxonomic coverage, or both. To address this problem, we assembled a large-scale plastid phylogenomic data set of angiosperms by sampling 80 genes from 2881 plastid genomes representing 2349 angiosperm species, 352 families (85% of the described families), and all 64 orders recognized in APG, with 163 species of gymnosperms comprising the outgroups. Our results yielded the diversification of eight major lineages in the early stages of angiosperm evolution. The ANA grade, i.e., Amborellales, Nymphaeales and Austrobaileyales, was confirmed to be the earliest-diverged set of lineages. However, the relationships of the mesangiosperms, including magnoliids, Chloranthales, monocots, Ceratophyllales and the eudicots, remain poorly resolved.

We estimated the divergence times of major angiosperm clades using 63 carefully verified fossils as calibration points. Our results support the origin of the crown angiosperms in the Rhaetian of the latest Triassic ca. 207 million years ago (mya). A rapid radiation of major lineages and orders of flowering plants occurred in the Middle to earlier Late Jurassic and Early Cretaceous. An early radiation may have occurred at the Callovian in the Middle Jurassic (ca. 165 mya) with the origin of three major lineages of mesangiosperms. Major radiations of the monocot lineages occurred in the Late Jurassic (ca. 157 mya) to the Valanginian in the earliest Cretaceous (ca. 133 mya). Core eudicots began to diverge at the Hauterivian (ca. 131 mya), mainly diversifying from the Aptian (ca. 121 mya) to the Turonian-Coniacian boundary (ca. 90 mya) in the Early Cretaceous, with the Paracryphiales and Dipsacales, the most recently diverged orders, at the mid-Campanian (ca. 79 mya) in the Late Cretaceous. Our divergence time estimates are generally congruent with others based on DNA sequence data and are corroborated by the phylogenomic-based estimate for the origin and diversification of major lineages and orders of holometabolous insects. However, they are significantly earlier than unequivocal fossil records of flowering plants. This may be attributed to the fact that even angiosperm-like pollen records from the Jurassic are spare and are thus difficult to find. The lack of resolution among mesangiosperms in our analysis may result from rapid radiation and extinction in the early stage of angiosperm evolution, particularly during the Jurassic.

Nuclear phylogenies of angiosperms at both deep and family levels: Implications on divergence times, morphological evolution and diversification rates

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Angiosperms are the most successful plant group, with an estimated total of over 350,000 species. Angiosperm phylogeny is a

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foundation for a variety of other studies, including evolutionary biology and ecology, comparative genomics, and translational agriculture and has been examined using chloroplast sequences for many years. Rapid divergences of multiple lineages, frequent whole genome duplications and other factors have posed major challenges to the resolution of angiosperm relationship. Recent advances in DNA sequencing have availed large quantities of nuclear gene sequences, greatly facilitating the acquisition of needed marker information for addressing difficult questions in angiosperm phylogeny. We have used transcriptome sequencing and other approaches to obtain dozens, even hundreds, of nuclear gene sequences, and performed multiple phylogenetic analyses of major deep lineages. These investigations have yielded highly supported relationships that are largely in agreement with chloroplast sequence-based phylogenies; at the same time, the new hypotheses according to nuclear genes support well-resolved relationships for taxa whose relationships were unclear before, illustrating the effectiveness of nuclear genes. Well-resolved relationships include five deep lineages of the mesangiosperm clade representing nearly all angiosperms and several major branches of core eudicots. We have also performed phylogenetic analysis of several groups including Brassicaceae, Asteraceae, and Rosaceae, and will summarize recent results from these analyses, and discuss their implications on the divergence times of relevant groups, reconstruction of ancestral character of key morphological features, and shifts in rates of diversification during angiosperm evolution.

Mapping Asia plants

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Because of the huge population and rapid growth of economy, biodiversity including plants are being seriously threatened in Asia. Both conservation and sustainable use of biodiversity need species distribution data. Asia is the continent with highest diversity of plants. However, the information of plant taxonomy and distribution in Asia are less available and more scattered, compared with other continents such as Europe and America. So, a better infrastructure for plant distribution in Asia is important. In 2015, Asia Biodiversity Conservation and Databases Network (ABCDNet) started a project entitled Mapping Asia Plants for cataloguing species of plants and collecting distribution data from the following aspects: 1) specimens, 2) observations, 3) species checklists, 4) expert maps, 5) inventories, 6) vegetation plots, 7) literatures and 8) on line datasets. Major achievements so far are as follows. 1) for China, national higher plant checklists including 35,835 species, 9 million records of occurrence; 2) for Southeast Asia, 120 thousands species names, 1.3 million records of occurrence; 3) for north Asia, 6,900 species of higher plants and their distribution in 13 sub-regions. Some of the data collected are shared with the websites of ABCDNet (www.abcdn.org/).

Thirty clues to angiosperm exceptional evolutionary diversification

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Angiosperms are the cornerstone of present-day terrestrial biomes, but little is known about the macroevolutionary processes underlying their diversification. A great controversy exists regarding the time when extant angiosperms lineages originated, and many questions remain regarding their evolutionary expansion: What is the phylogenetic position and temporal distribution of radiations leading to extant megadiversity? Are these radiations concentrated in particular phylogenetic regions or times? Are all angiosperm lineages radiating, or are some undergoing evolutionary depletions? Has a limit to angiosperm evolutionary expansion been reached? To investigate the absolute timing of angiosperm crown origin and lineage diversification, we integratively consider molecular sequence data of living species and information from the fossil record. Because sampled extant species with molecular data and fossils that provide absolute times are part of the same evolutionary process, we estimate divergence times and diversification parameters across angiosperms with the Fossilized Birth Death (FBD) Process, a model that estimates the rates of speciation, extinction, fossilization, and sampling probability of extant species, as a prior for divergence time dating. We contrast FBD age estimates for the origin of angiosperms with those derived from relaxed molecular clocks, and from two previous estimates derived from quantitative evaluations of the angiosperm fossil record. As the FBD directly incorporates information from the fossil record, it has a great potential for accurately estimating diversification and relative extinction parameters for angiosperms as a whole. To investigate radiations within angiosperms, we implement a Bayesian method (BAMM) that allows to identify significant diversification shifts across phylogenetic branches and through time. We conduct six alternative analyses that differ in the magnitude of the prior for the expected number of diversification shifts across the tree, and provide both technical and biological results. In all analyses, the posterior distribution of the number of shifts differs substantially from the prior. Moreover, a set of thirty diversification shifts highly supported by the data were congruently identified across the six analyses. Biologically, the placement of core diversification shifts suggests that extant angiosperm diversity is the result of several independent and partially overlapping radiations, interspersed with a few evolutionary depletions. Radiations are phylogenetically scattered and took place over a substantial length of angiosperm evolutionary history. Estimates of their long-term diversification trajectory suggest that angiosperm species accumulation has not reached a limit, but species turnover is increasing. The finding that several diversification shifts are distributed on two or more adjacent phylogenetic branches is congruent with the possibility that underlying causes of diversification may derive from synergic interactions among biological, ecological and physical factors.

How do plants age and die? – Some new insights

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Aging followed by death is an inevitable fate in most organisms including human. Our group has been studying the molecular mechanisms of aging and death over two decades to solve the question 'How does living organisms know when and how to die?' We have been revealing that aging and death in plants are highly regulated at the chromatin, transcriptional, post-transcriptional, and epigenetic levels. In this presentation, we will report some new insights on the regulatory mechanisms of aging and death in plants with a comparative view on aging in animals.

Aging involves transitions of complex regulatory networks across lifespan. Yet, limited is understanding of causal relationship between age-associated networks and aging processes. Here, we built age-evolving causal regulatory networks of *Arabidopsis* NAC transcription factors across lifespan using mutants of 49 age-associated NACs. These age-evolving networks revealed a regulatory inversion at pre-senescent stage from activating to repressive regulatory modes, which was governed by three hub NACs that negatively regulated key pro-aging pathways, ROS and salicylic acid pathways. Knockout and overexpression of the three hub NACs (the NAC troika) delayed and advanced the regulatory inversion, respectively, rendering earlier and delayed aging. Thus, the network transition by the NAC troika at pre-senescent stage determines initiation and rate of the following aging process in *Arabidopsis*.

Meristems are small population of pluripotent stem cells located at shoot and root tips of plants. They have remarkable ability to maintain themselves for a long period and serve as the constant source of cells for newly developing tissue and organs, allowing the long lifespan often observed in many tree species. We are striving to reveal the mechanisms behind the long lifespan stemmed from the long-time maintenance of meristem cells, employing the root apical meristem (RAM) as a model. *Arabidopsis* RAM consists of mitotically inactive quiescent cells (QC) and surrounding active stem-cells. While active stem-cells are susceptible to various stresses, QC cells are highly stress resistant. We have found that *ORE15* has important role in maintenance of RAM. We will report the underlying mechanisms.

Light is one of the most important environmental factors which affect plants growth and development. The critical roles of red light through phytochrome-mediated light signaling has been reported recently. We examined the role of far-red light on leaf senescence and found that leaf senescence is modulated by red (R): far-red (FR) ratio (R/FR). We also found a strong correlation between FR-responsive leaf senescence of various ecotypes and latitudinal cline, suggesting that FR signal is used for latitudinal adaptation of *Arabidopsis* ecotypes. 'Climatome' analysis using climate information around ecotypes' habitat revealed that temperature in winter and spring is strongly associated with FR-responsive leaf senescence. We will report a possible mechanism on how *Arabidopsis* adapted to latitude-dependent environmental changes.

We are also comparing the regulatory mechanisms of aging between plants and animals. We will report the differential roles of tRNA fragments in regulating aging in *Arabidopsis*, *C. elegans* (worms), and mouse.

Phytochrome photosensory signaling and transcriptional networks

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Plants constantly monitor the ambient light environment for signals that enable them to adapt to the prevailing conditions. The phytochrome (phy) family of sensory photoreceptors plays a central role in this process. Light absorption induces conversion of the phy molecule to its active Pfr conformer which then migrates rapidly into the nucleus where it induces expression changes in target-genes within minutes. Photoactivated phys signal to their target transcriptional network by binding to, and inducing the phosphorylation and degradation of PIF transcription factors (phy-Interacting Factors), thereby evoking these changes. We have been examining the mechanisms underlying the signaling transactions at both the phy-PIF and PIF-genome interfaces in this process. Previously, we have shown that signaling requires direct binding of the phy Pfr conformer to the PIF protein, that this results in multisite phosphorylation of the bHLH factor, and that this in turn leads to polyubiquitination by Cullin3-BTB E3 ubiquitin ligases (called LRBs), and, consequently, proteasomal degradation. Recently, we have identified a small family of Photoregulatory Protein Kinases (PPKs) that are collectively necessary for normal phy-induced PIF3 phosphorylation in vivo. These kinases are recruited by photoactivated phyB to a nuclear-localized trimolecular complex with PIF3, where they catalyze multisite PIF3 phosphorylation. This discovery fills a longstanding mechanistic gap in our understanding of the short transduction process from the light-activated photoreceptor to transcriptional regulation. We have also identified a unique negative feedback signaling-attenuation mechanism, directly at the phy-PIF interface, whereby the phy-induced phosphorylation of, and LRB recruitment to, the PIF molecule, results concurrently in ubiquitination and degradation of the interacting phy molecule in a mutually-assured destruction (MAD) configuration. This coordinated PIF and phy degradation thus results concomitantly in both transcriptional regulation of target genes and immediate feedback attenuation of signaling intensity. Using genome-wide transcriptome analysis, we have identified PIF-regulated genes that respond rapidly to phy photoactivation, and using integrated ChIP-seq and RNA-seq analysis, we have identified a diverse network of these genes that are direct targets of PIF-quartet (PIF1,3, 4 and 5)-regulated transcription. Moreover, the evidence unveils an intriguing dual-layered mechanism of regulation, whereby both the level of promoter-binding-site occupancy, and in situ modulation of bound-transcription-factor intrinsic activity, together generate a complex matrix of shared, but quantitatively differential, gene expression patterns, under the control of the phy-PIF signaling pathway.

Endocytosis and the regulation of signaling in plants

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Receptor-mediated endocytosis is an integral part of signal transduction, because, besides signal attenuation by removal of activated receptors and their bound ligands from the cell surface, it allows the spatial and temporal regulation of the signaling outputs from the endosomes. After receptors have been activated and internalized, they can be separated from their ligands and recycled back to the plasma membrane or transported for degradation. Crucial in the study of the interplay between endocytosis, recycling, and signaling of plant receptor kinases is the development of imaging tools (bioactive fluorescent probes) to visualize membrane-associated signaling events at a high spatiotemporal resolution. Recently, we visualized endocytosis of different receptor kinases in living *Arabidopsis thaliana* cells using fluorescent small-molecule and peptide ligands. The bioactive fluorescent probes together with genetic, biochemical and pharmacological analyses revealed distinct dynamics of endocytosis and differences in regulation of signaling outputs.

Mobilizing and integrating big data in analyses of phylogenetic and spatial patterns of biodiversity

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The pace of biological discovery is staggering in this era of “big data.” We are at a crucial juncture when it is more important than ever to mobilize and integrate massive amounts of data to address fundamental issues in the study and preservation of biodiversity. The enormous challenges presently faced in biodiversity science require novel approaches that not only employ massive data sets, but also combine bioinformatics, phylogeny reconstruction at an unprecedented large scale, use enormous amounts of digitized specimen data, as well as large, highly complex, multi-faceted down stream phylogenetic analyses. These post-tree analyses include niche modeling, niche diversification, and ancestral niche reconstruction, as well as other ecological analyses. Recent tool development in multiple disciplines including phylogenetics, ecological niche modeling, combined with rapidly developing, powerful cyberinfrastructure coupled with the availability of multiple new data sources afford unique and powerful opportunities. Together these resources provide novel possibilities for mobilizing and integrating enormous quantities of biological, geological, climatic and other related biodiversity data, ultimately facilitating the discovery of complex patterns and new hypotheses for further study. These multifaceted and interdisciplinary developments are major advances--biodiversity data on the global scale now being collected and analyzed are inherently complex. This tool integration is transforming what we broadly term biodiversity science, enabling numerous new research opportunities that we refer to as “next-generation” investigations in biodiversity science that couple systematics, ecology, and evolution. The growth in tool building and use also demands new training for our students that integrates domain knowledge in biodiversity as well as data science skills –such connectivity and training is needed to accelerate biodiversity research. This next generation Integrative biodiversity science is of crucial importance--the future of global biodiversity will be greatly impacted by these efforts. We as researchers cannot continue to simply react to continued worldwide threats to biodiversity. In contrast, through the use of integrative, multifaceted research relying on big data, we now have the ability to make biodiversity projections—we can now provide crucial data to scientists, as well as to the public, land managers, policy makers, urban planners, and those in agriculture.

Thinking through the e- in e-Floras; or, Floras old, new, and not-yet

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When faced with a disruptive new technology such as the World Wide Web, it often takes a lot of work, and time, to escape conventional ways of thinking through a problem and to fully realise the implications of the new. So-called e-Floras are no exception. How much does the e- in the name really reflect a new way of approaching a Flora’s core task (to communicate authoritative, synthesised taxonomic knowledge to as wide an audience as possible in the best way achievable)? If modern taxonomy and systematics were invented, or re-invented, now (in the age of the internet, social media, citizen science and the block chain), rather than in the 18th Century, would we do it all differently? And, what near-future disruptive technologies and paradigms do we need to be aware of when thinking about, or planning, an e-Flora project? Taking the new e-Floras of Australia and New Zealand as starting points, this talk will explore current and future thinking about e-Floras, and map out some ideas for, perhaps, the next wave.

Developing integrative systematics in the informatics and genomic era

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Systematics is the science of discovering, organizing and interpreting the diversity of all living organisms on Earth. Recent developments in genomics and biodiversity informatics are transforming systematics and have opened up many new opportunities. Major digitization efforts have made available large amounts of biodiversity data. Developments in biodiversity informatics in the last decade have helped the systematics community explore ways to enhance the efficiency in organizing, publishing, and utilizing systematic information. We are now constructing the tree of life and classifications at various levels using information from hundreds to thousands of genes and sometimes the entire genomes. The new data and tools are bringing systematics into an exciting age of new discovery and interpretation of biodiversity and its assembly. Integrative systematics in the informatics and genomic era needs to be developed with biological collections continuing to serve as the foundation of the field. Discovering, documenting and analyzing the patterns and processes of biodiversity remain the core of systematics, yet the community needs to enhance the efficiency of systematic research and practices with user-friendly pipelines. We especially call for new efficient workflows for taxonomic revisions, monographs and floras, that utilize both the traditional strengths of synthesizing diverse collections-based taxonomic data and the capacity of online resources and biodiversity informatics tools. A new global community-wide cyberinfrastructure, Biodiversity Cyberbank, comparable to GenBank for genetic data, needs to be established as the repository of all biodiversity data, as well as the means to effectively organize and disseminate systematic data to biologists and the public, to ensure the long-term sustainability of the vast biodiversity data. Integrative systematics requires the training of the next-generation of systematists with integrative skills, to address grand questions about biodiversity and its assembly at different scales. Finally integrative systematics must proactively educate the public and policy makers on the importance of systematics and collections in the biodiversity crisis of the Anthropocene.

Establishing New Botanic Gardens in Indonesia: An ecoregion approach

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The development of new botanic gardens has been included in the 2015-2019 National Priority Programs and the Indonesian Biodiversity Strategy and Action Plan (2003-2020). Within international contexts, the development of new botanic gardens is in line with the global commitments such as the Convention on Biological Diversity and the target VIII of the Global Strategy for Plant Conservation. The ecoregion concept has been applied in the new botanic gardens development process in order to save and manage effectively the plant diversity occurred in different ecosystem types. This strategy should not only be valued as a protection effort of plant diversity per se, but also considering the need for empowering local communities. By conserving the plant diversity in their own habitat characteristics we can expect higher success, particularly for endemic, threatened species. This strategy is also less costly because the collected-selected plants are brought to and managed in the closest, most suitable gardens. Since Indonesia consists of 47 different ecoregions, at least one botanic garden should be located in each ecoregion in order to conserve the representatives of the Indonesian plant diversity. By the end of 2016, there have been 30 botanic gardens both developed and developing gardens, located in 17 (out of 47) different ecoregions. The implementation of the Indonesian Presidential Regulation No. 93 in 2011 has strengthened the development phases of the new botanic gardens by engaging the Ministry of Public Works and the Ministry of Internal Affairs. The establishment of new desirable botanic gardens and the relevant roles to conserve plant species ex situ will serve the public needs, provide fruitful visitation, as well as create social and economic benefits. Social welfare is indeed a key factor that must be taken into account if botanic gardens are to succeed in their goals of plant diversity conservation and the sustainable utilization.

International developments and responsibilities for the botanical community in plant conservation

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The development of the Global Strategy for Plant Conservation (GSPC) through the U.N. Convention on Biological Diversity can trace its origins back to a resolution of the International Botanical Congress held in St Louis, Missouri in 1999 and its 2002 adoption by the international community. Since then the GSPC has been of fundamental importance not only to provide a strategic framework for plant conservation undertaken by the world's governments, primarily within the context of the U.N.'s Strategic Plan for Biodiversity and its related Aichi targets, but also a guiding document used by numerous botanical institutions worldwide. The 16 targets of the GSPC are wide-ranging and highlight goals that are due to be achieved by 2020. These goals relate to understanding and documenting plant diversity, conserving and using plants sustainably, as well as public education and building capacity for plant conservation throughout the world. The strategy has had a transformative impact on many botanical institutions, particularly botanic gardens, helping to consolidate conservation as a primary purpose for many.

In this presentation, ways in which individual institutions have been contributing towards the achievement of the 2020 goals will be outlined. Examples from the Missouri Botanical Garden will be included where its most recent strategic plan has made a formal commitment to contributing to the GSPC through research in conservation biology, ecological restoration, community conservation and education programs, horticulture and ex situ conservation, as well as building conservation capacity with institutional partners worldwide.

A widely accessible Flora of all known plant species is a fundamental requirement for plant conservation. Without it, we have no framework to assess the diversity of plants or the threats they face on a worldwide scale. The creation of such a Flora was included in the GSPC as its first target. Subsequently, in response to this target, a World Flora Online (WFO) project was launched in 2012, which is now guided by an international WFO Consortium, involving almost 40 botanical institutions and organisations involved in plant systematics. The progress in the development of the project since 2012 will be outlined, including the preview of a new WFO portal that is planned to be available at the Congress.

The likely future of plant conservation beyond 2020 will be reviewed when greater integration between biodiversity conservation and sustainable development can be anticipated, within the context of the achievement of the U.N.'s Sustainable Development Goals for 2030.

Big data in plant genomics to connect nature-derived evolutionary innovations with future precision agriculture

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DNA is the basic source code of life, connecting nearly all species in this planet during the past billions of years that produced magnificent biodiversity and remarkable innovations. To study the crucial role of plants in ecosystems, medicine development, and resources in food production and nutrition, big data of plant genomics by large-scale species sampling along the entire evolutionary line is an unprecedented but powerful way to understand the fundamental genetics and evolution. To achieve this goal, sampling and digitizing various non-model plants particularly by genome sequencing technology is the key, further to manipulate or edit/synthesize new life forms (or natural products) that are beneficial to human healthcare. From our pilot studies with careful scientific designs in algae, early land plants, and angiosperms that with innovative traits of interest, we have gained extensive novel insights in evolutionary forces that drive genome complexity and adaptation. However, all of these efforts are just the beginning, data is still far from enough, and our understanding to life is still much limited. Sequence most if not all of the species on this planet should be the final approach (every species/data is valuable indeed), just like what we have been working on the 'plant tree of life' project and even for the 'digitize the planet' project. All of which have been facilitated by the recent development in sequencing technologies (like BGISEQ-500/50 platform), through extensive global collaborations following a Data-Knowledge-Application mode in BGI. Through big data study of plant genomics, we have the opportunity to connect all valuable data/species together, explore biodiversity and gain inspirations from nature, and thus design 'super species' that are nutrition-rich and production-effective, and further guide future precision agriculture.

Diversity of tropical forests in South East Asia under the threat of rapid loss

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In the world, 60,065 species of trees have been recognized (Beech *et al.,* 2017) and tree species richness is the highest in tropical rain forests of Brazil and SE Asia. Tree species richness in SE Asia is, however, largely underscored because taxonomic inventories of trees in SE Asia remain rather incomplete and thus many new species have been described every year. On the other hand, tropical rain forests of SE Asia are rapidly converted to plantations at the rate of 0.36% per year. Therefore, we need to speed up taxonomic inventories of tropical trees.

To obtain more accurate documentation of species richness in SE Asia, we have carried out a series of field surveys in 35 locations of SE Asia where we placed a total of 136 plots of 100 m x 5 m and collected 32,015 samples of the vascular plant species found in or near each plot even for sterile plants. To identify those collections, we used DNA sequences of three regions widely used for plant DNA barcoding: rbcL, mat K and ITS. However, those regions did not always give sufficient discrimination of closely related species and thus sequencing of three regions for more than 30,000 samples is not cost-effective.

A new DNA barcoding using MIG-seq, a recently developed method of genome-wide SNP genotyping with the next generation sequencing platform (Suyama and Matsuki 2015) enables us to discriminate closely related species more efficiently. We applied this method for some genera of Lauraceae, the most species-rich family in SE Asia. Consequently, we discovered at least the following 104 new species; 7 of *Actinodaphne*, 20 of *Cinnamomum*, 10 of *Cryptocarya*, 42 of *Machilus* and 25 of *Neolitsea*. There are more new species, possibly 100 more, in other genera including *Alseodaphne*, *Beilschmiedia*, *Dehaasia*, *Lindera*, *Litsea* and *Phebe* on which taxonomic studies are in progress.

A reliable estimate of the proportion of new species is available in Mt. Bokor, Cambodia where we published the tree flora as a Picture Guide and enumerated 770 species of 108 families. Among the 770 total, 65 species (8.4%) were undescribed; the family having the richest new species was Lauraceae in which 11 of 33 species (33.3%) was undescribed. Our 32,015 specimens from 35 locations of SE Asia include approximately 24,000 species among which ca. 2,000 species (24,000 x 0.084 =2016) would be undescribed. Because the cumulative number of species we collected are still linearly increasing, this is likely to be underestimated.

New species are often found as one or a few individuals in disturbed and fragmented stands of lowland forest. Considering the rapid loss of tropical forest in SE Asia, many species may be becoming extinct before those are collected and described. We need more intensive efforts to discover and describe species in tropical forest of SE Asia and the method we employed provide an efficient way of responding to this need.

Sequence assembly, evolution and functional application of the cotton genomes

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We used the highly homozygous *G. hirsutum* (AtDt, $2n = 52$) genetic standard line TM-1 for DNA sequencing. We crossed a tetraploid *G. barbadense* cv. 3-79 with TM-1 to obtain 167 RIL individuals to construct the high-density genetic map. To successfully assemble the genome, we combined 180-fold whole-genome shotgun (WGS) sequences with a total of 100,187 BAC end sequences. Repeat sequences accounted for 57% of the D genome, 68.5% of the A genome and 67.2% of the AtDt genome. The AtDt genome displayed high degrees of conserved gene orders with the two diploids. Insertions of long terminal repeats (LTR), especially *Gypsy-type* retrotransposons, seems responsible for the two-fold size differences between the D (775.2 Mb) and A (1,694 Mb) genomes. Transposable elements (TEs) originated from Dt are more active than those from At. In-depth genome-wide analysis helped to resolve the puzzle with regard to gossypol biosynthesis and ethylene production in various cottons. The expression patterns of a large number of functional genes, especially both primary cell wall-specific and secondary cell wall-specific CesA or CesA-like (CSL) genes, are significantly different in the two subgenomes, suggesting that genome variation, caused likely by TE, especially by retrotransposon insertion, may play a crucial role during the concerted cotton genome evolution.

General Symposia

Theme	Symposium numbers
1: Biodiversity, Resources & Conservation	T1-01~T1-38
2: Taxonomy, Phylogenetics & Evolution	T2-01~T2-68
3: Ecology, Environment & Global Change	T3-01~T3-26
4: Development & Physiology	T4-01~T4-39
5: Genetics, Genomics & Bioinformatics	T5-01~T5-33
6: Plants & Society	T6-01~T6-15

THEME I: BIODIVERSITY, RESOURCES & CONSERVATION

T1-01: Developing priorities for the conservation of Karst flora (two sessions)

T1-01-01

Characteristics of carbon cycle and the effect of carbon sink in karst ecosystem

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Carbonate rock is the basic material of karst ecosystem, and its formation in geological era has an important role on the atmospheric CO_2 decreasing, about 99.5% of the carbon in the earth is sealed in carbonate rock; Karst ecosystem is characterized as rich calcium, alkaline, impacting carbon cycle: carbonate rock is the sedimentary rock deriving from clean ocean, its acidic insoluble matter is usually less than 10%, which lead shortage of the soil resources in karst area, therefore the total amount of limestone soil carbon in karst area is small. The high calcium makes more organic carbon stocked in shape of the slow and recalcitrant carbon pools, and improves the stability of limestone soil organic carbon. The proportion of underground biomass of vegetation in karst area may increased, because of shortage of soil resources, nutrients and water resources, the ration of underground/above vegetation biomass in the karst can be got to 30-50%, higher than non-karst area. The high content of HCO_3^- in karst water stimulates the photosynthesis of aquatic plants, inorganic carbon can be converted to organic carbon, this process improves the stability of the carbon in water flow. This paper try to clarify the state, migration and transformation of carbon in the interface of system of carbonate rock-soil-vegetation-atmosphere- water in karst ecosystem, meanwhile put forward the approaches to promote karst processes and carbon sequestration.

T1-01-02

Rooting depth determined with caves and DNA, and implicating water use under karst terrain

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The solute carbonate rock of karst in south China develops an epikarst zone with a high fissure and rich spatial structure underground, which is favor for root growth and extend to deep karst layer. Belowground vertical community composition and maximum rooting depth of karst terrain in three sites of Guangxi, China were determined by using DNA sequence variation to identify roots from caves 0–40 m deep. 21 sample sites including 15 sites at Guilin secondly forest, 3 sites at Nonggang national park primary forest, and 3 at Mulun National Park primary forest, were investigated. Roots from caves were identified by comparing their DNA sequences for the internal transcribed spacer (ITS) region of the 18S–26S ribosomal DNA repeat against a reference ITS database and above cave ground species. The result presented root belong to 7 species extend to 10m, and two species to 29m. The result of stable isotope δD and δ18O analysis showed that water uptake by tree species *Cyclobalanopsis glauca* sourcing mainly shallow to depth relevance with wet to drought season, less source was from ground water. Even in the driest October, only 20% water use was from ground water. However, soil moisture did not change greatly except subsoil which was influenced by evaporation; meanwhile transpiration in March, July and October was fairly high. These result may implicate soil water must be supplemented by other source besides rain water. Consequently, combining root depth result, which rooting of *C. glauca* could be found at 5-10m and reaching to cave water, suggesting there would be the hydraulic lift by root which transport water from deep epkarst fissure water or ground water and deliver it at soil.

T1-01-03

Developing priorities for karst conservation

Alice Hughes

Xishuangbanna Tropical botanical garden, Chinese academy of sciences

Karsts represent a challenging ecosystem for conservation. With almost unparalleled levels of endemism, and an estimated 90% of cave fauna undesribed in some countries they represent priorities for further research. However growing demand for cement in many regions, and especially parts of Southeast Asia where accelerating cement demand caused China to use 6.6 gigatonnes in just a two year period, exceeding US historical construction and leading to an exponential increase in the demand for cement and consequently the increasing destruction of limestone karsts to meet that cement demand. Thus to ensure karst diversity is adequately protected, we require both an understanding of hotspots of biodiversity, and how karst landscapes can be effectively managed to maintain karst ecosystems. Here we review a recently initiated project which sets to do that. By initially developing methods to map karsts, then develop stratified inventories of karst biodiversity, hotspots of endemism and also evaluate how landscape configuration changes the microclimate and species present on karst ecosystems. We discuss how such stratified approaches can be implemented and scaled across the landscape to generate standardized information on karst biodiversity and understand how to manage karts and develop effective priorities to account for diversity and endemism. Given the almost unparalleled endemism in these systems it is essential that we rapidly develop priorities that most effectively protect biodiversity in these challenging systems.

T1-01-04

Genetic diversity and structure of *Primulina renifolia* (Gesneriaceae), an endangered Karst cave plants

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2. School of Resources and Environmental Engineering, Anhui University
3. Gesneriad Conservation Center of China (GCCC)

We used 10 polymorphic microsatellite loci from transcriptome analyses data to assess the levels of genetic diversity and structure for 24 individuals from 3 populations in the type location. The result of genetic diversity showed that 63 alleles were identified across the ten microsatellites analysed while 3-10 alleles, as the mean value of 6 alleles, per locus. The mean number of alleles per locus (MNA) in all individuals was 6.3± 2.16; the values of observed heterozygosity (H_O) and expected heterozygosity (H_E) were 0.702 and 0.634 respectively, and *FIS* <0 showed few inbreeding. *Fst* result indicated a wide genetic variation between the No. 1 and No. 3 populations, however, no significant genetic differentiation were showed on No. 2 population, which were in the geographic middle position to other two populations; *Fst* analysis (0.0413) and AMOVA result supported small significant result as 76% of the total variance to the within-individuals component based on the microsatellite data, and the PCoA and structure analysis indicated that there just one clusters in total. Bottleneck analysis revealed that there was no historic bottleneck in these populations under TPM model, while Mode-shift test showed all the alleles form all populations are the L-shape.

T1-01-05

Conservation and biological implications of a diverse vascular plant flora in karst caves of SW China

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3. The Natural History Museum, London

Despite scientists long fascination with caves their diversity remains poorly documented, studied and understood with respect to both mineral and biological diversity. Alexander Von Humboldt was the first to document non-vascularized plants in caves in the 18th century. Surprisingly no vascular plant floras for caves have been documented since, despite several species having been documented as new to science and some investigation into adaptations of vascular plants to the low light environments in caves. Whilst documenting the karst flora of SW China field observations lead us to test the following hypotheses, 1) SW China caves contain a diverse flora, 2) which is a relic of a largely absent forest type lacking endemic species and, 3) that the light environment plants occupy is not distinct from that in forest understory. We surveyed 63 caves and used species accumulation curves (SAC) to estimate the total diversity of this flora. We evaluated the habitats and conservation value of the species documented using regional floras and existing species conservation assessments. We used a subsample of 14 caves to characterize the light environment. Four-hundred-and-eighteen vascular plant species were documented. SACs predict a total diversity of 529-846. Ninety-three percent are known from karst forest, 7% are restricted to caves. The proportion of threatened species is similar to that for the region. Forty-five percent of caves surveyed were disturbed. We documented a subset of species growing in the lowest light levels recorded for vascularised plants. We suggest that this flora is an extension of karst forest understory present prior to catastrophic deforestation and that in SW China caves serves as a refuge and germplasm source. We propose that caves represent a distinct plant biome, most similar to forest understory, differing in the absence of trees, leaf litter, root mats and low diurnal and annual variation in temperature and humidity, and elevated levels of CO_2. We highlight desertification, tourism and the absence of legislated protection of caves as threats and predict that plant-bearing caves are likely occur elsewhere in SE Asia.

T1-01-06

Patterns in karst colonisation by *Elatostema* (Urticaceae) inferred from a molecular phylogeny suggest roles for genome size, seed volume and range-size

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Elatostema is one of the most species-rich genera in the Urticaceae, encompassing ca 550 species that are distributed in Africa, Madagascar, Asia and Australasia. The centre of species-richness is in Asia. There are ca >200 species adapted to karst in SE Asia. In China, 184 species, 65% of the total diversity are associated with the SW China karst leading Wang (2014) to propose that the karst of this region is the centre of distribution and diversification for the genus. Karst is characterized by shallow soils deficient in N and P, but with excessive Ca and Mg and prone to seasonal droughts (Hao *et al.,* , 2015). Soil N is a limiting factor for genome size in karst (Kang *et al.,* 2015). This extreme habitat is associated with high species diversity and endemism for many groups of organism (Clements *et al.,* 2006; Schilthuizen & Clements, 2008). It is also very sensitive to deforestation which frequently results in desertification and the loss of this associated biodiversity. Adaptations which facilitate karst colonisation could include those which mitigate the impact of low N, high Ca, high Mg and water stress. In *Primulina* (Gesneriaceae), genes associated with calcium-permeable channels have been demonstrated to be under positive selection pressure in karst species of Tao *et al.,* (2106). Other possible adaptations to karst could include seed-volume (low N & P), small genome size (low N) and others associated with drought tolerance such as cyclic electron flow in photoprotection, pigments, osmotic adjustment and antioxidant enzymes (Liu *et al.,* 2011; Huang *et al.,* 2012). We use a phylogeny of 120 *Elatostema* species from SW China and SE Asia to explore patterns of karst colonisation within the genus. For all species we recorded the substrate on which they occur (karst versus non-karst) and their range size. For species in SW China we also measured genome size and ploidy level using flow cytometry and seed-size using SEM. Our results show contrasting patterns of speciation on karst between the basal clades and the most species-rich '*core Elatostema*'. By mapping a suite of characters on our tree we identified genome size, range-size and seed volume as being correlated with the colonisation of karst by *core Elatostema* but interestingly not for the basal clades of the genus. This has lead us to develop a series of hypotheses to explain karst colonisation and its consequences for range-size and species number that if confirmed will be important for the design of conservation strategies.

T1-01-07

Plant community retrogression leads to functional homogenization: Implications for Karst desertification

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Effects of ecosystem retrogression on functional diversity are poorly studied. Retrogression is generally characterized by a loss of biomass, structural diversity and taxonomic diversity (TD). It has not been studied in the context of functional diversity (FD), which can affect community assembly and ecosystem function. Transformation of black forest into *Kalmia* dominated heath in eastern Canada has parallel to shrub-dominated karsts in southern China. We hypothesized that post-fire *Kalmia* heaths possess lower TD than forest communities and heath formation selects for a narrow range of species traits that can tolerate habitat stress resulting in biotic homogenization. We tested the hypothesis by i) assessing overall alpha and beta diversity of post-fire heath and forest communities and also individual life form groups to determine if ecosystem retrogression results in a loss of biodiversity or biotic homogenization and ii) comparing FD metrics and plant traits to null models to detect community assembly mechanisms. We measured TD and FD in nine heath and four forest chronosequences in Newfoundland, Canada. We detected significant loss of alpha and beta diversity for both TD and FD. A loss of functional alpha diversity for trees and herbs in heaths indicates a filtering or exclusion of particular traits. Functional beta diversity was lower in heaths than forests overall as well as for trees and herbs, indicating that retrogression through heath formation results in functional homogenization of the majority of life forms. Individual traits of three life forms either converged or diverged in both communities but to different extents. We conclude that heath formation leads to biotic homogenization in retrogressive succession through filtering of traits and that assessment of FD can better reflect ecosystem states of post-fire plant communities than just TD. Our results have implications for any long-lasting simplified vegetation resulting from ecosystem disturbance such as degraded karst vegetation after anthropogenic disturbance.

T1-01-08

Profiling elevational diversity patterns between microbes and plants in a typical karst Tiankeng of China

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Karst Tiankeng, being discovered and named at the end of the 20th century in karst areas of China, has enjoyed increasing attention owing to its unusualgeologic processes, unique ecological communities and high-value for conservation and tourism in recent years. In such unique ecosystems, the distribution and composition of plants and animals in karst tiankengs are quite different from related external environments. Correspondingly, microorganism communities in karst tiankeng ecosystems may greatly differ from the related external environments since soil microorganisms and plants are dependent on each other. In this study, we examined the microbial abundance and composition in Shengmu Tainkeng, a typical mestone tiankeng of China. The results reveal that there are strong site differences in microbial community composition (including Bacterial and Fungi). However, diversity of the total microorganism community did not correlate with elevation. By contrast, as expected, plant diversity at the same sites declined along our elevational gradient. These results together suggest that elevational diversity patterns exhibited by eukaryotic microorganisms are fundamentally different from those of plants in Tiankeng ecosystems.

T1-01-09

Fossil records from Southwest China reveals a long existence of Karst vegetation

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Karst region occupys a large proportion of China's land area and is especially in in the Southwest China. Its vegetation ecology is very fragile and difficult to recover after over land-use, and even develops into "rocky desertification". The Wenshan and Maguan basins in southeastern Yunnan Province are Cenozoic sedimentary basins in the karst plateau, a large number of woody plant fossils were found in the Miocene strata of the basins. The flora seems represents an appearance of subtropical evergreen broad-leaved forest vegetation, but its composition are not as same as the typical modern evergreen broad-leaved forest. Combined with modern vegetation research we found that: many taxa in fossil flora also have a high importance value in today's limestone vegetation. For example, Calocedrus, Itea, Cladrastis, Dalbergia, Albizia, Rosa, Berchemia, Zelkova, Ulmus, Quercus miovariabilis, Carpinus, Coriaria, Mallotus, Cornus, Fraxinus, Pittosporum and *etc.* These plants above are main components of karst forest top-level community today. There are also some taxa bearing large amount fossils are sun-bearing and shrub-specific such as Pterolobium, Bauhinia, Paliurus ramosissimus, Ficus miochapaensis, Burretiodendron, Myrsine and *etc.* These findings suggest that in the early Miocene, due to soil and site constraints, there were karst forests in southeastern Yunnan distinct from typical evergreen broad-leaved forest, which was characterized by mixed of Calocedrus and many deciduous taxa. In addition, although the bare rock surface in the karst area can reach the forest community after a long succession, there was still a considerable area will have a long-term stability in the shrub status because of the environmental constraints. Since karst vegetation has been adapted to the environment from Miocene, this type of vegetation will still be available in this area if the human activities are reduced, the disturbed vegetation and biodiversity can also be restored gradually.

T1-01-10

Cycas fairylakea D.Y.Wang in danger and efforts made in its rescue

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Shenzhen Meilin Reservoir Management Office

Cycas fairylakea D.Y.Wang, of Cycadaceae and *Cycas*, is a kind of ancient relict plant and one of the rare and endangered plants in the world now. Under the "New International Grade of Endangered Species" by the International Union for Conservation of Nature (IUCN), *C. fairylakea* is involved in Endangered species. In China, it is listed as one of the National First-class Key Preserved Wild Plants. At present *C. fairylakea* only exists in small amount in Tanglang Mountain of Shenzhen City and Qujiang County of Shaoguan City, Heshan County of Jiangmen City in Guangdong Province, China. Due to development construction, recently the number of wild *C. fairylakea* is still dwindling, and till 2008, the total amount is even less than 2,000. Within the first-class drinking-water source reserve of Meilin Reservoir in Shenzhen City survive 1,013 wild *C. fairylakea*. But by 2007, most of the *C. fairylakea* in Meilin Reservoir was on the verge of death with the whole species in risk of extinction, because of their weak competitiveness, insect harm and other reasons. Since 2008, Shenzhen Meilin Reservoir Management Office has been constantly conducting the rescue and conservation of *C. fairylakea*, including the study of species properties, nurture technology, pandava prevention technology, the effect of light transmittance on the growth of *C. fairylakea*, pollination ecology, artificial pollination technology, seed transmission, seed seedling technology, *etc.*, exploring the threatening elements and protection methods for *C. fairylakea*. Through eight years of site protection and conservation, success has been achieved in saving *C. fairylakea.* Furthermore, with the growth status of *C. fairylakea* in Meilin Reservoir improving year by year, the vegetative growth and reproductive growth has fully restored, the species tends towards healthy from the brink of extinction, and the species size is rapidly expanding. To October 2016, in Meilin Reservoir, there are 1,107 *C. fairylakea* over 80cm in height, 4,126 seedlings less than 80cm, with species number totaling over 5,233 and species size expanding four times. At the meantime, more than 1,300 seedlings are planted in other placesprovided by us. To our pride, the rescue of endangered *C. fairylakea* is claimed as a success. In Shenzhen City, *C. fairylakea* is the unique species which belongs to the National First-class Key Preserved Wild Plants, and Meilin Reservoir is its largest habitat over the world. Thus Meilin Reservoir becomes the name card of ecological civilization of Shenzhen City for the rescue of *C. fairylakea*. *C. fairylakea* conservation breaks a new ground for the rescue of rare and endangered plants.

T1-01-11

Conflicts for plant conservation, *Delphinium caseyi* B.L. Burtt case.

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Biodiversity loss is one of the main concerns of the international scientific community. *Delphinium caseyi* is an endemic perennial in *Cyprus* and is distributed on two locations along Pentadactylos mountains. *D. caseyi* is a protected plant species both under local and international legislation and is categorized as "Endangered" by IUCN (1997) due to its' declining population on the island and is listed under EU Habitats Directive (92/43/EEC) as an Annex II plant species. The goals of this study are to determine the population growth areas and estimate the number of individuals. The next one is to update the IUCN category with the aid of the acquired data and to determine the reproductive biology. And the final goal is to develop specific conservation strategies.

T1-01-12

Tourism in Karst ecosystem: Economic development and sustainability of Lijiang river tourism

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The spectacular karst landscape with abrupt eroded limestone hills and winding rivers of southern China attract tourists from all over the world. The four-hour cruise along the Lijiang river is one of the top five tourist attractions in China. With recent economic success there has been a big surge of tourists and concomitant increase in tour boats in the river despite a dramatic hike in ticket price. Rapid urban expansion and agricultural development has been drawing large amounts of water from the river. Hydro-electric dams constructed upstream interferes the natural flow of the river, which in turn interferes the navigation of tour boat. Extensive river engineering is employed to keep the river navigable at low water level. To determine the sustainability of Lijiang river tourism we examined i) mean monthly rainfall pattern, ii) stream flow, iii) number of tourists and tour boats per year, ticket price, and iv) river engineering expenditures from 1960-2010. The number of tour boat increased from 1 in 1963 to 500 in 2010. During this time the number of passengers increased from 400 to 2,210,000. Ticket price increased from < US$1 - $34 and river excavation costs increased from $< 1,000 to over $230 million. From 1990 to 2010 the number of irrigation pups increased from < 100 to 14,200 and water drawn for urban use increased exponentially. Seasonal water flow changed from pre-dam time with larger pick flow during mid-raining season and lower flow during late rainy season causing navigation difficulty. Since the GDP of greater Guilin, Guilin City and Yangshuo County all show a dramatic increase from 1990 to 2010 increase in tourism in the area will likely continue. We predict that tour boat traffic in Lijiang river at the current level is unsustainable. Furthermore, any significant climate change related shift in monthly rainfall pattern in the watershed will likely aggravate the situation even further.

T1-03: Crowdsourcing herbarium digitization: partnerships, platforms, and the worldwide engagement for digitizing biocollections (WeDigBio) event

T1-03-01

Four years of label transcription: What makes volunteers efficient on the web

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Museum national d'Histoire Naturelle (MNHN)

The renovation of the Museum national d'Histoire Naturelle (MNHN)'s Paris Herbarium building was the opportunity to conduct a massive digitization of the P and PC collections. This resulted in almost 6 million images becoming available online in 2012. There is an important gap between the rich information available on the photographed specimen labels and the very few attributes indexed in the database. One of the ways to reduce this was to open the participation to the general public on the web. A first citizen science website, http://lesherbonautes.mnhn.fr/ was opened in late 2012 and the success of the operation has led to an improved and more flexible site in 2015. Both the quality and the extent of participation exceeded all initial expectations. The motivations of the volunteers to participate may vary, but a balance between gamification, scientific feedback and social interaction have structured a strong community who completed more than 60 transcribing missions, each with its own taxonomic, geographical or historical specifics. Quality was enforced by a progressive increase in newcomer's abilities, automated training via online quizzes, a strong redundancy in questions and some incentives to discuss special cases in a public forum to improve the collective knowledge. The site communication is more focused on finding answers than transcribing. The initial fixed set of questions on label content and georeferencing was extended in a more flexible way in a second version, thus enabling original workflows and the ability to transpose for use in zoology or paleontology. The heart of the community is a very active minority of power contributors. Their efforts provide the majority of the contributions. Despite some turnover, these volunteers have a long term involvement of several years. The Herbonautes community developed an original expertise about many aspects of the history of the Herbarium collections. Les Herbonautes also benefited from the patronage of the Fondation de la Maison de la Chimie, Paris and is now connected to hundreds of collections on the Recolnat infrastructure.

T1-03-02

Worldwide engagement for Digitizing Biocollections (WeDigBio)—The biocollections community's citizen science space on the Calendar

Elizabeth Ellwood[1], *Paul Kimberly*[2], *Simon Chagnoux*[3], *Paul Flemons*[4], *Edward Gilbert*[5], *Robert Guralnick*[6], *Kevin Love*[7], *Austin Mast*[1]
1. iDigBio; Florida State University
2. Smithsonian Institution
3. Muséum national d'Histoire naturelle
4. Australian Museum
5. Arizona State University
6. University of Florida
7. iDigBio

Digitization of biocollections, including herbarium specimens, is an ongoing and critical task that has been galvanized by technological advances and new resources, including innovations in crowdsourcing and citizen science. Involving citizen scientists in the digitization process increases their awareness of the number, kinds, and value of biodiversity specimens in collections, advances scientific literacy, increases support for biocollections, and builds sustainability for digitization activities. In turn, growing digital biocollections databases have direct implications for the global community who make use of those data for botanical research, education, and to inform policy. To build support for biocollections and their digitization activities and to increase digitization rates, we organized the annual Worldwide Engagement for Digitizing Biocollections (WeDigBio) Event. In the two years of the event, dozens of museums and classrooms have hosted onsite digitization events where participants transcribed specimen labels using one of five online platforms (DigiVol, Les Herbonautes, Notes from Nature, Smithsonian Institution's Transcription Center, and Symbiota). Thousands of additional citizen scientists also contributed online from more than one hundred countries, completing tens of thousands of transcription tasks. Planning and executing WeDigBio events required us to find efficient ways to integrate disparate transcription and participant data across platforms and projects. Here, we present information on the process of organizing an international citizen science event, an analysis of the event's effectiveness (e.g., transcription rates before, during, and after the event), lessons learned, and future directions.

T1-03-03

Building crowdsourcing campaigns for collections digitization using Biospex

Austin Mast, *Greg Riccardi, Robert Bruhn, Elizabeth Ellwood*
Florida State University

Biospex is an online basecamp for launching, advertising, and managing targeted efforts to digitize biodiversity specimens.

Biospex provides tools for curators, amateur enthusiast groups, and others to mint a webpage for a project, package specimens into one or a series of digitization "expeditions" associated with the project, launch the expeditions at crowdsourcing tools (e.g., Notes from Nature), widely recruit others to participate, and follow crowdsourcing progress. We will illustrate the functionality of Biospex using the WeDigFLPlants project, a collaboration between southeastern U.S. herbaria, the Florida Native Plant Society, and other Florida-focused amateur-enthusiast groups to transcribe > 50,000 herbarium specimens collected in Florida and held at > 50 herbaria. WeDigFLPlants is a WeDigBio Interest Group designed to maintain the momentum of the 4-day WeDigBio event throughout the year.

T1-03-04

WeDigBio: A two year tale: Unlocking over a century of natural history collecting and exploring; Accelerating increased accessibility to biodiversity data at The Field Museum, Chicago

Matthew Von Konrat[1], *Marilyn Alam*[3], *Megan Bradley*[1], *Laura Briscoe*[4], *Elana Carpinone*[1], *Lucille Carver*[1], *Robin Delapena*[1], *Christine Giannoni*[1], *Anni Glissman*[1], *Sharon Grant*[1], *Zoe Greenfield*[2], *Kate Golembiewski*[1], *Lauren Hancock*[1], *Kaccee Kellum*[1], *Jeff Konker*[1], *Crystal Maier*[1], *Christine Marsh*[1], *Joann Martinec*[1], *Paul Mayer*[1], *Susan Mochel*[1], *Angie Morrow*[1], *Caleb Mcmahan*[1], *Diana Ramirez*[2], *Yarency Rodriguez*[1], *Gretchen Rings*[1], *Pam Phelps*[5], *Jennifer Slate*[3], *Chloe Takala*[2], *Tom Thomas*[3], *Caitlin Vaughn*[6], *Kate Webbink*[1], *Barajas Sergio*[3], *Greif Matt*[3], *Alexandra Lopez*[7]

1. The Field Museum
2. Roosevelt University
3. Northeastern Illinois University
4. New York Botanical Garden
5. Oswego High School
6. DePaul University
7. Wilbur Wright College

Alarmingly, the world's biodiversity is diminishing rapidly and undergoing an extinction crisis. Natural history collections of museums and academic institutions, documenting the fossilized and living members of the world's ecosystems and their changes over time, are uniquely poised to inform the stewardship of life on Earth. The National Science Foundation (NSF) program Advancing Digitization of Biodiversity Collections (ADBC) is enhancing and expanding the national resource of digital data documenting biological and paleontological collections at a rapid pace. We provide a case study founded on these digitization efforts partnering with the annual "Worldwide Engagement for Digitizing Biocollections" (WeDigBio) Event to accelerate the pace of scientific discovery. Digitization of natural history collections is an ongoing and critical task that has been galvanized by technological advances and new resources, including innovations in crowdsourcing and citizen science. Involving citizen scientists in this process increases awareness of the number, kinds, and value of biodiversity specimens in collections, advances scientific literacy, increases public support for natural history collections, and builds sustainability for digitization activities. In turn, growing digital natural history collections databases has direct implications for the global community who make use of those data for research and education, including investigating climate and other environmental changes, conservation biology, population genetics, and even public health and safety. To build support for natural history collections, digitization activities and to increase digitization rates, the Gantz Family Collections Center of The Field Museum invited members of the public, including teens and young adults, to participate in a "transcription party" in 2015 and 2016. Partnering with college, university and high schools - especially those with a high proportion of students underrepresented in science - students received class credit for their work. Volunteers joined museums around the world as part of a global four day effort to digitize centuries of data about life on Earth organized by WeDigBio. The Field Museum was one of the most significant on-site event hosts, globally, with the support from over 400 citizen scientists working the equivalent of over 1,000 hours spanning the two events and producing over 12,000 collection records and pages of transcribed field notes. Projects spanned the entire collections including bryophytes, fungi, ferns, insects, fishes, fossil invertebrates, field notebooks and historical exhibit labels using a variety of online platforms. The majority of specimen and object data remain difficult to access, locked in the cabinets of museum and university collections in analog format, presenting the natural history collection community with many years of digitization work. Enlisting the public's help in transforming historic data labels into an open, globally accessible, digital resource is helping unlock valuable information that can help scientists accelerate the process of biodiversity discovery. We will discuss our experiences, assess performance and examine the balance of costs and benefit including quality of data and investment of staff time. We conclude that with careful training, planning, and targeting specific audiences these projects remain a cost-effective way of collecting data while also providing an excellent opportunity for people to become engaged with natural history collections.

T1-03-05

The notes from nature project: Evolutions and revolutions in enhancing engagement with data providers and citizen scientists

Julie Allen[1], *Michael Denslow*[2], *Austin Mast*[3], *Robert Guralnick*[1]

1. University of Florida
2. Southeast Regional Network of Expertise and Collections
3. Florida State University

Notes from Nature (http://www.notesfromnature.org; NFN) is a citizen science tool focused on public engagement and label transcription of natural history specimens. The project was developed collaboratively by biodiversity scientists, curators, and experts in citizen science, within the well-established Zooniverse platform. This project currently brings together digital images representing a wide variety of biodiversity records such as ledgers, herbarium sheets, pinned insects and fossils. Volunteer citizen scientists transcribe textual data contained in the specimen images. Since its launch in 2013, NFN has amassed > 1.2 million transcriptions from tens of thousands of volunteers worldwide. This talk focuses on innovations aimed to improve the platform, some incremental and others totally changing the way Notes from Nature works. We discuss improvements such as: (1) Tools to build consensus and quality metrics for the transcriptions to further enhance data quality for museum curators; (2) Better workflows to support image loading and repatriation of data to our providers; (3) Improved engagement about biodiversity by bringing in rich content from external sources such as Encyclopedia of Life and Map of Life. We also discuss engagement efforts and interoperability with other biodiversity informatics tools and databases that are important to our partners' curation needs. Such improvements help NFN take its place as a critical component of an ecosystem of tools needed to unlock the vast legacy biodiversity data for broad public good.

T1-03-06

Diversification in a transcription platform—costs and benefits

*Paul Flemons, **Rhiannon Stephens***

Australian Museum

Transcription of natural history collections through crowdsourcing has many benefits not just associated with the capture of data connected to the specimens of these collections. Benefits include broadening the understanding of the specific collections involved through participation; increasing scientific literacy particularly in relation to scientific collections; increasing the profile of the collection institutions and the specific collecting events involved; providing a true citizen science engagement for non professionals who have a passion for science. An ongoing challenge for transcription centres like DigiVol is the recruitment and maintenance of the transcription community that captures the data. DigiVol has pursued a strategy of engaging a broad range of institutions and types of data capture in an effort to provide a diverse engagement experience for transcribers. I will discuss the costs and benefits of this approach.

T1-04: Arboreta and botanical gardens as major centers for research and conservation of global trees and forests

T1-04-01

Plant diversity: What do we need to conserve?

Richard Corlett

Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences

Botanical collections have traditionally included as many plant species as possible in order to provide both an attractive and interesting display, and a diversity of living material for botanical research. In recent decades, the increasing focus on plant conservation has resulted in repurposing of these living collections as *ex situ* conservation. A botanical garden that previously claimed to display 5,000 species now claims to conserve the same number. Seed banks make similar claims for their collections and protected areas are often assumed to provide *in situ* conservation for all the species recorded there. However, while even a single individual, perpetuated by cloning, may save a species from extinction, species are not the only level of diversity that we need to conserve. If we want to have more than a living museum specimen we need to save more than one individual. But, how many? And which ones? Modern high-resolution genomic techniques can detect differences between almost any pair of plants or plant populations, raising the question of when these differences are considered to be of conservation relevance. It is not practical to treat every genetically diagnosable difference as significant. Provenance trials with economic plants, show that local adaptation is widespread, suggesting that plant conservation needs to target the full range of adaptive variation within each species, rather than a single local population. In practice, information on adaptive genetic variation is rarely available and overall genetic variation is commonly used as a proxy for adaptive variation. However, restricted gene flow produces spatial genetic structure even if populations show little or no local adaptation, so this practice may exaggerate the scale of the conservation problem. New methods for the functional interpretation of genetic variation are being developed, but current best practice is still to use neutral markers and/or phenotypic information as well to ensure that adaptive variation has not been overlooked. These issues apply to both *in situ* and *ex situ* conservation, but the best solution will vary depending on the costs. *In situ* conservation is expensive, particularly if it requires land that that has alternative economic uses. The high costs limit the number of separate populations of each plant species that can practicably be protected. *Ex situ* conservation of orthodox (drying tolerant) seeds in seed banks is much cheaper, so it may be practical to conserve large samples from enough populations to allow for the uncertainties outlined above. For the minority of (mostly tropical) species with recalcitrant (drying intolerant) seeds, however, living collections are so far the most practical *ex situ* technique. As a result, conservation costs are again high and space constraints limit both the number of individual plants that can be grown and the number of populations that can be spaced far enough apart to maintain genetic isolation. High conservation costs mean that collections must be optimized, but there is currently no consensus on what this optimum is.

T1-04-02

Role of botanic gardens in conservation and management of rare and endemic Indian trees

Paramjit Singh

Botanical Survey of India

Botanic gardens play an important role in conservation of rare species, plant recovery programmes and phenological studies. Strengthening *ex situ* conservation is one of the essential components of India's National Biodiversity Strategy and Action Plan (NBSAP), which is based on the principles built in the National Environment Policy of the country. Being a signatory to Convention on Biological Diversity, India is committed towards conservation and sustainable utilization of plants of our country. Botanic gardens conserve the plant species in areas outside their natural habitats and serve as repositories of many threatened indigenous plant species. Botanical Survey of India (BSI), a premier research organization of this Ministry has been contributing immensely towards the *ex situ* conservation of threatened, rare and endemic plants. It is estimated that, Botanic gardens harbor about 30 per cent of country's indigenous threatened plants. Botanic gardens play an important role as centers for plant conservation, preservation of endangered species, seeds and propagules, cultivation of rare, vulnerable and endemic species. They are also centers for exchanging information and plant materials and act as a platform for creating awareness through education programme. Significant role of botanic gardens in developing the Global Strategy for Plant Conservation (GSPC) and its ultimate adoption by the Parties to the Convention on Biological Diversity (CBD) is well recognized. Indian Botanic Gardens plays a special and remarkable role in contributing to the targets of GSPC in conservation, education and public awareness. In India, for conservation of rare species in botanic gardens 'Assistance to Botanic Garden' programme is implemented through Botanical Survey of India (BSI) which is managing this programme through various initiatives. The presentation highlights recent initiatives undertaken to conserve endemic and rare trees in various botanic gardens in India. The success story of rehabilitation of some of the endemics in the wild like *Bentinckia nicobarica, Gymnocladus assamicus, Stapletonia arunachalense, Rhododendron* species is discussed in detail.

T1-04-03

Botanic gardens in developing countries: Challenges and opportunities in urban ecosystems

Mauricio Diazgranados

Natural Capital and Plant Health Department, Royal Botanic Gardens, Kew

Some of the most biodiverse countries have often serious social and environmental problems. A common element of developing nations is inequality between rural and urban communities, fuelling rapid urbanization. Growth of many the cities in these countries brings further social and environmental problems, and often they become massive overpopulated centres, acting as barriers for the surrounding ecosystems, and even holding introduced exotic species (sometimes invasive) in their green spaces. Under this context, botanic gardens have a great opportunity, beyond *ex situ* and *in situ* conservation: they can lead processes of conservation, propagation and research of native endangered trees and plants in general, using cities as experimental arenas. The challenge is obtaining the required funds in these countries. However, serving the city is a powerful formula, especially when botanic gardens become a vital part of cities administrations, with a more active involvement in improving livelihoods of citizens. Bogota, capital of Colombia, is a great example of analysis: a city of 8.5 million, that despite being in one of the most biodiverse countries, holds green spaces with exotic (and invasive) species. The city is home of the Bogota Botanic Garden (JBB), Colombia's oldest and most important botanic garden. In the past years, while I was JBB's director for science, a powerful strategy of service was developed, based on five large approaches for the city: 1. tree management (planting, pruning, monitoring); 2. restoration of strategic ecosystems within the city district (wetlands, rivers and creeks, Andean forest); 3. greening urban spaces (green roofs and walls, green public furniture, green bus stops); 4. leading urban agroecology (public urban orchards; orchards of medicinal plans); and 5. gardening with native (often threatened) species. When these approaches are combined with the use of native and endangered plant species, this model provides a great opportunity to contribute to their *ex situ* conservation, propagation and research. The JBB maintains an open access database with the trees from the city's public space (~1.7 million records), and over the last three years has planted more than 200,000 trees of 90 species, and has restored 16 areas planting more than 45,000 trees. Besides the success of the implementation, the strategy became an economically sustainable model, representing more than 80% of the income, and allowing a solid investment in the JBB's infrastructure: construction of new laboratories, research and public greenhouses, herbarium, conference centre, *etc.* While many other botanic gardens in developing countries are struggling to increase funding, the JBB's model not only secured funding for a historic development of its facilities, but also had a relevant role in transforming the green spaces of the city, with native and often endangered species of trees. May this be a model for botanic gardens in other developing countries?

T1-04-04

How to preserve native flora? A case from Chinese botanical gardens

Jin Chen

Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences; Chinese Union of Botanical Gardens

Plant species extinction is among the most challenging threatens to the planet while effective actions, both globally and locally, appears to be limited. The information update for species distribution, the failure to convince decision makers and various conservation activities work separately without collective efforts, could be the challenging for solving the problem. To address biodiversity loss crisis, Xishuangbanna Tropical Botanical Garden (XTBG) has initiated a program termed 'Plant zero extinction program' in Xishuangbanna, a biodiversity hotspot in China's tropics. The concept for the program is trying to use the entire tools in conservation toolbox to achieve plant species to zero extinction in coming 5-10 years in the area. The preliminary results have shown it could be an effective and measurable approach to battle against local plant extinction. In 2014, by meaning of the network of the Chinese Union of Botanical Gardens (CUBG), this protocol has been expended to large areas throughout China, which is recognized as a key project of CUBG termed 'Complete coverage conservation program'. Totally, eight BGs (i.e., Xishuangbanna, Kunming, Wuhan, Tulupan, Guiling, Nanjing, Beijing and Shenyang), joined the program, targeting an area about 28% of total country territory. In this talk, I will present some of the progress. The weakness and challenging for achieving the safely conserve China's native flora will be discussed. As a whole, I wish to show a new and possibly workable model for botanical garden community acting as a leading role for collecting all efforts for effective conservation for native flora.

T1-04-06

The strengths and expertise botanical gardens can provide to the successful management and conservation of trees in the Anthropocene

Charles Cannon

Center for Tree Science, The Morton Arboretum

Human activity now dominates basic Earth processes, from the cycling of nutrients and energy in the environment to the fate of each species. As we make this transition into the Anthropocene, we must assess whether the traditional approaches to management and conservation are appropriate going forward. We need to anticipate the novelty and unpredictability of future climates and habitats, both of which pose unique challenges to long-lived sessile organisms like trees. In this talk, I will focus on the unique attributes and strengths that arboreta and gardens can bring to these challenges and how we can exploit the natural tendencies of tree reproduction and growth to potentially accelerate their adaptation and diversification, utilizing both traditional techniques like grafting and cutting-edge emerging technologies like genomics and metabolomics. I will also provide examples of how pro-active engagement can create opportunities for direct input into management and conservation of trees across the urban to wild spectrum.

T1-05: Climate change, conservation and the future of Asian pine species

T1-05-01

The influence of origin and climate upon tree growth and survival in a 100-year-old provenance trial in northern Arizona, USA: Lessons for managers

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Early in the 20th century, a provenance trial of ponderosa pine (*Pinus ponderosa*) was established on what is now the Fort Valley Experimental Forest, 14.5 km north of Flagstaff, Arizona, USA. The purpose of the study was to assess genetic diversity and adaptation patterns with the goal of improving the management of ponderosa pine forests. Seed was collected from 18 National Forests throughout the western United States and planted on the study site from 1913 to 1917. The seeds were identified as belonging to one of two varieties, *Pinus ponderosa* var. *ponderosa* and *P. ponderosa* var. *scopulorum*. Only *P. ponderosa* var. *scopulorum* provenances have survived to this day. Annual survival was monitored until 1919, and then subsequently remeasured in 1928,1951,1964, and 1995. Tree height was measured in 1928, and height and diameter were measured 1995-1996. In 2014, we undertook a remeasurement of height, crown density, and diameter and, in 2016, we added surrounding tree density to the dataset. Selected trees were cored in 2014 to examine provenance × climate interactions with radial growth. We compared climate at the source location to that of the study site. We also looked at different variables of annual weather (precipitation, growing season, temperature) for clues as to their influence on individual tree performance. In this paper, we present results of our analyses, including the relationship between recent tree growth and climate and local weather patterns at the seed source site. We also evaluate the influence of irregular mortality and the residual effects of survivor density upon growth. Finally, we offer suggested field applications of these results and some plans for future studies.

T1-05-02

Adaptation and introgression between two conifers from the Qinghai-Tibet Plateau

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Climate change is the single largest threats to forest tree populations. To survive the rapidly changing environments, they may migrate to track favorable ecological niche spatially, persist through plasticity in shorter term, and adapt to new environment via generating of new genetic variation and mutation. Hybridization and introgression may also contribute to the adaptive responses of forest tree populations to changing environmental conditions by transferring standing genetic variations and mutations from one species (population) to another. Such cases have been reported for animals and model plants, yet very rare for non-model forest tree species.

Here, we examined the role of introgressive hybridization in transferring adaptive genetic variation between two cypresses in the Qinghai-Tibetan Plateau. We collected samples for 35 (11 populations) and 30 individuals (12 populations) of *Cupressus duclouxiana* and *C. gigantea*, respectively, and performed RNA-sequencing. The transcriptome of one *C. duclouxiana* invidivual were assembled and clean reads of the other individuals were then aligned to this reference transcriptome. After stringent quality filtering, 878,614 single nucleotide polymorphisms (SNPs) were identified in all 65 individuals, and 1,208,905 in *C. duclouxiana* and 1,204,335 in *C. gigantea*. Both principal component analysis and phylogeny analysis suggest that the two species were clustered into two monophyletic clades, respectively, and *C. duclouxiana* comprises of two groups which correspond to its northern and southern populations. Genetic clustrering indicates introgression occurred from *C. gigantea* to the northern population of *C. duclouxiana*. This hypothesis is also supported by the pairwise FST values as well as the identical-by-descent (IBD) analysis. FST between *C. gigantea* and *C. duclouxiana* southern population is higher than that between *C. gigantea* and *C. duclouxiana* northern population (0.3126 vs. 03060); 50 comparisons between *C. gigantea* and the *C. duclouxiana* northern population were found get shared haplotypes, while only six comparisons between *C. gigantea* and the *C. duclouxiana* southern population got the IBD haplotypes. Meanwhile, the best-fitting demographic model (out of 16 models) in our Fastsimcoal2 simulations suggests much higher gene flow from *C. gigantea* to *C. duclouxiana* northern population (1.42×10-6) than the opposite direction (5.02×10-8) or than that between *C. gigantea* and *C. duclouxiana* southern population (2.10×10-7,7.35×10-8). To further identify the introgressed loci in the *C. duclouxiana* northern population, we calculated the D statistic and the fdM-statistic, and finally identified a subset of 71 candidate introgression loci, 47 of which got annotations from Swiss-Prot database. Meanwhile, we used HKA (Hudson-Kreitman-Aguade) test to identify genes under natural selection, a total of 2831 candidate of positively selected unigenes were identified and 19 of them were potential introgressed loci. Differential expression analysis suggested that 700 and 1148 unigenes expressed at a significantly higher level *in C. gigantea* and *C. duclouxiana* southern population, respectively. Three candidate introgressive genes showing selection signature, ABA3, TR6055|c0_g1_i1 (best hit to At1g55270) and SPHK2, were detected to show significantly different expression levels between the two lineages ($P \leq 0.05$).

Putting together, multiple lines of evidence support introgression from *C. gigantea* to the northern population of *C. duclouxiana*, a set of 19 candidate introgressive loci under positive selection were identified and three of them show significant expression level difference between high and low altitude lineages. These candidate introgressive loci from *C. gigantea* to *C. duclouxiana* most likely have assisted the northward and probably upward expansion of *C. duclouxiana*. Our findings will shed light on the geographical and genetic consequences of forest tree population in the mountainous regions in the face of global climate change, and more importantly, emphasizes the conservation values and important roles of introgressive populations and hybrid zone in adaptation to changing climate.

T1-05-03

Phytogeography of Korean Pinaceae

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Despite of ecological and landscape importance and public popularity of conifers, especially in case of Pinaceae in the Korean Peninsula, not much scientific information are known. Present work aims to understand the phytogeography of Korean native conifers, especially the family of Pinaceae or pine tree family, i.e., phylogeny, origin, life form, distribution, dispersal and migration. Korean native Pinaceae consists of five genera and fourteen species. Pinus or pine tree is systematically closely related to Picea or spruce and Larix or larch, but Abies or fir is related to Tsuga. Cold-tolerant boreal conifers which have migrated from NE Asia during the Pleistocene glacial epochs in search of glacial refugia and successfully survived, but now confined its ranges to the alpine and subalpine belts of the Korean Peninsula mainly due to climate amelioration since the Holocene period. Since the Last Glacial Maximum, species, such as Picea pungsanensis and Abies koreana have gradually adapted to local environment, and later became an endemic species of Korea. Disjunctive distribution of Pinus parviflora and Tsuga sieboldii on Ullung Island, which also occur in the Japanese Isles needs further study. Major dispersal agent of pine trees with winged seed is wind, but wingless pine tree seeds might dispersed by birds and rodents. Pine trees with bigger wings are easily dispersed by wind, and now show broader distribution. Species of Pinaceae with disjunctive distribution on the alpine and subalpine belts of the Korean Peninsula, which includes both ROK or South Korea and DPRK or North Korea seems to be more vulnerable to global warming.

T1-05-04

Phylogeography of *Abies koreana* and *A. nephrolepis* (Pinaceae) in Korea

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Genetic variations of *Abies koreana* and *A. nephrolepis,* taxonomically confusing taxa in *Abies,* were assessed using three mitochondrial DNA regions (*nad*5 intron 4, *nad*5 intron 1, and *cob*_pre), a chloroplast DNA region (trnV-UAC/trnT-GGU), and a nuclear DNA region (ITS) for 247 individuals from 19 natural populations to understand their phylogeographic relationships. In the mtDNA analysis, twelve polymorphic sites of the combined tree regions resulted in the resolution of five haplotypes (M1-M5). The average gene diversity within population (H_S) was 0.072, and total gene diversity (H_T) was 0.620. The populations were geographically divided into four groups (group1-4) by the SAMOVA analysis. In the cpDNA analysis, twelve polymorphic sites of a region resulted in the resolution of ten haplotypes (C1-C10). Average gene diversity within population (H_S) was 0.552, and total gene diversity (H_T) was 0.768. However, no variation was found in the nrDNA data. The populations of northern (group1) and southern (group2) areas were nearly structured for M1 and M2, respectively. The presence of the single mtDNA haplotype in the southern areas suggest that the current widespread populations could have expanded to central areas from a specific refugium following the last glacial periods (20,000 years ago). Based on the results from this study, the distribution of Abies koreana is postulated below Mt. Songni in Goesan-gun, Chungcheongbuk-do (group1 region). Additionally, the populations of M4 and M5 types (Mt. Worak in Jecheon-si, Chungcheongbuk-do and Mt. Yeongchuk in Yangsan-si, Gyeongsangnam-do) should be studied with closely related species of adjacent regions for specific taxonomic identify.

T1-05-05

How will subalpine conifer distributions be affected by climate change in Japan?

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This study aimed to assess the climate change impacts on Japanese keystone subalpine conifers (*Tsuga diversifolia* and *Abies veitchii*) incorporating the uncertainties of general circulation models (GCMs) for spatial conservation planning. Potential habitats for *T. diversifolia* and *A. veitchii* under current and 20 GCMs in 2080-2100 were projected using generalized additive models. The number of GCMs under which current potential habitat was projected to lose was counted by a spatial unit and was used as indices of vulnerability and prediction uncertainty. We identified gaps between protected areas and potential habitats under future climates. Reasonably accurate models were developed. We revealed that *T. diversifolia* and *A. veitchii* require cool and wet summers with low winter precipitation. Less than 1% of potential habitats under current climate were projected to be sustainable with low uncertainties of GCMs. These certainly sustainable habitats were mostly designated as protected areas. About 88 and 97% of current potential habitats for *T. diversifolia* and *A. veitchii* were projected to be non-habitats under future climates with low GCMs-related uncertainties. The southern and low elevated northern range limits of the species were included in these certainly vulnerable habitats, of which 70% were designated as protected areas. Our results suggest that subalpine conifers in Japan are vulnerable to the climate change irrespective of GCMs variation. Strengthening of monitoring may be necessary at unprotected and certainly vulnerable habitats in lower subalpine areas where influences of the climate change will appear first. Active management, such as assisted regeneration, may be necessary for conserving the vulnerable and genetically endemic populations.

T1-05-06

Climatic control of zonal vegetation in Northern Asia and its phytogeographical background

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Large-scale vegetation studies are one of the fundamental aspects of ecology and biogeography and a key to the clarification of modern as well as past processes in vegetation cover. This study focuses at the problem of indication of climatic gradients by vegetation complexes at local and regional scales and aims to quantify the local and regional scale relations of vegetation units, their complexes and climatic parameters within boreal vegetation zone in Northeast Asia. We used phytosociological (over 5,000 releves), climatic (2,200 climatic stations) and floristic (distribution records for 22,000 species) databases. Identification of bioclimates was made in accordance with Rivas-Martínez *et al.,* (1999) approach using different climatic parameters that include Kira's warmth (WK) and coldness (CK) indices, continentality index (CI), ombro-evapotranspirational (OEI) index and winter precipitation (WP). Analysis of indices calculated with an aid of developed models showed the significant differences of vegetation units of the order rank in bioclimatic ranges. WK decreases from values over 75 in the middle temperate zone (Saso-Fagetalia, Aceri-Quercetalia) to 20 in subarctic zone. Among boreal vegetation units the orders of Betulo-Ranunculetea have the lowest warmth index in the circumboreal zone that can be explained by cool summer in conditions of oceanic climate. CK varies between values of -25 and -150 within boreal and temperate zones with prevalence of deciduous broadleaved, mixed and evergreen broadleaved forests. The boreal orders Lathyro-Laricetalia and Ledo-Laricetalia representing boreal deciduous coniferous forests are characterized by very low values of coldness index that, in this case, is comparable to that of subarctic orders. The ranges of orders along the continentality gradient are reflected by changes of vegetation types within a zone with proximity to the ocean. In boreal zone the lowest values of CI are characteristic to the forestless and not yet described class of Aleutian meadows, followed by *Alnus fruticosa* scrub and *Betula ermanii* forests of the class Betuletea ermanii. Highest values belong to deciduous coniferous orders Lathyro-Laricetalia and Ledo-Laricetalia. The snow affects vegetation strongly in the conditions of continental climate within northern subzone of temperate zone, where lack of snow in winter causes a shortage of moisture in spring and early summer, and in oceanic regions of boreal zone, where the strong accumulation of snow causes 2-3 week delay of its melting and a considerable shortening of growing season. The communities of *Betula ermanii*, *Alnus fruticosa* and tall-forb meadows are characteristic to the regions with slower-melting heavy snow deposits. Zonal subarctic vegetation is represented by communities of provisional class Betuletea glanduloso-divaricatae composed of species complexes of Pacific coastal mountainous regions. In boreal zone, vegetation of Asian ultracontinental to maritime sectors is represented by communities of Vaccinio-Piceetea, Asian suboceanic to hyperoceanic sectors—by communities of Betulo-Ranunculetea. The boreal classes were likely differentiated in Pre-Pleistocene time due to the well developed floristic centers under the different climatic situations.

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T1-06: Reintroduction and management of rare plants in response to global change

T1-06-01

Conservation meets restoration - Rescuing threatened plant species by restoring their environments and restoring environments using threatened plant species

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Kunming Institute of Botany

Plant conservation biology needs a new approach to cope with the rapid disappearance of species and ecosystems. I introduce a conceptual integration of conservation biology and restoration ecology in what can be called conservation-oriented restoration. The usage of this term is limited to the cases when restoration is applied to a still functioning ecosystem, excluding cases when the destroyed ecosystem must be recreated or altered to a desirable state. The underlining idea of this approach is the importance of habitat restoration for the majority of threatened species, and, on the other hand, usefulness of threatened plant species for restoration of natural habitats. It is proposed that threatened plant species should become an important part of many restoration projects and be introduced not only into locations where they currently grow or grew in the recent past, but also into suitable locations within their potential distribution range. Because the number of potentially suitable locations can be close to zero if we consider only untouched natural habitats as suitable, the re-introduction sites should include those that require restoration efforts. The general methodological guidelines are provided to show why and how ecological restoration using threatened plant species should become an integral part of the conservation biologist’s armory.

T1-06-02

On the edge of a continent – An exploration of comprehensive rare plant conservation and restoration in Southern Florida, USA, over two decades

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The Institute for Regional Conservation

Southern Florida has undergone rapid transformation in just over a century. With barely 25,000 residents in 1900 the area now contains about 8 million people spread over an area of about 4 million hectares. The greater Everglades ecosystem, home to myriad tropical and subtropical species, was wracked by massive engineering works and expansion of the agriculture frontier. Coastal and upland ecosystems, containing many endemic species, were radically transformed to accommodate growing human populations. The entire region is now subject to many ecosystem and species-based restoration projects, including the Everglades Restoration, but population and other pressures continue to grow. Although there were some existing programs to conserve rare plants, in the mid-1990s The Institute for Regional Conservation (IRC) embarked on an ambitious and collaborative program to document and improve management of the region's native flora – a total of more than 1,400 species. Beginning with the cataloging of all existing information on plant distributions and rarity, intensive field surveys in dozens of protected areas, and the collection and databasing of herbarium specimens, IRC then assessed and ranked the entire native flora of southern Florida using a modification of the Heritage Program ranking system (now NatureServe). Results of this work were published online as the Floristic Inventory of South Florida (Gann *et al.,* 2001-2017) and as the book Rare Plants of South Florida: Their History, Conservation and Restoration (Gann *et al.,* 2002). Based on major findings in these publications, IRC then collaborated with multiple agencies at the local and national level to: 1) set up long-term monitoring programs for rare plants in fragmented ecosystems; 2) actively restore degraded habitat for several species listed by the U.S. Endangered Species Act and numerous other rare plants; 3) conduct baseline studies to evaluate the impact of the Everglades Restoration on rare plants in Everglades National Park (ENP); and, 4) conduct reintroduction and augmentation trials for several depleted species in ENP. This presentation will summarize the important results of IRC's work and how it has been used by the scientific and conservation communities to improve rare plant conservation and restoration efforts in southern Florida. It will also explore emerging concepts of plant conservation in the region given the reality of climate change (including sea level rise), fragmentation, invasive species and other factors. Finally, we will discuss how this work contributes to global initiatives, such as the Global Strategy for Plant Conservation, and serves as a model for other plant conservation initiatives in the Caribbean region.

T1-06-03

Conservation and introduction of *Camellia changii*, an endemic and rare plant in south China

Hai Ren

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Camellia changii is an endemic and rare species in south China with an extremely narrow range. It grew under the discontinuous canopy of secondary evergreen broad-leaved forest with well-drained acidic sandy loam. It is an insect-pollinated and self incompatible species. This species had characteristics of high blossoming rate but low seed setting rate. We found a total of 1039 wild individuals of this species during 2009-2013. The population structure was that of a declining population because no seedlings and few young plants were found. We confirmed the Critically Endangered status of this species, therefore, we established a nursery of plants generated by grafting of short cuttings (2-3 cm each) from all wild individuals to conserve the genetic diversity in the wild. We also conducted two types of conservation translocation experiments, augmentation and conservation introduction at two sites, Ehuangzhang and Tianxin, respectively, using 4-year-old grafted *C. changii* reproductive-size plants. The survival rate of transplanted plants was 100% after two years at both sites. The individuals at the augmentation site grew significantly faster than those at the introduction site ($P<0.05$). Transplanted plants at the augmentation site produced 26–28 flowers per plant per year, significantly less than did those at the introduction site (35–38 flowers) during the two-year (2013–2014) observation period ($P<0.05$). The content of anthocyanin, a main pigment in the petal of flowers, at the introduction site was less than that at the augmentation site. Compositions of the pollinating species of *C. changii* at both sites were similar, but the dominant pollinating species and their visiting frequency were different. Butterflies dominated the augmentation site and bees the introduction site. Nevertheless, the annual fruit and seed productions of both translocations in 2013 and 2014 were very similar. Our findings suggest that *C. changii* subject to the out-of-range conservation introduction did not suffer a decline in reproductive success when compared with augmentation. These findings suggest that certain rare and endangered plants may have the ability to cope with challenges that are brought on by conservation introduction in the face of climate change, at least in the short term.

T1-06-04

Orchid conservation translocation efforts in China and Florida: Which, why, who and future priorities

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Orchids are a highly diverse group of plants that are threatened due to habitat destruction and overharvesting. Globally, conservation translocation (sensu IUCN 2013) - the deliberate movement of organisms from one site for release in another with an intended conservation benefit – has been an important recovery strategy for orchids. In this study, we review current conservation translocation efforts of orchids in China and the United States to investigate variation in 1) the choice of plants for translocation; 2) the length of monitoring time; 3) the success of translocation efforts and 4) the type of agencies invoking translocation as a management action. We based our analyses on information gained from peer- and non-peer reviewed publications. We hope to unveil the motivation for orchid translocations between these two countries; both of which have notable contrasting socioeconomic and political systems. Based on our review of the literature we found 61 species (38 genera) targeted for conservation translocation across China (21 genera; 36 species) and the United States (17 genera; 25 species). We found that 2.6% (36) and 12.2% (25) of the national orchid species have been subjected to conservation translocation in China and the US, respectively. Five (13.9%) of the Chinese species and 11 (44%) of the US species were threatened on the global level while 22 (61%) of the Chinese species and the same number of US species (88%) were threatened on the national level. The type of species selected (habit) for translocation varied slightly among countries: for China, 42% (15) were epiphytes, 2.8% (1) were semi-terrestrial and 55.6% (20) were terrestrial while in the United States 40% (10) were epiphytes, 4% (1) were semi-terrestrial and 56% (14) were terrestrial. Furthermore the majority of plants used for reintroduction in China were adults (83%) while in the United States saplings were the most used growth stage (57%) in reintroduction efforts. The majority of the translocations had no or very short (1 year or less) monitoring periods. One year post transplanting survival was high in general, but survivorship declined with time since transplanting. Percentage survival was significantly higher for China (72.2%) compared to translocation efforts in the United States (35.2%) even though the mean number of propagules used in each reintroduction case did not differ significantly between China (mean = 267.5) and the United States (mean = 238.3). The majority of the translocation cases in China were carried out by state institutes. In contrast, the majority of the orchid translocation actions were carried out by private entities, such as nonprofits or universities, in the US. Overall, our results indicate that species are chosen for reintroduction more on the basis of threats at the national or local level than on a global level, and some were not chosen based on their biological rarity, threats or habit in both countries. We propose that social value (cultural and/or educational) be incorporated as a priority ranking along with more biological endangerment rankings of species.

T1-06-05

Domestication of endangered trees in economic forest plantation in Southeast Asia for restoring regional biodiversity

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The conversion of natural virgin forests into agriculture and forestland has continued to a severe extent in Southeast Asia due to the economic development and population increase. Tropical evergreen forest, one of hot spots for global diversity and climate change, is highly affected area where the natural forests are converted into para rubber, pulp, palm plantation and agricultural crops. *Aquilaria* species is one of critically endangered trees in Asia because the damage of *Aquilaria* trees by second-metabolism process might produce economically highly valuable resin-deposited tree called agrawood. People sometimes cut down the natural *Aquialria* trees to pursue the economic benefits without some adequate knowledge and practices in natural forests. Under these circumstances, the domestication of *Aquilaria* species in para rubber trees is investigated for restoring the forest lands. Since the late 1990s, smallholding farmers of Trat Province of eastern Thailand close to the Cambodian border covered by tropical evergreen forest has faced the problems that mono-cultural para rubber plantation make their livings and environments unsustainable due to loss of biodiversity, low price of para rubber sheet and soil degradation. We assess the life cycle of mono-cultural para rubber system and mixed forest system of *Aquilaria crassna* and para rubber based on field experiment data and modelling in terms of ecological, economic, social characteristics in Trat Province of eastern Thailand. It is realized that the aboveground species and nutrient richness and economic production of mixed forests will be significantly higher than that of the mono-culture in a life cycle. However the belowground component is not significantly different. The mixed forests system will be one of restoring regional biodiversity in mono-cultural para rubber plantation.

T1-06-06

The role of botanic gardens and partnership in plant conservation in the changing landscape of the southeastern United States of America

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Increasingly, botanic gardens and arboreta are highlighted as effective partners for conserving plant species diversity and restoring natural communities at a time when the need for these activities is urgent. Capacity for restoration and conservation at botanic gardens comes directly from staff expertise in horticulture and research, but also from the role that gardens can take as good partners. Gardens are in a position to communicate information about rare plant species to owners and managers of public and private lands, and they can be instrumental in creating networks for effective conservation action. Several examples from the southeastern United States of America illustrate how this has been put into practice: 1) Mountain bog habitats have been reduced to less than 3% of their original distribution and they harbour a suite of rare species, including the mountain purple pitcher plants, *Sarracenia purpurea* var. *montana*. After more than two decades of restoration efforts, survival rates of augmented plants are more than 76% and population genetic diversity is not significantly different between restored and original populations. 2) Dwarf sumac, *Rhus michauxii*, is an endangered shrub that has become extinct in some states and critically rare in others. Through multiple partnerships, our on-the-ground management efforts have resulted in exponential increases in plant numbers and documented high levels of genetic diversity in the last population of this species at the southern most edge of its range. 3) Florida torreya, *Torreya taxifolia*, is considered one of the most endangered conifers in the world. Recent renewed efforts to survey, study and restore this species have resulted in significant new insights for understanding existing threats and available tools for preventing extinction of this species. Not only are botanic gardens achieving successes in plant conservation, through networked partnerships, they are setting priorities for conserving at-risk plants species. In November 2016, the first Southeastern Partners in Plant Conservation meeting convened at the Atlanta Botanical Garden. More than 160 people from 24 states and territories representing, federal, state and local government agencies, botanic gardens, universities and other partners met for three days to set priorities for 279 at-risk plant taxa across eight subregions. Priority actions were identified for nine areas of conservation need including, land management, *ex situ* conservation, and genetic and taxonomic research. What was unexpected was the identification of taxa that did not need conservation action or regulation due to new information or confirmation that they are stable on protected lands. Ultimately it will take continued coordinated efforts between agencies and botanical gardens to address priority needs for plants species and prevent loss of plant biodiversity.

T1-07: Urban parks: effects of management and planting schemes on insect diversity

T1-07-01

Using DNA metabarcoding to understand diet and pollination role of Cave Nectar Bat

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Cave Nectar Bat, *Eonycteris spelaea*, is the largest nectarivorous bat in Southeast Asia. Due to its specialised diet of nectar and pollen, *E. spelaea* is the important pollinator of many plants including the monotypic *Oroxylum indicum*, the economically important *Durio zibethinus*, *Parkia speciose* and *P. timoriana*, and mangrove plants: *Soneratia alba* and *S. caseolaris*. The diet of *E. spelaea* was previously determined by identifying the pollens found (i) on the body and (ii) in the faeces of the bats based on the morphological characteristics of the pollen when examined under the microscope. However, this conventional method failed to identify many of the collected pollens to species level mainly due to the limitation of morphological identification which requires high level of plant taxonomic expertise. Therefore, we attempted to use DNA metabarcoding which utilises High-Throughput Sequencing at *rbcL* and *ITS2* regions to mass sequence the pollen and digested plant material in the faeces of *E. spelaea* roosting at Batu Caves, Kuala Lumpur city. We discovered plants including ferns that have not reported in diet studies of *E. spelaea*. Therefore, our study demonstrated the potential of DNA metabarcoding in dietary study of bats with specialised diet and provided insights into pollination role of *E. spelaea* in urban area.

T1-07-02

Butterfly diversity at a tourist hotspot; Sukhothai Historical Park, Sukhothai, Thailand

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Sukhothai Historical Park in Thailand is a popular attraction and UNESCO World Heritage Site. The historical park is a 70 km^2 with a diversity of plants and offers the opportunity for the visitors to interact with wildlife besides the cultural sites. Butterflies play an important role in the natural food chain and act as nature's gardeners through their pollination of flowers. In addition butterflies are biodiversity indicators and ambassadors for insect conservation. However, to our knowledge no studies have been conducted to record butterfly communities in historical parks in Thailand. The aim of the present study was to survey the butterfly communities in historic town of Sukhothai. Such information is essential as the park serves as an important study area for many schools and park managers. We sampled butterflies using sweep nets at Sukhothai Historical Park on 23rd to 25th June 2016. A total 143 individuals from 25 species were recorded, comprised of the families Nymphalidae (35.7%), Pieridae (35.7%), Papilionidae (14.0%), Lycaenidae (8.4%) and Hesperiidae (6.3%). The results of this study could be useful for park managers planning to enhance butterfly diversity through their planting schemes and is expected to support the information on the conservation management and the ecological role of the butterfly's species in Sukhothai Historical Park.

T1-07-03

Butterfly assemblage in Kuala Lumpur's urban parks

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Kuala Lumpur is the capital city and urban centre of Malaysia. The city is experiencing rapid urban sprawl across the Klang Valley conurbation and continues to experience strong population growth. Given the location of Kuala Lumpur and the Klang Valley in a highly threatened biodiversity hotspot, understanding the biodiversity carrying potential of urban habitats and the associated influencing parameters is critical, but so far has received little attention. We examined the species diversity of butterflies in urban parks in the Federal Territory of Kuala Lumpur and investigated the relationships between butterfly species richness and three park variables: i) park size, ii) distance from the central business district and iii) park age. Standardised butterfly sampling was conducted across different microhabitat types at each park: i) groves, ii) hedges, iii) flowerbeds and iv) unmanaged areas. We sampled 572 butterflies belonging to 60 species from five families. In total, 58 (97%) of the species sampled were considered common species in peninsular Malaysia. The two rare species belonged to the genus *Taractrocera* (Hesperiidae); 4 species from *Taractrocera* are known from peninsular Malaysia and all are rare. Although species richness was positively correlated with park size and age and negatively correlated with distance from the central business district; the correlations were weak and not statistically significant. However, species richness of host-specialist species was significantly positively correlated with park size and age. The highest species richness was recorded in the unmanaged microhabitat. It is likely that both park planting scheme and the presence of early successional plants in unmanaged microhabitat led to highest butterfly species richness in parks that contained all four microhabitat types. Whether a diverse planting scheme and increased size and number of unmanaged areas in urban parks can improve the ability of parks to sustain populations of rare butterflies in the face of future urbanisation remains to be seen.

T1-07-04

Urbanization alters bee assemblages through species turnover rather than species loss

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It is generally observed that species assemblages between urban and non-urban habitats are very different. However, it is unclear that if this pattern is mainly driven by species loss or species turnover. The evolutionary core hypothesis suggests that the more historical-persistent the stressor is, the more native species can respond to it. Compared with natural stressors such as altitudinal gradient, urbanization gradient is a relatively new stressor. Thus, dissimilarity between urban and non-urban habitats should be mainly driven by species loss rather than species turnover. In this study, we tested if the loss component is significantly higher than the turnover component in 13 non-urban and urban bee assemblages across Europe and North America. Surprisingly, we found that the turnover component is significantly higher than the loss component ($P = 0.01$), suggesting non-urban species are replaced by other species in urban areas. Removing exotic species from the analysis does not alter the conclusion. We also found that there is no significant difference between bee density and rarefied species richness between urban and non-urban habitats. Our results do not support the hypothesis that urbanization leads to beta diversity pattern through species loss. We speculate that while urban area is a relatively new habitat to species, its physical and biotic stress are still within the tolerance range of most native species.

T1-07-05

Structure and diversity of plants associated to *Medicago sativa* agro-ecosystem and its function as a refuge for butterfly and ant communities

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Several agro-ecosystems can serve as alternative sites of refuges and conservation of species diversity. Many studies have reported that the structure and diversity of plants associated with farmland can support a high species richness of butterfly and ant species, which are important pollinators and soil fertilizers, respectively. In this study, we characterized the structure and diversity of vegetation found on the edges of *Medicago sativa* farms in central Mexico. We also monitored and identified the species of diurnals butterflies (Rhopalocera) and ants associated with this vegetation. We worked under the hypothesis that, even in farms under intensive cultivation, the diversity of plants on the field edges is enough to support high species richness of butterflies and a stable community of ants. Butterflies, ants and plants were counted along three transects at the edges of a cultivated field during 12 months. Twenty-two pitfall ant-traps and Van Someren Rydon butterfly traps were distributed across the length of each transect, which was 250 m. The plants surrounding each trap (area of 1 m x 1 m) were counted and identified. We found 2,700 individuals of plants, belonging to 48 different species from 24 families; 1,490 individuals of diurnal butterflies, belonging to 57 species from six families; and a total of 6,454 individual ants belonging to different 15 species. The most abundant plant species were Poaceae, Fabaceae, Asteraceae, Solanaceae and Malvaceae. Most than half of the plant species found (58%) were native flora of central Mexico, 91.66% of them with ethnobotanical use (as medicine, forage or food). Even though the Simpson's diversity index showed no significant differences between transects, the Jaccard index showed that the compositional similarity between species is very low. ANOSIM analysis showed no significant differences, confirming that three transects have slow compositional similarity. In agreement with this, the beta diversity values (Wilson and Schmida) were high. The Canonical Correspondence Analysis showed that plant families explain 60% (first ordination axis) of the abundance of Rhopalocera species and 77% of the abundance of Formicidae species. The abundance of both butterfly and ant species can be divided into three groups associated with groups of plant families. Eight butterfly species were migratory, most of them associated with a group formed by Amaranthaceae, Euphorbiaceae, Annonaceae Lamiaceae Apiaceae and Fabaceae families. Most ant species were also found associated with these plant families. We have not finished conducting the necessary multiple regression analyses yet, but we hope to identify some key plant species that have made this particular agro-ecosystem a very important reservoir of diversity of butterflies and ants. This could provide useful information to implement eco-sustainable strategies for the management of similar agriculture lands.

T1-07-06

The role of urban plants in supporting diverse urban butterfly communities

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Butterflies are among the most charismatic insect groups. Butterflies are closely dependent on plants. In the larval, or caterpillar, stage, most butterfly species are specialized for herbivory on a restricted group of related host plant species (although the degree of specialization varies). In the adult stage, most butterfly species feed on nectar and are less specialized, although proboscis length may restrict feeding to flowers of suitable corolla depth and nectary positions. These relatively predictable food-related dependencies suggest that plant selection in horticultural landscaping can be carefully tailored to support butterfly diversity and populations of targeted butterfly species in heavily human-modified areas, such as urban areas. In the urban parks of Singapore in Southeast Asia, we found evidence that flowering intensity and the diversity of native cultivated trees were positively associated with butterfly species richness. This suggests that phenology and the use of native species are important considerations when designing urban park plantings for butterflies. We also found that insectivorous bird species richness is associated with butterfly abundance, most likely due to the availability of caterpillar prey. Managing plant composition for butterflies could therefore also have knock-on effects for higher trophic levels. This could be generalized to other groups of insect herbivores, which tend to be specialized consumers of plant species. However, the landscape context of cultivated host plants also matters. Placement in unfavourable environments may result in the creation of population sinks and ecological traps that would reduce, rather than enhance, populations. I will report some preliminary results regarding this from an on-going study.

T1-08: Hot forests and cold storage

T1-08-01

The tropics: Hot, wet and recalcitrant

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The Tropics, parts of North and South America, Africa, Asia and Australia that lie between the latitudes of the Tropics of Cancer and Capricorn, account for only c. 36% of Earth's landmass yet host c. 80% of the world's terrestrial biodiversity. The tropical climate is best described as warm to hot and moist year-round (with only a limited dry season) and often supports lush vegetation. However, temperature and rain can vary greatly from one area to another within this zone, and so too can biodiversity. The meta-analysis conducted in the present study revealed that whilst most authors are non-committal regarding the exact biological richness of the Tropics, data available for plants suggest that they house the highest richness for numerous lineages; host most of the global centres of species richness; and contain regions that differ greatly in terms of species diversity. Importantly, they also host a number of species that produce recalcitrant seeds that exhibit desiccation sensitivity, a biological phenomenon that is antipodal to the desiccation tolerance observed in resurrection plants and the majority of plant seeds (labelled orthodox). Desiccation sensitivity has been extensively investigated in recalcitrant seeds largely because its consequences preclude their storage using conventional seed storage methods. Our analysis of primary and secondary data suggests that there are c. 600 recalcitrant-seeded taxa reported to date but this figure is ever-increasing. Of these reported taxa, 433 species (431 terrestrial and 2 aquatic) have been shown to be found in the Tropics. The 431 terrestrial tropical recalcitrant-seeded (TTR) species belong to 62 families (199 genera), 13 of which have ≥ 10 species with this seed storage physiology: Dipterocarpaceae (86), Meliaceae (30), Arecaceae (26), Lauraceae and Myrtaceae (21), Fabaceae (20), Moraceae and Saptoaceae (19), Fagaceae, Rhizophoraceae and Sapindaceae (15), Clusiaceae (11) and Anacardiaceae (11). Furthermore, six genera were shown to possess ≥ 10 TTR species: *Shorea* (40, Dipterocarpaceae), *Artocarpus* (17, Moraceae), *Dipterocarpus* (13, Dipterocarpaceae), *Hopea* (12, Dipterocarpaceae), *Quercus* (10, Fagaceae) and *Trichilia* (10, Meliaceae). Using these data as a backdrop, the current contribution identifies TTR species (as well as genera and families) that represent conservation priorities based on their International Union for Conservation of Nature (IUCN) status (e.g. certain Dipterocarpaceae species) and discusses some of practical challenges and successes associated with the short-, medium- and long- term seed germplasm conservation of TTR.

T1-08-02

Personalization of cryoprotection: A systematic approach to optimize chemical and osmotic effects

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Recent advances in developing cryopreservation methodology has underpinned the wider application of cryogenic storage for the long-term conservation of important agricultural crops and further has been applied for endangered wild species. Although an appropriate cryopreservation protocol is a prerequisite for basic studies, cryobanking and further cryopreservation studies, the process relies on trial and error. Among the vitrification-based cryopreservation techniques, droplet-vitrification produces high post-cryopreservation recovery. However, the protocol cannot universally solve all challenges in plant cryopreservation. Specifically, not all materials have the same (in) tolerance to desiccation and cytotoxicity to the most widely used cryoprotectant solutions. This presentation suggests alternative approaches to the development of cryopreservation protocols based on the initial characteristics of plant materials, by introducing specific steps in a droplet-vitrification method. Firstly, the osmotic tolerance of samples can be classified based on their response to overnight preculture at various final concentrations of sucrose. Secondly, some alternative vitrification solutions to the popular plant vitrification solutions 2 and 3 (PVS2, PVS3) can be considered based upon the cytotoxicity and size of the samples. Using this systematic approach we can identify whether the material is tolerant or sensitive to the osmotic stress and chemical toxicity of cryoprotection with vitrification solutions, thus revealing which is the main barrier to the successful application of vitrification methods. Based on the sensitivity of samples we can design a droplet-vitrification procedure, i.e. preculture, osmoprotection, cryoprotection, cooling and rewarming. This enables the development of appropriate, 'personalised' droplet-vitrification protocols that can be used to facilitate the preservation of plants, particularly endangered species for which there may be limited material available.

T1-08-03

Frozen architecture: Preserving cellular and tissue structure of explants for an optimal cryopreservation

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Recalcitrant seeds cannot be dried and cooled to the standard conditions of seed banks and cryopreservation of small explants is currently the most feasible option for their long term storage and conservation. But cryopreservation of recalcitrant materials (particularly the embryo axes) presents diverse challenges during, for example, the excision of the selected explants, partial drying, cryoprotection, and cooling. Aside from oxidative stress, ice crystal formation and growth, cell and tissue shrinkage during partial desiccation and/or cryoprotection, and uneven drying among diverse tissues of the explants are some of the major problems. They often involve the collapse of the cell and tissue architecture of the explants throughout the drying and cooling steps, which ultimately affects function (i.e., development and growth) during the *in vitro* recovery phase. Diverse tools have been employed in the last 25 years to investigate these processes, contributing to the understanding of the mechanisms that lead to the failure or success of the cryopreservation process from a structural perspective. With these specialized tools that combine comparative plant biology, biophysics, microscopy, and mechanics, researchers have been able to measure and observe the changes that happen while the explants are dried and cooled. This paper will review this topic, focusing on the most current research, and identifying future areas for investigation. By knowing the mechanisms that confer protection to partial desiccation, cryoprotection and exposure to cryogenic temperatures of diverse explants during the cryopreservation process, we should be able to better predict optimal cryopreservation protocols for the rich tropical (and temperate) flora that produce recalcitrant seeds.

T1-08-04

Cryobiotechnology for the long-term conservation of life

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Life of many world's plant species is seriously threatened by different biotic, abiotic and anthropogenic reasons, which are provoking important losses at increasing rates. According to FAO, the highest rates of woody species loss are in the tropics, and forest species are the most affected by the anthropogenic impact. At present, the main challenge of plant cryobiotechnology is dealing with the preservation of highly endangered species, particularly as there is limited material to work with, little knowledge of their behavior, and the likelihood of material that is recalcitrant for storage and *in vitro* growth. The advances in plant cryobiotechnology associated with the use of *in vitro* culture techniques and cryogenic storage for different type of germplasm have provided new tools to face these difficulties and created new opportunities for multiplication, recovery, micrografting, and long-term *ex situ* conservation of such species at ultra-low temperature (-196°C). Current development of plant cryopreservation can be associated with the strategic adjustments in key cryoprotective steps. For vitrification-based protocols, these include: the exposure of material to highly concentrated formulations of osmoprotectants (loading and plant vitrification solutions); the encapsulation of specimens in calcium alginate; and the use of aluminum foil strips or cryoplates instead of cryovials to achieve ultra-rapid cooling in liquid nitrogen. These procedures have significantly enhanced the effectiveness of cryopreservation of plant germplasm. Genetic transformation technologies can also be an innovative approach for improving cryostorage and longevity of less stress tolerant species. They may be used to induce intracellular accumulation of organic osmolytes increasing the tolerance and protection of cells against stress, and consequently improving cryopreservation outcomes.

T1-08-05

Cryopreservation of some Thai orchid species

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Thailand is the origin of about 1,300 tropical orchid species and 180-190 genera. Deforestation and over-collection of wild Thai orchids for trade has placed orchid species at a risk of extinction, such as the genus *Paphiopedilum* and *Dendrobium cruentum* which are listed in Appendix I of CITES. Therefore, the conservation, as well as sustainable use is urgently needed to conserve orchids by various means. Various methods of cryopreservation of Thai orchid species are implemented. Recent methods were used, namely vitrification [dehydration in PVS2 solution, consisted of 30% (w/v) glycerol, 15% (w/v) ethylene glycol, and 15% (w/v) dimethyl sulfoxide, prepared in modified Vacin and Went liquid medium], encapsulation-dehydration (encapsulation in calcium alginate beads followed by air-drying in a laminar air-flow cabinet), encapsulation-vitrification (encapsulation in calcium alginate beads followed by dehydration in PVS2 solution), droplet-vitrification (fast freezing from small drops of PVS2 solution on aluminium strip), and cryo-plate (a combination of encapsulation and droplet on very fast freezing aiuminium plate) dehydrated with silica gel and drying beads. Application of these methods in seeds was successful in *Dendrobium chrysotoxum* (99%, vitrification). *Dendrobium cruentum* (32%, virification), *Dendrobium draconis* (95%, vitrification), *Dendrobium hercoglossum* (80%, encapsulation-vitrification). *Doritis pulcherrima* (62%, vitrification). *Rhynchostylis coelestis* (85%, vitrification), *Vanda coerulea* (67%, vitrification), as well as in protocorms of *Dendrobium cruentum* (33%, vitrification; 27%, encapsulation-dehydration), *Dendrobium cariniferum* (15%, encapsulation-vitrification), *Grammaytophyllum speciosum* (14%, encapsulation-vitrification), *Rhynchostylis gigantea* (19%, vitrification), *Vanda coerulea* (40%, encapsulation-dehydration) and *Seidenfadenia mitrata* (67%, vitrification) and *Arundina graminifolia* (76% and 74%, cryo-plate dehydrated with drying beads and silica gel, respectively; 33% droplet-vitrification; 64% encapsulation-dehydration with drying beads or silica gel), and protocorm-like bodies of *Paphiopedilum niveum* (22%, vitrification). Cryopreserved seeds, protocorms, and protocorm-like bodies were able to develop into normal plantlets. These methods appear to be promising techniques for cryopreservation of some Thai orchid species.

T1-08-06

Current progress in understanding cryopreservation effects on oxidative stress and metabolism of Western Australian species: A combined biophysical and metabolomics approach

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The Australian continent hosts a unique assemblage of plants with many endemic species. Post-European settlement including agriculture, grazing and development of urban centres has resulted in drastically diminished native habitats with their attendant floras in both coastal and inland arable regions, hence many indigenous species are now in danger of extinction. Cryopreservation of somatic tissues of critically endangered species where there are few individuals (hence little or no seed), or seed axes of recalcitrant species provides the safest long-term *ex situ* conservation approach, however post-cryopreservation survival and regeneration varies substantially between plant species. In order to better understand this variability in cryo-capability, fundamental aspects affecting cryopreservation were assessed in a variety of Australian species, such as membrane stability and composition, oxidative stress and respiration rates. Membrane damage is a leading cause for a lack of cryogenic success, with the application of preconditioning treatments linked to improved membrane stability. The composition of membrane phospholipids, sterols and sugars was assessed before and after preconditioning to identify changes that may be beneficial for surviving the cryopreservation process. Oxidative stress during the cryopreservation process is another important factor that can alter cryopreservation success. Antioxidants, as the major defence against oxidative stress, were assessed throughout the cryopreservation process to determine if excessive oxidative stress was a factor in reducing post-cryogenic success. Mitochondria are the powerhouse of the cell, hence any damage done during cryopreservation will have a detrimental effect on ATP production, thus reducing synthesis of new compounds and repair of damaged components of the cells. Respiration rates in recovering shoot tips were analysed to determine if mitochondrial function correlates to cryogenic success.

T1-09: Exploration history on eastern Asian floras

T1-09-01

A hundred years of exploration on flora of China (1916-2016)

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A hundred years has passed since the first modern taxonomic paper was published by a Chinese botanist in 1916. The general history of Chinese plant taxonomy can be roughly divided into the following periods: hard beginnings from 1910-1920s, wartime from 1930-1940s, restarting again from 1950-1960s, looking ahead from 1970-1980s, resplendent time from 1990-2000s, and finally flowering gloriously from 2000-2010s. The representative works of Plant Taxonomy in China are Flora Reipublicae Popularis Sinice (FRPS, Flora of China, Chinese edition, 1959-2004), up to 80 volumes, and Flora of China (FOC, Flora of China, English edition, 1993-2013), up to 25 volumes and 24 illustration respectively, plus more than 30 different local works both in provincial and higher levels, particularly from southwest China, such as Flora of Yunnan (20 volumes), Flora of Guizhou (10 volumes), and Flora of Xizang (Tibet, 5 volumes). There are about 300 families, more than 3,435 genera, and over 31,000 species of vascular plants from China, with more than fifty percent of them being endemic, which includes many relic elements, such as *Cathaya*, *Taiwania*, *Davidia*, as well as living fossils, such as *Ginkgo* and *Metasequoia*. Particularly, there are hundreds and thousands of ornamental plants either native or originating from China, such as *Rhododendron*, *Meconopsis*, *Primula*, *Gentiana*, *Camelia*, as well as Peony, Roses, Tea, which have been cultivated worldwide, which is why China has been called "Mother of Gardens" by Ernest H. Wilson (1929), a famous explorer from England who visited China during 1899-1911. This work is the first time that the general history of Plant Taxonomy in China has been summarized with various widely consulted publications both in China and abroad, including many archives which have never been reported before; and many facts have been confirmed for the first time. All in all, the first herbaria, gardens and arboreta, research institutions, journals and magazines, university along with their departments of Biology, Agricultural and Forestry, as well as the first graduates both from abroad and from China; all were established, founded, finished and completed during 1910-1930. However, since the wars as well as other issues, most of the taxonomic works, both research papers and monographic floras, have mainly been published in the last third of the century from 1980-2010s, including many young taxonomists as well as Ph.D. students have been trained, only very recently.

T1-09-02

Present data on Vietnam orchid inventory

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The orchid flora of Vietnam is the richest among all other regional floras of mainland Asia. Successive inventories show an increasing orchid number from 411 species in the year 1934, 718 spp. in 1992-1994, 897 spp. in 2003-2005, 1005 spp. in 2009 and 1090 species & 160 genera figured in 2011. Since last inventory, 116 species and 13 genera have been additionally discovered and documented in Vietnam. The present report summarizes published and new original data for the last 5 years. When the new data presented here were included, the orchid flora of Vietnam should comprise at least 1213 documented species from 174 genera. One genus discovered for this period in Vietnam has broad distribution in mainland subtropical and temperate Asia (*Cephalanthera*), 8 genera have broad Malesian distribution (*Abdominea*, *Hymenorchis*, *Cordiglottis*, *Grammatophyllum*, *Macropodanthus*, *Microtatorchis*, *Octarrhena*, *Tuberolabium*) and 4 genera are endemics of Indochina (*Bidoupia*, *Drymoda*, *Lockia, Vietorchis*). At least 116 species were discovered in Vietnam during last five years. Among them, 63 were discovered and documented as a new species for the flora of the country. These species form 4 groups: species with Mainland Asia – Himalayan distribution (19 species), species of South-Chinese floristic province (9 species), Malesian species (15 species), species of Indochinese Peninsula (20 species). Other 53 discovered species are local endemics of following floristic provinces: South-Chinese floristic province (6 species), North-Indochinese floristic province (15), Central-Annamese floristic province (4 species) and South-Annamese floristic province (28 species). All newly described endemics well illustrate unique character of local aboriginal floras of eastern Indochina and reveal some peculiar specific evolutionary threats in the orchid family. Brief illustrated data on floral morphology, distribution and ecology are provided for all newly discovered species. Expected IUCN Red List national status is identified for these species as well. Largest part of discovered species (67 species, or 58%) belongs to threatened categories: VU (20 species, or 17%), EN (25 species, or 22%) and CR (22 species, or 20%). Other discovered species (49 species, or 42%) belong to non-threatened categories: DD (32 species, or 28%) and LC or lover risk (17 species, or 15%). Totally for the last five years in studied area were discovered and described three new genera – *Bidoupia* Aver., *Lockia* Aver. and *Vietorchis* Aver., as well as at least 54 species new for science –

*Aerides phongii**, *Bidoupia phongii**, *B.khangii*, *Bulbophyllum bidoupense*, *B.blaoense*, *B.glabrichelia*, *B.phitamii*, *B.setilabium*, *B.striatulum*, *B.tipula*, *Cheirostylis glandulosa*, *Cleisostoma dorsisacculatum**, *C.lecongkietii**, *C.phitamii**, *C.tricornutum*, *Cordiglottis longipedicellata*, *Cymbidium repens**, *Dendrobium congianum*, *D.thinhii**, *Eria grandicaulis*, *E.praecox*, *Flickingeria xanthocheila*, *Gastrochilus kadooriei*, *Hymenorchis phitamii**, *Liparis caudata*, *L.honbaensis*, *L.longibracteata*, *L.longispica*, *L.ngoclinhensis*, *Lockia sonii**, *Luisia parviflora*, *Malaxis subtilis*, *Miguelia cruenta**, *Nervilia mekongensis*, *Oberonia dolichostachys*, *O.janae*, *O.ngoclinhensis*, *O.rhizoides*, *O.sonlaensis*, *O.tatianae*, *Octarrhena minuscula*, *Odontochilus aureus*, *Paphiopedilum tamphianum**, *Podochilus rotundipetala*, *Sarcoglyphis tichii**, *Schoenorchis hangianae**, *S.scolopendria*, *Taeniophyllum phitamii*, *Tainia acuminata, T.cornuta*, *Trichoglottis canhii*, Vanda gracilis*, Vanilla atropogon** and *Vietorchis furcata.* Almost all these taxa are strict endemics of Vietnam. Orchids, names of which are marked above by asterisk (*) stand presently on the verge of full extinction.

T1-09-03

The early exploration of the Nepal Himalaya: 1793-1850

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The early scientific botanical studies of the flora of Nepal were undertaken by British natural historians, mostly in connection with the developing relationship between the British East India Company and Nepal. The first botanist to visit Nepal was the Scottish medic Adam Freer, who accompanied William Kirkpatrick on a short mission to Kathmandu in 1793. However, no botanical collections are known from this time and so the start of the scientific study of the Nepalese flora is credited to Francis Buchanan-Hamilton, who made extensive collections of herbarium specimens and living plants during his year in Kathmandu as surgeon to the diplomatic mission led by William Knox in 1802-3. Buchanan-Hamilton recorded over 1200 plant species, but most were never published at the time and so his achievements as the Father of Nepalese botany have not been fully appreciated. Botanical research resumed around 1818 when Nathaniel Wallich sent his local collectors to work with Edward Gardner, the first permanent Resident to the Nepalese Royal Court. Wallich sent specimens back to London, where David Don published the first floristic account of Nepal in 1825 - Prodromus Florae Nepalensis. Wallich later spent a year in Kathmandu and collected over 2000 species. These, together with many thousands of S Asian plants, were distributed by him around 1830 and documented in the 'Wallich Catalogue' (now online http://wallich.rbge.info). Joseph Hooker made extensive botanical collections in the Sikkim Himalaya in 1848, and from his base in Darjeeling he journeyed into eastern Nepal and made the first collections from this, the botanically richest part of the country. It would be another 100 years before foreign botanists ventured into Nepal, when this secretive Himalayan kingdom opened its boarders to foreigners. The achievements of these early botanical explorers will be discussed, along with a consideration their role in laying the foundation of botanical research in Nepal, the Himalayas and wider eastern Asia.

T1-09-04

Russian explorers of East Asian flora

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Russian botanists made a great contribution to the study of East Asian flora—both the territory of Russia itself as well as neighboring countries. Karl Maximowicz in 1853–1857 for the first time explored the south of the Russian Far East and also travelled along the rivers Amur (Hēilóng-jiāng) and Ussuri (Wūsūlǐ Jiāng). As a result the famous "Primitae Florae Amurensis" (1859) have been published. In 1859–1862 he continued his exploration of the Russian Far East and also visited Japan. Maximowicz became one of pioneers of the study of Japan plants, especially flora of Hokkaido where he successfully worked together with his assistant Sukawa Chonosuki. In 1854 Russian naval officer Alexander Schlippenbach made first small plant collection from the Korean Peninsula. Famous explorer Nikolay Przewalski in 1867 visited the Ussuri area and Manchuria. Later, in 1870–1886 he led several expeditions to China and Mongolia, mainly their desert parts, but also visited several areas with East Asian flora. His activity was continued by expeditions led by Grigory Potanin, Mikhail Pevtsov, Vsevolod Roborovski, Pyotr Kozlov, Veneamin Ladygin and others. Collections of mentioned above botanists and explorers have formed a special section in the Herbarium of the Imperial Botanical Garden in St. Petersburg (now—Komarov Botanical Institute). Since that time Russian botanists have the continued interest to the study of the flora of China, Mongolia, Japan and Korea. Vladimir Komarov during 1895–1897 made a great field exploration in Manchuria and also visited North Korea. The results of this expedition were published in 3-volume "Flora of Manchuria" (1909). Komarov not only presented a complete species description of the area but also made very important phytogeographical suggestions. This study can be assigned as a significant step in developing of his original approach to species definition (known as Komarov species concept) which later became basic for the "Flora of the USSR". Ivan Palibin prepared "Conspectus flora Coreanae" (1899-1901) — the first summary of the flora of Korea. During 1955–1957 a series of Soviet-Chinese biological expeditions visited badly explored areas of Yunnan and Sichuan. Some collections were made also in North Korea in 1957. The part of Russia with East Asian flora (current Primorsky and partly Khabarovsk Territories, South Sakhalin and part of Kuril Islands) was intensively studied during the whole XX century. Vladimir Komarov and Evgenia Klobukova-Alisova prepared and published "Manual to plants of Far East Territory" (1931-1932). Their activity was continued in 1950-1985 by Vladimir Voroshilov, Dmitry Vorobyev, Pyotr Gorovoi, Alexander Schreter and others: a number of field guides, articles on taxonomy and floristics were published. The greatest achievement in the study of this area is 8-volume series "Vascular Plants of the Soviet Far East" (1985–1996) led and edited by Sigizmund Kharkevich. The preparation of this series facilitated developing of teams of Russian botanists working on Far East flora in several centers (Vladivostok, St. Petersburg, Moscow, *etc.*).

T1-09-05

The forerunner of Chinese plant collecting—A study on the

collecting history of Mr. K.K. Tsoong

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Through a carefully literature and specimen research, we studied the collecting history of K.K. Tsoong who was the Chinese plant collecting pioneer. After the study, we clarified that the earliest specimens collected by K.K. Tsoong were in 1903, Tianmu Mountain, Zhejiang Province, and are also the earliest plant specimens collected by Chinese scholars. Mr. K.K. Tsoong was the first Chinese scholar who large-scale collected plant specimens in China. From 1918 to 1921, he collected about 15,000 specimens from 11 provinces of China, e.g., Fujian, Guangdong, Yunnan, Zhejiang, *etc*. Specimens collected by K.K. Tsoong distributed in major herbariums from the United Kingdom, the United States and China. In particular, Museum of Biology, Peking University (PEY), Herbarium, Institute of Botany, CAS (PE), Beijing Normal University Herbarium (BNU) stored most of the specimens. His collections were the basement for the build of PEY, and play a great role in plant taxonomic review, the compiling of Flora of China, Flora of Hebei, and so on.

T1-09-06

New taxonomic bibliography of Chinese vascular plants

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The Chinese and English version of the national flora completed in 2004 and 2013, respectively, cover more than 30,000 kinds of vascular plants, with most of the taxa published for decades ago. However, new taxonomic treatment, either new taxa or new nomenclatural data have been changed continuously. Therefore, there is an urgent need for the acquisition of the dynamic information of Chinese vascular plants. Due to the relatively loose requirements of ICN (International Code of Nomenclature for algae, fungi, and plants) on effective publication, many taxa published in regional journals and books, especially in a local or less important publications, are difficult to be included in the international retrieval mechanism (such as IPNI, Tropicos) timely. And incognizance of Chinese characters for foreign researchers also affects the efficiency for Chinese information retrieval. The project of Bibliography of Chinese Vascular Plants for New Taxonomic Data aims at to solve the above problems systematically. The objectives of this project is dealing with the taxonomic literature of new taxa, name changes, synonyms, records and typifications for all of the vascular plants distributed in China by steps, systematically collecting the information from the literature and digitally publishing the information. Specialized researchers will be able to retrieve the specific information after registration. These documents will be browsed, downloaded, borrowed, or accessed using the document delivery services according to the policy of the publisher company or database services. The database of Bibliography of Chinese Vascular Plants for New Taxonomic Data contains the following items: a, Publication information: the name of book or journal, publishing year, volume, number and the page number range; b, Article information: author (s), title and language; and c, Taxa information: name of taxon, category of taxonomic treatment, page of taxon, type specimen and the herbaria in where the specimens preserved, distribution in the world and China, and other pertinent information if possible. The database will be opened to professional scholars, and information retrieval of all items can be realized through a database entry. As a part of the renewal of A Bibliography of Eastern Asiatic Botany (1936) and A Bibliography of Eastern Asiatic Botany Supplement I (1958), the project of Bibliography of Chinese Vascular Plants for New Taxonomic Data only includes the work of vascular plants distributed in China, and will be completed in about five years. Two books in the series will be published before July 2017, covering the taxonomic literatures from 2000 to 2015. In the next five years, six books will also be added to fill gaps in the previous literatures, thus forming a complete database of plant taxonomic literature in China since 1958.

T1-10: Spatial phylogenetics as applied to floras around the world I and II (two sessions)

T1-10-01

Spatial patterns of phylogenetic diversity and endemism in the vascular flora of Chile

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Phylogenetically-based methods are increasingly important tools for conservation. The combination of ancient and more recent geological processes makes evolutionary history crucial to the understanding of distributional patterns. This study addresses questions about phylogenetic diversity and endemism across Chile, using Phylogenetic Diversity (PD), Phylogenetic Endemism (PE), Relative Phylogenetic Diversity (RPD), and Relative Phylogenetic Endemism (RPE) along with statistical tests based on spatial randomizations. Endemism is one of the main considerations when defining conservation priorities; an endemic territory is tightly correlated with the idea of irreplaceability and consequently with how vulnerable a place is. Chile is a highly endemic country, since it acts as a biogeographic island, concentrating unique evolutionary lineages with different spatial and temporal origins. When incorporating phylogenetic information, endemism can be seen as the geographic concentration of evolutionary history of lineages in any portion of a phylogenetic tree. Spatial patterns of phylogenetic diversity and endemism were studied for Chile using a genus level phylogeny of the native vascular plants of the entire country. Distribution for each genus was determined using georeferenced information from fieldwork, literature, and herbaria. While Central Chile (a recognized biodiversity hotspot) showed the highest concentration of taxon richness, PD and RPD were significantly higher than expected in the south of the country (below 35° latitude), while the north showed less PD and RPD than expected. Many areas of significant PE were found in the north of the country, with a concentration of neo-endemism. The south of the country showed areas of significant paleo-endemism. These patterns of endemism make sense from a geological point of view and considering the origins of the flora that inhabit the different areas. These analyses provide a new perspective on conservation since they highlight important areas that might not be conservation priorities. To study this, the areas of significant phylogenetic diversity and endemism were geographically compared to the country′s protected areas (PAs) to see if and how much of the state system is currently protecting places that concentrate the most phylogenetic diversity and/or areas of significant paleo and neo-endemism.

T1-10-02

Spatial phylogenetics of the native California flora

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California is recognized as one of the world's top biodiversity hotspots and has a rich history of research on its vascular flora. Terms such as neo- and paleo-endemism were first used to describe significant elements of the California flora by Stebbins and Major and later adopted worldwide. Raven and Axelrod proposed various hypotheses as to how the diverse Californian flora has assembled over time, and much has been written on the biogeography and vegetation. All of these studies were carried-out using pre-cladistic taxonomy and species as the operational unit. A re-examination of the flora using newly available herbarium databases and novel phylogenetic approaches is critical given the need to prioritize conservation efforts in the face of rapid habitat loss and human-induced climate change across the state. We built a large spatial dataset comprised of specimen-based distributional data from the Consortium of California Herbaria and other collection databases, at a scale of 15 km x 15 km grid cells. Considerable effort was made to clean the spatial dataset to represent the natural range of the full California vascular flora; 1.38 million geo-referenced records remained after cleaning. We assembled a phylogenetic data matrix for 1,083 OTUs (i.e., terminal clades representing genera or monophyletic parts of genera) for 9 genes, beginning with data from GenBank and adding 1,182 new gene sequences targeted to fill in data gaps. A maximum likelihood phylogeny was constructed. These two datasets were used to find regions of significant phylogenetic diversity (PD) and phylogenetic endemism (PE) within California. We applied the recently developed metrics Relative Phylogenetic Diversity (RPD) and Relative Phylogenetic Endemism (RPE), as well as Categorical Analysis of Neo- And Paleo-Endemism (CANAPE). Observed biodiversity is not necessarily an indicator of significant biodiversity and therefore should not be used alone. We used a spatial randomization to develop null hypotheses used for statistical tests of the above indices. Using these tests we can determine whether more or less of the phylogeny occurs in an area than expected (PD), whether branch lengths in an area are longer or shorter than expected (RPD), and whether significant concentrations of long or short-branched endemism occur in an area CANAPE). We found: (1) extensive phylogenetic clustering in the southern coast ranges, southern Great Valley, and deserts of California; (2) significant concentrations of short branches in the Mojave and Great Basin Deserts and the south coastal ranges, and long branches in the northern Great Valley, Sierra foothills and the northwestern and southwestern parts of the state; (3) significant concentrations of paleoendemism in northwestern California, northern Great Valley, and western Sonoran Desert and neo-endemism in the White-Inyo Range, northern Mojave Desert, and southern Channel Islands. Multiple analyses were run to observe the effects on significance patterns of phylogenetic uncertainty, using uncalibrated trees (with raw molecular branch lengths) vs. time calibrated (ultrametric) trees, and artificially filling sampling gaps through species distribution modeling. We found that results are largely robust to phylogenetic uncertainty and time calibration, but ecologically modeled ranges tend to affect the ability to detect localized centers of endemism.

T1-10-03

A new spatial phylogenetic method for distinguishing neo-endemism, paleo-endemism, and meso-endemism: Range-weighted branch length difference (RWiBaLD)

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It is important to understand spatial patterns of phylogenetic endemism (PE), both for academic studies of the ecological, biogeographic, and evolutionary causes of endemism, as well as for practical conservation applications. We propose a new method called range-weighted branch length difference (RWiBaLD) that extends a previously published method called categorical analysis of neo- and paleo-endemism (CANAPE). A range-weighted tree (RWT) starts with a phylogenetic tree with branch lengths and divides the length of each branch by its range size, thus the wider ranging branches are disproportionately shrunken down and most of the length of the tree is in range-restricted branches. The RWT is the basis for PE: PE is PD measured on a RWT. The RPE metric is a ratio comparing standard PE measured on the RW original tree to PE measured on a RW comparison tree (that retains the same topology but has all branch lengths adjusted to be of equal length before range-weighting). CANAPE is a two-step method that employs RPE and a spatial randomization to allow a clear, quantitative distinction between centers of neo- and paleo-endemism. Step one of CANAPE is to establish that a location is a center of high PE: it must be significantly high (one-tailed test) on either the RW original tree, the RW comparison tree or both. Step two is to examine the significance of the RPE ratio itself (two-tailed test). If the ratio is significantly high (greater than one), that means the range-restricted branches in that location are longer than expected (indicating a concentration of paleo-endemism). If it is significantly low (less than one), that means the range-restricted branches in that locality are shorter than expected (indicating a concentration of neo-endemism). When the ratio is not significantly high or low, there is some unknown mixture of lengths of range-restricted branches in that location that is not dominated by either neo-endemism or paleo-endemism. RWiBaLD delves into this mixture of branch lengths in a location by examining each branch of the phylogeny (both terminal and deeper branches), determining the amount and type of phylogenetic endemism (PE) it contributes, allowing a clear understanding of which branches on the tree are responsible for the broad, summary pattern given by CANAPE. The branches contributing the most PE are selected by taking those with the smallest ranges, and the type of PE is determined by the difference between the length of the branch on the range-weighted original tree and the length of the branch on the RW comparison tree. Where this difference is strongly positive, the branch is a paleo-endemic; where this difference is strongly negative, the branch is a neo-endemic. High PE branches that show only a small difference belong in an unrecognized category in the study of endemism: we here coin the term "meso-endemism" to refer to range-restricted lineages of intermediate length. We show how these categories of endemism can be mapped on the phylogeny as either continuous or quantitative traits for use in comparative methods, with an illustration using the genus *Acacia* in Australia.

T1-10-04

Spatial phylogenetics of the vascular plants of Florida: The effects of tree uncertainty and ultrametricity

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The recent data explosion in biodiversity and geophysical data, along with novel analytical methods and tools, has enabled an unprecedented capability to both model species distributions across space and time and integrate those results into broad-scale diversity assessments. As part of a larger project to understand plant biodiversity in Florida, U.S.A., we report on the development of phylogenetic trees explicitly built for the purpose of integration with spatial ecological and biodiversity knowledge. We chose Florida as a focus for study because it is home to a hyper-diverse biota, encompassing a broad range of terrestrial and aquatic habitats. Florida's ecosystems include over 4,000 species of vascular plants alone, and there is a unique floristic transition in Florida from temperate forests in the north to tropical elements in the south. We compare the results of phylogenetic diversity analyses based on a phylogenetic tree constructed specifically for this purpose with those derived from a common approach of pruning a subset tree from a pre-assembled supertrees using Phylomatic and the Open Tree of Life. Further, we compare the results from both phylograms and chronograms and highlight the differences in interpretations between these two types of trees. Finally, we take into account uncertainty in the phylogenetic process and how that may affect measures of spatial phylogenetics. We provide evidence that careful consideration of phylogenetic tree construction and use is essential for proper interpretation of spatial patterns.

T1-10-06

Spatial phylogenetics of the Australian flora

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Biodiversity is usually measured by examining changes in the number of species across a region to identify areas of high species diversity, endemism and turnover. Our research group is applying a novel suite of spatial phylogenetic tools including two new metrics, Relative Phylogenetic Diversity and Relative Phylogenetic Endemism, and new methods called categorical analysis of neo- and paleo-endemism (CANAPE) to measure biodiversity. CANAPE searches for centers of endemism, and classifies them by the branch lengths of the rare taxa within them, allowing, for the first time, a clear, quantitative distinction between centers of neo- and paleo-endemism across an area. We apply these metrics on newly developed near species level phylogenies for *Acacia, Banksia,* and *Eucalyptus* and genus level phylogenies for the entire Australian flora (>1,900 genera). When we combine these phylogenies with the detailed spatial data from the Australian Virtual Herbarium available through the Atlas of Living Australia, we can understand patterns of endemism through time, space and clade. While patterns of phylogenetic diversity and endemism are similar to patterns of taxonomic diversity and endemism, in depth analysis of the distribution of long and short branch taxa provide compelling insight and significance tests of evolutionary patterns of endemism. Areas with an overrepresentation of short branches are primarily found in the arid interior of Australia while areas with an overrepresentation of long branches are found in southern and coastal areas. As expected the areas of significant endemism change depending on the phylogenetic scale of terminals and when different components of the flora are analyzed. For example the Southwest Australia is a significant area of phylogenetic endemism at the genus level in *Acacia*, *Banksia* and *Eucalyptus*. However the southwest is much less important than the Wet Tropics of Queensland in the analysis of the entire flora at the genus level. Observed PD and PE, as well as taxonomic richness and endemism, are inadequate to fully understand patterns of biodiversity. The combination of statistical tests applied here can be used to better explain biodiversity patterns and the evolutionary and ecological processes that have created them. The spatial phylogenetic methods used can also be used to identify conservation priorities at any geographic scale or taxonomic level. Understanding patterns of biodiversity on the landscape is important for conservation planning, given the need to prioritize efforts in the face of rapid habitat loss and human-induced climate change.

T1-10-07

High-resolution phylofloristics reveals evolutionary constraints on the assembly and future migration of a regional vascular flora

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The emerging discipline of phylofloristics analyzes the ecological and evolutionary processes driving past and future patterns of community assembly across landscapes. Here we integrate a phylogeny and ecological niche modeling and present a phylofloristic analysis for all members of the Wisconsin vascular flora to quantify fine-scale variation in potential species richness and phylogenetic diversity. We calculated the mean phylogenetic distance (MPD) of species in over 50,000 geographically structured assemblages throughout the state, and tested whether these assemblages are phylogenetically clustered or over-dispersed. A shift from phylogenetically conserved to over-dispersed assemblages occurs over only 4.5° of latitude, in a flora shaped by Holocene dispersal following deglaciation. Spatial phylogenetic turnover is most strongly related to changes in annual minimum temperature and secondarily to spatial distance. Species ranges and assemblage composition appear driven primarily by phylogenetic niche conservatism. Over the next 50 years, our models predict large-scale extirpation of northern species within Wisconsin and widespread increases in ranges of southern species. Substantial species turnover is expected throughout the state, with a net increase in regional diversity as Wisconsin becomes more climatically suitable for southern and eastern species. However, our models predict that the geographic structure of phylogenetic diversity will be largely lost as a result of this turnover. These analyses should provide a useful baseline for targeted conservation strategies, by identifying specific lineages and geographic assemblages that are most at risk. Our datasets and approaches should also be directly applicable to the analyses of similar patterns throughout North America.

T1-10-08

Delineate phytogeographical regions of China: A phylogenetic approach

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Biogeographical regionalization provides an indispensable background for research of biodiversity and conservation. Previous regionalizations were proposed mainly based on taxonomic dissimilarity and/or endemism, while ignoring the evolutionary relationship among taxa. We used a mega-phylogeny of 2,591 genera of angiosperms and their geographical distributions to quantify both taxonomic and phylogenetic dissimilarity (or beta diversity) between grid cells. We applied a hierarchical cluster analysis on the phylogenetic beta diversity matrices to generate a phylogenetic regionalization of the Chinese flora. We also explored the differences of environmental variables among biogeographical regions and the correlations between environmental variable and phylogenetic turnover. An indicator genus analysis was used to characterize the genus composition of each of the biogeographical regions. Phylogenetic relatedness has significant correlation with taxonomic composition for floras in China. The lowest spatial turnover in phylogenetic relationships of the floristic assemblages occurred in Hengduan Mountains, Qin Mountains, Taihang Mountains and Yan Mountains, whereas the highest spatial turnover in both taxonomic and phylogenetic composition was inferred to be in the southernmost part of China. Two major groups (South Cluster and North Cluster) and five subgroups representing five floristic regions were recognized based on phylogenetic beta diversity matrices. The regions differ significantly in three environmental variables and indicator genera. Mean annual precipitation (MAP) is the most important environmental variable distinguishing between regions; Paleotropical region has the most indicator genera while Holarctic region has the least indicator genera. The five regions we recognized can help develop strategy to effectively conserve the taxa and floristic regions with different origins and evolutionary histories.

T1-10-09

Phylogenetic composition analysis of the world's tropical forests suggests that two major tropical forest types existed during Gondwanan breakup

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Based on an extensive data set containing more than 4,000 plots, about a million trees and approximately 15,000 tree species, we compare the world's tropical forests based on the phylogenetic similarity of their angiosperm tree communities. We identify a basal division in the world's tropical forests that corresponds to the breakup of Gondwana in a western and eastern portion, with Africa containing elements of both. We also find a connection between Asia and America that likely represents remnants of the once extensive Boreo-tropical forests. Our analysis shows that Gondwana may already have contained two distinct tropical forest types prior to or shortly after breakup (ca. 100 million years ago), a time when angiosperms were still a relatively young group of plants. Our analysis shows the usefulness of including phylogenetic information in floristic analysis for biogeographic inference.

T1-10-10

The spatio-temporal patterns of diversification of global gymnosperms

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The evolutionary history of gymnosperms has long been a con-

troversial issue for plant evolutionary biologists. Although this group is thought to be older than angiosperms, increasing number of recent studies have revealed that many gymnosperm species may be much younger. Here, we use 21 genes and 46 fossil calibrations to infer an dated phylogenetic tree including all 1,118 extant gymnosperms. We used this complete species level tree of the extant gymnosperms to study the historical patterns of their diversification. We also assess the effects of past diversification dynamics on the present day diversity patterns. We found that most extant gymnosperm species are rather young and have diverged in the Neogene. A strong increase in net diversification rate was observed from 10 Ma to present, which may be the results of rapid diversifications of cycads and conifers. Geographically, gymnosperm species diversity and evolutionary rates were found to vary between continents, which imply distinct evolutionary patterns across continents. We speculated that the paleoclimate and tectonic events rather than the modern environmental factors are the main determinants of gymnosperms' contemporary spatial pattern. Our study is the first to depict the global evolutionary history of gymnosperms in a spatial context and reveals the important role of Cenozoic climate and deep-time tectonic events in shaping the contemporary diversity pattern of gymnosperms.

T1-10-11

Quantitative geospatial data and area definition in modern hstorical biogeography: The continental Australian flora as a case study

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The study of historical biogeography requires the definition of biotic areas (areas of endemism). Areas are core to the entire research program of historical biogeography, whether for model-based or more traditional approaches, yet their definition remains problematic. With recent shifts towards model-based biogeographic analyses there is renewed interest in biotic areas, or rather methods to define barriers or turnover of biodiversity in a landscape – which are all related concepts. This is at its most challenging within a continental landmass, where there are few sharp barriers or boundaries to define biotic areas. For example, in Australia vast areas are arid or semi-arid environments, and these have traditionally been treated as a single huge area in many analyses. In addition, the definition of biotic areas has been inconsistently applied across Australia, with some regions comprised of many small biotic areas and others much larger in size, with no consistent methodology used to define scale or boundaries. Therefore, using Australian plants and the quantitative geospatial data now available through large-scale databasing of the Australian flora (through Australia's Virtual Herbarium and Atlas of Living Australia projects), we examine problems with the prevailing existing area definitions in Australia and discuss possible newer methods to define areas, include some recent results and a new bioregionalisation of Australia.

T1-10-12

The recent and rich high elevation flora of the southern south American at risk under climate change

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Historical records around the globe show high altitude species can contend with global warming by migrating into cooler conditions. However, in seeking cooler conditions, the amount of suitable habitat available in the case of many species is expected to be reduced on account of the geometry of mountainous terrain, the orientation and degree of isolation of mountain ranges and their peaks, and regional changes in precipitation. High elevation, above-treeline or bioclimatically-equivalent habitats in South American Andes, found at progressively lower altitudes toward higher latitudes, support a rich alpine flora. Dated phylogenies and the nested positions of some genera within larger clades indicate a fairly recent origin for many clades. Distributional modelling employing Ensemble Forecasting (BIOMOD), georeferenced herbarium occurrence data, two climate models and two climate change scenarios on a large pool of high altitude species from the southern Andes (27°-56°S), predicts there will be far more losers than winners under climate change, even when unlimited dispersal capacity is assumed and a more conservative temperature increase is considered. Whether the severe habitat loss predicted for many species by these models becomes fully manifest will depend on the ability of high altitude species to hold on at the trailing edge and reach favorable thermal microsites within their present altitudinal ranges. Better knowledge of dispersal capacity and of establishment success at the leading edge of alpine species′ altitudinal ranges is critical to our understanding of the impacts of global warming on high altitude floras, as is knowledge on physiological traits such as frost resistance and reproductive traits such as flower longevity.

T1-11: Biodiversity informatics and Mapping Asia Plants (MAP)

T1-11-01

Tri-trophic interactions among pubescent oak (*Quercus pubescens* Willd.), green oak leaf roller (*Tortrix viridana* L.) and their parasitoids

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In this study, tri-trophic interactions between green oak leaf roller (*Tortrix viridana* L.) which causes complete defoliation in oaks, heavily defoliated pubescent oak (*Quercus pubescens* Willd.) trees distributed in Akdağ Nature Park (Turkey) and parasitoids of *T. viridana* were investigated. The green oak leafroller (*T. viridana*) is a major defoliator of oak woodlands. Within the beneficial entomofauna accompanying the leaf rollers (Lepidoptera: *Tortricidae*) a big role is played by parasitic insects from Hymenoptera. They attack the larvae and pupae of tortricids, limiting the density of their population. As a result of this study many adult parasitoid individuals were emerged from *T. viridana* pupae. These parasitoids are members of Hymenoptera belonging to Ichneumonidae family *Itoplectis maculator*, Pteromalide family *Cyclogastrella simplex* and *Pteromalussemotus*, Torymidae family *Monodontomerus aer-*

eus and Chalcididae family *Brachymeria tibialis*. In Turkey, green oak leafroller pest mainly affects *Q. pubescens* L. Interactions between trees and their pathogenic, parasitic and herbivorous enemies are among the driving factors of co-evolutionary processes in forest ecosystems. Whether there is an interaction between the species that characterized with regard to morphological and biochemical was investigated on the basis of various statistical analyses. As a result of statistical analyses there is a significant relationship, which validated with observed species number from all stations, between parasitoid *I. maculator* habitat selection and amount of volatile 2 Hexenal, *T. viridana* and amount of trans Caryophyllene. This relationship is emerging as an S.O.S signal that released from *Q. pubescens* trees against *T. viridana* attacks. As a result environmental changes not only have an indirect importance in plant defense mechanism but also have an importance place in volatiles releasing mechanism in attacked plants by herbivore. If oak trees can take enough essential elements from soil they can activate signal mechanisms that are related with defense.

T1-11-02

An introduction of Southeast Asia Plant Biodiversity Information Infrastructure (SEADiv) from Asia Biodiversity Conservation and Database Network (ABCDNet)

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Southeast Asia represents four global biodiversity hotspots, Sundaland, Wallacea. Philippines and Indo-Burma. Its complex and diverse topography, and varying climates, provide a partial explanation for the high biodiversity. So far, the rich flora is still largely unknown, and many taxonomic inventory work still needs to be done. As part of Asia Biodiversity Conservation and Database Network (ABCDNet), we aim to build a platform for sharing biodiversity information in Southeast Asia—Southeast Asia Plant Biodiversity Information Infrastructure (SEADiv), a pilot project. Our main aim is trying to integrate current available data based on flora books and herbaria collections, and then synonymize all scientific names to make a preliminary checklist for higher plants of Southeast Asia. First step, we made an inventory of 328 Southeast Asia flora books or book series and other taxonomic literatures, based on the collections of 32 libraries from Southeast Asia, and several other libraries from Netherlands, London, New York *etc.* where significant plant taxonomic work has been carried out in the colonial times. Based on literature analyse and key plant taxonomist interview, 35 flora books or book series are listed as key floras, the taxonomic names, and distributions are typed into a database. Second step, an inventory of 1,350,000 specimens from 105 herbaria of Southeast Asia and other countries were obtained through online resources such as GBIF, Tropicos, USDA, JSTOR, HerpNET, MaNIS, OBIS, ORNIS, REMIB or acquired based on directly inquiry. Third step, TNRS (Taxonomic Name Resolution Service v4.0) based on The Plant List program are used as the basis for synomyomize the data gathered from key flora books and specimen data. Final step, the online platform is being prepared to share information about the prelimanary higher plant checklist of Southeast Asia.

T1-11-03

Mining hidden biodiversity data which were collected in North Korea: Based on the herbarium collections before 2nd World War

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The Korean Peninsula has been recognized as a biodiversity corridor that would connect in a consistent manner critical habitat in Northeast Asia. Six decades after the end of the Korean War, Korea remains trapped in mutually reinforcing security dilemmas, so called cold war. It has been hard to imagine a direct South-North scientific exchange as well as an indirect cooperation through the international platform such as the GBIF. As Peter Raven recommended, it is urgent to establish a regional biodiversity network which maintains all records of the occurrence of organisms that the organizers have been able to gather. The available data in North Korean region are inaccessible and not well managed and formatted. For publishing, harvesting and using biodiversity data from Korean Peninsula, which aims to document and synthesize knowledge on the botanical richness of Korea peninsula. The potential for improved knowledge in the form of well documented biodiversity information is now recognized as essential for a better understanding and more effective conservation of Korean plants. The Korean Peninsula Flora (KPF) database builds on earlier work that used data aggregated from heterogeneous sources before the end of the 2nd World War, such as herbarium collections from TI, KYO, TUS, E, A, and other diverse literatures. KPF database comprises ca. 65,000 accessions of vascular plants collected from Korean peninsula from 1850 to 1945. Georeferencing was to be accomplished on a largely individual place name and record basis, by using numerous maps, gazetteers, and spatial databases, and also through consulting collectors' itineraries. Among these, material from People's Republic of Korea (DPRK) is represented with ca. 33,000 accessions. The largest part of this material originates from Russsian botanist, Komarov, V.L and Japanese, Nakai, T. that are followed by other collectors, such as Imai, H., Mills, R. G., Furumi, M., Nomura, N., Saito, T., and Okuyama, S.

T1-11-04

Impact of future climate change on the distribution patterns of seed plants endemic to the Tibetan Plateau

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Evidences have shown that many species have already shifted their distributions as a response to climate change, which may greatly impact the function of local or even global ecosystems. However, few studies have discussed the species vulnerability on a regional basis. As one of the most sensitive regions to the global climate change and unique for its rich biodiversity, the Tibetan Plateau has undergone an earlier and faster warming process compared to the global mean along with changes in precipitation during last decades and may continue in the future at a faster pace. Here, we collected the distribution information for 1,672 endemic seed plants of Tibetan Plateau and assessed the effects of future climate change on the diversity and distribution of 1,095 species using an ensemble species distribution modeling framework. We projected range shifts of these species at year 2050 and 2070 under two RCP scenarios (RCP 2.6 and RCP 8.5) and further evaluated the threatened status for these species based on the International Union for Conservation of Nature criteria. We also evaluated the protection effectiveness of national and provincial nature reserves on these species. Main conclusions are summarized as follows: (1) Species distribution models performed well in modeling the current distribution of endemic seed plants on the Tibetan Plateau. Species richness is high in the mountainous area of south-eastern plateau and low in the north-western part of the Tibetan Plateau. Temperature seasonality is the main factor controlling the distribution of most species. (2) Average richness across the plateau would increase in the future if consider full dispersal. The center of richness will move upward and to the center of the plateau. Severe species loss may happen in the southern edge of the Tibetan Plateau, especially the southern part of the Hengduan Mountains. The turnover rate of species is high in the north-western part of the plateau, indicated the vulnerability of this region under future climate scenarios. (3) It was predicted that the range of about 30% species would decrease in the future even when considering full dispersal. Taken species dispersal into count may aggravate the risk of threat. National nature reserves on the Tibetan Plateau may not be so efficient in protecting these endemic species under current climate condition, but could better protect them in future climate change. Our study provided insights for biodiversity conservation management in this region under climate change.

T1-11-05

Moscow Digital Herbarium and the National Depository Bank of Live Systems initiative (Russia)

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Imaging of specimens is the modern trend in the herbarium management. Today, at least 62 herbaria have 1 M+ specimens and at least eight herbaria have estimated their collections as 1 M. Even in the larger herbaria mass digitisation is still not a common practice. In late 2014, the Moscow University Herbarium (MW) had received direct investment for digitisation within the grant #14-50-00029 from the Russian Science Foundation as part of the National Depository Bank of Live Systems initiative launched by the Moscow State University. As of November 2016, MW Herbarium holds 1,011,253 specimens being the 60th largest herbarium in the world and the second largest in Russia. An average annual increase in the last 12 years was 15,100 specimens. We employed new technical staff to facilitate further growth of the collections, intensified the collection management and 22,013 specimens were added in 2016 as a result. The MW Herbarium holds 37,100 species and subspecies of vascular plants and 2,223 species and subspecies of mosses and liverworts. Having the stable budget for the next four years, we decided to scan 1 M specimens at 300 dpi (TIFF + JPG) in 2015-2018, to digitise 4.6 K type specimens at 600 dpi, to scan 78 K labels from bryophyte capsules, and finally to database and georeference label data from as much specimens as possible until exhaustion of the budget. As of 6 January 2017, the Moscow Digital Herbarium consists of 785,887 specimens—712,925 images of vascular plants and 72,962 images of bryophyte labels. Vascular plant specimens are originated from eastern Europe—357,951 images; Asian Russia—159,663 images; Caucasus—97,400 images; the Crimea—31,790 images; Mongolia—27,323 images; South Asia—22,649 images; Africa—7,559 images; types at 600 dpi—4,630 images; *Herbarium Alchemillarum*—3,960 images; and labels from capsules of mosses—72,962 images. Collections from Middle Asia, western Europe, Americas, Australia and Oceania, liverwort labels, and historical collections are not yet digitised. At the moment, we operate the sixth largest digital herbarium in the world. The Moscow Digital Herbarium is the largest Russian biodiversity database. The Moscow Digital Herbarium publishes its data on the web portal of the National Depository Bank of Live Systems, a collaborative network of the Moscow University biological collections. Since October 2016, a fast public version of the botany portal with some functional limitations is available at http://plant.depo.msu.ru/, whereas an operational version is available at https://plant.mitotech.ru/. The National Depository online system is open for contributions from other Russian biological collections and soon will be visible via GBIF. We are nearly ready to start in 2017 data capturing from labels. To avoid mistakes and to make the first portion of label capturing easy, we will not enforce the operators to capture data from every single sheet. We will ask the operators to avoid capturing data from labels with at least one unclear word or to tick any uncertain entries. Also, we are ready to pay modest remuneration for professional botanists from small institutions and universities for capturing the data which might be interesting for them using the forms on our web portal.

T1-11-06

World vascular plant checklist in the Catalogue of Life

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The Catalogue of Life (CoL, www.catalogueoflife.org/col) is a global taxonomic catalogue of valid species through all domains. It is built as a curated assembly of expert based Global Species Databases around the world. Today, the CoL contains basic information on 1.6 million valid species arranged in global classification, plus 1.5 million synonyms and over 400,000 common names. In 2015-2016, the CoL has aggregated world checklists for all families of vascular plants with 335,566 accepted species. This is about 99% of Tracheophyta species diversity recently recognised by taxonomists. Data from 18 plant databases has been assembled into the synonymic catalogue. Classes *Equisetopsida* (38 spp), *Lycopodiopsida* (1,393 spp), *Marattiopsida* (133 spp), *Polypodiopsida* (11,530 spp) and *Psilotopsida* (139 spp) have been provided by World Ferns database (Hassler M., 2017). Cycads (class *Cycadopsida*, 353 spp) has been taken from The World List of Cycads database (Calonje M., Stanberg L. & Stevenson D. (eds), 2015). Conifers (class *Pinopsida*, 615 spp) have been supplied by Conifer Database (Farjon A., Gardner M. & Thomas P., 2014). Classes *Ginkgoopsida* (1 spp) and *Gnetopsida* (112 spp) were taken from World Checklist of Selected Plant Families (Govaerts R. (ed), 2014). Flowering plants have been compiled from an array of 15 databases: AnnonBase (2,440 spp; Rainer H. & Chatrou L.W. (eds), 2014), Brassicaceae database (3,778 spp; Warwick S.I., Francis A. & Al-Shehbaz I.A., 2009), ChenoBase (2,007 spp; Walter J., 2014), Droseraceae Database (179 spp; Culham A. & Yesson C., 2008), Global Compositae Checklist (32,280 spp; Flann C. (ed), 2014), ILDIS World Database of Legumes (16,371 spp; Roskov Y., Zarucchi J., Novoselova M. & Bisby F. (eds), 2014), IOPI Global Plant Checklist (1,845 spp; Wilson K.L. & Berendsohn W.G. (eds), 2007), The Integrated Taxonomic Information System (Orrell T. & al., 2016), The Lecythidaceae Pages (311 spp; Mori S.A., Prance G.T. & Tulig M., 2006), Lacistemataceae Holistic Database (24 spp; Young F.E., 2009), Geranium Taxonomic Information System (344 spp; Aedo C., 2012), Solanaceae Source (1,082 spp; Knapp S., Walley L., Bohs L., Nee M. & Spooner D. (eds), 2008), World Checklist of Selected Plant Families (120,279 spp; Govaerts R. (ed), 2014), World Plants (133,957 spp; Hassler M., 2016) and World Wide Wattle database (1,469 spp; Maslin B., 2015). Global checklist of flowering plants in the CoL consists of 321,252 accepted species in total. CoL standard dataset includes accepted scientific names, synonyms, position in the classification, common names, references, distribution, ecological environment and credits to data providers and authors. We are updating CoL monthly. However, each database decides to have own schedule for updates depending on a progress. All data are available online as dynamic retrieval system, webservice, customised download service in DwC-A format and on annually published DVD (Roskov Y. & al., eds. (2017). Species 2000 & ITIS Catalogue of Life, 2017 Annual Checklist. Digital resource at www.catalogueoflife.org/annual-checklist/2017. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405-884X).

T1-12: Contribution of Brazilian private natural heritage reserves to Atlantic forest biome flora conservation

T1-12-01

Are there more original species in remnants with greater phylogenetic diversity?

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Greater phylogenetic diversity in a community is related to more complementary resource use and application by different groups. The increase of complementarity is associated to the presence of species that present rare phylogenetic traits, with unique functions in the community. These species are known as ‘original species’, and can be identified through an originality index. This metric can describe the structure of the community and assist in the determination of priority conservation areas. In the Chaco, a seasonal domain, exclusive to South America (Argentina, Bolivia, Brazil and Paraguay), species composition varies throughout its territory, according to abiotic and biotic factors, resulting in different phytophysiognomies. The aim of this study was to analyze the phylogenetic diversity in two remnants of Chaco and to verify if the most diverse one presented a greater percentage of original species. Our experiment was carried out in two remnants, with an area of 1ha in phytophysiognomy of forest and in woodland chaco. For phylogenetic analysis we calculated the Net Relatedness Index (NRI) and Nearest Taxon Index (NTI) and to calculate originality the distance between each pair of species, by the sum of the branches, needed to bind the pairs in the phylogenetic dendrogram. The most original species were those with a sum of decreasingvalues around 50%. In both areas, phylogenetic diversity was lower than expected at random (W = 1069, p = 0.44), indicating aggregation, possibly due to environmental filters acting on a regional scale, such as climate. However, the lower phylogenetic diversity in the woodland chaco (W = 1523, p = 0.01), may be related to more intense environmental filtration due to the dystrophic soil that selects for the establishment of certain groups. In the forest chaco, the eutrophic soils favor the establishment of species belonging to different groups, also increasing species richness. We collected 75 species, of these 66 found in the forest chaco and 26 in the woodland one. Of these only 17 species (21.3%) found in both remnants represented 51.5% originality. Twelve of them in the forest chaco and five in the woodland, proportionally the woodland chaco presented more original species (19.2%) than the forest one (18.2%) considering the richness. Furthermore, the less restrictive environment had the capacity to maintain species belonging to different phylogenetic groups, while the other selected for related groups with less parity between species. However, the higher percentage of original species, where the environment was more restrictive, allowed unique functions to be performed in the community, minimizing local redundancy and contributing to the maintenance of its functioning. With this, we conclude that the Chaco community, under local filtering, contains species belonging to groups less related to each other than the community in which the environment is less restrictive and allows the establishment of several groups.

T1-12-02

Woody plant diversity in the seasonally dry tropical forests of the Urubamba valley, a threatened biodiversity hotspot in southern Peru

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The woody flora of the seasonally dry tropical forests of the Middle Urubamba Basin, located in Cusco, southern Peru, was studied. Twenty plots of 50 m x 20 m (0.1 ha) were sampled from 700 to 1,300 Msnm; (DBH) ≥ 2.5 cm, representing all life forms (lianas, trees, shrubs, hemiepiphytes and cacti). 5,259 individuals have been recorded including 552 species, 303 genera and 82 families. The quantitative sampling reveals the presence of 92 species/0.1 ha, on average compared to the diversity already found in BTES of 150 species/0.1 ha with an alpha Fisher diversity index of 133.6 (Managua locality). The richest families were Fabaceae (69 species), Bignoniaceae (30), Moraceae (27), Apocynaceae (23) and Sapindaceae (22); the richest genera being *Inga* (13 species), *Aspidosperma* (11), *Ficus* (11), *Machaerium* (10) and *Eugenia* (9); the five most abundant species were *Allophylus punctatus* (142 individuals), *Pogonopus tubulosus* (122), *Warszewiczia coccinea* (111), *Annona neoulei* (106) and *Anadenanthera colubrina* (95). Half of all individuals (2,593,49.4%) were in the small DBH class (2.5-5 cm). The analysis of vegetation and abiotic variables show the existence of three types of BTES, the Amazonian pluviseasonal tropical dry forest, sub-Andean pluviseasonal tropical dry forest, and savanna-like pluviseasonal tropical dry forest. Initial data on phytogeographic relationships show that the BTES of the Urubamba are better related to the BTES nucleus of the Chiquitania of Bolivia and an almost null relation with other BTES nuclei Peruvian such as those of Tarapoto and Marañon. The state of conservation of these forests shows that much of these have been hugely deforested, and dry neotropical dry forest species such as *Amburana cearensis*, *Myroxylon balsamum* (Fabaceae), *Cedrela saltensis*, *Swietenia macrophylla* (Meliaceae), and other woody species have almost completely disappeared. Therefore, it is concluded that the high diversity of wood taxa, endemism's and particular phytogeographic relationships, indicate that the BTES of the Urubamba basin is part of the Andean-Amazon hotspots and forms an important nucleus of diversity for the BTESs. It has been concluded that these dry forests need urgent protection and conservation because of their rapid and alarming destruction.

T1-12-03

New species to science discovered at private nature reserves in the Brazilian Atlantic Forest Biome

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In Brazilian Private Natural Heritage Reserves located at the Atlantic Forest Biome and Hotspot some new species to science have been discovered. Publications in the Atlantic Forest Biosphere Reserves Booklets and Botanical Congresses have already mentioned some of these discoveries. In this presentation will be shown an updated compilation of the new species described in private nature reserves such as: 1. *Ficus lagoensis* C. C. Berg & Carauta – Figueira (Moraceae) - RPPN Fazenda Lagoa/Minas Gerais 2. *Neoregelia pernambucana* – Siqueira Filho & Leme - Bromélia – (Bromeliaceae) RPPN Frei Caneca/Pernambuco 3./ 4 *Couepia monteclarensis (Chrysobalanaceae),* e *Asterostigma lombardii*. (Araceae) - RPPN Feliciano Miguel Abdalla/Minas Gerais 5./ 6 - *Byrsonima pedunculata e Bunchosia pernambucana* RPPN Fazenda Bituri - Pernambuco.

T1-12-04

Conservation *in situ* of endangered species in private nature reserves in the Brazilian Atlantic Forest Biome

Maria Cristina Vieira, *Andressa Lima*

Associação de RPPN e outras Reservas Privadas de Minas Gerais

In this presentation the existing knowledge on flora conservation *in situ* in Private Natural Heritage Reserves in the Atlantic Forest Biome in Brazil shall be described. This information has already been partially published and presented in the Atlantic Forest Biosphere Reserve publications and also in several scientific events, such as Latin American and Brazilian Botanical Congresses. Several endangered species quoted in the Red Book of Brazilian Flora edited by the National Center for Plant Conservation of Rio de Janeiro Botanical Gardens (CNCFlora; cncflora.jbrj.gov.br) have been identified in private nature reserves in the Brazilian Atlantic Forest Biome. The following RPPN case studies will be presented: Fazenda Lagoa/MG; Frei Caneca/PE; Mata do Sossego/MG; Mira Serra/RS; Reserva Natural da Serra do Teimoso/BA; Santuário do Caraça/MG; Rizzieri/SP.

T1-12-05

Plant species richness in private nature reserves in the Brazilian Atlantic Forest Biome

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The Atlas of Forest Remnnants of the Brazilian Atlantic Forest Biome published by the Foundation SOS Atlantic Forest points out that only 8.5% of the original forest cover has survived and considers this Biome as a Hotspot. Considering that 80% of the this Biome remaining forest ecosystems are in private land and that there is still a lack of information concerning the Flora Diversity the Floristic Surveys done in RPPNs are of utmost importance. In this presentation the results of floristic surveys developped at RPPNs and published in the Atlantic Forest Biosphere Reserve publications and presented in several scientific events, such as Latin American and Brazilian Botanical Congresses shall be described. The following case studies of RPPNs revealing high flora diversity shall be presented: RPPN Salto Morato/P ; RPPN Caraça/MG; RPPN Fazenda Lagoa/MG; RPPN Feliciano Miguel Abdalla/Caratinga, MG; RPPN Veracell/BA.

T1-13: The evolution redwoods of event in botany sciences

T1-13-01

Fossil histories of redwoods from China and their implications for plant conservation

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Redwoods is general name for three genera of *Metasequoia, Sequoia*, and *Sequoiadendron*. Fossil histories of *Metasequoia,* and *Sequoia* have fossil records from China are reported and their implication in conservation are discussed in the current presentation. Dawn redwood, *Metasequoia* was widely distributed in the Northern Hemisphere since the Late Cretaceous and more than 500 fossil records were reported but native living dawn redowood growing in center China only. In China, most Metasequoia fossils were reported from Paleogene strata of Northeastern China.

Recently, fossil leaves and corns were found from the middle Miocene of Zhengyuan, Yunnan province. This is a southernmost fossil record of *Metasequoia*. Coast redwood, *Sequoia,* is endemic to Pacific Coast Range, however it has widely distribution regions in the geological time. Most *Sequoia* fossils have been recognized from the Paleogene and early Neogene sediments of mid-northern latitudes. Fossils of *Sequoia* after the late Miocene are scarce and absent from Asia and Europe in the late Pliocene. New *Sequoia* fossil reported from the middle Miocene of Yunnan. These fossils are morphologically similar to *S. sempervirens*, indicating a close affinity to their modern relative. The genus probably disappeared from Yunnan some time after the late Miocene. Here may arise a question of why *Sequoia* have gone extinct in Yunnan but sustain in other regions. Investigations demonstrate that aridification of the dry season (winter and spring) associated with monsoon intensification might have played a role. Today, Yunnan has dry conditions from the late autumn to next early spring, whereas the Mediterranean regions and the western coat of America have wet winters and springs. As seeds of *Sequoia* are recalcitrant, they germinate immediately upon their maturity in autumn. Unfortunately, dry conditions after the autumn may prevent their seeds from germinating or their seedlings from developing new leaves. In contrast to the modern pattern, Yunnan was shown to have relatively humid conditions in the dry season during the Neogene. Humid and suitable habitats might explain the past existence of the two genera in the region. As the precipitation in the dry season decreased linked to the Asian monsoon intensification as is elaborated below, the dry winter and spring ultimately led to their extinction in Yunnan. The similar case can be observed in *Metasequoia* as well. So monsoon climate would cause extinction or relic of *Metasequoia* and *Sequoia* from China.

T1-13-02

The present status and future prospect of American coast redwood planting in China

Minghe Li

Central China Agricultural University

In 1972, the former US President Richard Nixon brought one small American coast-redwood tree to China as a gift to Premier Zhau Enlai. By rooting cuttings from that small gift tree, about 4,000 stecklings were propagated and planted in many localities in China. That Nixon redwood clone and other redwood trees from introduced seeds have grown very well in southern China, so far much better than local major planting species *Cunninghamia, Cryptomeria* and *Metasequoia*. It thus appears that coast redwood has great potential importance for China's and thus Earth's timber economy and for the enjoyment and well-being of future visitors to redwood parks in China as well. However, redwood has not yet been planted widely in China. This is mostly because China does not have reliable pedigreed redwood seeds, rooting redwood cuttings is not yet operationally satisfactory, redwood tissue-culture is complicated and costly, and redwood somatic embryogenesis is still in early stages of development. In 2014, the author tried an idea: to establish a redwood 'sprout orchard', in which many sprouts grow up from still-attached stems shallowly buried in soil. Those sprouts are cut underground and used as juvenile cuttings thus predisposed for rapid rooting. By 2016, average rooting percentages of over 90% have been obtained, the stecklings raised from them are apparently satisfactory, this process can be cycled 2 or 3 times in a year and the cost is less than 1/3 that of tissue-culture. China has land, labor, economic strength and technology, and therefore, may play an important role in Earth's future redwood planting.

T1-13-03

The basic situation of original dawn redwoods conservation

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As Hu Hsen-Hsu said in 1947, "the discovery of dawn redwoods is the most important and interesting new finding in Botany in China". In 1941, *Metasequoia* was described as a fossil genus by Shigeru Miki. In 1943, Wang Chan collected some specimens from an unidentified tree in Hubei, China. In 1946, Cheng Wan-Chun and Hu Hsen-Hsu connected these specimens to *Metasequoia*. In 1948, Cheng and Hu named the species as *Metasequoia glyptostroboides* and published a paper entitled "On the new family Metasequoiaceae and on *Metasequoia glyptostroboides*, a living species of the genus *Metasequoia* was found in Szechuan and Hupeh". This discovery shocked the world, as "living fossils of *Metasequoia* was found". However, after recent years of extensive introduction and cultivation, *M. glyptostroboides* has been distributed almost all over the world. Studies on *M. glyptostroboides* had provided significant information to paleobotany, paleoclimate, paleogeography, and the phylogeny of gymnosperms. Li Jianhua and Ban Jide classified dawn redwood-dominated communities into three types: (1) *Metasequoia glyptostroboides-Camellia cuspidata-Parathelypteris nipponica* community, (2) *Metasequoia glyptostroboides-Camellia cuspidata-Bigonia-Pilea* community, and (3) *Metasequoia glyptostroboides-Eurya hebeclados-Iris japonica-Parathelypteris nipponica* community. The largest *M. glyptostroboides* population is in Xiaohe, an open river valley in weastern Lichuan. Xiaohe is located at N30˚10', E108˚45', to the south of Fubao Mountain, east of Qiyue Mountain, west of Maqian Town, and north of Zhonglu Town, with altitudes range between 900 m and 1,350 m. Xiaohe has an area of around 60,000 ha, a length of about 30 km (from south to north), and a width of about 20 km (from east to west). It's an ideal place for dawn redwoods to grow, with short sunshine duration, low evaporation, high precipitation (1,500.9 mm per year), high relative humidity, and 230.9 growing days per year. Fubao Mountain and Qiyue Mountain block the coldness coming in winter and the heat in summer from the plain, which is to the southeast of Xiaohe. The temperature in Xiaohe fluctuates between -15.4°C and 35.4°C, with an annual average of 12.8°C. Dawn redwoods grow along both sides of the valley on alluvial soil and irrigated by foothill creek. There are approximately 5,000 dawn redwoods grow in this area with an average diameter of 20 cm. In 1972, a dawn redwood orchard was established in Hubei, China. In 1981, the Provincial Government of Hubei approved the establishment of Lichuan Xiaohe County Dawn Redwood Nature Reserve, aiming to protect the original dawn redwoods, especially "Lichuan Modaoxi dawn redwood type tree". In 2003, the State Council of P. R. China approved the establishment of Hubei Xingdoushan National Nature Reserve. The National Government of P. R. China has been directly investing the conservation of original dawn redwoods and their associated ecosystems. More than 5,700 dawn redwoods are included in this conservation. In 2012, Chinese Dawn Redwood Botanical Garden was established, with an area of more than 400 acres. A database for the original dawn redwoods has been established to facilitate the conservation.

T1-13-04

A whole-plant reconstruction of a Cretaceous redwood with novel morphology and ecology

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2. Texas State University, San Marcos

The three living redwoods (*Sequoia*, *Sequoiadendron*, and *Metasequoia*) are the sole remnants of a diverse group that was once widespread across both hemispheres. Redwoods (the Sequoid clade of the Cupressaceae) have a rich fossil record that leads back through the Cretaceous, yet there are relatively few whole-plant reconstructions of fossil species from which to understand their complex evolutionary history. We have previously reported an extinct Late Cretaceous redwood from the Jose Creek Member, McRae Formation, of New Mexico based on associated shoot impressions, pollen cones, and *in situ* permineralized stump. Additional field collections have recovered seed cones and pollen cones at various stages of maturity in organic attachment to foliage shoots. Here we reconstruct the entire plant, assess its relationships through phylogenetic analyses of living and fossil redwoods, and interpret its ecology based on morphological traits and our ongoing reconstruction of the fossil flora. The Jose Creek redwood is represented by over 500 specimens, collected primarily from one extensive ash-fall deposit that preserves a 74.7 million year old subtropical to paratropical forest. Shoot impressions preserving multiple orders of branching show heteromorphic leaves of two distinct morphologies, similar to *Sequoia*. The thickest axes bear highly reduced (scale-like) leaves with short pointed free tips. These axes give rise to up to 3 orders of planate branchlets bearing bifacially flattened leaves with narrowly elliptic shape and decurrent attachment to the stem (taxodioid leaf form). However, similar to living and fossil *Metasequoia*, the Jose Creek redwood exhibits opposite decussate leaf phyllotaxy, with leaves secondarily oriented into one plane by torsion along the basal decurrent portion of the leaves. Pollen cones are ellipsoid with spirally arranged microsporophylls and are shed individually. Similar to *Sequoia*, they are borne terminally on ultimate shoots, or on highly reduced axillary shoots that bear only a few scale-like leaves. Seed cones are ellipsoid in overall shape, borne singly at the tip of shoots with either scale-like leaves or taxodioid leaves. Mature bract-scale complexes are peltate, with centrally located tip that is recessed in a distal transverse groove with ridges that radiate from the groove. Overall, the Jose Creek redwood shares characteristics with both *Sequoia* and *Metasequoia*, but its combination of characteristics is unique compared with living and other known fossil redwoods. Phylogenetic analysis clearly places the Jose Creek redwood within the Sequoioid clade, but not in the distinct group formed by modern and fossil *Metasequoia*. The Jose Creek redwood was one of the dominant taxa in highly diverse sub- to paratropical forest that included palms, *Zingerberopsis*, a few other gymnosperms, and diverse magnoliids and eudicots. The absence of bud scales or scale-like leaves at the base of the branchlets and the absence of growth rings in the wood suggest absent or poorly developed dormancy mechanisms, unlike Cenozoic and modern relatives. This is consistent with other evidence suggesting a regional paleoclimate with low seasonality, and demonstrates that the ecology of the Jose Creek redwood is unlike that of other known redwoods.

T1-13-05

California coast redwood park and nature reserve: Protecting redwoods since 1918

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The first people to see the coast redwoods and the giant sequoias were the ancestors of today’s native Americans-the nomadic hunters, fishermen and gatherers who migrated across North America about ten thousand years ago. These first discoverers were probably less astonished by the coast redwoods than were the first Europeans to see them, because they came to the redwoods through the ancient coniferous forests of the Pacific Northwest. Some of these trees were almost the equals of the coast redwoods for height, although none had ever been seen which rivaled the Giant Sequoia for sheer mass. The California Pacific Redwood Park and Nature Reserve has been protecting 36 very beautiful parks. Americans have long experienced the struggle to protect redwoods. Finally in 1918 an outstanding non-profit organization was set up: Save-the-Redwoods League. For nearly 100 years, the organization has educated generations for the benefit of mankind about protecting the excellent redwoods forest and guided California in the establishment of Redwood Park and its park protection guidelines. In 1928 the park and guidelines were consolidated into the California National Park System. Today, the California National Parks System is one of the best in the world, preserving vast natural resources and historic sites that provide education and entertainment for the benefit of the people and visitors from all over the world. Save-the-Redwoods League protects sequoias by purchasing sequoia forests and the surrounding land to nurture them. The principles of conservation biology guide our efforts to protect large, continuous mahogany land with forest diversity. With League’s donors’ support, Save-the-Redwoods League has protected over 200,000 acres (the size of 16 Manhattan Islands) and helped develop dozens of redwood parks and reserves for everybody to enjoy. League protection for redwood forests is unwavering and the vision for the future of redwoods is one in which the entire redwood forest ecosystem — public and private lands alike will continue to thrive and adapt to change. As our forests are increasingly threatened by unsustainable logging, development and climate change, it is essential for conservationists and foresters to work together.

This cooperative approach, in which active forests are managed alongside parks and reserves, allows for the permanent protection of more old-growth redwoods and wildlife habitat than would be possible otherwise. Science 1980's a Chinese American researcher at the University and Jepson Herbaria at UC Berkeley has initiated a new series of collaborations between Chinese and American botanists including Magnificent Chinese and American Redwoods, a bilingual book. Developments in the modern studies of molecular genetics, physiological ecology, and phylogeny -- underway in both the United States and China -- will continue to enhance this long tradition of collaboration and advance our ability to conserve these amazing trees and their forest ecosystem.

T1-13-06

The ancient redwoods: Where are the oldest, largest, and tallest redwoods?

Momei Chen
University of California, Berkeley

The three keystone species of redwoods have always been esteemed. (Taxonomy: The family Taxodiaceae traditionally comprises the redwoods (*Metasequoia*, *Sequoia*, and *Sequoiadendron*)). Modern molecular phylogenetic studies show that they are a monotypic genus of the family. The new family scientific name is Cupressaceae, which has nomenclatural priority over the traditional Taxodiaceae. The dawn redwood (*Metasequoia glyptostroboides* Hu et Cheng) was a "living fossil" widely distributed in high latitudes of the Northern Hemisphere in the early Cretaceous period (K2). During the Paleogene Period (E) and Neogene Period (N) of the Cenozoic, its distribution was extended and the genus included more than 10 species. In the Quaternary ice age, the genus became nearly extinct, with only one species surviving in central China within Shizhu (Chongqing), Lichuan (Hubei), and Longshan (Hunan). The range of the genus is broad at North Temperate Zone latitude 29º5′-31º32′, east longitude 108º-110º). Here, the sole surviving species, dawn redwood, occurs in extremely wonderful landscape areas. It persists on only one modest-sized stand and several scattered occurrences near the juncture of three provinces: Lichuan in West Hubei, Longshan in Hunan, and the deep valleys of Sizhu County of East Sichuan. The Cupressaceae includes two other related genera: the coast redwood (*Sequoia sempervirens* (D.Don) Endl) found in Pacific Coast Range. Redwood forests have been present in California for about 20 million years. They represent a unique and beautiful relic flora from the days of the dinosaurs. About a million years ago, advancing ice sheets confined the coast redwoods to their modern range. The glaciers left untouched a well-defined area, now known as the Redwood Belt, where the tall trees flourish, undisturbed. Giant sequoia (*Sequoiadendron giganteum* (Lindl.) J.Buchholz) are the tallest, most massive, and oldest trees in the world. They are found in the Sierra Nevada Range in California, which is 640 km long and 60–130 km wide, located mostly in E Calif. It rises to 4,418 m at Mt. Whitney, the highest peak in the United States outside Alaska. The range not only includes the renowned groves of massive giant sequoia, but the richness of the Sierran flora mirrors that of the state as a whole—of nearly 6,000 species of vascular plants known to occur in California, Calaveras Big Trees State Park, Yosemite National Park. The giant sequoia has the distinction of being the largest living thing in the world, past and present. The individual with the record size is the General Sherman Tree in Sequoia National Park. It has a volume of about 51,000 cubic feet (14,441,592 cubic meters) which, if made into a 2-by-4, would yield a board almost 175 miles (281,635 kilometers) long. To date, the bristlecone pines of the southwestern arid mountains are accepted as the oldest known living things; giant sequoias amongst the ancient ones. The oldest known giant sequoia is represented by a cut stump in the Converse Basin which, when the annual rings were counted, was determined to be at least 3,200 years of age.

T1-15: Building a global system for the conservation of all plant diversity

T1-15-01

The Millennium Seed Bank Partnership: A global seed conservation network

Jonas Muller
Royal Botanic Gardens Kew

Conserving plants *ex situ* as seeds in seed banks is a cost-effective conservation strategy intended as an insurance policy for the survival of plant species faced with threats in their natural habitats. The Millennium Seed Bank (MSB) Partnership is the world's largest and most ambitious *ex situ* conservation initiative of its type. The MSB Partnership is managed by the Royal Botanic Gardens, Kew (United Kingdom) and is a global network with currently 140 partner institutions in over 97 countries and territories covering all continents. More than half of those countries have active seed collecting projects running. The network is steadily growing, with more countries and institutions joining in, particularly from biodiverse countries. Over the last year, 14 partnership agreements have been renewed, and 13 new partner institutions have joined the MSB Partnership since 2015. Building on previous achievements and in line with the strategic outputs of Kew's Science Strategy, the MSB Partnership aims to collect 25% of the world's bankable flora by the end of 2020. Over 37,500 of the world's floral species are now secured in *ex situ* conservation, held in country of origin and in duplicate storage at Kew's Millennium Seed Bank. The majority of those seeds is available for restoration and research. Seeds held at the Millennium Seed Bank come from 187 countries worldwide, reaching beyond the geographical boundaries of the partner countries. The MSB Data Warehouse was established as a way of data sharing across the MSB Partnership. Currently, the MSB Data Warehouse holds data of 100,200 collections of 47,400 unique taxa (41,700 species) which are freely available to view for registered MSB Partnership users. 115 different users have consulted the MSB Data Warehouse in 2016. Training and capacity building is yet another important activity in MSB Partnership. During 2016 alone, Kew trained 245 individual partners from 27 countries. Our seed conservation experts provided 5,884 hours of training in 22 training courses.

T1-15-02

The Global Oak Conservation Partnership

Nicole Cavender[1], *Murphy Westwood*[1,2], *Kirsty Shaw*[2], *David Gill*[3], *Magin Georgina*[3]

1. The Morton Arboretum
2. Botanic Gardens Conservation International
3. Fauna & Flora International

Oaks (genus *Quercus*) are keystone species in a wide range of ecosystems around the world, such as oak-hickory forests and oak savannas in North America and subtropical broad-leaved evergreen forests in Southeast Asia. Globally, oaks are ecologically valuable for promoting terrestrial biodiversity, performing critical ecosystem functions, and providing food and habitat for countless species of animals. However, many oak species are threatened with extinction and oak-dominated ecosystems are declining worldwide. Furthermore, oak acorns cannot be seed banked through conventional methods, so long term *ex situ* conservation of threatened oak species must be through living collections. In order to prevent extinctions and ensure healthy oak populations for the future, conservation efforts must integrate tailored *in situ* protection and management activities in coordination with genetically diverse *ex situ* collections of living trees. In response to the global threats facing oak species, The Morton Arboretum, Botanic Gardens Conservation International, and Fauna & Flora International launched the Global Oak Conservation Partnership in 2015. The Partnership works with and supports organisations in regional oak diversity hotspots such as Mexico and Indochina to develop and implement conservation strategies and initiatives that directly support the vision and mission of the Global Trees Campaign – the only global conservation program dedicated to saving all of the world's threatened tree species. The goals of the Partnership are: (1) Oak species of greatest conservation concern are identified and prioritised for action; (2) The world's most threatened oak species are secure and their wild populations are recovering through conservation action; (3) *Ex situ* conservation collections of threatened oak species are scientifically informed, efficiently managed, genetically diverse and representative, and climate change resilient; (4) Partners, practitioners and the wider community are empowered to act for oak conservation. The Partnership will work towards these goals by creating a multi-disciplinary program of conservation activities driven and implemented by a multi-national group of partners. These partners include local and international stakeholders from botanic gardens, academic institutions, government agencies, conservation NGOs, protected area managers, land owners, local communities, policy makers and the private sector thus leveraging the expertise, knowledge and networks of a variety of sectors. Through coordinated action at a global scale, we will ensure that oak species diversity is secure for the future.

T1-15-03

PSESP (Plant Species with Extremely Small Populations) conservation in China

Weibang Sun
Kunming Botanical Garden, Kunming Institute of Botany, Chinese Academy of Sciences

The new concept of PSESP (Plant Species with Extremely Small Populations) developed for rescuing most of the globally threatened plant species was firstly promulgated in Yunnan province of China in 2005, and it has being globally recognized and accepted. PSESP is characterized by small remaining populations (far lower than Minimum Viable Population, MVP), restricted habitat, extremely high risk of extinction, and exposure to serious human disturbance. Scientifically, there is no MVP threshold for plant species, and therefore 5000 mature individuals with less than 500 mature individuals for each isolated population has been proposed for qualification as a PSESP in China, which was based on both a review of literature addressing global MVP, and conservation practices in China. For such species with an extremely high risk of extinction, rescuing populations together with comprehensive study is undoubtedly urgent. During the past 12 years, especially from 2011, several national and regional-level conservation strategies for actions have been planned (120 species have been targeted by the State Forestry Administration and National Development & Reform Commission, 62 species have been approved by Yunnan provincial government, and in total some 143 species in Southwest China are targeted for action). Meanwhile, activities such as field surveys, creating the in-situ conservation sites, propagation for both *ex-situ* conservation and population restoration, and PSESP germplasm banking, have all made great advances in some parts of China. Recently, with particular support from the National Natural Science Foundation of China (NSFC) and Ministry of Science and Technology of China (MSTC), several major PSESP projects have been fruitful. This presentation will take an overview of PSESP conservation programs throughout China, and will highlight the most current outcomes of our work on typical species such as *Craigia yunnanens*is, *Acer yangbiense, Manglietia ventii* and *Manglietiastrum sinicum*.

T1-15-04

Capacity building for red listing of plants

Malin Rivers
Botanic Gardens Conservation International (BGCI)

There are nearly 400,000 plant species in the world, but less than 7% of these have been given a global conservation status on the IUCN Red List of Threatened Species. Yes, there are further species assessed in national, regional, non-IUCN conservation assessments-but despite these we are still missing conservation status information for nearly half of the world's species. We therefore need to step up red listing for plants in order for us to know which species are in need of conservation action and how to prioritise this action. Botanic gardens are ideally placed to be involved in red list assessments. They are hubs of information on plants in terms of the biodiversity collection they host: living collections, herbaria, libraries, seed collections, *etc.* They are also centres of the human expertise on plants. Botanic gardens are already very active in the production of red list assessments on large, medium and small scales, and many of their staff, volunteers and research associates are involved in red listing. However, we need to better organise the red list effort for plants to ensure that no plant is forgotten. We therefore need a coordinated network of plant red list experts with the goal to enhance plant red listing-a community of practice for plant red listing. This community of practise can share knowledge, share expertise, share data, share tools, share training and share outputs. This approach to share information and experiences will ensure that best-practises are established and continue to evolve. The approach also needs to be cost-effective and efficient. Through a coordinated effort, plant conservation prioritisation through red listing will work towards the prevention of plant species extinction.

T1-15-05

Saving our charismatic megaflora: Conservation of the world's most threatened tree species in their natural habitats

***David Gill**, Georgina Magin, Victoria Price*
Fauna & Flora International

The world's estimated 60,000 tree species are one of humanity's most important natural resources. Trees provide a huge variety of timbers, medicines and foods for millions of people and enrich our daily lives by shaping many landscapes and cultures. However, extensive habitat loss and degradation, unsustainable harvesting and climate change threatens at least 9,600 different species with extinction. For many species, the long-term future of their wild populations now requires urgent and targeted conservation action. However such action is often limited in scope and the effectiveness of conservation interventions *in situ* is often constrained by limited understanding on how best to address the threats faced by individual species. Furthermore, many of the people responsible for managing the sites where threatened tree species occur have never had the opportunity to develop the technical skills required for tree conservation. The Global Trees Campaign, a partnership between Fauna & Flora International and Botanic Gardens Conservation International was established in 1999 to prevent tree species extinctions in the wild and ensure their benefits for people, wildlife and the wider environment. This presentation describes examples from FFI's field programme for threatened trees, as a contribution to the Global Trees Campaign. Various approaches used in the field to reduce threats, ensure sustainable use and reinforce and reintroduce some of the world's most threatened tree species will be described. Lessons learned from individual case studies will be shared, before opportunities to catalyse greater action for the world's threatened species are put forward for discussion.

T1-15-06

Sud Expert Plantes Développement Durable: A multilateral programme toward the development, the scientific cooperation, the support to collections, the capacity building and the avocacy for plant biodiversity in the Global South

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In keeping with the 2011-2020 Strategic Plan adopted by the Tenth Meeting of the Conference of the Parties to the CBD in Nagoya, Japan in October 2010, France scaled up its biodiversity action with a new National Biodiversity Strategy (NBS) for 2011-2020. Target 16 of the NBS aims at strengthening international solidarity and ensuring greater mainstreaming of biodiversity into French development assistance. Target 17 of this plan intents at reinforcing green diplomacy and international governance for biodiversity. In this context, France launched in 2015, the Sud Expert Plantes Développement Durable programme (SEP2D), a 5 years multilateral programme aiming at sustaining the scientific communities in 22 francophone countries in West and Central Africa, the Indian Ocean and South East Asia. Focussing on the sustainable management of plant biodiversity, SEP2D is supported by the French Development Agency (AFD), the French Global Environment Facility (GEF) and the French Ministry of Foreign Affairs and International Development (MAEDI) as well as other public and private partners among which the "Institut de Recherche pour le Développement" (IRD) and the "Muséum National d'Histoire Naturelle" (MNHN). The programme strategy is guided by a collegial expertise on the following themes: Forestry & Mines, the Valorisation of plants for medicinal, cosmetics & agro-biodiversity purposes, Research & collections and Training & Awareness. The scientific research on plant biodiversity is a cornerstone of the sustainable development in the Global South. It enables the identification and the building up of potential innovations derived from these countries' rich plant diversity but also provide the National policy makers the tools to preserve their sovereignty. Furthermore, a valorisation of the research on plant biodiversity is an essential pre-requisite to the development of a strong scientific expertise. A particular attention is needed on the appreciation of the enrichment of the collective knowledge, the individual expertise, the know-hows, the data collecting, their analysis and their valorisation to which the scientific community contributes. SEP2D is making every effort to bolster partnerships and collaboration in this regard, building bridges between research, education and societal demands. It encourages the scientific community to work on applied topics, such as the major challenges associated with tropical plant biodiversity assessment and conservation. It aims to reinforce the scientific research on plant biodiversity and the collections' and the botanical gardens' activities in these countries. With this approach, SEP2D not only aims to rely on natural resources markets as leverages for economic development, but also envisions to encourage long term mutually benefitting partnership inclusive of every stakeholders needs and aspirations. SEP2D seeks to participate to the development of a scientific research that would also be inclusive of national, sub-regional, regional and international public policy issues. A science, based on this South-North and South-South partnerships, that will advocate the National authorities, public stakeholders, private sector operators and associations in order to meet societal demands.

T1-16: Pollination and breeding systems of endangered plant species

T1-16-01

Fly pollination and rarity in the genus *Ceropegia* (Apocynaceae)

Jeff Ollerton
University of Northampton

Pollination by flies (Diptera) has been important to the diversification and ecology of the flowering plants, but is relatively neglected and poorly understood in contrast to pollination by (arguably more charismatic, better known and popular) groups such as bees, butterflies and birds. Species within the genus *Ceropegia* (Apocynaceae) employ a strategy of temporarily trapping their small (usually less than 4mm in length) fly pollinators, only releasing them following pollination and attachment of pollinaria.

The genus contains approximately 200 species, all of which were, until 2000, CITES listed due to the trade in wild collected plants. In the wild *Ceropegia* species tend to form very low-density populations and many are rare and endangered. Recent research on *Ceropegia* has resulted in some significant new findings in relation to the diversity of pollinators exploited by the genus, and the functional ecology of interactions with those pollinators. In this talk I will assess what we understand of these plant-pollinator interactions in relation to what is known about rarity and distribution of *Ceropegia* species. The analyses will be framed within the context of a more accurate understanding of phylogenetic relationships between the major groups than has previously been available. *Ceropegia* species have evolved a specialised trapping strategy to exploit atypical, "non-standard" pollinators. This strategy is ecologically (and evolutionarily) successful despite generally low population densities, raising the interesting question that rarity and pollination system may be causally linked in this fascinating group of plants.

T1-16-02

Reproductive ecology and conservation challenges of sub-alpine orchids in Yunnan after a decade of drought

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Yunnan, southwest China, at the eastern edge of the Himalayas, has a far higher diversity of temperate, terrestrial orchids compared to their better studied, congeners distributed through Europe and North America. However, rapid increases in ambient temperature and changes in precipitation rates are recorded in the Himalayan Region as symptomatic of Global Warming. Consequently, during the past decade of vernal drought, most populations of montane orchid species in Yunnan have experienced a dramatic retreat. To make the status of these species worse there remains a large gap between efforts to protect remaining populations vs. a lack of information on their reproductive ecology. We monitored the reproductive ecology of populations of sub-alpine orchids on mountains in northern Yunnan using a series of field and lab techniques including fluorescence microscopy, insect pollen load analyses, flower-insect morphometrics, and floral scent analyses (GC-MS). This produced new data sets that determined breeding systems, flower-pollinator interactions and seed viability analyses to represent species in eight genera (*Amitostigma*, *Calanthe*, *Cypripedium*, *Epipactis*, *Habenaria*, *Herminium*, *Orchis* and *Spiranthes*). Results showed that population sizes decreased in most species over 3-5 years due to spring droughts at higher elevations with a decline the frequencies of those plants producing flowering stems. Some species, e.g. *Cypripedium margaritaceum*, remained at a higher risk of regional extirpation than others. While all species studied were self-compatible but pollinators were needed to produce the greatest numbers of viable seeds. Pollinating insect species differed between Yunnan species vs. their European/North American congeners and highly specialized pollination systems were found in some *Cypripedium* species. The fecundity of some species appears limited by their pollinator reward systems (nectar or food mimesis), pollinator guilds and the desynchronization of the flowering periods of the orchids, their sympatric co-blooming flora and the emergence of their primary pollinators. Severe Spring drought delayed the flowering time of some *Cypripedium* species, delayed fertilization and arrested fruit maturation by winter time. Seed set rates in some multiple-flowering species were also inhibited by a combination of inbreeding depression, low temperatures, herbivory and anthropogenic stress.

T1-16-03

The reproductive biology of Asteracae on Oceanic Islands: Coping with rarity

Daniel Crawford[1], *Gregory Anderson*[2]

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2. University of Connecticut

Asteraceae are well represented on oceanic islands, often with the highest number of lineages and/or the most endemic/native species in an archipelago. This indicates that the members of the family are not only good dispersers and effective colonizers but also have the capacity to radiate and speciate in the island setting. Several reproductive attributes of the family contribute to their capacity to cope with rarity at different stages of the evolution of a lineage in the island setting. Initially, the colonizing ancestors will be very rare and face two major challenges: lack of compatible mates and/or pollen vectors; and insufficient genetic diversity for radiation and diversification. Two of these challenges can be met by self-compatible (SC), self-pollinating colonizers. However, SC colonizers will have been derived from highly selfing source populations, and thus will bring with them little genome-wide heterozygosity. As a consequence, the colonizers may well have little potential for diversification. Self-incompatible (SI) colonizers would have higher diversity but would lack both compatible mates (especially if derived from 1 or few disseminules) and likely lack pollinators. There is another alternative: some of the large insular lineages of Asteraceae arose from SI colonizers, but there is growing evidence that their colonizers may have been able to set some selfed seed (i.e., leaky, or pseudo-self-compatible, PSC). Thus, the SI-derived colonizer could establish a sexually reproducing population with higher diversity. In addition, dominance relationships among S-alleles in the sporophytic SI system of Asteraceae could increase the number of compatible mates in small populations. And, Asteraceae can be pollinated by a variety of vectors, making it feasible for them to utilize new pollinators in the islands. Despite high SI, low levels of PSC may result in populations with mixed mating systems, providing reproductive assurance in the colonization of disturbed areas on islands. We will discuss why the breakdown of the SI locus and associated origin of the selfing syndrome, both often associated with the colonization of new continental ranges, are rare occurrences in island lineages. Furthermore, habitats are fewer on young islands, and regular disturbances on islands may bring differently adapted species into contact, with resulting hybrids. Asteraceae are particularly amenable to hybridization because of their generalist pollinators. For rare species, hybridization could either increase genetic diversity, introduce new variations in reproductive biology, or drive them to extinction. Dioecy on islands has been considered advantageous because it promotes increased outcrossing. However, if populations decrease, skewed sex ratios may reduce effective population sizes and eventually drive species to extinction. Research on Asteraceae endemic to the Juan Fernández Archipelago and the genus *Tolpis* from the Macaronesian archipelagos will be used as examples. In addition, the reproductive biology of Asteraceae in the Juan Fernández and the Canary Islands will be contrasted, to show the possible roles of abiotic factors in shaping differences.

T1-16-04

Pollination syndromes in a biodiversity hotspot

Kingsley Dixon
1. Curtin University
2. Missouri Botanical Garden
3. Kings Park and Botanic Garden

The southwest Australian biodiversity hotspot contains approximately 8,000 species comprising 6 endemic families and one endemic order. Twenty million years of genetic isolation from eastern Australia and bounded by the Indian Ocean to the west combined with no glaciation for the past 250 million years has resulted in a region where the processes of evolution have played out to their fullest. This is seen in the diversity of life forms and ecological attributes from acute levels of fire resilience, the greatest diversity of carnivorous plants on earth to the remarkable attributes developed by plants in the region to grow, in fact thrive, on some of the most infertile landscapes on earth. But perhaps the most remarkable evolutionary force has been development of an array of pollination syndromes rarely matched by any other single biogeographic region. With 17% of plants pollinated by birds and the highest level of sexual deceptive pollination syndromes in orchids, pollination has driven levels of evolutionary specialisation across a wide variety of groups. My presentation will review some of the latest research into pollination including resolving one of the first cases of cryptic speciation in the Orchidaceae.

T1-16-05

How do population size and pollinator availability affect reproductive success, mating system and population genetics of a red-listed plant, *Polemonium caeruleum* L. (Polemoniaceae)?

Marcin Zych[1], *Justyna Ryniewicz*[1], *Katarzyna Roguz*[1], *Emilia Brzosko*[2], *Ada Wróblewska*[2], *Izabela Tałałaj*[2], *Beata Ostrowiecka*[2], *Edyta Jermakowicz*[2], *Paweł Mirski*[2]
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Polemonium caeruleum L. (Polemoniaceae) is a boreal plant species producing dichogamous and predominantly bee-pollinated flowers. Literature data show that occasionally the plant may be pollen limited and suggest that the species may exhibit mixed-mating system. We surveyed 15 *P. caeruleum* populations and, working in the field, over two years conducted a five-variant pollination experiment and studied pollinator abundance and identity. Our study populations showed various degrees of compatibility: from clearly self-incompatible (SI) to fully self-compatible (SC) ones. In most SI populations, however, self-pollination occurred via geitonogamous pollen-transfer rather than autonomous selfing, since isolated flowers (those with restricted pollinator access) hardly produced any seeds. Only the smallest of the surveyed populations, composed of <20 flowering individuals, exhibited significant pollen limitation. This population was also clearly self-compatible, producing significant seed set following induced (geitonogamous) self-pollination. To relate this data to the plant's population genetics we performed AFLP analyses for all studied populations, which will be discussed in the paper.

T1-16-06

How general is the relationship between fitness and abundance in wind-pollinated plants?

James Rodger[1], *Cang Hui*[2], *John Pannell*[1]
1. University of Lausanne
2. Stellenbosch University

Pollinator visitation, pollen receipt and seed set were shown to be generally lower in smaller plant populations in a now decade-old meta-analysis. This is a threat to rare plant populations but may make it easier to manage invasive ones. However, the conclusion of the previous meta-analysis that fitness is generally positively related to population size in plants only applies to animal-pollinated species, as insufficient studies on wind-pollinated species were available to form conclusions for this group. We will present a meta-analysis of the relationship between abundance and fitness in wind-pollinated species, and a comparison of the effect of abundance on fitness between wind- and animal-pollinated species. We will also assess results for wind-pollinated species for consistency with models of transport of pollen by wind. In a preliminary dataset, positive relationships between abundance and fitness were found in 17 out of 18 wind-pollinated species.

T1-17: The World Flora Online – a response by the international plant taxonomic community to the global strategy for plant conservation（two sessions）

T1-17-01

Introduction and progress on developing the World Flora Online

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1. Missouri Botanical Garden
2. Conservatoire et Jardin botaniques de la Ville de Genève

In 2010, the updated Global Strategy for Plant Conservation (GSPC) of the U.N. Convention on Biological Diversity (CBD) included as its first target (Target 1) the need for "An online flora of all known plants." Its aim was to ensure that a coherent, comprehensive, accessible and authoritative baseline of knowledge would become available to support efforts being made worldwide to conserve plant diversity. Therefore, in January 2012 in St. Louis, Missouri, USA, representatives from the Missouri Botanical Garden, the New York Botanical Garden, the Royal Botanic Garden Edinburgh, and the Royal Botanic Gardens, Kew — all members of the Global Partnership for Plant Conservation, took the initiative to organise a new global project to achieve this target. First steps included a proposed outline of the scope and content of a World Flora Online (WFO), as well as a decision to create an international Consortium of institutions and organizations to collaborate on providing its content. The WFO project was subsequently launched in October 2012 in India, at an event held during the 11th Conference of the Parties (COP) to the CBD, where the COP also adopted a decision welcoming the WFO initiative. In January 2013, a Memorandum of Understanding (MoU) on the WFO was opened for signature. To date, 35 institutions and other organizations have signed the MoU and are participating in the project. A range of other institutions and organizations worldwide is also invited to participate in the WFO Consortium. The project is managed by a WFO Council which meets twice a year, supported by several expert Working Groups. The WFO aims to be an open-access, web-based compendium of the world's plant species. It will be a collaborative, international project, building upon existing knowledge and incorporating information drawn from published Floras, checklists and taxonomic and other revisions. It will also require the collection and generation of new information on poorly know plant groups and plants in unexplored regions. At its core is a taxonomic 'backbone' including a consensus classification of known plant genera and species, including accepted names and synonyms. Originally based on 'The Plant List', (version 1.1) (www.theplantlist.org), the taxonomic core referential of the WFO is being actively reviewed, moderated and updated by a growing network of taxonomic experts worldwide. Other data included are data sources, geographical distributions and information on the current status of each species. Where possible, existing keys and other identification tools are being incorporated, as well as vernacular names. Work had progressed to deliver the WFO through a web portal, using software donated by RBG Kew and adapted by the Missouri Botanical Garden and other partners. The project aims to launch this the first public, albeit incomplete, version of a World Flora Online in mid-2017. The project represents a major step forward in developing a consolidated global information service on the world's flora. It also provides an important forum and mechanism where the world's plant taxonomists can ensure that the results of their work are acknowledged and presented for use by the global community.

T1-17-02

Transforming hard copy to digital for the World Flora Online

William Thomas, *Melissa Tulig*
New York Botanical Garden

We estimate that there are 350,000-400,000 plant species and that descriptions for as many as half of these are only available in hard copy. Therefore, one of the greatest challenges in achieving the goals of the World Flora Online will be to make available the huge amount of botanical information that is not yet digitized. The New York Botanical Garden Press has been publishing floras and monographs for the past 125 years and most of the treatments are not freely available online. As part of our commitment to the WFO project, we used the Flora Neotropica Monograph series to test the conversion of a print series to marked up digital treatments of the individual taxa contained across all volumes. Optical character recognition software was used to convert images of the publications to text. Then project staff reviewed and edited the output and parsed treatments into fields that comply with DarwinCore standards. We then proceeded to do the same for the Memoirs of The New York Botanical Garden. Combined, these publications contain over 22,000 treatments that are now freely available online.

T1-17-03

The e-Flora of South Africa: An alternative method to compiling an online flora as a contribution towards the World Flora Online Initiative

Margaretha Marianne Le Roux[1,2], *Ronell Klopper*[1,3], *Janine Victor*[1]
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2. University of Johannesburg
3. University of Pretoria

Floristic accounts are useful products that provide taxonomic information for all plant species within a defined geographic region. Traditionally, these accounts were compiled by one to several contributors and published in hard copy volumes. These projects often took long to complete and soon became outdated with difficulty of publishing updated versions. However, with the availability of electronic tools and a developing digital environment, Flora compilation has embraced new and innovative ways to overcome some of these challenges. In South Africa, the first floristic account was compiled by Thunberg in Flora Capensis in 1823. This was followed by Harvey and Sonder's Flora Capensis between 1859 and 1933, which was completed through contributions from multiple authors. In 1955 the Flora of southern Africa (FSA) project commenced with the aim to replace the outdated *Flora Capensis*. This project was never completed and as an alternative, a regional/provincial conspectus programme was initiated. To date, three volumes covering *ca*. 67% of South Africa's flora has been published (Flora of the northern provinces, 1996; the Greater Cape Floristic Region: core Cape Flora, 2012, and the extra Cape Flora, 2013), with the Flora of the Free State, Eastern Cape, KwaZulu-Natal and the Nama Karoo to follow. With the development of the updated Strategy for Plant Conservation (2011–2020), South Africa has committed to contribute a country-level Flora towards the World Flora Online (WFO) initiative (Target 1). After considering the remaining time in which to make such a contribution and the availability of published information (FSA volumes, published regional/provincial conspectuses and published taxonomic revisions), it was decided to compile an electronic Flora using existing information and following an aggregator portal approach. Available information for this endeavour covers *ca*. 85% of the South African flora. The remaining 15% of the flora will be dealt with in the conspectuses that are currently being compiled. The aggregator portal approach involves the use of published information by acquiring permission from the copyright holders, digitising the material if it does not exist in electronic format, mining the required data from the publication and aggregating it into a database from where it will be made available online in open access. Two disadvantages of this method are: (1) multiple descriptions for each taxon may be collected; (2) the format of descriptions will be inconsistent within and among species. These disadvantages may temporarily be overcome by presenting the most recent or appropriate description first and by displaying the data in a standard template. The main advantages of this method are: (1) existing information will be aggregated into one central portal and; (2) an e-Flora for South Africa will be completed by 2020. An overview of the history of Flora compilation in South Africa will be presented, along with the method currently followed to compile the e-Flora for the country. Advantages and disadvantages of this method and progress to date will be highlighted.

T1-17-04

The Flora of North America: Moving from hard copy to digital information sharing

Geoffrey Levin
Canadian Museum of Nature

The Flora of North America North of Mexico (FNA) is a 30-volume work that includes taxonomic treatments of all the native and naturalized plants growing in the region. All taxa are described and included in dichotomous keys, distributions of all species and infraspecific taxa are mapped, and about 20% of species are illustrated with line drawings prepared specifically for FNA. Producing FNA has created an international community of authors, reviewers and editors, integrating broad-scale taxonomic knowledge with regional floristic expertise, and fostering further collaboration. By providing keys and technical descriptions for numerous rare taxa and introduced species, FNA is a vital tool for botanists and others concerned with conserving the continent's flora. Soon after volumes are published, they are made available electronically through the efloras website and data are shared with JStor's Global Plants. As we near publication of the final volume in 2018, we are preparing to transition to a new website that moves beyond replication of the printed information. This new site will allow users to use either dichotomous or interactive keys and to search on any combination of characters, including morphology, phenology, habitat, and geographic distribution. We envision additional data sharing, including with the World Flora Online. The site will also include tools for moderated and versioned authoring and editing of treatments, allowing the Flora to remain up-to-date by continuously incorporating results of new research.

T1-17-05

World Flora Online—Technology & techniques to create a comprehensive data portal for all plants

Mark Watson[1], *Chuck Miller*[2], *William Ulate*[2]
1. Royal Botanic Garden Edinburgh
2. Missouri Botanical Garden

The World Flora Online (WFO) is an international collaborative project aiming to bring together floristic and monographic information on all the world's plants in fulfilment of Target 1 of the Global Strategy for Plant Conservation 2020: 'An online Flora of all known plants'. This builds on The Plant List (www.theplantlist.org) - a working list of all known species of vascular plants and bryophytes. The rationale of WFO is to meet the need for baseline information to support plant conservation and sustainable development, and so the main target audience is both those engaged in these activities and the taxonomic community providing authoritative information. The technical aspects of how the WFO will be achieved is handled by a Technical Working Group established by the WFO Council, and includes people with a range of skills from computer programmers to biodiversity informaticians to taxonomists. The requirements of the system were established through a Use Case study, where stakeholders and their needs were documented and prioritised. The data elements needed to fulfil the prioritised use cases were characterised and aligned with Darwin Core, Dublin Core and Plinian Core - new data elements with definitions were created only when absolutely necessary. The database and internet portal of the eMonocot system (http://e-monocot.org) was used as the basis for the WFO system. The WFO comprises of two logically separate components: the Taxonomic Backbone (nomenclature and classification) and the Descriptive Content of previously published floristic and monographic treatments linked to the Backbone. Contributors to either of these components send in their data conforming to the defined data standards and these are harvested into the WFO system. A crucial step in the ingestion is the resolution of and linage to the taxonomic names used in WFO and the contributor dataset, and for this WFO has established new identifiers for all names from subspecies to order and for all vascular plants and bryophytes. A demonstration portal (http://demo.worldfloraonline.org) is being used to test the system, and a production portal will be launched at IBC.

T1-17-06

Plants of the World Online Portal (POWOP), how the Royal Botanic Gardens, Kew will contribute to the World Flora Online

Abigail M Barker, *Daniel Borg, James Crowe, John Iacona, Don Kirkup, Nicola Kuhn, Nicky Nicolson, Isabelle Sims, Robert Turner*
Royal Botanic Gardens, Kew

In 2015, the Royal Botanic Gardens, Kew committed to delivering nine strategic outputs which aim to disseminate Kew's scientific knowledge of plants and fungi to maximize its impact in science, education, conservation policy and management. The Plants of the World Online Portal (POWOP), is one of those outputs. POWOP will enable users to access information on all the world's known seed-bearing plants by 2020. A key function is to ensure that Kew's floristic data can be easily harvested and ingested by the World Flora Online (WFO) portal enabling Kew to support the Global Strategy for Plant Conservation (GSPC) Target 1 2020 "An online Flora of all known plants". POWOP is a single point of access for authoritative plant species information, providing a multi-dimensional catalogue of plant life, including information on identification, distribution, traits, threat status, molecular phylogenies and uses. It uses Kew's extensive data resources including its Floras, alongside images from the digitisation of the collections. The portal has been designed to maximise accessibility and enables dissemination of plant information to users via mobile, tablet or desktop computer. The codebase is open source and available for use by partners in other countries to set up their own portals, which can be linked through to Kew and the WFO. Kew will help to enable this process through knowledge transfer and support, building on expertise in institutions across the globe to further develop a network of botanical data hubs. Ultimately, this will lead to a resource with global coverage which can empower and inform citizens, policy makers, conservationists and farmers everywhere, about the importance of plants and fungi to everyday life. POWOP will provide Kew's flora data to the WFO in Darwin Core Archive format linked by persistent identifiers from the International Plant Names Index (IPNI) and The Plant List. POWOP was launched in mid-November 2016 with a focus on the Floras of Tropical West and East Africa. It is these Floras which will form the basis of Kew's initial contribution to the WFO.

T1-17-07

The South African National Plant Checklist: History and utility

Ronell R Klopper, *M Marianne Le Roux, Brenda Daly, Janine E Victor*
South African National Biodiversity Institute

A national species checklist is an essential resource that forms the foundation for a range of research and biodiversity-related activities. South Africa has had the benefit of access to an updated national and regional plant checklist for many decades, which has underpinned botanical research in the country. The South African National Biodiversity Institute (SANBI) is mandated by the South African Government to co-ordinate taxonomy in South Africa, which includes maintenance, updating and dissemination of country-level checklists. Although at present the checklist only includes South African species, in the past it covered the entire Flora of Southern Africa (FSA) region (Botswana, Lesotho, Namibia, South Africa and Swaziland). Additionally, other southern African country-level plant checklists (for Angola, Botswana, Lesotho, Malawi, Mozambique, Namibia, Swaziland, Zimbabwe and Zambia) were compiled and published under the auspices of the Southern African Botanical Diversity Network (SABONET) lead by SANBI. Furthermore, SANBI was a key partner in compilation of the African Plant Checklist and Database. This monumental project was a collaboration between SANBI and Conservatoire et Jardin Botaniques de la Ville de Genèva, Switzerland, and later Tela Botanica and Missouri Botanical Garden. It initially combined two major sources of data: the Flora of Southern Africa dataset and the Lebrun & Stork Enumération des plantes à fleurs d'Afrique tropicale publications covering tropical Africa. This produced a global first inventory of the flowering plants of sub-Saharan Africa. Subsequent inclusion of names for angiosperms in North Africa and Madagascar in the online database has seen this resource grow to a continent-level catalogue. Plant checklists for South Africa and the FSA region were originally published in hardcopy and later made available in pdf format. However, the checklist is now available online as both a downloadable spreadsheet and in a searchable platform. Checklist and specimen data are housed in a BRAHMS database that enables the online platform to link the checklist to specimen records held in the SANBI herbaria [Compton Herbarium (NBG and SAM), Cape Town; KwaZulu-Natal Herbarium (NH), Durban; National Herbarium (PRE), Pretoria]. Future developments will expand coverage to include specimen level records from other South African herbaria. This is in line with global efforts to mobilise plant biodiversity data. Additional descriptive data, images and distribution maps will be linked to the checklist as part of the eFlora of South Africa project. This will enable country-level input to the World Flora Online Initiative that aims to fulfil Target 1 for the 2020 Global Strategy for Plant Conservation. This presentation will briefly outline the history of compiling and disseminating the South African National Plant Checklist. Information will be provided on the family-level classification followed, and the current processes and procedures applied in terms of data management, prioritisation, and quality control. The application and utility of the checklist as backbone for several foundational and applied biodiversity disciplines, such as herbarium specimen curation, conservation assessments, and biodiversity policy and planning activities, will be discussed. In addition, the importance of an updated national plant checklist during compilation of an eFlora for South Africa will be highlighted.

T1-17-08

Contribution to the WFO of completely digitized plants natural history collections: The case of Paris Muséum national d'histoire naturelle's vascular plants collection

Visotheary Ung, *Marc Pignal, Maïté Delmas, Serge Muller, Marc Jeanson, Germinal Rouhan, Véronique Schäfer, Thomas Haevermans*
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Large herbaria, i.e. global plant knowledge depositories constitute a suitable tool to address targets one and two of the Global Strategy for Plant Conservation: obtaining a complete list and conservation statuses for all known plant species. Paris Herbarium, one of the largest in the world has complete the digitization of its vascular plants specimens holdings, with more than 6,000,000 specimens, and 170,000 taxa. All images are freely available online. We have published a data paper in Scientific Data, where we described and explained precisely the whole digitization process. Besides, we have developed a citizen-science program: Les Herbonautes (lesherbonautes.mnhn.fr, Recolnat program - ANR11-INSB-0004) to gather additional data through its platform to complete our dataset as well as involving a large community of scientific volunteers. This ever growing amount of data could be integrated into the WFO portal to help accessing it while contributing to document plant biodiversity.

T1-17-09

Flora of North America: Nomenclature review

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Since the beginning of the 20th Century almost all floras have relied upon nomenclatural and/or taxonomic indices/databases of various magnitudes serving either global (IPNI and Tropicos), or regional (e.g., *Fl. Eur.*), or individuals (e.g., *Selaginella* L.) for nomenclatural assessments. Each of the indices presents some problems, e.g., showing: a) misapplied names as later homonyms; b) prosynonyms as validly published. As the International Code of Nomenclature became stabilized since the 1930s, application of the Code enhanced nomenclatural accuracy asserting authorship, validity, legitimacy, and priority of names. For the 30-volume Flora of North America (FNA; 1993-2018?), we are making an effort to check the protologue of the included names to achieve nomenclatural accuracy, a worthy goal, which FNA has been seeking. Such verifications have led to corrections of even recently published names, e.g., "*Leptosiphon montanus* (Greene) J.M. Porter & L.A. Johnson (2000)" was found to be invalid because of the failure to provide a direct reference to the basionym. Occasionally, what have been perceived to be validly published names were found to be invalid making it time consuming to ascertain the first place of valid publication, e.g., "*Canotia* Torr. (1857)", originally proposed as a provisional name ("… there can be but little doubt of its constituting an undescribed genus, we may bestow upon it the provisional name of *Canotia*"), is believed to have been first validated by Asa Gray (1861). However, the boundary between a taxonomic doubt vs. a provisional name or a polite way (to express oneself that was all but customary in some periods/countries) is fluid, e.g., *Lespedeza angustifolia* var. *brevifolia* Britton 1893 ("… specimens of a peculiarly short-leaved form … may claim recognition as var. *brevifolia*"); the preceding remark is considered to be polite. For apple, because of the uncertainty of the acceptance of the proposal to conserve the name *Malus domestica* Borkh. (1803, nom. illegit.) over *M. pumila* Mill. (1768), the latter name has been recognized in FNA. Citations of synonymy, if any, usually include legitimate names above the rank of forma; exceptions are basionyms at any rank and illegitimate homotypic replaced names. For well-established illegitimate names, if accepted for FNA, proposals were made for conservation, e.g., *Heteromeles* M. Roem., nom. cons., *Prunus serotina* Ehrh., nom. cons. If the rank of a name was uncertain in the original publication, the name was cited as unranked, e.g., *Zizia* [unranked] *Taenidia* Torr. & A. Gray (1840); the authors applied the sign § for both subgenus and section. Furthermore, for each genus name, etymology is provided; in this regard, pre-Linnaean, Linnaean, and post-Linnaean literature has been consulted; however, for a few names, the given or intended etymology is uncertain, e.g., *Causonis* Raf., *Nekemias* Raf., and *Oenothera* L. When nomenclatural inaccuracies were found in the published volumes, appropriate corrections have been made in the online versions.

T1-17-10

E-Flora India initiative and prospects

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Since 1990, Flora India has published its products as family and genus treatments in terms of 10 volumes and 27 Fascicles. An e-Flora will complement printed formats with electronic editions, offer unlimited access and instant updates, and increase the cost-effectiveness substantially by streamlining the production work flow. It will allow structured data entry, interactivity, multimedia, and enhanced accessibility. Moreover, it will also allow collaboration between several e-Flora projects (e.g., Australian eflora initiative and World Flora Online Initiative), users of taxonomic data, and other databases. This increases the possibilities for innovative scientific co-operation, also between other research fields and attracts worldwide contributors using services offered by the e-Flora. An overview is presented of available e-taxonomic products and ongoing projects contributing to Flora India. This is presented in the context of a strong plea to strengthen the implementation of state-of-the-art e-taxonomy tools to speed up the generation and publication of Flora India information. The increasing array of electronic taxonomy tools available for the elaboration and dissemination of floristic information has brought many advantages. It enables a shift from the traditional Flora concept as a static, printed account to a dynamic and interactive format, allowing for rapid updating and multiple uses of information. It provides taxonomists with means for instant interactive and remote co-operation, including continuous processes for evaluating preliminary results by peers and updating existing information. It also allows taxonomists to forge better links with their user-communities by making the products of their research more tailor made and accessible via internet. In order to strengthen and speed up Flora India related activities, that the Flora India community needed to adopt a more pragmatic and flexible attitude—flexible in terms of formats and publication strategy, and standards of robustness and confidence in the results. The Flora India project has recently gained new momentum by starting a new website (http://efloraindia.nic.in), by adopting e-taxonomy tools and by joining a broadly supported initiative that wants to promote the adoption of richly interactive and truly collaborative systems for the production and presentation of floras. Flora India work can be strengthened by increasing its global accessibility, facilitating efficient remote collaboration, making use of databases to safeguard data, changing the work flow in data preparation and presentation, and creating institutional commitment.

T1-18: Pollination by non-flying mammals

T1-18-01

When flowers smell cheesy: An investigation into functional floral traits of African and Australian mammal-pollinated proteas (Proteaceae)

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Mammal-pollinated plants commonly produce inflorescences that emit very strong odours. Evolutionary shifts from bird to non-flying mammal pollination in the African plant genus *Protea* (Proteaceae) are most notably associated with modifications in inflorescence size and olfactory cues. Using headspace sampling and gas chromatography-mass spectrometry of inflorescences and dissected floral parts, we compared the emission rates and chemical composition of floral scents between several species from each of the mammal, bird and beetle pollination systems in this genus. Bird-pollinated species with larger tall colourful inflorescences emitted small amounts of "green-leaf" volatiles and benzenoid compounds, including benzaldehyde, anisole and benzyl alcohol. Beetle-pollinated species produce shorter and more open inflorescences that emit fruity scents that are more complex and on average 10-fold greater in the amount of scent emitted than odours sampled from bird pollinated species. These fruity scents are dominated by linalool, other monoterpenes and a variety of benzenoids. Floral scents of small dull-coloured non-flying mammal pollinated *Protea* species shared more similarities with those of beetle-pollinated species, with nectar emitting cheesy, buttery odours (diacetyl and acetoin in combination with dimethyl disulphide, several ketones and alcohols). Numerous choice tests with common rodent pollinators revealed that these mammals are significantly attracted to certain concentrations of ethanol and other fermentation volatiles, and preferred the sour-milk/cheesy odours of nectar from a rodent-pollinated *Protea* species over that of a solution of the same sugar composition, indicating that floral odours play a functional role in pollinator attraction. Remote cameras have revealed that these species are visited not only by a variety of rodents, but also elephant shrews and small carnivorous mammals such as mongooses and genets. As mammal-pollinated *Banksia* and *Grevillea* species in Australia also attract pollinators with incredibly strong floral odours, current collaborative work and preliminary results on the evolution of floral traits in the Proteaceae family across Africa and Australia will be discussed.

T1-18-02

A unique case of floral specialisation for pollination by elephant-shrews

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Pollination of plants by non-flying mammals (therophily) is a rare phenomenon. In South Africa, therophilous plant species investigated thus far are pollinated by mice (Rodentia) and sometimes secondarily by non-related elephant-shrews (Macroscelidea). In this study we investigated the pollination ecology of the holoparasitic South African endemic *Hyobanche atropurpurea* (Orobanchaceae). Its long-tubed and black flowers are visited most frequently by long-tongued elephant-shrews (*Elephantulus edwardii*) and to a lesser extent by short-tongued mice (mainly *Acomys subspinosus*). Because of the close mechanical fit between the long-tubed flowers and elephant-shrews' snout and tongue morphology, *H. atropurpurea* appears to be adapted to elephant-shrews as pollinators. Elephant-shrews carry much more pollen than mice in the fur around their snouts and in their scats. Extensive observations, video monitoring and pollen transfer experiments showed that elephant-shrews contact the plants' reproductive organs during almost every flower visit whereas flower visiting mice rarely make contact with the stamens and stigma. Compared to mice, the elephant-shrews transferred pollen more often and in significantly larger numbers to the stigma of the flowers. Moreover, flowers were often damaged or eaten by mice, but never by elephant-shrews. These results suggest that *H. atropurpurea* is specialised for pollination by elephant-shrews.

T1-18-03

Flying and non-flying mammalian pollinators of *Mucuna macrocarpa* (Fabaceae) and their effect on pollination

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The genus *Mucuna* (Fabaceae) has a pantropical distribution and comprises approximately 100 species. Most species of *Mucuna* have inflorescences hanging down from vines. The stamens and pistil of the *Mucuna* flower are completely concealed within the keel-shaped petals (carina). Exposure of the reproductive organs within the carina is termed "explosive opening." It has been reported that the agents that trigger this step are specific bats or birds. Previous studies have suggested that the animals that open the flowers may also be the major pollinators of *Mucuna*, although there is no experimental evidence. This study targeted *Mucuna macrocarpa,* which is an evergreen vine distributed from Southeast Asia to Kyushu, Japan. The objectives of this study were (1) to confirm the necessity of the explosive opening step for successful pollination in *M. macrocarpa*, and (2) to identify the explosive openers in several habitats of *M. macrocarpa* with/without fruits bats. Bagging and crossing experiments indicated that *M. macrocarpa* was a self-compatible species but never sets fruit without explosive opening. These results demonstrate that explosive opening is indispensable for successful pollination in *M. macrocarpa*. A video camera trap survey showed that explosive openers were Ryukyu flying foxes (*Pteropus dasymallus*) on Okinawa (subtropical, Japan), whereas in the regions without fruit bats, Japanese macaques (*Macaca fuscata*) and Japanese martens (*Martes melampus*) in Kyushu (temperate, Japan), and red-bellied squirrels (*Callosciurus erythraeus*), Formosan striped squirrels (*Tamiops maritimus*), and masked palm civets (*Paguma larvata*) in Taiwan (subtropical) were openers. Among these, Ryukyu flying foxes, Japanese macaques, and red-bellied squirrels were effective openers in each region. When these mammals opened a flower, many pollen grains adhered to their body surface, suggesting that the explosive openers are the effective pollinators of *M. macrocarpa*. These explosive openers include both flying and non-flying mammals; however, with the exception of Japanese macaques, their explosive opening behaviors are common. The similar behaviors regardless of the difference in species may ensure the pollination success of this plant in each region. In terms of the flying ability of the opener and its effect on pollination, it is predicted that there are differences in flower visiting height and locomotory ability. Ryukyu flying foxes visited flowers at a height of 2 m or higher, whereas Japanese macaques and red-bellied squirrels visited flowers from ground level to the canopy. Consequently, fruits were observed only on the upper vines on Okinawa where the flying fox is the only opener. In contrast, fruits were observed from the ground to canopy in Kyushu and Taiwan where non-flying mammals were the openers. In addition, previous studies have shown that flying foxes have the largest home range size among the three effective openers, whereas that of squirrels is only one-fiftieth that of flying foxes. Furthermore, flying foxes can move more directly between feeding sites regardless of any terrestrial barriers. These characteristics may affect pollen transfer distances. Therefore, regional differences in the pollination of *M. macrocarpa* can be attributed to the flying ability of the explosive opener.

T1-18-04

Rodent responses to volatile compounds provides insights into the function of floral scent in mammal pollinated plants

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Flowers pollinated by mammals such as bats and rodents have evolved in dozens of plant families. Volatiles are considered to play a key role in the attraction of mammals to these flowers. Vol-

atiles that attract bats to flowers have been identified in previous studies, but the chemical ecology of rodent-plant interactions has scarcely been investigated. Volatile signals are likely important in rodent pollination systems because rodents are generally nocturnal and rely heavily on olfaction to locate food. The aim of this study was to identify compounds that may play a functional role in the attraction of rodents to flowers. Eighteen volatile compounds were identified in headspace samples taken from rodent-pollinated flowers in southern Africa and used in experiments that involved four native rodent species (*Aethomys namaquensis, Rhabdomys pumilio, Rhabdomys dilectus* and *Mastomys natalensis*). Compounds were tested individually in unrewarded y-maze experiments to determine their attractiveness to rodents. In addition, associative conditioning experiments using artificial nectar as a reward were conducted to determine the learning abilities of rodents when their food is associated with particular volatiles. Rodents generally responded positively to aliphatic compounds (ketones, esters and acids) and responded negatively to aromatic compounds and a sulphide compound that is known to be attractive to bats. Associative conditioning generally resulted in little change in the attractiveness of compounds. It was concluded that oxygenated aliphatic compounds, particularly ketones and esters, are key attractants of rodents to flowers adapted for pollination by these animals.

T1-18-05

Floral visitation and reproductive success in the rare *Grevillea obtecta* (Proteaceae)

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In Australia vertebrates are ancient and important pollinators, birds occupy diurnal niches and small mammals prevail at night. While these pollinators seek similar rewards they rely on different cues. This study examined pollination of *Grevillea obtecta* which belongs to a clade of mostly ornithophilous species however, unlike most of its relatives, it produces a strong scent that also attracts nocturnal marsupials. Flowers produced unpredictable volumes of high quality nectar in dense, partially hidden inflorescences. Bird and mammal visitors were recorded using motion-triggered cameras. Antechinus and brush-tailed phascogale were the main nocturnal visitors while yellow-faced honeyeaters pollinated during the day. Excluding vertebrate pollinators reduced fruit set significantly, from 8.4% to 1.4%. The flowers of *G. obtecta* are yellow-pink to red-brown and mostly reflect long wavelengths of light, consistent with its close relatives. However, floral scent is unusual within the clade and GCMS revealed a mix of 31 compounds dominated by camphene (35.89%) and limonene (10.82%). We conclude that in *G. obtecta,* the separate pollinator suites (day and night) may be interpreted as double specialisation because the nocturnal and diurnal cues evolved independently and specialisation does not drive fitness trade-offs between these floral traits.

T1-18-06

Bees, birds, bats–and mice? Pollination syndromes and floral traits in Merianieae (Melastomataceae)

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Bee buzz-pollination constitutes the most widespread pollination system in the tropical plant family Melastomataceae. While bee pollinated species reward their pollinators only with pollen, approximately 3% of the ca. 5,500 Melastomataceae species have evolved additional reward systems (nectar, food bodies) and are pollinated by hummingbirds, bats and passerine birds. Furthermore, rodents have been observed visiting flowers in three cloudforest species of the Neotropical tribe Blakeae; their role as pollinators is under debate, however. Recent observations on one species *(Meriania sanguinea)* in the also Neotropical tribe Merianieae suggest another incidence of flower visitation and possible pollination by rodents in the family. Flowers are red, pendent, produce nectar and emit strong, sweet scent. Irregular diurnal visitors are hummingbirds while rodents have been detected in nocturnal video observations taking up nectar from the flowers. Following the concept of pollination syndromes, which assumes a convergence of floral phenotypes to the species' most important functional group of pollinator, rodent pollinated species are usually inconspicuous in colouration and emit yeasty or cheesy scent and thus stand in stark contrast to floral traits observed in *M. sanguinea*. Detailed floral morphological studies of 59 Merianieae species including representatives of all pollination systems place *M. sanguinea* within the hummingbird pollination syndrome. However, the species differs from other hummingbird pollinated species in inflorescence architecture, stamen structure and stamen arrangement, mechanism of nectar secretion and scent. Further analyses of pollen transfer, nectar and scent profile are planned to clarify if rodents constitute effective pollinators or rather are occasional secondary pollinator or mere nectar thieves.

T1-19: Ethnobotany of mountain regions

T1-19-01

Traditional knowledge in the Caucasus - changing knowledge patterns in Georgia after the end of soviet occupation.

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The territory of modern day Georgia has been continuously inhabited since the early Stone Age, and agriculture was developed during the early Neolithic era. In Georgian the name of the country is "Sakartvelo", and "Georgia" is semantically linked to Greek meaning "agriculture". Due to its long tradition, agriculture in Georgia is characterized by a great diversity of landraces, and endemic species of crops. These show a high level of adaptation to local climatic conditions and often high disease resistance. Fieldwork was conducted in Khevsureti, Khevi, Samtskhe, Javakheti, Tusheti, Svaneti, and Racha in July-August 2013, July-August 2014, and September-October 2015. Interviews using semi-structured questionnaires were conducted with over 175 participants after obtaining their oral prior informed consent. Our research indicates that while traditional crops like wheat, barley and rye have almost disappeared, a large number of species is traditionally also grown in home gardens, and a large part of the wild flora is used for food, medicine and cultural purposes. However, the related traditional knowledge is mainly held in the generation above 50, while younger people have started to loose traditional knowledge. No gender differences in knowledge could be observed. Overall, home gardens serve mostly as source for food, while wild plants are especially important fro medicine, and traditional pickles and jams. The main reason for genetic erosion of ancient crop varieties is the demographic decline in mountain regions due to harsh economic conditions and lack of modern infrastructure. The shift from ancient cultivars to modern high-yielding crops such as maize and potato, began in mountain villages after the end of Soviet occupation, when local inhabitants who had been forced to the lowlands, returned to their original villages.

T1-19-02

Changing markets – medicinal plant ethnobotany in the Andes of Bolivia, Peru and Colombia

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Given the importance of local markets as a source of medicinal plants for both healers and the population, literature on market flows and the value of the plant material traded is rather scarce. This stands in contrast to wealth of available information for other components of Andean ethnobotany. The present study attempts to remedy this situation by providing a detailed inventory of medicinal plant markets in the La Paz-El Alto (Bolivia); Lima, Trujillo and Chiclayo (Peru) and Bogota (Colombia). Both species composition, and medicinal applications, have changed considerably over time. From 2001-2015, semi–structured interviews were conducted with hundreds of plant vendors in order to elucidate more details on plant usage and provenance. The results of the present study were then compared to previous inventories of medicinal plants in the region, as much as available, to elucidate changes over time and impact of interview techniques. Over the years we we encountered and documented over 800 plant species. This indicates a great wealth of ethnobotanical knowledge in the Andean region of South America. All markets show a considerable change over the last few decades, with new species being introduced into the market chain, and other species being replaced. In course of the present study it became apparent that even well known species might often be replaced by other apparently similar but botanically unrelated species due to environmental and market forces. The present data indicate that, while the floristic composition of often remained relatively constant over the last decades, the number of indications for which certain species were used increased tremendously, and that profound differences exist even between markets in close proximity. The dramatic increase in previously not used species used per indication might pose serious risks for consumers. We found serious problems due to species replacements. Even plants that have a well established vernacular name, and are easily recognizable botanically, can be replaced by other species that can pose a serious health risk. Vendor education and stringent identification of the material sold in public markets are clearly needed.

T1-19-03

Ethnobotany of climate change in the greater Himalayan Region: a 3000 km transect

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The Himalaya are experiencing rapid climate change: temperature increases, changes in precipitation, and changes in permanent snows and glaciers. The region is a center of temperate biodiversity and includes plants of utilitarian and cultural value to Himalayan peoples. Our Himalayan subnetwork of the Global Observation Research Initiative in Alpine Environments (GLORIA) monitors climate change, alpine vegetation, and ethnobotany across a 3000km transect. The subnetwork spans the greater Himalayan region from Swat Valley in Northern Pakistan, through the Manang, Langtang and Kanchenjunga areas of central and Western Nepal and the Jomolhari and Tampela areas of Bhutan to the Hengduan Mountains in southwest China. At each monitoring area, we sample four mountain summits on an elevational gradient from the treeline ecotone to the uppermost limit of plant life – in these areas 4000-5000 masl. To this basic methodology, which we share with other researchers in GLORIA, we add ethnobotanical interviews to determine alpine plant uses. Baseline data from all sites in our Himalayan transects show species diversity following the steep east-west precipitation gradient, with the easternmost sites, in “Medicine Mountains” of China, the most species-rich. These sites also exhibited the greatest percentage of medicinal and aromatic alpine plants of any Himalayan sites. At these sites – the three from which we’ve completed both baseline and resurvey data – useful plant diversity, species composition in general, and response to climate change over the seven-year period between surveys were all structured on a strong North-South aspect gradient. Understanding the importance of topography can inform our predictions for climate change effects on species composition in this biodiverse and culturally valuable flora. Across the entire subnetwork, mountain regions show high endemism. Many of the plants sampled, including indicator species, are ethnobotanically important. For these high alpine useful plants, we anticipate climate change threats both from increasing temperatures and from competition with lower-altitude plants extending their range. Indigenous Himalayan communities in each of the regions, already coping with the direct effects of climate change, also see traditionally used and marketed medicinal and aromatic plants becoming harder to find, alpine pastures declining as vegetation patterns change, and seasonal indicators falling out of synchrony. Adaptation and mitigation strategies vary in each location. In China and Nepal, mountain villages are experimenting with new crops to take advantage of warming temperatures. In Nepal and Pakistan, we work with local medicinal and aromatic plant gatherers to evaluate procedures for the sustainable collection of wild plants and the possibilities for their cultivation. These data that will continue to emerge from this subnetwork will deepen the understanding of climatic factors in shaping medicinal and aromatic alpine vegetation in the Himalayan ecosystems, and contribute to national sustainable development measures and international efforts to understand and address climate change and its ethnobotanical impacts.

T1-19-04

Landuse changes and plant biodiversity conservation in traditional mountainous agroecosystems

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People in Andes, Caucasus, Himalayas or other mountainous regions have domesticated local plants and animals for many centuries. In these traditional mountainous agroecosystems there is great biodiversity of plants from genetic level, species level to farming system level. In recent decades, however, the plant diversity in mountainous communities had been affected by various reasons, especially by landuse changes resulted from policy or market enforcements. To examine the loss of plant biodiversity in traditional mountainous agroecosystems in terms of landuse changed, villages from Asian countries including Bhutan, China, India, Laos, Malaysia, Myanmar, Nepal and Thailandhad been tentatively investigated. Xishuangbann, a tropical area in southwest China, had been selected as a case study site, taking its shifting cultivation as an example. The semi-structured interview, participatory observation, focus group discussion and ethnobotanical methods had been used in the investigations. Investigations had been conducted in Xishuangbanna to compare the changes of upland crop landraces. Data were collected from 1992, 2006 and 2016 in different ethnic groups (Dai, Jinuo, Hani, Yao, Miao, Bulang, Lahu & Yi) and Han Chinese. Crops investigated in swidden agroecosystems are upland rice (*Oryza sativa*), taro (*Colocasia esculenta*), Xishuangbanna cucumber (*Cucumis sativus* var. *xishuangbannaensis*), melon (*Cucumis melo*) and others. Shifting cultivation or swidden or slash-and-burn agriculture was the most important landuse type in agricultural history. It has been argued for many decades in the context of economic development and biodiversity conservation. Because of too many people and too little forest available, Chinahas issued policies of landuse to reduce and ban shifting cultivation since 1980s. Swidden agriculture had been stopped completely in the country by the end of 2010s. The results revealed the number of crops at both species and landrace level decreased rapidly in 1992-2006, at an average rate of 46.5% and 71.8%, respectively. Millet, flee herb (*Adenosma buchneroides*), sorghum, Xishuangbanna cucumber, and *Amorphophallus kachinensis* could not be found in the swidden systems. Almost all landraces in traditional swidden agroecosystems of Xishuangbanna had completely disappeared by the end of 2015. Only some individual households in a few remote villages of Yao, Hani, Miao or Bulang ethnic groups still maintain one or two landraces of these crops. Some new crops including passion fruit, rubber tree, macadamia and *Pennisetum sinese* were introduced into the local systems, which resulted in the loss of biodiversity. The results implied that more investigations should be conducted before landuse policy was issued, otherwise the biodiversity would be threatened or damaged by the unreasonable policies. In the Xishuangbanna case, the genetic resources should be collected and preserved in seed banks when the government issued the swidden ban. Lessons from our case study will be beneficial for other communities in traditional mountainous agroecosystems.

T1-19-05

Introduction of new analytical and quantitative approaches for the production and conservation of medicinal and aromatic plants for economic development of Pakistan

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The project is being implemented by University of Swat in collaboration with Missouri Botanical Garden under the Pak-US Science and Technology Promotion Program, the program of HEC Pakistan. The current project has been evaluating opportunities to maximize farm income through introduction of high value medicinal and aromatic plants (MAPs) in the war-stricken district Swat of Pakistan, directly supporting new *Framework for Economic Growth* of the country. The hypotheses is the establishment of *ex-situ* experimental production plots, leading to the development of skills in horticultural production and marketing among people in the valley and help rebuild commercial connections between this area and the rest of Pakistan. District Swat host about 90% of its population depend directly or indirectly on agricultural and forestry products. This translate the importance of the present project for maximize farm income and other livelihood to uplift the socio-economic conditions of the farmers through introduction of high value MAPs and make them self sufficient to achieve the goal of poverty alleviation and to move the country towards economic stability. This project has covered a range of interventions such as local awareness campaigns, capacity-building training, market survey, and MAPs production in the farms land of community. Local and indigenous communities have evolved traditional wisdom about the cultivation of conventional crops with very low economic return. This paper focuses on the cultivation of ten high value MAPs with farmers in three different locations/villages. This study also focus on the economic analysis and profitability of selected MAPs production and to explore the prospects of MAPs cultivation as a potential economic venture in the region and a way of ensuring the long-term conservation of these plants in the wild. The study has also incorporate evaluation of its performance in introducing standardized production technology and appropriate post-harvest management, which represent the prime 'engines of growth' for the local economy. These strategic economic development areas are entirely based upon, and closely interlinked, with the management and conservation practices of high value MAPs, and intact landscapes. It was suggested that the cultivation of important and threatened MAPs is very effective means to satisfy market demand, to provide income to communities dependent on the wild natural resource, and to reduce pressure on the wild population of plants species. Moreover, the available marginal, sub-marginal and cultivable wasteland can be developed and planted with selected MAPs species. It was also recommended that the farmers should be further guided through extension visits in order to educate them regarding the adoption of new technologies related to MAPs production and conservation.

T1-19-06

Ecological calendars: Enabling the participation of indigenous and civil society to cope with climate change in the Asian highlands

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One of the greatest challenges of climate change is the difficulty in predicting its effects at the local community level due to erratic and unprecedented weather patterns. It is therefore an urgent need to develop anticipatory capacity in indigenous and civil society, as this capacity will provide these peoples with the ability to envision possible futures and develop a dynamic plan to deal with these uncertainties. We argue that development of a local adaptation strategy to climate change that is culturally and ecologically grounded will require re-conceptualization of the notion of time within an ecological framework. This will require the characterization of ecological calendars that are place-based and integrated with indigenous knowledge that has grown over generations and is sensitive to the biophysical signals. Such ecological calendars need however to be tested, validated and possibly recalibrated due to shifts in ecological and climatic regimes. These recalibrated calendars, together with climate scenario will enable us to anticipate and plan activities for future conditions and outcomes. While recognizing that some local groups are more resilient than others, the adaptive capacity of indigenous people and local groups can be strengthened through the revival, validation and recalibration of ecological calendars when appropriately assisted in partnerships with universities, research academies, government and non-government organizations. Intercultural dialogue must be entrenched in these partnerships, and is best achieved by interaction between both local and scientific knowledge systems. Thus, we call for establishment of the Ecological Calendar Network in Asian Highlands as a seed for change, as they develop innovative solutions to livelihood and climate challenges in the world's mountainous regions. We endorse the IPCC's recognition of the importance of local communities in climate change adaptation, and the necessity of respecting cultural and spiritual values in efforts to achieve sustainable development in mountain regions where local communities live and work.

T1-20: Biodiversity and conservation of aquatic plants

T1-20-01

Development of a global conservation strategy for freshwater plants

Richard Lansdown

IUCN SSC Freshwater Plant Specialist Group

Freshwater plants are low on the global conservation agenda. To counter this, the IUCN SSC Freshwater Plant Specialist Group (FPSG) was established with the aim to promote and further the conservation of freshwater wetland-dependent plants. The remit of the FPSG includes vascular plants, bryophytes, algae and some lichens which could not survive if the wetlands in which they occur were drained and probably includes more than 30,000 species worldwide. The FPSG takes a mainly species-focussed approach, using the IUCN Red List of Threatened Species to derive lists of taxa which are threatened, then developing conservation strategies for individual species, genera or families. One of our aims is to use Red List Assessments to establish a global Red List Index of freshwater plants which will enable monitoring of trends both in the conservation condition of individual species and in that of the wetlands which they support. Another aim of the FPSG is to raise the profile of freshwater wetland species, habitats and sites which are overlooked by the conservation industry, such as seasonal wetlands, flushes and river or stream headwaters. In addition we are working to identify taxa which are particularly vulnerable to climate change and to develop protocols by which such species may be used to monitor climate change in wetlands. However, we still must concentrate on fundamental issues which together form the foundation of all conservation. The taxonomy of many groups is still so poorly elucidated that we cannot start to address their conservation needs, while in some regions the taxonomic expertise necessary is lacking and field botanists use working names for species yet to be formally described. Similarly, we still lack the basic ecological information necessary to understand the conservation requirements of many wetland plant species and further to this, we have only a very limited experience of application of field conservation methods to wetland-dependent plants. A major emphasis within the FPSG is to support and promote research to acquire the information needed to provide credible, informed conservation. Through IUCN and other avenues, such as the publication of Species Conservation Strategies, the FPSG disseminates information on freshwater wetland-dependent plants to a wider audience from policy makers to the general public.

T1-20-02

Overlooked and undervalued: Diversity and conservation of Australia's seasonal freshwater wetlands

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Australia's dynamic climate and ancient landscape have produced one of the world's largest suites of seasonal aquatic habitats. These ecosystems harbour an immense assemblage of species ranging from specialist to generalist, and from widespread to highly restricted. Seasonal freshwater wetlands in Australia display unusual features in a global context, with a high proportion of species occurring in rain-fed, rapidly desiccating habitats experiencing high ecological stress. Plant endemism in these challenging habitats is remarkable, driven by geological antiquity, insularity, and their comparative long-term stability compared with other aquatic ecosystems, and their floras display distinctive ecological passport features associated with bet hedging, reproductive resilience and niche exploitation. However, data relating to the ecology and biogeography of Australia's seasonal aquatic flora are sparse, particularly in the remote tropical north where many wetland plants are actively growing when conditions are least conducive to accessibility. Seasonal freshwater wetlands have been poorly surveyed, face increasing pressure from a number of pressing threats, and our understanding of their true biodiversity is hampered by high levels of cryptic and undescribed diversity across many taxonomic groups. Historically, conservation initiatives and land management projects in Australia have focused predominantly on large wetland systems such as lakes and river deltas, and high-profile communities such as mound springs and swamp-based rainforest. As the frequency of range-restriction and ecological specialisation appear to increase with a decrease in habitat scale, the strong emphasis placed on these ecosystems only partially captures wetland biodiversity, and an increased attention to rain-fed wetland habitats of reduced size and local catchment must become an integral part of future conservation planning in Australia.

T1-20-03

Air, water, soil and soil pore water, a review of the four dimensional challenge for wetland plant translocation

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Translocations (*sensu* IUCN) can be effective conservation tools only if they are undertaken with underpinning science to support the actions and outcomes. Translocations are rarely a simple exercise of moving species and/or populations for conservation purposes, the so-called 'gardening approach', rather, behind each translocation there should be a level of pragmatic and focused science to support the actions and to interpret the long-term viability of the action. Various authors have shown that the success of reintroductions can be at risk with even minor gaps in knowledge of the target species biology, ecology, interactions with other species, threats, *etc.* leading to failure. In the last decade important improvements in translocation techniques have increased their general rate of success. However, there is limited information available on the rate of success of translocation in wetland plants. Wetland plants are often grown *ex situ* but, unlike terrestrial plants, the interplay of different ecological factors such as the climate, the water quality, the soil and the water circulating in the soil, make acquiring a real understanding of the target species ecology, the identification of threats and their removal, the selection of suitable release site, *etc.* more challenging. A worldwide review of the rate of success in wetland plants translocation, including strictly aquatic plants and other wetland-dependent species is presented. The review takes into account how translocations were carried out, the types and aims of ecological studies performed, how threats were identified and removed, reasons for success or failure and very importantly, how the post-translocation success has been evaluated. Specific examples of successes and failures are also presented. In particular, failures usually remain unpublished, but understanding why a translocation failed can be as useful as a successful story.

T1-20-04

The road ahead for the *ex situ* conservation of aquatic plants: A project to restore the extinct species *Eriocaulon heleocharioides* to the wild

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The IUCN Red List of threatened species includes 61 species classed as extinct in the wild. An organism is a component of the ecosystem and can be maintained as a species only in the wild; therefore, returning extinct species to the wild is important. Stable and long-term *ex situ* conservation, conservation biological research, voluntary conservation activities, building of co-operative relations with concerned parties and social understanding are essential for the successful return of a species to the wild. However, *ex situ* conservation generally faces some problems in terms of supplying populations to return to the wild. Decline in the genetic diversity of ex-situ populations is a particularly serious challenge. As the cultivation period increases and/or as the cultivation condition differs from the wild, the genetic diversity and fitness may decline, preventing a successful return of the species to the wild. To solve these problems, in 2008, we pioneered a conservation project to return the extinct species *Eriocaulon heleocharioides* (Eriocaulaceae) to the wild. This species had disappeared from its last habitat, the Sanuma Lake, Japan, after a change in the lake's water management plan in 1994 and has only been preserved *ex situ*, such as the Tsukuba Botanical Garden. In 2008, the project team first persuaded the concerned parties to return to the previous method of water management in Sanuma Lake. Subsequently, optimal growth environment conditions, pollinators, reproductive traits and seed storage and germination conditions were studied. In 2009, the plant flowered and bore fruit in the wild–after a period of 14 years. Nevertheless, the conservation team still cannot maintain the population without repeated introduction. Moreover, our preliminary results suggested that there has been genetic degradation of the species in *ex situ* conservation. Here, we describe the project and discuss problems and solutions regarding the *ex situ* conservation of aquatic plants with a view to reintroduction to the wild.

T1-20-05

Challenges and risks of conservation of an aquatic macrophyte extinct in the wild

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The reintroduction of a plant species regionally extinct in the wild poses a stimulating conservation challenge. However, when the issue concerns an aquatic dioecious macrophyte, with vigorous asexual reproduction forming very dense floating mats that can alter aquatic communities (through competition and allelopathy), each step must be very well planned. This is the case of *Stratiotes aloides* L. (water soldier), a keystone species that is declining in Europe, but is recognized as invasive in North America. The species, once abundant in the wetlands of North-Eastern Italy, is currently extinct in the wild in Italy. Only female Italian plants have been saved from extinction and are cultivated *ex situ*. To assess the feasibility of the de-extinction of *Stratiotes aloides* in Italy we investigated the water quality, the genetic diversity, and the potential spread of the species in its European range. The high concentrations of surface water inorganic nitrogen (especially nitrates) were identified as the likely cause of decline and extinction of *S. aloides*. The analyses of genetic diversity revealed the presence of different genetic patterns across Eurasia, that could be explained through long-distance bird-mediated dispersal events. Moreover, chromosome counts revealed the presence of diploid (2n = 24) and tetraploid (2n = 48) populations. According to our results the reintroduction of vital populations of *Stratiotes aloides* in Italy could be carried out using male individuals from the same genetic cluster of the Italian females, to avoid outbreeding depression. High-density stands must be reintroduced to permit self-facilitation of *S. aloides* under high nitrogen loads. However, considering the presence of high nitrogen loads in historical growing sites of *S. aloides*, there is a high risk of failure. Though in case of successful reintroduction, the risk to spread vegetatively downstream and the long-distance bird-mediated dispersal of seeds with unknown consequences on already threatened freshwater ecosystems cannot be ruled out. Therefore, considering the risks and benefits of the de-extinction of *Stratiotes aloides,* chaperoned managed relocation of female individuals in isolated water bodies basin (like gravel pits) could be the best solution.

T1-20-06

Degradation of aquatic vegetation in lakes in the Middle and Lower Reaches of Yangtze River in the past 60 years: Implications for the conservation of aquatic ecosystems

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There is widespread degradation in aquatic vegetation by a mixture of human activities. But it's difficult to evaluate the relative contribution of each factor to the vegetation degradation, because of data lacking. Such knowledge gap will limit our ability to make effective policies to conserve aquatic vegetation. To address these uncertainties, we compiled published data of aquatic vegetation in lakes along the middle and lower Yangtze River, China, and data of environmental properties and indexes of human disturbance during 1950s-2010s. (1) Aquatic vegetation of almost all lakes suffered significant degradation and succession in the past 60 years. For example, species richness had decreased by 16%, vegetation area decreased by 88%, and productivity decreased by 79% for aquatic vegetation. (2) Plant functional groups explain most of inter-species variations in the temporal trend, with submerged plants most sensitive to human activities. (3) For changes in aquatic vegetation, extensive aquaculture was the dominant factor, and water pollution and hydraulic projects were less important, while reclamation showed much inferior effect. We also found that disconnection between lakes and Yangtze river triggered community succession, with the dominant species shifting from submerged species to floating-leaved species or algae. To mitigate the degradation of aquatic vegetation, we should minimize the scale of aquaculture in natural lakes and keep the natural interconnected river-lake networks.

T1-21: Green digitization: online botanical collections data answering real-world questions

T1-21-01

From field collections to digital data: A workflow and digitization pipeline for reconstruction of a fossil flora

Dori Contreras

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The integration of curation and digitization with project-focused data collection is a key component to performing time- and resource-efficient studies from new fossil collections. Currently, standard workflows for processing fossil specimens starting from initial field collection and continuing through digitization are not widely established. Field work, data collection, curation, and digitization are often widely separated in time, involving different teams of personnel and separate funding. In many cases this follows the necessity of bringing older museum collections into the digital age. For new collections, however, more efficient use of personnel and monetary resources can be made by integrating these processes into a streamlined workflow carried out by researchers during the course of their study. Such workflows simultaneously meet the short term needs of a scientific study and the long term needs of the museum for archival of specimens and enhancement of public access to collections. Here I present my workflow for reconstruction of a diverse Late Cretaceous flora from plant macrofossils that are preserved in an extensive recrystallized volcanic ashfall deposit. This project ultimately aims to address fundamental questions about Late Cretaceous forests during the rise of angiosperms, including taxonomic and function diversity and spatial structure. Over the course of four years, I have made extensive new collections, establishing 26 quarries that span the >1.2 km length of exposure. Field censuses of leaf specimens have been performed at each quarry, and over 2,000 fossiliferous slabs with voucher specimens have been collected for further study and curation at the University of California Museum of Paleontology. Basic tasks involved in the reconstruction of the flora include differentiation and description of unique leaf types, identification and census counts of leaf specimens at each quarry, and measurement of leaf characteristics from digitized specimens. I will discuss how these tasks have been worked into a pipeline to streamline data collection and curation, including both specimen imaging and generation of geo-referenced individual specimen records as part of the digitization process. I will delve into parts of the workflow that have caused processing bottlenecks, and what measures have been taken to resolve them, especially how physical re-organization of space and both people and physical resources have been key to developing the workflow. Lastly, I will show how the digitized specimen data is being used in the context of this study.

T1-21-02

Developing standards for scoring phenology from herbarium specimens

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The digitization of herbarium specimens has advanced our ability to understand complex and changing biological systems. However, when a digital record consists only of a taxon name, date, and location, we are limited in the types of research questions we can address. While basic data such as these can be used to address changes in species distributions, herbarium specimens are rich with additional information regarding plant health, reproductive condition, and morphology that is generally not captured in digitization workflows. Phenology—the timing of life-cycle events such as flowering—in particular, has cascading effects on multiple levels of biological organization from individuals to ecosystems but data about these events are rarely interpreted, captured, or systematically scored in digitization efforts. Community wide data standards for scoring phenology from herbarium specimens are currently lacking. This talk will address the current efforts being taken by the Plant Phenology Ontology and the Phenology Standards Working Group. We will present the draft standards developed by the working group. The goal is to incorporate these standards into the Apple Core and potentially the Darwin Core, and develop the training tools necessary for phenological digitization to be included in all future digitization workflows.

T1-21-03

Virtual Herbaria tracking usage and benefits for biological collections an example from Australasia

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The Australasian Virtual Herbarium (AVH) makes available nearly 6 million records from all major collecting institutions in Australia and New Zealand. Traditionally loan records and research visitor information have provided data on the usage and importance of the collection. However, having released herbarium specimen data freely into the digital world the challenge for collecting institutions is to understand how their records are being used. Tools developed by the Atlas of Living Australia provide important insights to overall usage that can be used to inform funding bodies about the importance of these collections. Individual case studies also provide insights about how the sum of the data is more valuable than the parts.

T1-21-04

Current status and the applications of online botanical collection data in China

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In recent years, the government of China pays more and more attention to the conservation and application of the biodiversity. However, one of the most important works to achieve the goal of some national biodiversity action plan is to obtain the vast high-quality biodiversity data. Luckily, people can more easily get these data from different sources in China. For specimen data, NSII (China National Specimen Information Infrastructure) is the largest online specimen data portal in current China, which is funded by Ministry of Science and Technology (MOST). It has more than 14 million records (about 12.64 million records can be accessed via the internet) from more than 120 institutes or universities. From the view of Kingdom, 9.24 million records (73.1%) are plant specimens and 3.27 million records (25.87%) are animal specimens. For field data of botanical collection, we can get them from CERN (Chinese National Ecosystem Research Network), SinoBON (BON is short for Biodiversity Observation Network) and CFH (China Field Museum). Besides, you can also get other botanical collection data from other government projects, internal cooperation projects, citizen science platforms, digital literatures and social media. In China, there are also many applications after the integration of the botanical collection data from different data sources. People can find these applications in research articles, APPs (like for flower identification with Baidu and Microsoft), national/regional/global projects (for example, Southeast Asia Plant Biodiversity Information Infrastructure, SEADiv) and national policy reports. From the applications, we can see that the botanical collection data has played a very important role in the activities of research work and government work. Furthermore, we also need more digitization and put them online.

T1-21-05

Rebranding botanical collections: Global societal and biodiversity data needs for novel research

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Building on centuries of research based on herbarium specimens and their digitized data collected across time and space, a new era of discovery, synthesis and prediction has begun. Aggregated, open access botanical and associated biological, environmental and ecological data sets, from genes to ecosystem, can be used to document the impacts of global change on communities, organisms and society, predict future impacts, and help to drive the remediation of change. Within the collections and research community there is a need for advocacy for botanical collections, their expansion, continued and sustainable digitization (specimen data, associated media and information), including non-traditional digitized data fields and annotations, and born-digital field data to enable the rapid access of rich, digitally available, datasets for research, education, informed decision-making, and other scholarly and creative activities. Researchers are currently getting enormous benefits from data type-specific aggregators like iDigBio and Biodiversity Heritage Library (BHL) but assistance is still needed when working with large and disparate data sets. One example of infrastructure development is the Global Unified Open Data Architecture (GUODA) collaboration, which approaches this problem with processes to automatically retrieve and format data from providers of large open access datasets for easy processing by researchers. Tools for data discovery, visualization, representation and analysis, along with skills training, are also becoming increasingly important for inspiring novel research, in turn increasing the intrinsic value of the physical collections.

T1-21-06

Invasive or Not? A collection-based investigation of a historically unseen, persistent green algal bloom on Pacific coral reefs

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In 2012, a coarsely-filamented, unattached, large-celled, tropical *Chaetomorpha* (hereafter, CULT *Chaetomorpha*) suddenly appeared on reef flats in Guam, Mariana Islands, and caused ephemeral, localized outbreaks. By 2014, the bloom turned into a perennial phenomenon and occupied most of the southeastern shoreline of Guam (approximately 22% of the island's coastline). A further expansion into the west coast became apparent in late 2014 and in 2016 similar blooms were documented for Saipan in the northern Mariana Islands. This study investigates the invasion history and origin of the CULT *Chaetomorpha* in the Mariana Islands by screening online botanical collections. DNA sequence analysis was used to identify the bloom-forming alga and to determine the probable origin of different strains of CULT *Chaetomorpha*s from the Mariana Islands, the Western Pacific, and online aquarium retailers.

T1-22: Microbiomes of bryophtyes

T1-22-02

Cyanobacteria-bryophyte associations

Silvia Pressel[1], Katie Field[2], Martin Bidartondo[3], Sebastian Schornack[4], Jeffrey Duckett[1]
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4. University of Cambridge

Land plants form associations with a great diversity of microorganisms. Partnerships with fungi, mycorrhizas or mycorrhizas-like, occur in over 80% of extant plant species, spanning the land plant phylogeny and including the earliest diverging bryophyte clades (liverworts and hornworts). The best understood and most actively researched interactions between plants and bacteria are rhizobia—the endosymbiotic nitrogen-fixing association of the Gram-negative soil bacterial genus *Rhizobium* with roots of legumes, not least because of their very important role in agriculture. Less well understood are the partnerships between nitrogen-fixing cyanobacteria (mostly in the genus *Nostoc*) and their host plants. These range from epiphytic associations, such as those reported in a few mosses, most notably *Sphagnum* and the feather-moss *Pleurozium schreberi*, which have been shown to contribute significantly to the N economy in the Arctic, the Antarctic, and boreal forest regions, to endophytic ones. Endophytic plant-cyanobacteria symbioses are rare in liverworts, restricted to two basal genera in the Blasiales (*Blasia* and *Cavicularia*), but ubiquitous in the hornworts. They are found sporadically in vascular plants: in one fern, *Azolla*, in all Cycads and in only one flowering plant family, the Gunneraceae. While liverworts harbouring cyanobacteria are notably fungus-free, hornworts are much more promiscuous, variably also forming associations with a broad diversity of Glomeromycotina and Mucoromycotina fungi, sometimes simultaneously. The significance of these tripartite associations remains unexplored, with key questions on possible nutritional and other interactions between fungi and cyanobacteria inhabiting the same host hornwort hitherto unanswered. We will present our novel insights into these tripartite partnerships and discuss these in the context of plant-microbe symbiosis evolution.

T1-22-03

Symbiotic options for the conquest of land

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The colonisation of Earth's continental land masses by plants > 475 Ma marked a turning point in the development of the terrestrial biosphere, with widespread consequences for the future of terrestrial life. It has long been hypothesised that the terrestrialisation of plants was facilitated by nutritional symbioses with Glomeromycota fungi to form arbuscular mycorrhiza-like associations. The establishment of this symbiosis would likely have enhanced access to mineral nutrients for early, rootless plants in exchange for carbon fixed through photosynthesis. Our recent discoveries that the earliest diverging clade of land plants (Haplomitriopsida liverworts) forms mutualistic symbioses with partially saprotrophic Mucoromycotina fungi challenge this long-standing paradigm. Using a combination of microscopic, molecular and isotope tracer techniques, we have shown that other lineages of early-diverging liverworts form simultaneous mutualistic associations with both Glomeromycota and Mucoromycotina fungi. Together with recent fossil evidence, these findings bring into question the identity, biology and functioning of the fungal symbionts of the earliest land plants. Here, we suggest that there were symbiotic options available to the earliest land plants and that such relationships may have been far more varied and transient than hitherto assumed.

T1-22-05

Notes on microbiomes of mosses from some active tufa deposits, China

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Tufa depositing sites are widely distributed in the southwestern China, and contain a number of rare and unusual microbiomes communities of mosses such as cyanobacteria, protozoa, diatoms, fungi and algae. Particularly, calcicolous mosses and cyanobacteria are often abundant on tufa deposits. Supported by the National Natural Science Foundation of China (NSFC. No.31360043; No.31360035; No. 31160042), the biodiversity of microbiomes of mosses from some active tufa deposits sites were investigated in central Guizhou, China.After our preliminary identification and analysis in laboratory, 39 biota taxa belonging to five groups have been found from karst deposits in research area. Among them, there are 3 species for cyanobacteria while 15 for protozoan, two for green algae, 10 for diatoms and 9 for mosses.The relationship between microbiomes communities, mosses and tufa deposition have been discussed in this paper, including (1) the surfaces of biota acting as sites for nucleation and crystal growth of calcium carbonate from fresh water; (2) the process of photosynthesis removes carbon dioxide from the water, favoring carbonate precipitation; (3) living microbiomes communities providing a framework for carbonate deposition (4)the morphology of karst deposition may be affected by the growth of living microbiomes communities. Biodiversity of microbiomes and mosses may play an important ecology and biogeological role in karst deposition process at active tufa sites.

T1-23: Plant biodiversity data mobilization and management in the 21st century

T1-23-01

The digitization of a large herbarium, new opportunities & new responsibilities

Quentin Groom
Botanic Garden Meise

Globally a large number of herbaria are digitizing their collections. Researchers are now able to access these images and data of specimens through web portals and aggregators. This process promises many new opportunities for new and improved botanical research. It will also improve access to collections and reduce the historical inequalities between scientists. Herbarium digitization should reduce the cost of taxonomic research, but also expand the use of herbaria in ecology, biogeography and evolution. Indeed, we have only began to imagine what will be possible once the world’s herbaria are digital. In 2015 the Botanic Garden Meise, Belgium, received a large grant from the Flemish government to fund the digitization of the Garden’s African and Belgian collections. This will result in the digitization of more than a million specimens that will be accessible on a new web portal. We also received funding to modernize our imaging equipment so that the complete digitization of our collections no longer remains a distant possibility. Nevertheless, digitization is not a mere adjunct onto traditional curatorial activity. It will change the way collections are managed and biodiversity informatics will be increasingly central to the functioning of a modern herbarium. Digitization opens up many questions on how data are managed. For example, who should have access to those data? What data standards should be used? How can data be referenced permanently and how can the data publishers get recognition for their work? This means we need to create new policies, plans and procedures to maintain the quality of our output. Though the possibilities of digitization are exciting, we must be attentive to the new responsibilities it presents, so that we can ensure the long term sustainability of this research infrastructure.

T1-23-02

Everyday operational issues associated with managing the digital collections of one of the largest herbaria in the World

Sarah Phillips, ***Alan Paton****, Marie-Hélène Weech, Laura Green*
Royal Botanic Gardens Kew

We will present challenges, related to prioritisation, data quality and data curation in the context of the daily operation of digital collections management at Kew and discuss possible solutions. Over the last decade, digitisation rates have increased dramatically with ca.13% of the herbarium now databased. However, these specimens are physically dispersed throughout the collection, having been selected for digitisation based on specific scientific research and digitisation projects or through image requests from external researchers. The priorities of these stakeholders have not always matched those of the institution, and has led to a bias for certain taxonomic or geographical units not necessarily representative of the whole collection. As any one cupboard can be searched through multiple times for different specimens, this digitisation approach is not the most efficient. However, the recent emergence of industrial scale imaging and processing means it is now a reality that the 7 million specimens could be imaged within a 5 year time frame, working sequentially through the cupboards. Securing funding for mass digitisation is challenging, the sheer volume of specimens making it expensive and funding has traditionally been easier to obtain for geographically scoped projects or projects targeting particular species to answer individual research questions. Currently digitised and un-digitised material are intermingled, which is not operationally efficient or strategic, providing a bias to digitally accessible records and limiting value to users if this bias is not understood. The increasing volume of digitised collections brings new challenges to curators of these specimens and corresponding digital records. Un-digitised material should not be stored amongst sections of already digitised material and the digital collection needs to stay aligned with the physical collection. As specimens are moved and filed under different taxonomic names their digital records need to be updated. This takes additional resource often using software not designed efficiently for these tasks. Feedback received from image and data end users through the herbarium catalogue and other aggregator portals are sent to curators for assessment and records updated accordingly.

Feedback is encouraged to enhance data quality but takes time to manage, the efficiency of which could be improved through provision of easy to use automated annotation tools and linking of duplicates in other herbaria. In the current digitisation workflow, full label transcription is completed from the images rather than from the physical specimens. Different methods have been employed to capture label data from images including outsourcing, crowdsourcing and working in collaboration with project partners and to a smaller scale individual researchers. These approaches all have their own challenges with differing depths of data capture, quality and format of data returned. However all require decisions on level of data cleaning necessary before records are imported into the collection management system and made publically available. Capturing label data post imaging lends itself to user prioritisation of transcription where data is enhanced for specimens when there is an identified need. Future options for consideration include sharing the transcription and associated data validation, working as a global community rather than at an institutional level.

T1-23-03

Integrated digitization workflow and management system for herbarium plant specimens

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Large amounts of herbaria specimens represent as invaluable assets in academic research and policy-making, attributing spatial-temporal distribution pattern and phenotypic traits of species against emergent problems of natural resource management, global food malnutrition and climate-related dynamics. Accordingly, less than one third of herbaria specimens were digitally transcribed, while the estimated cost of sheet photographing and label recognition was between 5 and 7 USD dollars per specimen. The current efficiency and maintenance of digitization workflow, despite various modules and strategies were already proposed, is mainly limited to the duration and scale of funding, time, and staff. In addition, lack of integration of distinct digitization steps from specimen bar-coding, followed by label recognition and manual typing, to final data upload and website management, into a total solution package further hinders the establishment of a cost-effective coordinated digitization system. Based on the primary scanning result at TNU, we present an advanced digitization system, whose functions combine specimen barcode reading, on-top scanning platform, Optical Character Recognition (OCR) of specimen label, and registration-verification-based system for specimen image archive and intuitive database management, specifically for the digitization of herbarium plant specimens. Compared to conventional convey-belt and camera-based photographing system, this system design can significantly cooperate with recruited volunteers with limited botany background and experience, economically maximizing image capture efficiency at an ideal rate of roughly 200 records of complete label information per hour. The intuitive interface of database management system offers registration-based parallel verification options on specimen images and corresponding label data for the convenience of internal file management and external control over public-access website. The default file format and database structure follow the JSTOR standard, facilitating the import/export of database information among GPI members.

T1-23-04

Data mobilization for the Global Tree Assessment

Malin Rivers
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The Global Tree Assessment is an initiative to provide conservation assessments of all tree species by the year 2020. This will be the first comprehensive assessments to effectively protect and safeguard the world's trees. The Global Tree Assessment is utilising a wide range biodiversity data sources, tools and platforms to achieve its goal. The Global Tree Assessment is led by Botanic Gardens Conservation International (BGCI) and the IUCN Species Survival Commission (SSC) Global Tree Specialist Group (GTSG), and to date we have already completed a complete list of the world's tree species, developed a robust procedure for identifying those that are not threatened according to international criteria and identified those tree species that are known to be threatened at national, regional or international level.

A global tree checklist with about 60,000 accepted tree species and their country distributions - GlobalTreeSearch - forms the backbone of the Global Tree Assessment. The assembly of this list of the world's trees relied on over 700 data sources, including various digitized biodiversity information, such as online floras, checklists, and specimen databases, as well as not yet digitized information. The expansion of the availability of floras online, and more detailed distribution information of species (including species status/specimen references/date of observation) would facilitate and improve this data source. Equally, much of this data is available, but is not open access. Increasing the amount of data freely available would be beneficial.

The Global Tree Assessment has also focussed on developing a methodology to identify widespread, not threatened species. An initial workshop determined data sources, tools and methodologies to identify not threatened trees. Open sourced data and data manipulation tools will be used for rapid identification and documentation of these species, this in turn will allow us to concentrate on collecting information on the species most at risk.

Another database, ThreatSearch, in which all known plant conservation assessments are collated, including national, regional and global levels, has also been used to identify tree species that are already known to be threatened.

The Global Tree Assessment has been made possible by the progress in plant biodiversity data mobilization. However, we still need to continue to develop an extensive global collaborative partnership for the completion of this project. This partnership will rely on biodiversity informatics tools and platforms in order to work efficiently across political, geographical and linguistic boundaries.

T1-23-05

Rapid response to invasive species in the digital era

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Rapid availability of observation data of invasive species is an important means to be able to prevent establishment and spread of these species and to help management for already widespread invasive species. Yet, these data have to be trustworthy and also have to be made available to management organisations fast to be able to adequately diminish the influence of invasive species in the ecosystem. In The Netherlands a vast majority of botanical observation data is collected in the National Database Flora and Fauna. In this database many private, corporate, scientific and governmental parties are working together to collect data on the distribution of plants and other organisms and recently comprises 100 million observations. These data are not only collected by professionals, but mainly through a network of volunteers. Data from different apps and web portals are send to the National Database. Data quality is assured through a validation protocol. This first automatically checks data integrity and the probability of a species' determination, based upon earlier and nearby observations of the species. Remaining doubtful observations are then manually checked by species group experts. In this way, more than 90% of the data becomes available to different types of end users within a few days after being collected. In the case of invasive species, local managers can rapidly act after receiving an e-mail alert or accessing the data portal.

T1-23-06

The Global Biodiversity Information Facility: A review of progress and challenges in plant data mobilization and integration

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GBIF—the Global Biodiversity Information Facility (http://www.gbif.org/) is the leading aggregator of species occurrence data, including botanical and fungal data. As of January 2017, GBIF had more than 700 million records harvested from over 32,000 data resources published by more than 800 data providers. Since its establishment in 2002, GBIF has provided leadership in delivering tools, standards and best practices for mobilizing biodiversity data for free and open use by the research community and to support policy applications. Established tools and processes now support the aggregation and integration of species distribution data from natural history collections, field research, environmental genomics, citizen science and literature into a single, unified resource. Modern approaches to linked-open data management point the way to this data resource becoming the focal point for connecting the full spectrum of recorded information about species and communities. GBIF strives to provide well integrated data of high quality and in large quantity to enable users to query and summarize data, with answers that are as complete and accurate as possible. This can only be achieved if data providers worldwide mobilize large, high quality data across a wide spatial, temporal and taxonomic spectrum and publish them openly using interoperable standard data formats. We review GBIF progress in plant data mobilization and integration, and provide insights on future directions. We also highlight the challenges encountered and invite a discussion on future expectations of the botanical community and the role the community can play in developing and curating the GBIF database to meet the needs and expectations of users.

T1-24: Systematics, population biology, morphology and conservation of the grape family Vitaceae: overview and recent advances

T1-24-01

Phylogeny and diversification of *Tetrastigma* L. (Vitaceae)

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Tetrastigma is one of the most species rich genera of the economically and agronomically important family Vitaceae. It encompasses ca. 95 species that are primarily distributed in the tropical and subtropical regions of Asia and Australia, and species of the genus exhibit great diversity in both vegetative and reproductive characters. Species of *Tetrastigma* can be distinguished from other taxa of Vitaceae by their 4-lobed stigmas. The genus has attracted a great deal of attention due to its unique host-parasite relationship with Rafflesiaceae, especially *Rafflesia* (the genus with the largest flower on the world). However, relationships among major clades of *Tetrastigma* remain contentious probably because of insufficient phylogenetic signals. With 10 chloroplast markers and expanded taxon sampling (73/95 species), we recognized six major clades within *Tetrastigma* and relationships among these six major clades were well resolved. With extensive field collections and herbarium work, we systematically documented seed morphology throughout *Tetrastigma* and traced the evolution of six key seed characters. Morphological synapomorphies for each major clade of *Tetrastigma* were detected based on character optimizations and traditional morphology-based infrageneric classifications of *Tetrastigma* were evaluated. Ancestral area reconstruction and molecular dating suggest that *Tetrastigma* might have originated from the Sino-Himalaya and the Indo-China region during the Eocene and it then reached Australia and Malesia several times independently. A series of diversification rate tests of *Tetrastigma* were conducted to identify potential diversification rate shifts. The relationships between diversification rate shifts, adaptive traits, and habitats of *Tetrastigma* were assessed. To provide a comprehensive infrageneric classification of *Tetrastigma*, further field investigations are needed especially in southeastern Asia, where many new species or forms of Vitaceae have been discovered in the recent decade. Nuclear phylogeny of *Tetrastigma* is in progress to test potential phylogenetic incongruence among plastid and nuclear genomes and investigate the underlying mechanisms concerning the adaptation and diversification of *Tetrastigma*.

T1-24-02

Genomic analyses reveal deep phylogenetic relationship, biogeographic history and adaptations to different ecological environments of Eurasian grapes

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The application of whole-genome resequencing based on next-generation sequencing (NGS) provides an unprecedented opportunity for researchers to resolve long-standing evolutionary questions. The taxa of grape genus (*Vitis* L.), especially the Eurasian grapes, are widely recognized as important genetic resources for improvement of cultivated grapes. However, resolving the deep phylogenetic relationships within *Vitis* is a significant challenge, which limits our understanding of the evolutionary history of *Vitis* and impedes the utilization of valuable wild grape resources. Meanwhile, the biogeographic history and the adaptive evolution of Eurasian species of subgenus *Vitis* are still poorly understood. Identifying the genetic basis of adaptive variations may be the key to understanding the genetic diversity and the speciation of Eurasian species of subgenus *Vitis*. In this study, we carry out whole-genome resequencing of 38 accessions (representing 37 taxa) in the genus *Vitis* based on the *V. vinifera* L. reference genome. A neighbor-joining (NJ) phylogenetic tree within the genus *Vitis* based on 2,068 single-copy orthologous genes are reconstructed with high resolution and bootstrap values of almost all branches are 100%. The phylogenetic relationship clearly reflects the continental distribution of taxa of subgenus *Vitis* (*i.e.* America, Europe and East Asia) and the sister-clade relationship of European and East Asian *Vitis* species is robustly supported for the first time. Four subclades from East Asia have been identified, in which three subclades can be recognized as early diverging in the East Asian clade: subclade I, subclade II and subclade III, while, subclade IV is the most derived subclade. The taxa of subclade I are mainly distributed in northern China and the Far East, and subclade II and subclade III are widely distributed from northern China/the Far East to the southern China. In contrast, the taxa of subclade IV are mainly distributed in southern China and Southeast Asia. We infer that the ancestral taxa of East Asian *Vitis* are distributed in northern China and the Far East and then migrated to the southern China and Southeast Asia. There are preferences to different ecologic environments within Eurasian grapes, i.e. the bushy, sprawling to low climbing species with small leaves generally occur in shrub habitats and the high climbing species with big leaves generally occur in forest habitats. Further analyses comparing the two groups based on selective sweeps identify 6,370,000 bp and 6,870,000 bp of selection regions in the high climbing species group and the low climbing species group, respectively. Among the genes embedded in selected regions of high climbing *Vitis* species group for the forest habitats adaptation, several selected genes involved in the regulation of stem elongation growth and increased apical dominance may play a significant role for high climbing species to get light at the forest canopy. In contrast, some selected genes of low climbing *Vitis* species group associate with reduced apical dominance with shortened primary stems and increased number of branches. We provide insights into both phenotypic- and genetic-level adaption to environments of Eurasian grapes.

T1-24-03

Evolutionary history of *Vitis* based on genotyping-by-sequencing data

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As concerns mount about sustainable agriculture in a changing climate, attention is focusing on understanding diversity and diversification in crop wild relatives. The domesticated European grapevine (*Vitis vinifera*) is among the most economically important berry crops in the world and is becoming an increasingly valuable component of rural agricultural development. In many areas of North America and around the world, grapevine cultivation includes not only *V. vinifera* but also abiotic and biotic stress resistant native North American *Vitis* species, which, aside from being grown outright, contribute to hybrid scions and rootstocks. Despite this, relatively little is known about geographic patterns of variation among natural *Vitis* populations or the evolutionary processes shaping them. The evolutionary history of the grapevine genus has been notoriously challenging, in part due to likely rampant hybridization of sympatric species. Within subgenus *Vitis*, a large North Temperate group that includes ~50 *Vitis* taxa (~18 in North America), it appears that most species are interfertile. In North America it is common for two, three, or up to five *Vitis* species to grow in sympatry, further increasing the likelihood of interspecific hybridization. In this study, we leveraged living germplasm collections maintained by the United States Department of Agriculture Agricultural Research Service (USDA-ARS) Grape Genetics Research Unit (Geneva, NY) and National Clonal Germplasm Repository (Davis, CA) to investigate relationships among *Vitis* species. Single nucleotide polymorphisms (SNP) were identified using genotyping-by-sequencing. After sequencing and filtering we generated a dataset of 358 individuals representing 17 *Vitis* species (12 from North America, the European *V. vinifera*, and four Asian species) and three *Ampelopsis* species. Individuals were genotyped at approximately 11,000 sites with a minimum mean depth of coverage of 10. To infer phylogeny, we used the Geneious Tree Builder software and the RaxML plugin with the GTRCAT model and rapid bootstrapping. Preliminary results provide support for previously recognized groups including 1) monophyletic *Vitis*; 2) monophyletic subgenus *Vitis*; 3) within subgenus *Vitis*, a monophyletic group of Eurasian species. In addition, these data suggest close relationships between *V. acerifolia, V. arizonica, V. riparia* and *V. rupestris*, and close relationships between *V. cinerea, V. palmata,* and *V. vulpina*. In some cases, sampled individuals from a single species did not cluster together in these analyses. This could represent misidentification within the USDA system; alternatively (or in addition), it is likely that interspecific hybridization is contributing to reticulate patterns of evolution within the genus *Vitis*. Future areas of work should include detailed population level sampling, and morphological and genetic analyses of *Vitis* populations where multiple species occur in sympatry as well as in regions where each of the species occur in isolation.

T1-24-04

Leaf anatomy and micromorphology of the New World *Vitis*: Implications for taxonomy and evolutionary shifts to new habitats

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The economically important grapes of *Vitis* subgenus *Vitis* occupy diverse habitats in North America and the Neotropics, but they are especially diverse in the mesic forest areas in the southeastern U.S. and the xeric scrublands in southwestern North America (e.g., the Edwards Plateau of Central Texas and adjacent areas). Species in subgenus *Vitis* comprise a clade (except for *V. californica*) according to ongoing molecular phylogenetic studies. Nevertheless, species delimitation within North American subgenus *Vitis* remains difficult due to limited variation in traditionally applied macromorphological characters, especially tendril frequencies along branches, tendril branching, floral sexuality, stipule size, leaf shape, inflorescence size, berry size and coating of berries. Therefore, we examined the c. 18 species of *Vitis* subgenus *Vitis* in the New World for leaf anatomical and micromorphological characters using light and scanning electron microscopy, and we analyzed our data using principal component analysis (PCA). We observed that micormoprhological characters of taxonomic importance include trichome type (prostrate, ribbon-like, unicellular; upright, multicellular); stomata morphology; mesophyll organization and midrib vascularization. Our PCA revealed seven clusters of species according to their morphological variation: (1) *Vitis labrusca* and *V. aestivalis*, (2) *V. acerifolia*, *V. riparia* and *V. rupestris*, (3) *V. cinerea* var. *cinerea*, *V. cinerea* var. *baileyana*, *V. cinerea* var. *helleri*, *V. biformis* and *V. tiliifolia*, (4) *V. palmata* and *V. vulpina*, (5) *V. mustangensis* and *V. shuttleworthii*, (6) *V. arizonica* and *V. monticola*, and (7) *V. californica* and *V. girdiana*. The PCA showed high loadings for the compactness or airiness of the spongy mesophyll on PCA axis one and of the height of the palisade layer on axis two. Leaf anatomical and micromorphological characters are often strongly related to the environmental preferences of species. However, when we represented the distributions of species within biomes in ordination space, we found little correlation with the clusters revealed in our PCA. According to micromorphological features, *Vitis labrusca* is more closely related to *V. aestivalis* than to *V. mustangensis* and *V. shuttleworthii* with the former two lacking a ridge above the midvein, and having four distinct bundles in the midvein, and raised stomates. *Vitis mustangensis* and *V. shuttleworthii* share a protruding stomatal apparatus abaxially and highly papillose epidermal cells adaxially. *Vitis cinerea* var. *baileyana* and var. *floridana* have similar trichome morphology and leaf anatomy. *Vitis biformis* from Mexico and the *V. cinerea* complex show simple trichomes with a multicellular base. *Vitis californica*, *V. arizonica*, and *V. girdiana* all have an airy spongy layer, two rows of cells in the parenchyma bridge of tertiary veins, and striate subsidiary cells. We hypothesize that adaptations to new habitats or biomes are reflected by the micromoprhological character states that we observed with xeric species possessing a spongy layer with large air spaces and well-developed, large vascular bundles, and the tropical rainforest species *V. tiliifolia* having thin leaves with a poorly-differentiated mesophyll and hyathodes adaxially.

T1-24-05

Taxonomic diversity and phylogenetic classification of Vitaceae

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Vitaceae (the grape family) consists of about 15 genera and 900 species primarily distributed in tropical regions. The family is most well-known for the economic importance of grapes and is also ecologically significant with many species as dominant climbers in tropical and temperate forests. Recent phylogenetic and phylogenomic analyses of sequence data from all three genomes have supported five major clades within Vitaceae: (1) *Cayratia* Juss., *Cyphostemma* (Planch.) Alston and *Tetrastigma* (Miq.) Planch., with the latter two nested within *Cayratia*; (2) the *Cissus* L. clade; (3) the grape genus *Vitis* L. and its close tropical relatives *Ampelocissus* Planch., *Pterisanthes* Blume and *Nothocissus* (Miq.) Latiff, with *Nothocissus* and *Pterisanthes* nested within *Ampelocissus*; (4) *Parthenocissus* Planch. and *Yua* C.L. Li; and (5) *Ampelopsis* Michx., *Nekemias* Raf., the African *Rhoicissus* Planch., and *Clematicissus* Planch. Based on the phylogenetic and morphological evidence, the new classification places the 900 species of the family into 15 genera: *Acareosperma* Gagnep. (1 sp., poorly known from Laos; never sampled in any of the recent phylogenetic studies), *Afrocayratia* gen. nov. (10 spp.), *Ampelocissus* (110 spp., including 20 species of *Pterisanthes*, and the type species of *Nothocissus*), *Ampelopsis* (15 spp.), *Causonis* Raf. (25 spp.), *Cayratia* (25 spp.), *Cissus* (350 spp.), *Clematicissus* (7 spp.), *Cyphostemma* (200 spp.), *Nekemias* (9 spp.), *Parthenocissus* (c. 15 spp.), *Rhoicissus* (12 spp.), *Tetrastigma* (80 spp.), *Vitis* (70 spp.), and *Yua* (2 spp.). *Causonis* and *Afrocayratia* are segregate genera from *Cayratia* s.l. *Nekemias* is a segregate of *Ampelopsis* s.l. *Clematicissus* is expanded to include five neotropical species in the *Cissus striata* Ruiz & Pavon complex, and represents a genus with an intercontinental disjunction between Australia and the Neotropics. The phylogenetic classification further recognizes three tribes in Vitaceae: (1) tribe Ampelopsieae trib. nov. (43 species in 4 genera: *Ampelopsis*, *Nekemias*, *Rhoicissus* and *Clematicissus*); (2) tribe Cissieae trib. nov. (665 species in 7 genera: *Cissus*, *Cayratia*, *Causonis*, *Afrocayratia*, *Acareosperma*, *Cyphostemma* and *Tetrastigma*); and (3) tribe Vitieae (207 species in 4 genera: *Ampelocissus*, *Vitis*, *Parthenocissus* and *Yua*).

T1-24-06

The fossol record of the native grape family (Vitaceae) from the Cenozoic of Australia

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The fossil record of the Vitaceae family from Australia includes pollen, seeds and wood. The oldest fossil pollen referred to the family from Australia is Late Eocene in age from the Bass and Gippsland Basin in south eastern Australia and Vitaceae pollen is also recorded from the Oligo-Miocene coal floras of south eastern Australia. In Australia, two generalised types of seeds are recognised in the Vitaceae, with pyriform and oval-shaped seeds present since the Eocene and this indicates that the family had already diversified by the Eocene in Australia. Re-examinaton of *Cissocarpus jackesieae* from the Oligocene Capella locality in central Queensland shows that the seed are oval in shape, have a linear chalazal scar, paired ventral parallel grooves (infolds) that are parallel but diverge slightly towards the apex, with a columnar, moderately thick sclerotesta. A phylogenetic position near the *Cissus*, *Cayratia*, *Tetrastigma* clade seems likely. The second undescribed taxon from this locality has a pyrifrom seed with short, ventral infold slits that are apically divergent. Fossil wood from the Capella locality is also recognized as belonging to the family and the bark and wood anatomy of a new genus *Austrovideira dettmannae* sp. nov. is also described. The species shares with the other species of Vitaceae described to date the presence of secondary xylem with two diameter classes of vessels, wide vessels usually solitary, narrow vessels forming radial chains, very wide and tall rays, scanty paratracheal parenchyma and septate fibres. The secondary phloem has a clear stratified structure, with fibre bands alternating with the other axial elements and rays rapidilly dilating towards the periderm. A new genus *Austrovideira* is proposed because the fossil wood from Capella differs from *Vitaceoxylon,* the only other genus of Vitaceae wood known, in having scalariform intervessel pits and homocellular rays composed of exclusively procument cells, which are characters not previously seen in fossil Vitaceae wood. The only known extant species of Vitaceae with these combination of features is *Vitis* and *Parthenocissus*. *Parthenocissus* is present in the Australian flora, while *Vitis* is a Northern Hemisphere genus occurring in temperate regions of North America, Europe and Asia. *Austrovideira* differs from *Parthenocissus* in lacking vessels in tangential groups. This new discovery is the first fossil Vitaceae wood from the Australian flora and the Southern Hemisphere. The fossil wood record indicates that the lianescent habit in the Vitaceae was established by the Eocene in the Northern Hemisphere, and by the Oligocene in the Southern Hemisphere. The fossil record, based upon pollen and seeds, shows that the Vitaceae were present in Australia from the Eocene and based upon variation in seed morphology the family had already diversified by this time. The family, based upon seeds and wood, is well represented in the Capella flora with at least two, possibly three taxa. The modern North Queensland Vitaceae Flora includes sympatric genera *Clematocissus*, *Cissus*, *Tetrastigma, Ampellocissus* and *Cayratia*, so the co-occurrence of a number of Vitaceae species in the same fossil flora could be expected.

T1-25: Science of the Jade Dragon Mountain

T1-25-01

Phenological progressions of Mt. Yulong Rhododendron flowering over season and elevation

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Phenology-the seasonal timing of life-history events-is a critical dimension of natural history. In plants, reproductive phenology is particularly important, affecting gene flow, population persistence and species boundaries. Phenology is also one of the earliest and most noticeable traits by which organisms respond to climate change. However, these responses are complex, and only beginning to be understood, especially in the montane and alpine environments that are among the ecosystems most vulnerable to climate change. Drawing from diverse data sets and employing multiple methodologies, we examine how climate affects phenology in Rhododendron spp. of Mt. Yulong, Yunnan, China. Mt. Yulong is a center of diversity for Rhododendron, home to a suite of species that are diverse, dominant, and ecologically and culturally salient. They are also extraordinarily well represented by a unique set of historical plant collections. We reassemble these historical collections to build a proxy record of Rhododendron phenology, and use this record to show that although increased annual temperatures are associated with earlier flowering, increased fall temperatures are associated with delayed flowering. These contrasting effects have resulted in opposing changes in flowering time, even during rapid recent anthropogenic warming. In addition, we comprehensively monitor flowering phenology in the most common Rhododendron species on Mt. Yulong over gradients of season and elevation. We measure individual response to inter-annual temperature change, contextualize the results within the herbarium record, and characterize the effects of phenology on reproductive success. Mt. Yulong Rhododendron spp. show an elaborate sequenced progression of flowering over season and elevation. Phenological response to temperature varies among species, elevation, and season. Both phenology and response to temperature directly impact reproductive success, making this progression vulnerable to future climate change. A community phylogeny shows how phylogeny affects this phenological progression. Uniquely among phenological traits, last flowering day shows a phylogenetic signal, and is associated with flower size and fruit size. We suggest that this pattern may be driven by the limited time for fruit development before the onset of cold temperatures in autumn and highlight the unique and asymmetric pressures on phenology at seasonal extremes. Finally, we explore the dynamics of ecological knowledge of Rhododendron phenology around Mt. Yulong in two ethnic groups: the indigenous Naxi and immigrant Nuosu Yi. Local knowledge is rich and local understandings of changes and drivers parallels results from herbarium and ecological methods. While these knowledge systems are dynamic and able to adapt to change, they are also threatened by urbanization and changing lifeways. In combination, these studies reveal the complexity of phenological response to climate change. Multiple methodologies allow a deeper exploration than simple ‘earlier spring’ models of phenological response, while similar patterns seen among the diverse data sources increases our confidence in each. Mt. Yulong Rhododendron spp. flower in a progression over season and elevation, constrained to some degree by phylogeny. Both phenology and response to temperature directly impact reproductive success. Their responses to temperature vary among species and across elevations, and the effects of warming in different seasons drive contrasting responses.

T1-25-02

Quaternary palaeoecology and climate change in the Jade Dragon Snow Mountain Region, Yunnan, SW China

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The Hengduan Mountains, with a distinct altitudinal differentiation and strong vertical vegetation zonation, occupy an important position in Southwest China as a global hotspot of biodiversity. Pollen analysis of lake sediments sampled along an altitudinal gradient in this region, enables us to understand how the vegetation zonation arose and how it has responded to climate change and human impacts through time. Here we present new findings on Late Quaternary palaeoecology and climate change in the Jade Dragon Snow Mountain, one of the highest peaks of the Hengduan Mountains region, based on pollen records from Haligu, Wenhai and Shuanghaizi Lakes. Our results show: 1) From 22.6 to 19.4 ka BP, vegetation in the southern Jade Dragon Snow Mountain was dominated by broad-leaved forest, accompanied by needle-leaved forest. The period 19.4 to 17.9 ka BP was a transition stage from broad-leaved forest to needle-leaved forest. Between 17.9 and 9.3 ka BP, needle-leaved forest declined and broad-leaved forest began to increase at first, while needle-leaved forest expanded and broad-leaved forest shrank towards the end of the period. From 9.3 ka BP to the present, the vegetation changed to evergreen broad-leaved forest, mixed needle- and evergreen broad-leaved forest, needle-leaved forest. Vegetation on the eastern side of the mountain was of coniferous and broad-leaved mixed forest between 11.2 and 1.3 ka BP, followed by needle-leaved forest dominated by *Pinus* and *Abies* since 1.3 ka BP. 2) Climate in the period of 22.6–17.9 ka BP was characterized by cold and dry conditions, possibly corresponding to the Last Glacial Maximum (LGM, the start of which perhaps occurred prior to the basal age). Between 17.9 and 9.3 ka BP, temperature and humidity increased at first, followed by a colder and drier climate at the end (YD: 12.9–11.2 ka BP). After 9.3 ka BP, climate showed unstable with three significant phases of rapid climate change (at 9.3–8.7 ka, 7.0–6.6 ka, 3.7–2.4 ka BP), accompanied by a significant decline in humidity. 3) Plant diversity variation indicated by Simpson index, Shannon-Wiener index, Pielou's evenness and rarefied species richness was congruent with the temperature changes over the Holocene. 4) The Jade Dragon Snow Mountain region was probably under cultivation and experiencing other anthropogenic activities since the past 10 ka BP, particularly human settlement and human impact increased in intensity over the last 2.4 ka BP. These new findings are helpful not only for us to understand the history of biodiversity in the Jade Dragon Snow Mountain Region, but also useful for taking measurements on biodiversity conservation in the future.

T1-25-03

Origins and objectives of the Jade Dragon Research Station

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Around the world, Research Stations occupy a special role in the infrastructure of science. They support long-term, site-specific and multidisciplinary studies that are critically important in an era of global change. They also play an important role in the training and development of new generations of scientists, providing practical experience of living and working in what are generally remote and challenging conditions. Beyond this Research Stations often make a direct contribution to the protection and conservation of biodiversity and the natural environment. With this in mind, the Kunming Institute of Botany and the Royal Botanic Garden Edinburgh began in 2001 to develop the Jade Dragon Research Station set in a surrounding landscape designated as the Lijiang Alpine Botanic Garden. The concept was to make the Jade Dragon Snow Mountain a focal point and centre for long term interdisciplinary research and conservation biology within alpine zone of the Hengduan Mountain Biodiversity Hotspot. From the beginning the project had an additional dimension of relationship building between China and the UK. In 2004 the Research Station was formally recognised by CAS and the UK Government as the First China UK Joint Scientific Laboratory and it has since been the focus of a wide diversity of international investigations. This introduction to the origin and objectives of the Jade Dragon Research Station draws parallels with other Research Stations around the world. It will also review recent developments and investments in scientific facilities at the research station and nearby, including the establishment and enumeration of a 25 hectare permanent monitoring plot following the methodology of the Tropical Forest Science and Forest Global Earth Observatory (CTFS-ForestGEO). The symposium will also discuss and review the prospects for future research and conservation activities on the Yulong Mountain and the wider Hengduan mountain region.

T1-25-04

Phylogeny and ecological processes on pollination networks across an elevation gradient in the Jade Dragon Mountain, SW China

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Ecological interactions are often phylogenetically conserved. However, species adapt to ecological conditions (i.e. competition and facilitation) could diminish or eliminate this phylogenetic constraints. Until now, changes in the strength of the effect of phylogenetic relationships on interspecific interactions (phylogenetic signal) across ecological gradients are poorly known. In addition, the adaptive mechanisms to promote species coexistence (i.e. shift partners, temporal segregation) are rarely studied at the network level. Here, we constructed 36 quantitative phenological plant-pollen vector networks by combining field observations and insect pollen load analyses at four sites along an extreme elevation gradient (2,725–3,910 m) in nine temporally distinct sampling periods. For each site, we also constructed eight temporal cumulative networks by pooling the phenological networks sequentially along flowering periods. To estimate the phylogenetic diversity and phylogenetic signal in the interaction networks, we reconstructed the phylogenetic trees of plant and pollen vectors using barcoding markers. We first test the effects of pollen vector to plant ratio (A/P ratio), resources density and phylogenetic diversity on phylogenetic signal in interactions of phenological plant-pollen vector networks. Then, we examined the role of temporal niche segregation in coexisting of plant and pollen vector species by comparing the phylogenetic signal between temporal cumulative networks and their corresponding phenological networks. For the first time, we found that plant phylogenetic signal in interactions tended to decrease with elevation, whereas pollen vector phylogenetic signal did not show systematically variation with elevation. The low strength of plant phylogenetic signal was related to low A/P ratio, low flower density and low plant phylogenetic diversity. However, pollen vector phylogenetic signal did not influenced by A/P ratio, pollen vector density and pollen vector phylogenetic diversity. In addition, results showed that the strength of plant and pollen vector phylogenetic signal tended to increase with the time accumulation of interactions in some communities. The phylogenetic signal of temporal cumulative networks pooled interactions of all the nine censuses higher than their corresponding phenological networks for both plants and pollen vectors across the four sites. Our results highlight that phylogenetic conservatism in plant interactions is low, and decrease with the increase in environmental stress. However, pollen vector interactions are mainly phylogenetic constrained and are not sensitive to variation in ecological processes. This study also indicates that temporal segregation among sympatric closely related species interacting with similar partners is an important mechanism to promote species coexistence.

T1-25-05

Survey and germplasm conservation of PSESP (Plant Species with Extremely Small Populations) in Southwest China: Role of China's Botanical Gardens of Kunming, Lijiang and Shangri-La

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PSESP (Plant Species with Extremely Small Populations) is a conservation concept developed for rescuing most of the globally threatened plant species, and it was firstly promulgated in Yunnan province of China in 2005. PSESP are characterized by small remaining populations (far lower than Minimum Viable Population, MVP), restricted habitat, extremely high risk of extinction and exposure to serious human disturbance. Survey and Germplasm Conservation of PSESP in Southwest China (2016-2021) is a national key project supported by the Ministry of Sciences and Technology of China in 2016. A total of 231 PSESP are targeted in this project which covers an area of 1459300 square kilometers in some 490 counties in Yunnan, Guizhou, Sichuan, Chongqing, west Guangxi and southeast Xizang. Based on the survey and investigation of the targeted species, at least 70 of them are required to be propagated and conserved *ex-situ* in Botanical Gardens, while the germplasm of over 100 PSESP will be stored in national germplasm banks. Survey and germplasm collection of 82 PSESP from 100 counties with 456100 square kilometers in the "Yunnan-Xizang" region of Yunnan plateau, north west Yunnan and southeast Xizang, has been planned as a sub-project. Undoubtedly, the resources of the botanical garden of Kunming, Lijiang and Shangri La located in this region are rather important for implementing the project. This presentation will provide an overview introduction of the project goals and its current progress, and emphasize the importance of the three botanical gardens for the success of the project. Special mention will be made of the Lijiang Alpine Botanic Garden, associated with the Jade Dragon Research Station, and its future development.

T1-25-06

Forest biodiversity monitoring in North-western Yunnan at the 25 hectare Yulongxueshan Forest Dynamic Plot and associated satellite plots

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One of the main goals of the 25 ha-Yulongxueshan Forest Dynamic Plot is to explain the high species diversity of subalpine forests. Spruce-fir forest is the best protected forest vegetation on the eastern slope of the Yulong Mountains. To better understand its species composition and community structure, a 25 ha permanent plot was established in the Yulongxueshan Provincial Nature Reserve by Kunming Institute of Botany, Chinese Academy of Sciences and Yulongxueshan Administration of Provincial Nature Reserves from 2012 to 2014 following the standards of the Center for Tropical Forest Science (CTFS). We analyzed the flora, community composition, structure, size class and spatial distribution based on data collected from all woody plants (except bamboo) with DBH⩾1 cm. A total of 47,751 free-standing individuals with DBH ⩾ 1 cm belonging to 62 species, 41 genera and 26 families were recorded with northern temperate plants being the main floristic elements. The Importance Values (IV) of 19 species were 1, and these species contributed the IV of 91.23% of the community. The forest community was divided into four layers, the dominant canopy species being *Abies forrestii, Picea likiangensis* and *Quercus guajavifolia*. *Gamblea ciliata* var. *evodiifolia*, *Acer pectinatum* and *Padus brachypoda* occupied the sub-canopy where the absence of small size-class trees suggests their populations may decline in the future. *Sorbus prattii, Viburnum betulifolium* and *Rhododendron yunnanense* dominated the sub-tree layer, while the shrub layer was strongly dominated by *Berberis fallax*. 30 satellite plots, containing three samples of 1000 square meters, were established in different mountains in the northwest Yunnan in the area upstream of three great rivers of Asia, recognized for its importance for biodiversity conservation, and as a security barrier for regional ecology. The aim of the satellite plots is to describe the differences in plant community structures in the many different vegetation types found in the area and to explore the mechanisms which maintain the plant communities. The dominant tree species include *Pinus armandii, Pinus yunnanensis, Pinus densata, Quercus pannosa, Larix gmelinii, Picea likiangensis, Picea brachytyla var.complanata, Abies georgei, Abies delavayi, Abies forrestii,* *Taxus yunnanensis* and *Alnus nepalensis*. The coordinates, DBH and height of trees were recorded together with surveys of shrubs, herbs, litter, deadwood and biomass. We found that random distribution of species was common in the mature forest, while in the young forest clumped distribution and uniform distribution were more typical. The reserves of carbon in alpine and subalpine coniferous forests and soils were richer than for forests at lower altitude, and had slower regeneration rates. Once the alpine and subalpine forest has been damaged it may face a long period of recovery, and more carbon from the forest and soil released into the atmosphere with important consequences for global change.

T1-26: Plants on inselbergs: speciation patterns, ecology and conservation

T1-26-01

New vistas on plant evolution: Lessons from terrestrial islands

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The study of oceanic islands has provided interesting hypotheses for our understanding of ecological and evolutionary processes that affect the biodiversity of ecosystems. Inselbergs are ancient monolithic outcrops mainly in tropical and subtropical regions, which rise abruptly above their surroundings, thus forming naturally occurring terrestrial islands. Nevertheless, these isolated environments have been so far overlooked concerning evolution and speciation. Due to harsh environmental conditions, both non-vascular and vascular plants of inselbergs are clearly distinguished from the surrounding matrix, represented by specialized species adapted to drought, heat and high irradiation. In southeastern Brazil, particularly in the *Mata Atlântica* domain, they occur in large numbers and in various degrees of isolation. This core area, which harbors a highly diverse inselberg flora, has been recently baptized as Sugar Loaf Land (SLL). Different plant communities are found on these outcrops, and mats are the most prominent habitat, consisting especially of long-lived monocots occurring on open rocky slopes, literally glued by dense wiry roots. In most cases mats are formed by angiosperms (e.g. within Bromeliaceae, Cactaceae, Cyperaceae, Orchidaceae, Poaceae, Velloziaceae) and ferns (e.g. *Selaginella*). Based on our newly generated data, Brazilian monocot-mats are among the richest in species in the world, and it can be expected that SLL is the hotspot of inselberg plant diversity concerning numbers of species and endemics. Particularly outstanding is the high beta diversity, i.e. the high species turnover over between neighboring rock outcrops in the above mentioned region. It can be assumed that the high alpha and beta diversity are due to the concentration of large numbers of inselbergs in the core area, the existence of phytogeographic links to other rock outcrop types (e.g. *campos rupestres*, ironstone outcrops) and a high degree of demarcation against the surrounding matrix. Also remarkable within the mat-formers is the widespread occurrence of species displaying a large spectrum of morphological variation, causing extreme difficulties in their taxonomic treatment. Highly variable (polymorphic) species, whose variation though partly correlated with ecology and geography, are called ochlospecies. In our study, broad intraspecific variation was found especially in populations of monocot taxa. The consideration of these species-complexes is important not only for solving taxonomic problems, but also for understanding processes of species evolution in terrestrial islands such as inselbergs.

T1-26-02

Patterns of plant species richness on inselbergs: A global overview

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In many parts of the world rock outcrops such as granitic and gneissic inselbergs form important landscape elements that contain numerous plant and animal species and provide important ecosystem services. They are millions of years old and rise often abruptly above their surroundings. Typical are extreme environmental conditions (e.g. lack of soil and water, high temperatures and insolation). Consequently their flora and vegetation clearly deviates from those of the surroundings. Throughout the tropics a typical set of plant communities and habitat types is present on inselbergs. Prominent are cryptogamic crusts, monocotyledonous mats and ephemeral flush communities. Many vascular plant species have developed particular adaptive traits in order to cope with life on inselbergs. In particular, desiccation-tolerant vascular plants are dominant mat-formers on tropical inselbergs with members of Cyperaceae and Velloziaceae being of particular importance which show a Gondwanian distribution. We estimate that globally 15,000-20,000 species of vascular plants occur preferentially on inselbergs. Particularly rich in inselberg specific species are southeastern Brazil (“Sugar Loaf Land”), Madagascar and southwestern Australia. Moreover, rock outcrops in these regions are characterized by high numbers of endemics and high rates of beta diversity. For certain countries and regions (e.g. East Africa, India, China) almost no data are available concerning the floristic composition of granitoid inselbergs. Due to negative human impacts (e.g. mining, fire, tourism, invasive weeds) inselbergs are threatened on a global level what could result in the loss of many unique and endemic taxa.

T1-26-03

Malagasy inselbergs: Floristic diversity and aspects of conservation

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Globally, inselbergs which are isolated rock outcrops, mostly consisting of granite or gneiss, constitute old terrestrial habitat islands which are widespread throughout the tropics. Madagascar is known to be rich and diverse in terms of species and ecosystems and is renowned for its high levels of endemism, unparalleled in the world. Specific habitats such as inselbergs which have been largely neglected hitherto contribute to this status and also provide various ecosystem services such as important sources of mineral water. They form prominent landscape features, mainly in the central highland regions and have clearly a global importance with regard to their high species richness of certain plant groups such as succulents, terrestrial orchids, resurrection and carnivorous plants. Most of them are still unexplored and biologically unknown but recent extensive fieldworks conducted on more than 40 Malagasy inselbergs have contributed to a better knowledge about their floristic diversity and habitats. Up to now, more than 800 species were recorded to occur on Malagasy inselbergs. The most important families with largest numbers of species are Orchidaceae, Cyperaceae, Rubiaceae, Asteraceae, Apocynaceae, Poaceae, Fabaceae, Asparagaceae, Euphorbiaceae and Lamiaceae whereas *Cynorkis, Cyperus, Euphorbia*, *Kalanchoe, Aloe, Cynanchum*, *Exacum, Angraecum, Senecio, Xerophyta, Utricularia* and *Jumellea* represent the most speciose genera. Inselbergs are physiognomically identical throughout the island, but differ significantly in respect of their floristic composition as well as the degree of endemism. With southeastern Brazil and southwestern Australia, Malagasy inselbergs have been identified as one of the three hot spots of inselberg plant diversity. Effectively, Malagasy inselbergs are currently threatened by habitat degradation due to various human pressures (mining, quarrying and fire, the common illegal collection of plants for commercial purposes) resulting in loss of floristic diversity and the ecosystem services they are providing. Many of their species are highly endangered now, for ca. 20% of the species which have been assessed for the IUCN Red List, ca. 50% have a threatened status. Moreover, inselbergs are under-represented within the Protected Area network of Madagascar. Conservation of this unique ecosystem is urgently needed at regional, national as well as international levels and the most efficient way could be the creation of additional protected areas. In addition, creating awareness of their importance for the local population is also urgent.

T1-26-04

Midland lateritic hills of South India: Cradle of neoendemism?

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The Western Ghats, which is one of the major global biodiversity hotspots, has been recently declared by the UNESCO as a World Heritage Site and the Western Ghats Ecology Expert Panel (WGEEP) constituted by the Ministry of Environment and Forests (MoEF), Government of India, submitted their report in August 2011, to address the issue of conservation of biodiversity of the Western Ghats. Unfortunately there has been never an effort to recognize the rare biodiversity of coastal lateritic plateaus and hills, parallel to the Western Ghats, which bore a large proportion of rare and endemic plants. Northern Kerala of South India, which is the type locality of laterite, is characterized by wide lateritic plateaus which provide extremely harsh physical environment for life leading to the development of specialized plant communities with large number of endemic and habitat specific species. Madayippara, a midland lateritic plateau located in the Kannur District of Northern Kerala, South India, is an icon of the rich floristic diversity and endemism shown by South Indian lateritic hills. The plateau forms a number of microhabitats due to the difference in geographic terrain and soil cover, thus forming a complex of peculiar habitats with diverse forms of plants, mainly ephemeral herbs. Out of the 665 flowering plant taxa recorded from the microhabitats of the plateau, within a limited area of 3.65 square kilometers, 161 (24.21%) are endemics. Most of the endemic species occur in specialized microhabitats. The plateau is the type locality of nine taxa, *viz., Rotala malabarica* (Pradeep *et al.,* 1990), *Nymphoides krishnakesara* (Joseph & Sivarajan, 1990), *Justicia ekakusuma* (Pradeep & Sivarajan, 1991), *Lepidagathis keralensis* (Madhusoodanan & Singh, 1992), *Coelachne madayensis* (Pramod & Pradeep, 2012), *Eriocaulon madayiparense* (Swapna *et al.,* 2012), *Lindernia madayipparensis* (Ratheesh Narayanan *et al.,* 2012), *Parasopubia hofmannii* (Pradeep & Pramod, 2013) and *Parasopubia hofmannii* var. *albiflora* (Pradeep & Pramod, 2013). Two recently described species from South India, viz., *Eriocaulon gopalakrishnanum* (Rashmi & Krishnakumar, 2013) and *Lindernia tamilnadensis* (Prasad & Sunojkumar, 2014) also occur in this plateau. Lateritic plateaus of Northern Kerala, which are associated with characteristic and rich biodiversity, are now under varied types of anthropogenic threats such as large scale mining for bricks and clay. Any slight disturbance in the micro ecosystems can easily take away a number of short-lived herbaceous species which cannot be easily conserved outside its natural habitat. For the conservation of the rich diversity and microhabitats of the plateau, *in situ* conservation of the entire habitat is the only solution as *ex situ* conservation measures cannot provide complex microclimatic requirements artificially.

T1-26-05

Conservation and exploration of Rio de Janeiro's inselberg flora, Brazil

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Inselbergs worldwide host a very particular vegetation, being a refuge for rupicolous plant communities which do not exist in the surrounding matrix. Inselbergs occur both in the tropics (e.g. southeastern Brazil, Madagascar) and in temperate zones (e.g. southwestern Australia). In many regions their unique biodiversity is threatened by human activities, such as quarrying, fires, illegal collection of ornamental plants, tourism and weed invasion. In the metropolitan area of Rio de Janeiro these negative effects have caused the degradation of many inselbergs. The region forms a biodiversity hotspot where inselbergs harbour many local endemics and threatened species. In the city of Rio de Janeiro more than 200 inselbergs occur in an urban agglomeration with c. 6.000 inhabitants/ km^2. Southeastern Brazil (incl. Rio de Janeiro) forms together with Madagascar and southwestern Australia one of the most important regions globally, with regard to the richness and degree of endemism of rupicolous species. Remarkably, little is known about the flora, vegetation and conservation status of inselbergs of the megadiversity country Brazil. Based on own fieldwork and data available from other sources we present an overview on the inselberg flora of Rio de Janeiro. In total 193 species of vascular plants occur on inselbergs in the study area. More than 80 species (> 40%) are restricted to the Atlantic Rain Forest domain. Richest families are Bromeliaceae (17 spp.), Orchidaceae (11 spp.) and Cactaceae and Araceae (6 spp. each). In total 25 species (13%) are endemic to the federal state of Rio de Janeiro with Bromeliaceae (10 spp.), Orchidaceae (3 spp.) and Gesneriaceae (2 spp.) comprising species that are often restricted to a few (< 10) inselbergs. In addition, 32 species (17%) are listed under particular threat categories. Based on the major risk criteria of the four lists checked (IUCN, CNCFlora, MMA, SMAC), 6 species are Critically Endangered (CR), 14 Endangered (EN), 11 Vulnerable (VU), and one Near Threatened (NT). The families with high numbers of threatened species are Bromeliaceae (12 spp.), Cactaceae and Begoniaceae (4 spp. each), and Orchidaceae (2 spp.), which also usually figure among the richest families on inselbergs in Brazil. Certainly many other species will have to be included in these lists, once their conservation status has been evaluated. Furthermore, as new studies on inselberg vegetation in southeastern Brazil are underway now, new species have been discovered, as well as new distribution records for the state. It becomes clear that inselbergs, even in the middle of one of the biggest urban areas of Brazil, still host important scientific novelties, since there is still a lack of knowledge regarding their vegetation, mainly in the steepest parts. Thus, the information provided by us can contribute to conservation actions, in order to prevent the extinction of the most vulnerable species. Seeking to guarantee the conservation and diversity of the rupicolous community as a whole, might help to guide public policies towards *in situ* conservation of species on inselbergs in Rio de Janeiro.

T1-26-06

Development of SSR markers for species of *Chresta* (Asteraceae, Vernonieae) from rock outcrops of the Brazilian Caatinga using genomic data

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Chresta is a genus of Asteraceae almost endemic to Brazilian *Cerrado* and *Caatinga*. Despite the relatively small number of species, the genus presents high morphological variation, which raises interesting evolutionary questions. From the 18 species of the genus, seven occur in the semi-arid *Caatinga* region, usually growing directly over quartzitic or granitic rock outcrops, forming distinct populations that are isolated from each other and spread apart in a dense semi-deciduous forest matrix. Five of these species present similar morphological features, which sometimes overlap. Usually populations in the extremes of the distributions present different morphologies from those found in the "core" populations. This variation led to the acknowledgment of some populations as different species, such as *C. hatschbachii* and two new species yet to be published, which were all segregated from *C. harleyi.* The morphology of *C. filicifolia,* which occurs in the Atlantic Forest, is closely related to those from *Caatinga* species, what may indicate a *Caatinga*-Atlantic Forest link that has been observed in other plant groups. In the present work, we used genomic data previously obtained by the sequencing of approximately 1000 nuclear markers from four different *Chresta* species and the whole chloroplast sequence from seven samples to design SSR markers that present variation among five *Caatinga* species and *C. filicifolia* and among individuals from the same species. The markers will be further used to evaluate genetic diversity and gene flow among populations from the same species and different species, in order to investigate processes of speciation and evolution in this species group and in the *Caatinga* as a whole. The chloroplast sequences of seven *Chresta* species were aligned using MAFFT, and the SSR finder Phobos was used to locate possible repeat regions, which were then selected based on putative differences in size among the seven sequences. The set of nuclear markers was assembled in HybPiper and then alignments for each locus were individually searched with Phobos. Also, contigs assembled with Spades in the HybPiper pipeline were searched with Phobos and contigs with possible candidates were then blasted in GenBank to assure they were not chloroplast sequences. Nineteen pairs of primers were developed, twelve from nuclear markers and seven from chloroplast; most of them amplified with all species, using the same PCR recipe and program. Fragment analysis was carried out in 56 individuals from different populations of six species (*C. filicifolia*: 4 individuals, *C. harleyi*: 28, *C. hatschbachii*: 4, *C. martii*: 10, *C.* sp1: 12, *C.* sp3: 2) with 12 pairs of primers, four pairs being chloroplast markers and eight nuclear. From the 12 tested primers, only 4 nuclear markers showed variation among all species. Three of the chloroplast primers presented variation among individuals of *C. martii*, but were not variable in the other species.

T1-27: The evolution of tropical biodiversity: a global comparative perspective (two sessions)

T1-27-01

Global signals of extinction and adaptation in megafaunal-fruited plants: Insights from a keystone tropical plant family (Palms, Arecaceae)

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Frugivory (fruit-consumption and subsequent seed dispersal by animals) is a key plant-animal interaction in tropical rainforests. Of particular importance for this mutualism are large-bodied frugivores because they disperse the largest seeds and contribute disproportionally to long-distance dispersal events. However, the consequences of global change on the diversification and adaptation of small- (< 4 cm) and megafaunal-fruited (> 4 cm) plants at large spatial and temporal scales remain largely unexplored. Here, we combine an all-evidence species-level phylogeny for all palms (Arecaceae, ca. 2600 species), global species distributions and a novel fruit size dataset to study the consequences of dispersal by megafaunal frugivores (mammals 44 kg) on palm diversification.

We show that palms (a keystone resource for frugivores) with megafaunal fruits have lower speciation rates than smaller-fruited palms, possibly due to frequent long-distance dispersal events that increase gene flow among populations and thereby limit allopatric speciation. Furthermore, a time-window analysis demonstrates that since the onset of the Quaternary (2.6 Mya), a period a dramatic global change, extinction rates of megafaunal-fruited New World palm lineages have increased whereas Old World palms show an increasing transition rate (evolving smaller fruits from megafaunal fruits). We suggest that high extinction rates in the New World could be triggered by climate-driven habitat fragmentation potentially coupled with a temporary decrease in megafauna diversity in the initial stages of the Great American Biotic Interchange. Increased transition rates in the Old World could reflect adaptation to dispersal by smaller-bodied, volant frugivores against a background of sea level fluctuations in island-dominated environments of Southeast Asia. Our results suggest that plant lineages with megafaunal fruits are disappearing, either due to increased extinction rates or rapid evolution to smaller fruit sizes.

T1-27-02

Exploring the evolutionary history of the Australian rainforest flora

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Despite representing <1% of the Australian land surface, rainforests have high biodiversity and biogeographic significance. Rainforest vegetation was once widespread throughout the supercontinent Gondwana. As the Australian continent drifted northwards on the Sahul shelf, its rainforests became isolated. The increasing continental aridification associated with these geological processes, also caused major distributional contractions and lineage losses. This loss of diversity was somewhat reversed as the Sahul continental plate approached and eventually collided with the Sunda shelf. The increased proximity between landmasses promoted biotic exchange between the two floras, with extensive immigration of Indo-Malesian rainforest lineages (i.e. with Sunda ancestry) to Australia. An increasing number of phylogenetic studies is revealing the contrasting biogeographical histories of these two floras within the Australian continent. Yet, so far, little is known about the impact that distinct ancestries have on the geographic distribution and landscape-level dynamics of rainforest species, as well as on the relative prevalence of specific functional attributes. I will present continent-wide data showing how these two biogeographic groups can be broadly distinguished based on a range of functional attributes, and on their geographic distribution. I will then discuss contrasting population dynamics patterns based on multi-species investigations using next-generation sequencing (NGS) technologies (*in silicio* chloroplast DNA assembly). These analyses detected significant differences in functional trait variation, and in continental dynamics between species with Sunda and Sahul ancestry. We also confirmed the location and relative importance of long-term refugias.

T1-27-03

Comparative approaches to studying plant diversification in tropical rain forests

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Warm and wet environments have played a key role in the diversification of plants. The vegetation of these environments, known as tropical rain forest (TRF), has been estimated to harbour 43-45% of extant vascular plant species despite only covering ~7% of the Earth's land surface. In addition, many plant lineages now occurring outside TRF trace back their origins to this biome. Although the history of TRF has been well studied, many fundamental questions are insufficiently answered. How important is the old age of TRF for their extraordinary diversity? Do plant species originate more readily, and/or disappear less frequently, in TRF compared to other biomes? Which factors promote speciation and regulate extinction in TRF? How much plant diversity did TRF receive from other biomes during their history? Is there a limit to the number of species that can exist in a biome at any time, and is this limit higher in TRF than elsewhere? Multispecies comparative studies can provide crucial answers to these questions, but rely on phylogenetic trees as an explicit evolutionary framework. Phylogenies link fossils to extant taxa, allow process-based modelling of evolutionary and biogeographic change, and facilitate the estimation of speciation and extinction rates. Critical insights can be gained at two levels: globally, diversification in TRF can be systematically compared to diversification in other biomes, modelling ancestral biome associations on a phylogeny and identifying the frequency and drivers of speciation, extinction, and biome shifts. Meanwhile, TRF is not a homogeneous entity, and differences in diversification dynamics within the biome can yield important insights, using a similar approach but focusing on environmental gradients and historical differences inside TRF. We illustrate these possibilities using case studies, mainly from the palm family (Arecaceae), which has been extensively used as a model for plant diversification and ecology in TRF. Based on this we point to areas where more research is needed, emphasizing the need for broad comparative studies across taxa and regions. The feasibility of broad comparative studies is, in spite of the "big data" revolution, still limited by the availability of basic biodiversity data across the dazzling diversity of TRF plants. Not least, the pace at which TRF plant taxa are included in reliable phylogenetic frameworks will determine how soon the comparative approach to TRF plant diversification comes to full fruition. Given the rampant destruction of TRF across its range, time to collect such data is pressing.

T1-27-04

Exploring the evolutionary and ecological drivers behind intracontinental geographic disjunctions: The African Rand Flora

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Compared to other biogeographic regions located at tropical latitudes, North and Central Africa remain surprisingly poor in species numbers. Sometimes termed as the "odd-man out" in the global Latitudinal Diversity Gradient, a species-poor Africa is not universal across all groups of organisms, but in angiosperms examples of lineages with low diversity in Africa and high diversity in other tropical regions (the Neotropics, South East Asia) abound. This pattern is often associated to striking intra-continental disjunctions, in which closely associated species are distributed at opposite margins of the African continent, and in most cases separated by geographic distances of thousands of kilometres. Many of these taxa are seemingly confined to their restricted distributions by environmental barriers, i.e., the presence of intermediate, inhospitable land that lies outside the set of environmental conditions in which species can reproduce and maintain viable populations. One example is the Rand Flora, a continental-scale pattern in which different angiosperm families share a similar disjunct distribution, with related species confined to the margins of Africa and adjacent islands such as Macaronesia and Socotra. The barriers in this case are the deserts of Sahara in the north and Kalahari and Namibia in the south, or the tropical lowlands of Central Africa. Whether such patterns were formed by dispersal in recent times and ecological constraints (niche-conservatism) or through the fragmentation of a wider distribution in the past by environmental barriers ("ecological vicariance") is unclear. For the Rand Flora, the interest lies in the possibility to study the effects of large-scale extinction associated to the ongoing aridification process that started in the Miocene and climaxed in North Africa c. 7 million years ago with the formation of the Sahara Desert. Rand Flora relicts would be in this case survivors of climatic extinction, confined to their current distribution ("climatic refugia") on the edges of the continent by the disappearance of populations in the intermediate regions. Unlike background extinction rates, intrinsic to each lineage and dependent on biological traits, large-scale climatic extinction is a process concurrently affecting multiple lineages that inhabit the same region. The difficulty to study this type of extinction lies in the need to integrate different evolutionary processes: Red Queen (biological, intrinsic) versus Court Jester (abiotic, extrinsic), and – since aridification in Africa is a long-term, ongoing process – contrasting evolutionary levels: from across species (macroevolutionary) to within-populations (microevolutionary). The latter has become feasible with the recent development of next-generation sequencing techniques that allow scaling up the number of genes, individuals, populations and species sampled from non-model organisms. We will present evidence for the use of such approach to unravel the origins of the Rand Flora pattern. Using molecular (genomic), palaeobotanical and palaeoclimatic data, combined with species-distribution models and novel cross-lineage Bayesian biogeographic methods, we demonstrate that climatic extinction associated with ecological vicariance is the most plausible explanation for the Rand Flora pattern.

T1-27-05

Amazonia is the evolutionary heart of Neotropical biodiversity

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The American tropics (the Neotropics) harbor the highest number of species on Earth and for centuries scientists have attempted to understand the origins and evolution of this biodiversity. It is now clear that different regions and clades have responded differently to climatic and geological changes, and that the Neotropical diversity is largely due to this historical complexity in addition to fine-scale ecological processes. Despite this, there is a lack of basic understanding of how Neotropical biodiversity was assembled over evolutionary timescales. Here we infer the timing and origin of the biota in all major Neotropical regions by performing a cross-taxonomic biogeographic analysis based on species from six major clades (angiosperms, anurans, birds, ferns, mammals, and squamates), species occurrence data, and dated molecular phylogenies. We show that Amazonia is at the heart of Neotropical diversity, serving as a source for thousands of lineages to other regions since the Late Cretaceous. Most biotic interchange has taken place between Amazonia and western South America, closely followed by exchange with Central America. The West Indies and the northern South American savannas are primarily sinks of diversity. In several cases, congruent patterns in the timing of biotic interchange across clades suggest that abiotic factors played a key role in shaping major patterns of biodiversity. These results demonstrate the complex origin of tropical ecosystems, and highlight the need of establishing biotic corridors across biomes and elevational gradients to safeguard future biotic interchange and diversification.

T1-27-06

Historical biogeography and diversification in the pantropical Bombacoideae

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The species of the pantropical clade Bombacoideae (Malvaceae) are characteristic elements of the vegetation in both Africa and South America and include such charismatic tree species as the baobabs (*Adansonia* spp.) and kapok (*Ceiba* spp.). Most species of the clade occur in South America, congruent with the commonly observed pattern of high species richness in South American lineages. Here we use fossil calibration from explicit morphological analyses to date a molecular phylogeny of 120 species of Bombacoideae based on nuclear and plastid data. We combine this phylogeny with large scale species distribution and morphological data to reconstruct the biogeographic history and trait evolution within the clade. Specifically, we date the dispersal events out of America and test the relationship of dispersal-related traits and climatic niche breadth with range size and diversification rates. Finally, we compare the results to analyses from other taxonomic groups and discuss the results in the context of recent studies suggesting high diversification rates and species turnover as causes for the extraordinary plant species richness in tropical South America, and proposing South America as global 'species pump'.

T1-27-07

Recent origin and diversification of the Andean orchid flora in the world's richest plant biodiversity hotspot

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Species richness is highly uneven across the globe, with both species-poor and mega-diverse areas. 'Biodiversity hotspots' could be areas of environmental stability where species accumulate through time or alternatively areas of major landscape change that directly or indirectly promote rapid species diversification. The Andean Mountains in South America host *ca* 15% of the world's plant species in only 1% of the world's land surface, hence it is the most species-rich biodiversity hotspot on Earth. One of the most prominent and diverse components of the Andean and neotropical flora are the orchids, yet the mode and tempo of evolution of their remarkable diversity are still poorly understood. We address these issues by inferring the biogeographical history and evolutionary dynamics of the two largest neotropical orchid groups (Cymbidieae and Pleurothallidinae, with *ca* 7,000 species). Using comparative phylogenetic methods, geological and biological datasets, and novel molecular sequences, we find that the majority of Andean orchid lineages only originated in the last 15 million years. Most Andean lineages are derived from lowland Amazonian ancestors, with additional contributions from Central America and the Antilles. This contrasts with previously reported patterns of mountain flora origins, where highland lineages were mostly derived from high-altitude, already pre-adapted immigrants. Species diversification is correlated with Andean orogeny, and multiple migrations and re-colonisations across the Andes indicate that mountains do not constrain orchid dispersal over long timescales. Our study suggests that landscape changes can greatly participate in the formation of anomalously rich areas – biodiversity hotspots.

T1-27-08

A community phylogenomics approach to the study of the origin and evolution of hyper-diverse Southeast Asian forests

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Most tree species are concentrated in tropical latitudes, with the Indo-Pacific region being as rich as the neotropics and each region estimated to harbor *c*. 22,000 tree species. Although tree species diversity is similar in these two regions, the level of threat they face is in sharp contrast. SE Asian forests have the highest deforestation rate of all tropical regions and, given that they are currently in a refugial state as a result of Quaternary Glaciations, these forests are particularly vulnerable to disturbance. As part of the ongoing research programme on "Global Tree Conservation through Seed Science" funded by the Garfield Weston Foundation, we present an overview of our project investigating angiosperm tree community assembly through time across SE Asia and neighboring regions. Our overall aim is to investigate how the drastic geological and climate shifts experienced throughout Earth's history have affected speciation, extinction, and migration processes in the tropical forest trees of SE Asia. This will be achieved by integrating new analytical methods and high-throughput sequencing techniques, with climate and niche modeling, to create a community phylogenomics framework of SE Asian forests at the genus level. We will then use this framework to explore a range of factors which may have contributed to the hyper-diverse SE Asian forests. For example, we aim to explore whether the SE Asian tropical tree flora has arisen predominantly by the immigration of species from neighboring areas or whether it is the product of *in situ* speciation/extinction processes modulated by climate shifts and geological processes (e.g. plate tectonics, volcanism). Since much of the lowland forests in SE Asia have experienced periodic changes in sea level which in turn have led to changes in niche availability, we will also examine what impact these changes have had on diversification rates (i.e., speciation, extinction, migration). This will be done by looking for evidence that changes in diversification rates in the lowland forests are linked to past sea level shifts. Similarly, the impact of changes in precipitation regimes (e.g., monsoons) will also be factored in. Finally, we wish to address the impact of past geological dynamics since we hypothesize that mountain formation as a result of plate tectonics will lead to opening of new niches and therefore to a biodiversity increase. Such a hypothesis would be supported if we were to observe increases in speciation or immigration across highland communities in relation to orogenic events in the region. Overall, the data generated by this study will highlight areas within the SE Asian region comprising the highest levels of unique diversity (e.g. phylogenetic diversity, phylogenetic endemism) and hence of most pressing conservation concern.

T1-27-09

The origin and phylogeography of mangroves *Xylocarpus* (Meliaceae) From the Indo-West Pacific

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The *Xylocarpus* genus is the only mangrove genus in family Meliaceae. Besides the ecological importance as mangrove species, plants of *Xylocarpus* are also valuable in medicinal usage that biological activities extracted from these plants show potent in antimalarial, antiallergic, antifeedant, antifilarial, antibacterial, antidiarrhoeal and antineoplastic properties. Recent molecular data placed it within the subfamily Swietenioideae, but its generic boundary between sister genera is unclear. By sequencing five chloroplast and five nuclear fragments, we aimed at resolving phylogenetic problems on both species and population levels. Phylogenetic trees indicated that *Xylocarpus* is closest to *Carapa* genus and diverged with *Carapa* in Africa within 16.39-27.52 million years ago during Miocene, and later diverged into three species, *X. granatum*, *X. moluccensis* and *X. rumphii*. Furthermore, *Xylocarpus* may have underwent a "out-of-Africa" dispersal, in which long distance dispersal (LDD) played important role. Phylogeographic analysis on the population level revealed that populations of *X. granatum* have split into three groups, Indian Ocean (IDO), Southeast Asia (SEA) and Australasia (AUA), whereas those of *X. moluccensis* split into West and East group with boundary along the Malay Peninsula. The chronological order of haplotypes suggested a west to east dispersal both in *X. granatum* and *X. rumphii.* Though closely related, barriers of land mass Sunda shelf and Ocean currents in Wallacea have different influences on *X. granatum* and *X. moluccensis,* which may attributed to the difference in their fruits' ability of LDD. Low level of genetic diversity was found on population level in all the three species, that reminds of conservative concern.

T1-27-10

Australasian (non-Orchidaceae) asparagales biodiversity

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Asparagales comprise about one third of all monocots in 12 - 14 families, predominant in the tropics (1,122 gen.; 36,205 spp.). The order includes Orchidaceae and economically important taxa (e.g. asparagus, onions and vanilla). Despite a high diversity within Australia (12 families; 48 genera and over 300 species), phylogenetic studies to-date have typically neglected sampling Australasian Asparagales taxa. This has led to difficulties in classifying Australian lineages and, as a result, evolutionary relationships among lineages remain uncertain. Additionally, extreme morphological heterogeneity in many lineages (e.g. Asparagaceae subf. Lomandroideae) makes accurate estimation of taxonomic boundaries difficult. In this newly initiated study we are using phylogenomic approaches and high throughput sequencing techniques, such as shotgun whole genome sequencing and exon capture, to generate a dated phylogeny, with dense sampling of Australian taxa. This study will investigate cryptic diversity at a fine evolutionary scale within *Lomandra*, one of the most complex genera of Australasian Asparagales, with c. 51 spp. found across a wide range of biomes. Phylogeographic analyses will enable investigation of the environmental processes that have shaped patterns of diversification in this genus, which includes widespread species and narrow endemics. The phylogenetic framework provided will be used to ask these questions: i) what are the biogeographical patterns of sister taxa among biomes? and ii) is there congruence in the patterns consistent with historical events? We will present a preliminary high throughput dataset, which comprises several hundred loci from chloroplast genome sequences from representative Asparagales families including lineages present in Australian mesic, arid and monsoonal tropics biomes. We will discuss trends in diversification of the Australasian Asparagales in the context of the expansion and contraction of Australian biomes. This study will provide insights into the influences of macroevolution and macroecology on Asparagales lineages by understanding diversification across multiple clades in historical climatic and geologic contexts.

T1-27-11

A new way to evaluate the evolutionary tempo of community assembly

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Understanding the age of plant community assemblies can greatly improve our understanding of landscape genetics and will improve conservation management plans. Adding a temporal component to community turn-over can greatly increase our understanding of the environmental change and fluctuations that a community has experienced, which components of the assembly has experienced recent rapid turn-over and which components may have formed part of the more distant past of the assembly. In this paper we will discuss a simple method to obtain assembly age profiles using full chloroplast SNP data. The example data set uses whole genome Illumina libraries obtained from over 70 rainforest tree species with samples collected from multiple important rainforest refugial regions along the east coast of Australia. For each species and each library Chloroplast DNA was extracted bioinformatically and for each species a chloroplast reference genome was assembled. We could then estimate a species specific substitution rate for each species by using the sequence data (along with other publicly available chloroplast genomes) in a fossil calibrated phylogenetic analysis using an MCMC approach. The estimated substitution rates for each terminal branch was then used to estimate the TMRCA for each species at each of the three regions using a coalescent approach. The estimates were used to obtain age profiles for each region and each species. The results from this exercise highlights that a simple method can be applied to a relatively simple data set in order to obtain complex background information. The results support the more recent findings that the rainforests of Australia are dynamic assemblages and indicate how each region has undergone unique species-turnover. As expected from a dynamic system the results indicate that for each region a different set of species forms the older and younger components of the region. This method can be applied to any vegetation type and can be applied to landscape level ecological studies as in the given example of the Australian east coast rainforests.

T1-27-12

Floristics of the "Andean *tepuis*" of Ecuador and Peru: Diversity, endemism and phytogeographical links to the Guiana Shield

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The ancient sandstone mountains of the Guiana Shield, the *tepuis* of southern Venezuela and adjacent countries, are relatively well-known floristically with numerous endemic genera and species that have been described since the early 19th century. Less well-known are the “Andean *tepuis*, a term we apply to the sandstone mountain ranges with a north-south orientatation located east of the main cordillera of the Andes, including the Galeras and Cutucú ranges in Ecuador, the Cordillera del Cóndor along the Ecuador-Peru border, and the Escalera, Azul, and Yanachaga cordilleras in Peru. The Andean tepuis are much younger geologically than those of the Guiana Shield, but share similar edaphic conditions with soils derived from the nutrient-poor quartzitic sandstone substrate. Whereas the Precambrian Roraima Formation that constitutes the tepuis of the Guiana Shield has been available for plant colonization at its present location and elevation since the beginning of the rise of the angiosperms, the Cretaceous sandstone of the Hollín Formation that constitutes the Cordillera del Cóndor and other Andean tepuis has been uplifted with the recent tectonic movements of the Andes and has been exposed at its present elevation only within the last 5 million years. Disjunct distributions between the Guiana highland tepuis and the Andean tepuis have been recorded for a number of plant families, notably within genera harboring 30-40 species in the Guiana Shield and one or two outliers on the Andean tepuis; examples are *Bonnetia* (Bonnetiaceae) and the *Crepinella* group of *Schefflera* (Araliaceae). Similar disjunctions between the Andean tepuis and the white-sand areas of lowland Amazonia include *Pagamea* (Rubiaceae) and *Guatteria* (Annonaceae). Numerous locally endemic taxa have been described from the Andean *tepuis* in recent decades; notable among these are species within species-rich genera which are mostly mid-canopy trees in Andean cloud forests such as *Clethra* (Clethraceae), *Weinmannia* (Cunoniaceae) and *Miconia* (Melastomataceae), for which the local endemics on the highest summits of the Andean tepuis are low shrubs growing in open shrublands on the oligotrophic sandstone outcrops. Many of the Andean tepui endemics share a characteristic of small, thick sclerophyllous leaves, evidently an adaptation to the nutrient-poor soils. Much more floristic and taxonomic work is required before the floras of the Andean tepuis can be adequately characterized and enumerated, and efforts are currently underway by botanical researchers in Ecuador and Peru to meet this challenge in coming years. Phylogenetic and phylogeographic analyses of the Guiana Shield-Andean tepui disjunctions are lacking at present but are an important goal for future research.

T1-28: The biology of mycoheterotrophic plants (two sessions)

T1-28-01

Comparative plastome genomics in Ericaceae: Plastid gene losses and rearrangements across all trophic levels

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Non-photosynthetic, heterotrophic plants often have a highly reduced plastome associated with the transition from an autotrophic to mixotrophic to fully heterotrophic nutritional mode. They are either parasitic via direct connection with other plants (i.e. haustorial parasites) or are epiparasitic on mycorrhizal fungi that are themselves in symbiotic relationships with autotrophic plants (i.e. mycoheterotrophs; MHT). There are at least 21 independent origins of heterotrophy among eudicots. Ericaceae, the heather family, is a large and diverse group of plants that forms elaborate symbiotic relationships with mycorrhizal fungi, and includes autotrophs as well as fully and partially mycoheterotrophic plants. Based on input from a broad Southern hybridization survey, and using a next generation sequencing approach, we sequenced plastomes of 14 Ericaceae species across the trophic spectrum. This includes plastomes of four autotrophic (*Enkianthus, Gaultheria, Epigaea, Arctostaphylos*), three mixotrophic (*Chimaphila, Moneses, Pyrola*), and seven (*Allotropa, Hemitomes, Hypopitys, Monotropa, Monotropsis, Pityopus, Pterospora*) genera. To investigate potential intraspecific variation, the latter group saw two individuals sequenced for *Allotropa virgata*, *Monotropa uniflora*, and *Pityopus californica*. Previously sequenced plastomes of autotrophic Ericaceae (*Arbutus, Vaccinium*) were also included in analyses as well as those of *Actinidia* (Actinidiaceae) and *Camellia* (Theaceae), as progressively more distant outgroups. Our results indicate that the rearrangements of the large single copy region of the plastome are commonplace for most member of Ericaceae. Associated with these extensive structural rearrangements of the plastome across the family is an increase in forward and palindromic repeats, including in fully mycoheterotrophic species. Additionally, most Ericaceae share the loss of the plastid *ycf1* and *ycf2* genes, which is rare even among other fully heterotrophic plant lineages. *clpP* is either absent or present as a highly divergent ORF. Mixotrophic plants retain most genes relating to photosynthesis but are variable for the plastid *ndh* genes. The plastomes of fully heterotrophic Ericaceae are highly reduced in both size (~33-41 kbp) and content, having lost a copy of the inverted repeat and all photosynthesis related genes; they are reduced to encoding housekeeping genes and an unusual plastid acetyl-CoA carboxylase subunit (*accD*)-like open reading frame. Intraspecific variation within MHT Ericaceae ranges from a few differences (*Allotropa*) to extensive population divergence (*Monotropa*). Despite an increase in the rate of their nucleotide substitutions, we found that the plastomes of Ericaceae are generally under strong purifying selection, including those of partially and fully mycoheterotrophic plants in their remaining protein-coding genes. However, several classes of genes are under positive selection, including the *ndh* genes. Furthermore, positive selection is acting on the branch leading to subfamilies Vaccinoideae and Ericoideae for the *rps*, *rpl*, and *rpo* genes. We also identify a number of ribosomal proteins under relaxed or neutral selection. Finally, we demonstrate and discuss the limitations of large plastid data sets in resolving the number of independent origins of mycoheterotrophy in Ericaceae.

T1-28-02

Partial mycoheterotrophy is more widespread among orchids than previously assumed: A multi-element stable isotope natural abundance approach

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Based on their isotopic positioning between autotrophic non-orchids and fully mycoheterotrophic orchids some green-leaved orchids have been identified during the last decade as gaining simultaneously carbon from two different sources, own photosynthesis and fungal supply. This mode of mixotrophic carbon gain is called partial mycoheterotrophy. All of these ^{13}C- (and ^{15}N-) enriched partially mycoheterotrophic orchids are mycorrhizal with fungi simultaneously forming ectomycorrhizas with forest trees, while the majority of green orchids worldwide are mycorrhizal with fungi of the phylogenetic heterogeneous assemblage of rhizoctonias. Recent work has documented that carbon and nitrogen isotope abundance is not suited to unequivocally identify partially mycoheterotrophic species among rhizoctonia-mycorrhizal green orchids. Here we provide an essentially new approach to identify species with partially mycoheterotrophic nutrition among rhizoctonia-mycorrhizal orchids. In addition to carbon and nitrogen stable isotope abundance we analysed hydrogen isotope abundance in dry tissues of a fully MH orchid, in green orchids associated either with ectomycorrhizal fungi or with rhizoctonias, in autotrophic reference plants and in sporocarps of ectomycorrhizal and saprotrophic fungi growing together in the understorey of a dark beech forest and of a broad range of orchids and autotrophic reference plants from sunny montane meadows. It turned out that almost all orchids were significantly enriched in heavy hydrogen isotopes relative to autotrophic reference species irrespective whether they were enriched in heavy carbon isotopes or not. In addition to carbon, nitrogen and hydrogen stable isotope natural abundance, we also analysed oxygen stable isotope abundance in order to rule out different transpiration rates of orchids compared to reference plants as responsible for the differences found for the hydrogen isotope abundances. Thus, partial mycoheterotrophy appears to be much more widely distributed among green orchids than previously assumed.

T1-28-03

Arbuscular mycorrhizal interactions of mycoheterotrophic plants

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More than half of all species of fully mycoheterotrophic plants—plants that live on fungi during their entire lifecycle—are associated with mycorrhizal fungi of the phylum Glomeromycota. These fungi form arbuscular mycorrhizas with over 80% of all land plant species, which makes this the most common and widespread mycorrhizal interaction on earth. Unlike the mutually beneficial interactions between photosynthetic plants and arbuscular mycorrhizal fungi, which are generally characterized by low specificity, mycoheterotrophic plants often show high specificity towards particular lineages of arbuscular mycorrhizal fungi. Here we investigate these mycoheterotrophic interactions in an evolutionary and ecological context. We demonstrate that not all mycoheterotrophic flowering plant species grow on an equally narrow range of arbuscular mycorrhizal fungi. In two mycoheterotrophic plant lineages with extremely specific interactions we measure a high level of phylogenetic niche conservatism, indicating that specificity is retained during evolution and diversification. Within one of these mycoheterotrophic lineages a comparison of the mycorrhizal diversity between roots of mycoheterotrophs and surrounding plants shows that mycoheterotrophic plants consistently target more narrow lineages of fungi than photosynthetic plants, despite the larger fungal pool available in the soil. These data indicate that host specialization is a common consequence of the evolution of mycoheterotrophy in the arbuscular mycorrhizal symbiosis. The tendency of mycoheterotrophic plants to target specific lineages of arbuscular mycorrhizal fungi supports the idea that different fungal lineages have different ecological functions.

T1-28-04

Global distribution of mycoheterotrophic plants

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Mycoheterotrophic plants are achlorophyllous, non-photosynthetic plants, with the exceptional mode of life of obtaining carbon from the mycorrhizal fungi in their roots. This ability of cheating mycorrhizal networks is relatively widespread in land plants and evolved multiple times independently. The diversity of fungi involved in this interaction is quite large, including members of the phylum Glomeromycota, Basidiomycota and Ascomycota. Mycoheterotrophic plants associated with arbuscular mycorrhizal fungi (Glomeromycota) generally occur in tropical rainforests, while the mycoheterotrophs associated with ectomycorrhizal fungi, and more rarely saprotrophic fungi, (Basidiomycota and Ascomycota) are restricted to temperate areas, with some exceptions in the tropics for the latter. While many mycoheterotrophic plants show a high specificity towards narrow lineages of fungi, the distribution of mycoheterotrophic plants is clearly more limited than that of their fungal partners. Thus, the occurrence of mycoheterotrophy is not explained by the presence of suitable host fungi alone. There is an insufficient knowledge on the ecological drivers that limit mycoheterotrophic plants' distribution. Here, we attempt to infer global ecological niches of these plants by using species distribution modelling. We focus on fully mycoheterotrophic plants in angiosperms, and include representatives of all lineages growing on arbuscular, ectomycorrhizal and saprotrophic fungi. Our results contribute to the understanding of ecological conditions where mycorrhizal networks are cheated at a global scale.

T1-28-05

Organellar phylogenomics and molecular evolution in mycoheterotrophic plants

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About 50 independent lineages of land plants have completely lost photosynthetic ability as a consequence of mycoheterotrophy —they obtain their carbon and other nutrients from mycorrhizal or saprophytic fungi housed in specialized root systems. These plants likely evolved from photosynthetic plants that complemented photosynthesis for all or part of their life cycle in this manner. Examples of the latter (partial and initial mycoheterotrophs) are known in several families, including orchids. Full-blown mycoheterotrophy can have profound effects on genome evolution, particularly the plastid (‘chloroplast’) genome, which is thought to degrade in a relatively ordered manner after loss of photosynthesis, according to recent evolutionary models of gene loss and retention. Long-term persistence of the plastid genome in non-photosynthetic plants is likely a consequence of delayed losses of multiple non-bioenergetic genes. Here we report on plastid genomes retrieved from one conifer family and nearly all of the 10-11 angiosperm families that include mycoheterotrophic plants. We document instances of gene loss and retention, changes in selective regime, and patterns of genome structural change and rate elevation across recent and ancient examples of photosynthesis loss, and several partially mycoheterotrophic lineages. Although often idiosyncratic, some general evolutionary patterns emerge. We also address the molecular evolution of the mitochondrial genomes of mycoheterotrophs, and apply these new organellar data in a phylogenetic context to address higher-order placements and relationships among mycoheterotrophic lineages of plants.

T1-28-06

Plastid genome evolution in mycoheterotrophic orchids

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Heterotrophic plants present numerous examples of extreme morphological and genomic reduction. Models of plastid genome (plastome) degradation have been put forth recently for these plants, focusing initially on gene loss/retention and pseudogene formation. The most recent models have evolved to incorporate additional genomic characteristics including base frequency changes, indel accumulation, genomic rearrangement, repeat accumulation, substitution rates (both synonymous and non-synonymous), and structural dynamics of the inverted repeat. Nearly half of the approximately 400 described holomycotrophs (HM) belong to Orchidaceae. The number of transitions from the partially mycoheterotrophic (PM) to HM lifestyle in Orchidaceae is over 30, and this number may increase as fine-scale phylogenetic and physiological studies are completed. Several researchers have published complete plastomes of holomycotrophic orchids, which display a range in the degree of degradation due to relaxed selective constraints of photosynthesis. However, most have focused on single plastomes, allowing only comparisons to (relatively) distantly related orchids. Aside from plastome evolution, very little is known about differences in gene expression or nuclear genome evolution across the spectrum of autotrophy, partial mycoheterotrophy, and holomycotrophy. We have developed the leafless, North American genus *Corallorhiza* as a non-standard model for studying morphological, physiological, ecological, and genomic consequences of obligate parasitism upon fungi. *Corallorhiza* is a promising system because the amount of green, putatively photosynthetic tissue varies significantly among its 12 species. Whole plastome sequencing has demonstrated that *Corallorhiza* is early in the process of plastome degradation, compared to some other parasites and mycoheterotrophs. The genus originated ~2.6 million years ago, and the two holomycotrophic lineages likely arose ~1.9 (*C. striata* complex) and ~1.3 (*C. maculata* complex) million years ago. Expanded sampling of 48 plastomes across the geographic range of the *C. striata* complex revealed substantial infraspecific variation in plastome size and gene content, with plastomes ranging from 141 kb in *C. striata* var. *striata* to 124 kb in the *C. bentleyi*-*C. involuta* clade. Furthermore, a phylogeographic approach to assessing plastome degradation in this species complex revealed two putatively independent transitions to HM in the complex, in addition to that in the *C. maculata* complex. Another North American genus containing HM species is *Hexalectris*. This clade of approximately 10 species represents a powerful system for comparison with *Corallorhiza* and members of Neottieae, the only orchid taxa for which both green, photosynthetic members and non-green, nonphotosynthetic members have been sequenced in a phylo-comparative fashion. Thirty plastomes were sequenced from *Hexalectris* and the related, photosynthetic *Bletia* and *Basiphyllaea*. Results will be compared with those from *Corallorhiza* and Neottieae, to test predictions within the context of recent models of plastome evolution, and to refine these models. Additionally, data comparing plastid and nuclear gene expression patterns between PM and HM *Corallorhiza* will be discussed. The ultimate goal of this research program is to take a comparative approach across all of Orchidaceae, in order to characterize patterns and processes of plastome degradation, and to similarly test hypotheses based on nuclear genome evolution and gene expression.

T1-28-07

Phylogenetic analysis of the mycoheterotrophic genus *Thismia* (Thismiaceae, Dioscoreales) based on molecular and morphological data

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Thismia Griff., comprising more than 60 species, is the largest genus of the family Thismiaceae. It is widely distributed in tropical regions with a few species in subtropical and temperate zones. Members of the genus *Thismia* are small mycoheterotrophic herbs with highly reduced vegetative structure and intricate floral morphology. Current views on phylogenetic affinities within this genus are controversial. Recent analyses have revealed paraphyly of the genus *Thismia*; however, they were based on limited sampling, and the genes used in these studies had high substitution rates. Thus, these results remain provisional and are still to be verified; besides, most of the species of *Thismia* have never been studied in terms of molecular phylogeny. We have examined the relationships between members of the genus *Thismia* on the basis of extended sampling which included originally collected specimens. A total of 21 species were used for phylogenetic studies, which were carried out by analyzing molecular and morphological data. The morphological dataset was compiled basing on careful consideration of available literature and original observations. The molecular phylogenetic analysis involved markers from the nuclear (18S, 5.8S rRNA genes and the internal transcribed spacers, ITS1 and ITS2) and mitochondrial (*atp1* gene) genomes. We have found that internal transcribed spacers show high level of interspecific divergence, which makes it impossible to use them for assessing phylogenetic relationships at the generic level. Nevertheless, ITS regions are revealed to be significant in resolving the phylogenetic affinities within groups of closely related species. Analysis of the other molecular markers allowed reconstructing phylogenetic relationships at the level of the whole genus *Thismia*. As in the previous studies, in our trees *T. panamensis* (Standl.) Jonker from Central America is distantly related to the Old World taxa. Then, several monophyletic groups were determined. One of them is a strongly supported group comprising five species: *T. annamensis* K.Larsen & Aver. fromVietnam, *T. filiformis* Chantanaorr. and *T. alba* Holttum ex Jonker fromThailand, *T. aseroe* Becc. and *T. inconspicua* ined. from Borneo. Another one consists of the Vietnamese *T. mucronata* Nuraliev and *T. puberula* Nuraliev and Thai *T. nigricans* Chantanaorr. & Sridith and *T. mirabilis* K.Larsen. Evolutionary pathways of morphological characters were studied by mapping their conditions onto our combined molecular phylogenetic tree. The following characters were examined: floral symmetry, presence of a mitre, leaves, appendages of tepals, a wing-like appendage of a connective, number of stamen appendages and some others. Evolution of some of them, including the presence of transverse bars inside the hypanthium and the number of stamen appendages, was found to be homoplastic. Our investigation provides important data for establishing a new taxonomic system of the genus *Thismia*. As they suggest, certain morphological characters traditionally employed in its taxonomy show more complicated evolution than it was believed before. We propose a list of characters which can be used for morphological description of the monophyletic groups supported by molecular data.

T1-28-08

Mycorrhizal diversity and nutritional strategies in the fully mycoheterotrophic orchid *Epipogium roseum*

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All orchid species completely rely on their mycorrhizal fungi for the provision of nutrients, at least during the non-photosynthetic protocorm stage. At the adult stage, most orchids develop a photoassimilating apparatus and become autotrophic, while fully mycoheterotrophic orchids are achlorophyllous and remain dependent on fungal sugars even at maturity. Recent advances in studying of orchid mycorrhizas are linked to the development of molecular techniques that enable identification of fungal symbionts, whereas the recent application of stable isotope analyses has represented a powerful tool for understanding nutrient exchange between orchids and fungi. In spite of the increasing number of studies describing fungal diversity in orchids, there is still more to be learnt about the identity and functional role of fungal partners and specificity in orchid mycorrhizal associations. We investigated the diversity of root fungal associates in the fully mycoheterotrophic forest orchid *Epipogium roseum* Lindl., using morphological and molecular techniques. We also applied stable nitrogen and carbon isotope abundance analyses in order to test whether nutrients are gained from the fungal symbiont. Orchid underground organs (stolons and tubers) were collected from fifteen *E. roseum* adult individuals, in three geographically distinct protected areas in Yunnan Province (South China). Total underground organ DNA was extracted and fungal ITS regions were PCR amplified using ITS1F/ITS4, ITS1/ITS4-Tul, and ITS1-OF/ITS4-OF primer pairs and sequenced. Microscopic observations of *E. roseum* hypogeous system sections revealed an extremely low level of fungal colonization. Molecular analysis uncovered a range of fungi, belonging to *Basidiomycota* and *Ascomycota*, in the rhizomatous system of the investigated orchid species. We did not find any dominant associated fungal taxon. On the contrary, fungal species belonging to different genera, such as *Tulasnella*, *Coprinellus*, *Ilyonectria*, and *Fusarium* were sporadically detected in the analysed orchid hypogeous organs. Carbon and nitrogen stable isotope abundances were measured from flower stalk samples of *E. roseum* and leaves of three non-orchid species collected around each orchid individual, maximum 1 m apart. All orchid plants were significantly enriched in both ^{13}C and ^{15}N compared with neighbouring reference species, showing that the investigated orchid species depends on mycorrhizal partners for nutrition. The analysis of mycorrhizal strategies in *E. roseum*, performed in our work, provides valuable information on the biology of this threatened orchid species that could be of great value for its conservation.

T1-28-09

Phylotranscriptomic analysis of mycoheterotrophic and non-mycoheterotrophic lineages in the Pandanales and Dioscoreales

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Mycoheterotrophy has evolved independently within the sister monocot orders Pandanales and Dioscoreales. Transcriptome data were generated for exemplars representing all APG III families for these two orders including mycoheterotrophic species *Gymnosiphon sp*. and *Burmannia biflora* within the Burmanniaceae (Dioscoreales), and *Lacandonia schismatica* and *Triuris* sp. within the Triuridaceae (Pandanales). Phylogenomic analyses of these data are resolving relationships among families and elucidating the timing of gene and genome duplication events in the Pandanales/Dioscoreales clade. Further, divergence of the molecular and biological functions of genes expressed in mycoheterotrophic and non-mycoheterotrophic lineages is being characterized. Results from these analyses are forming a firm foundation for investigations of the origin of mycoheterotrophy in the Pandanales and Dioscoreales.

T1-29: Ethnobotany and ethnopharmacy

T1-29-01

The mission and development of molecular pharmacognosy

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Traditional Chinese medicine (TCM) resources which is a kind of special resources, has a great value in utilization and development. It is related to the matter of people's livelihood, social stability, ecological environment protection and development of the emerging strategic industries. As the embodiment of national advantages in global competition, TCM resources has important strategic significance. With the development of society, the demand of medicinal materials keeps increasing. However, the awareness on how to develop and use TCM resources rationally is far from enough, which leads to that the sustainable development and utilization on TCM resources faces enormous pressure. With the continuous development of molecular biology, more and more technologies have been applied in sustainable development and utilization on medicinal plants. The biosynthetic pathway of secondary metabolites from medicinal plants has gradually been discovered. Using synthetic biology approaches by heterologous expression of the entire biosynthetic pathway in microorganism and channeling metabolic flux, could produce plant natural products with a high productivity. This strategy could be used for production of rare active ingredients, and provide an alternative route for the production of single-component from Chinese herbal medicine and its extract. This biosynthetic pathway analysis and the strategy of synthetic biology production have played a more and more important role in the production of plants' natural products, such as the famous Artemisinin, Opioid Analgesics, Paclitaxel, *etc.* Since 1995, when the molecular pharmacognosy was put forward for the first time, molecular pharmacognosy has become a mature inter-discipline which has a stable research direction, leading technology level, innovative theory, widespread academic influence and reasonable discipline team during 20 years of development. At present, more than 20 universities have opened the course of molecular pharmacognosy for undergraduate and graduate students. In 2012, molecular pharmacognosy (English version) which was published by Springer press, marked that molecular pharmacognosy has gained the international recognition. Currently, TCM synthetic biology has become the key research area in molecular pharmacognosy. Moreover, getting the medicinal active component by using the strategy of TCM synthetic biology is becoming the one of the most potential way for achieving resources recognized by international authority in this research are. It will become an important approach on TCM resources sustainable utilization.

T1-29-02

The specific use of wild species as medicinal plants of small ethnic group of the North

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Li Shizhen ideas set out in of the Compendium of Materia Medica are the basis for the development of Ethnobotany and ethnopharmacy. Currently, it is very important to have time to collect endangered folk details about the features of the use of small nations species of local flora as useful species (medicinal, food, hygiene). Collection and analysis ethnobotany data about using of wild species of local flora as medicinal plants people from small ethnic population on the North of Russia. Flora of North-West part Russia includes approximately 2700–3000 species of vascular plants. Small ethnic groups look like as Veps, Karelians, Izhora, Vodes, Seto-Estonians, Komi-Zyrian, Saami-they using as helpful (medicinal and edible) plants only some species from local flora (not more than 1-2% from the total number of species from flora of North-West of Russia). Modern information technologies penetrate deeper into the lives of different segments of the population. This leads to a rapid loss of traditional knowledge accumulated by many centuries. Everything is quickly erased the memory of the use of different kinds of plants to treat diseases, using them as amulets, and their role in many ceremonies. Only in small remote villages can still find people who preserve old knowledge about the use of plants as useful. It is important to have time to collect and store grain of people's knowledge and skills in the use of wild plant species as useful (food, medicinal, ceremonial, construction, ritual). Fewer young people who pass native folk knowledge about the use of plants. Particular attention should be paid to informants (who to interview and how). Many healers, who keep native people's knowledge, often do not go to the contact, and do not share their knowledge to use as a medicinal plant. In many countries, especially in the last decade, a lot of attention to the collection of ethnobotanical and ethnopharmacy data. These works are important to and among minorities of the North, whose lives are still largely dependent on the connection with Mother Nature, the ability to use the plant as useful. In the data collection process it has been revealed that a single national group, but residing remotely from each other, one and same species are not equally used. Different people often use various types of plant for the treatment of diseases or as edible. Currently requires the organization of a wide collection of information on the use of wild species of local flora of the local population as useful, especially as food and medicines. The most complete knowledge about potential new sources from local floras for the development of new drugs of plant origin, derived from data collected ethnobotanical, will identify promising new kinds of complex medical and biological research and development of new highly effective drugs.

T1-29-03

Using a bioassay-guided approach to investigate the medicinal properties of plants routinely utilized in traditional Chinese medicine (TCM)

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The Tennessee Center for Botanical Medicine Research (TCBMR) at Middle Tennessee State University (MTSU), Murfreesboro, Tennessee, USA, in collaboration with the Guangxi Botanical Garden of Medicinal Plants (GBGMP), Nanning, China and the Institute of Medicinal Plant Development (IMPLAD), Beijing, China, has employed a bioassay-guided approach to investigate the medicinal properties of 40 plants that have been utilized routinely in traditional Chinese medicine (TCM). The study served two purposes; (1) to evaluate the effectiveness of bioassay-guided screens and (2) to evaluate the correlation between the properties of the plants that were identified in the bioassays versus what was predicted according to the Chinese pharmacopoeia. The TCBMR is an interdisciplinary center that has expertise in using rapid *in vitro* cellular screening approaches to develop antibacterial, anticancer, antifungal, antiprotozoal, antiviral, or immunosuppressant agents and in the purification and identification of compounds. In this study a series of extracts were prepared from each of the 40 plants and then the extracts were tested for antibacterial, anticancer, antifungal, antiprotozoal, antiviral, or immunosuppressant activities. A number of plants with antibacterial, anticancer, antifungal, antiprotozoal, antiviral, or immunosuppressant activities were identified and while there was good correlation with the bioactivities that were identified versus what is predicted by the Chinese pharmacopoeia, a number of new yet unknown bioactivities were discovered as well. Pure compounds were isolated from some of the more promising potent plant extracts using bioassay-guided fractionation. Some of the compounds that were isolated, such as apigenin, an antiviral agent, hederagenin, an anticancer agent, and pandanusphenol, an anticancer agent, have been identified before, but some of the compounds that were identified were novel, such as *cis*- and *trans*- gnetin H, two anticancer agents and iso-iridogermanal, an antiprotozoal agent. This study further demonstrates the importance of known botanicals with medicinal properties as sources for new pharmaceutics.

T1-29-04

From comprehensive analysis to quality standards of Chinese herbal medicines

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Chinese herbal medicine is an extremely complex system, which contains hundreds or even thousands of chemical components in one herb, not to say compound formulas composed of several or tens of herbs. This generates great difficulty to clarify its chemical composition, which is the fundamental basis for the research of quality control, mechanism, pharmacological and toxicological actions, PK/PD, clinical trial, new drug discovery, *etc.* Thanks to the new analytical technique advancement, comprehensive analysis for traditional Chinese medicines and other herbal medicines becomes feasible. In the past several years, we dedicated ourselves to the herbal analysis by using some of the new techniques or instruments, such as UPLC, 2DLC, HRLCMS, UPC2, *etc.* to facilitate the elaboration of holistic quality control standards of herbal complex systems. In this lecture, several typical herbs were exemplified by using this approach. For examples, similar herbs of Panax species named *Panax ginseng*, *Panax quinquefolium*, *and Panax notoginseng* were analyzed by LCQ Advantage ion-trap mass spectrometer to profile their ginsenosides. To fully understand the chemical diversity of Tumeric (the rhizomes of *Curcuma longa*), multiple neutral loss/precursor ion scanning combined with substructure recognition and statistical analysis was utilized. Using this approach, 846 terpecurcumins (terpene-conjugated curcuminoids) were discovered from turmeric, including a number of potentially novel compounds. In addition, some new developed techniques as 2DLC and UPL2 were also applied in the herbal analysis. Based on the comprehensive analysis results, holistic quality standard of TCM complex systems was elaborated. The strategy and key factors of holistic standard were established and the quality monographs of a number of TCM herbs were elaborated and adopted by several mainstream pharmacopoeias including Chinese Pharmacopoeia, United States Pharmacopoeia and European Pharmacopoeia. It can be concluded that with the new development of analytical techniques, more and more chemical components in the complex herbal system will be clarified to facilitate their quality control and clarification of therapeutic substances.

T1-29-05

Beyond GAP -- Ecological agriculture considering the entirety of an ecosystem in the cultivation of medicinal plants

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Cultivation of medicinal plants is an important issue across the globe. In countries such as China, India, and some other nations, large tracks of land are used for this increasingly important and economically valuable resource. However, in our rush to serve this need, we often do not use sustainable agricultural practices in the management of this land. Meanwhile throughout the world the harvest of wild medicinal plant resources is putting enormous pressure on biodiversity, both of the harvested species and the entire ecosystem that depend on these species. The vast majority of medicinal plants used globally still come from the wild, while most of those in cultivation have only been domesticated for a relatively short period of time. Finding ways to improve the yield of those already in cultivation, as well as understanding how to bring wild plants into an agricultural settings is a serious challenge. Putting aside the exceptions of weedy species used in medicine, i.e. *Taraxicum* sp., *Plantago* sp., *etc.,* the vast majority of medicinal plants are found in relatively specific ecosystems and under specific bio-regional conditions. However, experience has taught us that simply growing a plant within that bio-region may be insufficient for long-term sustainable cultivation, and, in fact, such methods often yield very poor results. China has a long history of a concept known as *Daodi* herbs, which is to say that a medicinal plant produced in a specific region/area (within its native habitat) is considered the best clinically therapeutic material, this concept was largely based on the wild material, but it has been transferred to modern agricultural needs. Furthermore, a review of the material on cultivation of medicinal plants, while extensive in many areas, generally overlooks the concept of the soil as an ecosystem, and thus compares data on plant available nutrients in the soil (N, P, K, *etc.*), pH, *etc.* as well as the above-ground ecosystem, i.e. temperature range, annual rainfall, average number of days without frost, elevation, *etc.* This has created a clash between archaic agricultural concepts used for growing food with a developing field of medicinal plant agriculture. However, food production has, largely, failed to consider the soil as an ecosystem consisting of bacterial, fungi, protozoan, nematodes, earthworms, and other micro- and macroarthropods that plants depend on in a multitude of ways. This dependence is especially true for plants being moved from wild habitats to cultivated habitats, because these wild plants have long developed a relationship with the soil ecosystem consisting of these organisms, and are essential for their healthy growth. Looking at past work that has considered these issues for the cultivation of various plants, we will discuss current research showing how to apply this concept to what can be truly called "ecological agriculture" of medicinal plants.

T1-29-06

Therapeutic Principles and Methods guided identification of renoprotective substances from Chinese medicines

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Chronic kidney disease (CKD) has become a global public health problem that accounts for more than 10% of the population in the developed countries. Renal fibrosis is known to be the inevitable consequence of CKD which could in turn lead to a progressive and irreversible loss of renal function, a process known as end-stage renal disease (ESRD) needing dialysis or organ transplantation. Thus finding new approaches aimed at attenuating renal fibrosis is of great importance. In the recent years, search for renoprotective substances from Chinese medicines has become our research focus. Inspired by the traditional uses of Chinese medicine, in particular, guided by unique therapeutic principles and methods of traditional Chinese medicine (TCM), we selected medicinal resins and *Ganoderma* fungi respectively with blood circulation promoting, tonic effects as our research materials, and structurally diverse renoprotective substances were successfully identified targeting TGF-β1/Smads, Wnt/β-catenin and Nrf2/Keap1 signaling pathways. Our practice justifies that therapeutic principles and methods based exploration of Chinese medicines might aid gaining new insights into the scientific connotations of Chinese medicines.

T1-30: TM and drug discovery

T1-30-01

Discovery of the effective components of Chinese herbal medicines

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Chinese herbal medicines are gaining increasing attention worldwide due to their significant therapeutic effects. However, the effective components, i.e. chemical constituents responsible for the therapeutic effects, are usually not well defined. This is challenging due to the complicated chemical composition. A comprehensive understanding on the chemical constituents and *in vivo* metabolism is critical to dissect the effective components of herbal medicines. We have developed a series of analytical methods, including LC/MS, 2DLC and SFC to globally profile the chemical constituents of herbal medicines, and to discover novel compounds with significant bioactivities. We have also proposed a strategy to monitor the multi-component pharmacokinetics of herbal medicines. Combined with biological evaluations, these new techniques have been used to elucidate the effective components of licorice (Gan-Cao).

T1-30-02

Discovery of novel chemical structures from some traditional medicinal plants and their bioactives

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In the Foreword of famous monograph "Natural Pruducts — Their Chemistry and Biological Significance", its authors, some famous professors said: Naturally occurring organic chemical compounds (natural products) have always fascinated chemists. Interesting and intriguing chemistry is involved in their *in vivo* production and their laboratory utilization, and their importance as structural material and biologically active molecules (substrates for life processes, toxins, hormones, drugs, *etc.*) is of unparalleled importance. It was very difficult that we got similar felling with them, because they are of great scholars, but we already were subdued by the natural product structures of fascinating and diversity. Additionally as well-known, new molecules from traditional medicinal plants are highly signi? Cant sources of new discovery and development process. Therefore, our research group have been focused on the discovery of novel chemical structures from traditional medicinal plants and their bioactives. In the presentation, I would like to share the three features of our results, which finished in investigation of chemistry and bioactives of some TM plants in a couple of years, as following: 1. discovery of novel cycloartane triterpenoids from Cimicifuga spp. and their bioactives as well as the mechanism of bioactive molecules; 2. discovery of novel structures of triterpenoids and meroterpenoids from Ganoderma spp. and their bioactives; 3. discovery of novel structures of triterpenoids and meroterpenoids from Meliaceae and their physiological effects.

T1-30-03

Chemical and bioactive constituents of wolfberry

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The fruit of *Lycium barbarum*, also called wolfberry and goji berry, has been used as a traditional Chinese medicine (TCM) to nourish liver and kidney and brighten eye. Wolfberry has also been marketed as food and dietary supplements in North America, Caribbean countries, European Union, Oceania, and Southeast Asia. A systematic study on wolfberry chemical constituents including their bioactivities was carried out. 87 chemical constituents were obtained and identified including 19 dicaffeoylspermidine derivatives, 15 phenylpropionoyl phenylethylamine derivatives, and 53 polyphenols, in which 31 constituents are new compounds. Their anti- Alzheimer's disease (AD), antioxidant, and hepatoprotective activities were assayed. The discovery of chemical constituents and their bioactivities is beneficial for understanding the scientific basis of the effects of wolfberry. Dicaffeoylspermidine derivatives, a rare kind of plant secondary metabolites, are primarily distributed in the family of Solanaceae. All dicaffeoylspermidine derivatives discovered in wolfberry are new compounds and named lycibarbarspermidines A-S. Lycibarbarspermidines A-S are the first glycosidic products of dicaffeoylspermidine derivatives, and lycibarbarspermidines N-O are the first cyclization products of dicaffeoylspermidine. Moreover, dicaffeoylspermidine derivatives were first isolated and identified from wolfberry and they exhibited anti-AD and antioxidant activities which are related to the effects of wolfberry. The content of lycibarbarspermidines in wolfberry is high and estimated to be beyond 2.1 g/kg, which are potential natural anti-AD drug candidates.

T1-30-04

Research progress on *Panax notoginseng* for medicinal purposes: Effects of *Panax notoginseng* in neurodegenerative diseases

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Panax notoginseng (sanqi), the root of *Panax notoginseng* (Burk.) F.H. Chen, is a well-known traditional Chinese herbal medicine which has been widely used for diseases related to the circulatory system, such as cardio- and cerebro-vascular disorders. Now it has been accepted as a functional food and is used globally as a natural medicine. Ginsenosides are responsible for various pharmacological properties in notoginseng, and have been shown to produce many beneficial effects in the nervous system. Our work reveals novel mechanisms involving in reducing Aß accumulation by activating PPARγ and up-regulating insulin degrading enzyme (IDE), increasing neuronal excitability and modulating cell strength, which contributes to the protective effects of notoginseng or ginsenosides against neurodegeneration and synaptic dysfunction. Our study suggests that notoginseng may potentially be useful as a therapeutic agent for Alzheimer's disease.

T1-30-05

Mapping the polysaccharides in rhizome and root of *Panax ginseng* by HPGPC-UPLC- CAD and UPLC-QQQ- MS/MS determination and chemometric analysis

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Ginseng, a wildly used herbal medicine in China and many other countries, is derived from the rhizome and root of *Panax ginseng* C.A. Mey. It has been well studied that ginsenosides are the main secondary metabolites and important bioactive ingredients of *P. ginseng*, however, the carbohydrates are often overlooked. In fact, phytosaccharidesare as wellmajor active components in herbal medicines and carry much weight in the total tonic effect of herbal medicines. It is therefore essential to investigate polysaccharides in *P. ginseng*, where chemical profiling and structure determination of the polysaccharides is fundamental. The objectives was to investigate distribution and accumulation of polysaccharides in *P. ginseng*; to characterize primary structures of tissue-specific polysaccharides in the medicine. Ten batches of *P. ginseng* were separated by tissues using laser microdissection (LMD). Polysaccharides were extracted and purified from the cut sections respectively. High-performance gel permeation chromatography on an ultra-high performance liquid chromatography with a charged aerosol detector (HPGPC-UPLC-CAD) and UPLC coupled to triple quadrupole mass spectrometry (UPLC-QQQ-MS/MS) was performed to analyze the polysaccharides both qualitatively and quantitatively. Principle component analysis (PCA) was conducted to give clear comparison of polysaccharides from different sections of the medicine. Three macro-sectioned parts, namely, rhizome, main root, and branch root, and five micro-dissected tissues, namely, cork, cortex, resin canal, phloem, and xylem, of ginseng were separated. The different parts can be distinguished according to their respective polysaccharide profiles and sugar compositions. The skin, especially the cork, contained polysaccharides with smaller molecular weights and higher compositional percentages of galactose and galacturonic acid; the flesh, including xylem, phloem, and resin canal, contained larger carbohydrates consisting glucose as a dominant sugar residue. The PCA pattern showed distinctiveseparation of the five microdissected tissues and unified tissue construction in both main and branch roots. It is therefore conjectured that the flesh of ginseng contained mostly neutral polysaccharides, while the skin had a higher compositional proportion of acidic pectins. The present work paves the way for further structural determination and pharmacological studies of ginseng polysaccharides. Combining the present results and previously investigated ginsenoside distribution, the ancient literature record and traditional usages of *P. ginseng* can be explained, inspiring more economical and efficient application of the medicine.

T1-30-06

Chemical constituents and their anti-AD activities of the medicinally important plants: *Hypericum perforatum* (St John's Wort)

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The genus *Hypericum* is one of the largest genera in Clusiaceae, consisting of 484 species throughout the world. *Hypericum perforatum*, also known as "St John's Wort", is widely distributed in Europe, North Africa, Asia, and North America. Since the early 1800s, extracts of *H. perforatum* (EHP) have been utilized externally for wound healing and internally to relieve the symptoms of neurological disorders. In addition to the use of EHP for alleviating mild to moderate depression that is mainly attributed to the inhibition of various neurotransmitter receptors, further studies indicated that EHP also exerts versatile biological properties, including antibacterial, antiviral, anti-inflammatory, cytotoxic, and neuroprotective properties. To date, types of organic substances such as flavonoids, proanthocyanidins, naphthodianthrone, prenylated acylphloroglucinols and xanthones have been previously isolated from this plant. Among them, the polycyclic prenylated acylphloroglucinols (PPAPs) typified by hyperforin, characterized by a highly oxygenated and densely substituted bicyclo[3.3.1] nonane-2,4, 9-trione or other related core structures, have attracted great interest from the scientific community due to their intriguing molecular structures and interesting pharmacological effects. Aimed to discover structurally unique and bioactive secondary metabolites from the genus of *Hypericum* collected in central China regions, our group reported the isolation of almost one hundred new PPAPs from *H. sampsonii*, *H. attenuatum*, *H. japonicum* and *H. ascyron*. Although an abundance of reports exist on the biological activities and many organic substances including 26 PPAPs were previously reported to be derived from *H. perforatum* by other research groups, the present chemical study by our research group on this species has led to the isolation of 40 new PPAPs, along with 15 known ones. Some of the newly isolated PPAPs exhibited potent inhibitory activities against acetylcholinesterase and beta-secretase (BACE1) enzymes in Alzheimer's disease. The finding will provide new insights into the skeletal diversity of PPAPs and more scientific evidences in favor of sustainable exploitation and utilization of the important plant resources of *Hypericum* for natural anti-AD agents.

T1-31: Plants in human health and well being

T1-31-01

An ethnobotanical perspective of traditional medicinal plants from Fairy Meadows, Western Himalaya, Pakistan

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Ethno medicinal traditional knowledge about the use of plants for various human ailments is known by the elders of the community members. This explorative ethnobotanical survey was attempted to document, build up and publicize the gigantic local knowledge of century's practiced restorative use of medicinal plants, their mode of preparation and administration by the inhabitants of this secluded territory. A total of 146 informants (101 males and 45 females) were interviewed through questionnaire method. Ethnomedicinal data was quantitatively analyzed through use value (UVi), frequency of citation (FC), relative frequency of citation (RFC) along with a Pearson correlation coefficient (PCC). All the plants were checked with previously published articles for their novel uses. The demographic inventory of the participants was also recorded. Collected specimen were preserved, mounted, labeled on herbarium sheets, cataloged and deposited with voucher numbers in Arid Agriculture University Rawalpindi (AAUR). 90 plants species belonging to 75 genera and 49 different families have been documented in the current study. These medicinal plants were used against 55 pathological disorders in the area. Among these, most commonly treated ailments, stomachic (23.3%), cough (17.7%), asthma and fever (16.6%) *etc*. Most of the collected plants were wild (86%) and (14%) were cultivated. Herbs were the dominant life forms contributing 63% followed by trees 26% and shrubs 11%. Asteraceae was the leading family contributing 10% of the total families. Leaves are the most widely used parts (27%) followed by fruit (19%), root (16%) and stem (12%) for curing different ailments. Most common method of herbal preparation was powder (27%), decoction (16%) and direct use (13%). Among these, 81.7% preparations were administered orally. The medicinal plant with highest use values were *Berberis lyceum* (5.47), *Thymus palmata* (5.07) and *Rhododendron anthopogon* (5) while the plants with greater relative frequency of citation were *Berberis lyceum* (0.97), *Thymus serphyllum* (0.89) and *Rhodendron anthopogon* (0.75). The pearson correlation coefficient was found 0.836 between RFC and UV showing high positive association. This is the first ethnobotanical study in Diamir district and comparative results with other districts of Gilgit Baltistan revealed that 25.5% of the plants have been documented ethnomedicinally for the first time in Gilgit Baltistan. In this study, we have reported plentiful indigenous knowledge about local communities.

T1-31-02

Uptake of heavy metals in the peel and flesh of potatoes irrigated with untreated wastewater in the presence of plantain peel biochar

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Consuming crops heavily loaded with heavy metals can lead to serious health damage in human body. Heavy metal loading in crops, in most cases, results from uptake from contaminated soils. Just like the essential nutrient that are taken up by plants during their growing season, these heavy metals, when present in irrigation water, can be available for uptake in the soil solution. Biochar, produced in the pyrolysis of organic waste (s), has been reported to reduce the uptake of heavy metals to crops grown on contaminated soils. The duration of this effectiveness of biochar as a biosorbent is uncertain. Some researchers have found biochar effective for just one year/season, while others have found it effective for more than one year/season. A study was conducted in six outdoor PVC lysimeters (1.0 X 0.45 m) following a randomized complete block design with 3 replicates. For two consecutive years (2015 and 2016), we investigated the effectiveness of plantain peel biochar applied to sandy soil and irrigated with untreated wastewater to study the uptake of six heavy metals (Cd, Pb, Cr, Zn, Cu and Fe) by the flesh and peel of Russet Burbank Potatoes. Biochar (1% w/w) was mixed in the top 10 cm with sandy soil only in the first year before planting the potatoes, while in the second year, no new biochar was applied. For both years, the potatoes were irrigated with untreated wastewater applied at the just ponding rate. Upon harvesting, the flesh and peel was sampled, washed (trice) with deionized water, oven-dried (60^{o}C for 48 hrs), digested with nitric acid, and analyzed for the heavy metals with an Inductively Coupled Plasma Mass Spectrometry (ICP-MS). In the first year, there was significant reduction ($P<0.05$) in only Cd and Zn, while in the second year there was significant reduction ($P<0.05$) in all six heavy metals in both the flesh and peel of the biochar amended treatments, compared to the control (without biochar). This suggests that biochar can reduce heavy metal uptake by the edible parts of Russet Burbank Potatoes. Likewise, it suggests that plantain peel biochar became more effective in the second year.

T1-31-03

Biosynthesis of major secondary metabolites in the well-known anti-aging wolfberry

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Lycium L. is a genus of Solanaceae containing about 80 species distributed in the temperate and subtropical zones. *Lycium* species are mostly found in dry, semi-saline environments. Chinese Pharmacopoeia (2010) recorded lycii fructus (*Gouqizi*, wolfberry, dry red fruit [RF] of *L. barbarum*), and lycii cortex (*Digupi*, dry root bark of *L. chinense* and *L. barbarum*). Black fruits (BF) of *L. ruthenicum* have also been used as folk medicine, especially in Tibetan and Mongolian medicine. Therefore, wolfberry (or Goji, Gouqi) nowadays in China refers to the products prepared from *L. chinense*, *L. barbarum*, and *L. ruthenicum*, which is one of the most famous anti-aging herbs. Genomic sequencing, transcriptome sequencing and chloroplast development have been investigated for identification of key genes involved in the biosynthesis and accumulation of carotenoids, especially zeaxanthin dipalmitate, and betaine. The failure of the chromoplast development in BF causes low carotenoid biosynthesis levels and continuous carotenoid degradation, which ultimately leads to undetectable carotenoid levels in ripe BF. In contrast, the successful chromoplast biogenesis in RF furnishes the sink necessary for carotenoid storage. The abundant zeaxanthin accumulation in RF is primarily determined via the high levels of carotenoid biosynthesis, transportation, and storage, as well as the lack of carotenoid degradation, which are regulated at the transcriptional level. Function of several key genes will be characterized and reported. Glycine betaine (GB) plays significant role in salinity tolerance in many plants. The last biosynthetic step of GB is catalyzed by aminoaldehyde dehydrogenase (AMADH) serving as betaine aldehyde dehydrogenase (BADH) activities. *LrAMADH1* is characterized as the true LrBADH which also responses to salt stress in *L. ruthenicum*.

T1-31-04

Ethnobotanical studies on medicinal plants traded in local periodic markets in rural Guizhou, China

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Guizhou, a province in Southwest China is well-known for its biological and cultural diversity. Miao, Dong, Buyi, Yao, Shui, Maonan and other ethic groups living in this region depend on local medicinal markets for medical treatment and family healthcare. However, information on the market traded medicinal plants is not well documented from traditional medicinal markets in southern Guizhou. The study aimed to document medicinal plants traded by local people in the periodic markets. Ethnobotanical market survey had been adopted for documenting herbal plants and associated traditional knowledge. A total of 387 medicinal plant species, belong to 309 genera and 123 families, were investigated and collected together with their medicinal uses by the local people. The plants were used to treat 256 human diseases. Of the 387 species, 234 (60.47%) were obtained from the wild whereas 71 (18.35%) were from home gardens, and 82 (21.19%) species were from both home gardens and wild habitats. Herbaceous species (247) were the most utilized plants, accounting for 63.82% of the species, followed by shrubs (59,15.25%). Fabaceae came out as a leading family with 25 medicinal species (6.46%) while Asteraceae (5.94%) followed with 23 species. The most frequently used plant parts were the whole plants (148), followed by roots (84). Decoction was a widely used method to prepare traditional herbal medicines. Our research results indicated that the study area might be probably one of the richest diversity centers in both species and cultural level of the country's ethnomedicines, to compare with other parts of China. The medicinal plants which spontaneously sold in the open air markets are collected by the local people from wild and their living environment, even the toxic plants. There is at risk of ingesting toxic plants sold in popular markets, because there is no hard distinction made between medicinal and toxic plants. The local government should regulate the traditional markets for misusing of the herbal medicine and protecting the local biodiversity and associated traditional knowledge.

T1-31-05

A friendly relationship between endophytic fungi and host medicinal plants

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Endophytic fungi or endophytes exist widely inside the healthy tissues of living plants, and are important components of plant micro-ecosystems. Over the long period of evolution, some co-existing endophytes and their host plants have established a special relationship with one and another, which can significantly influence the formation of metabolic products in plants, then affect quality and quantity of crude drugs derived from medicinal plants. Our research group focused on the endophytic fungi and their correlations with the host medicinal plants. The following contents were carried out: (1) Research on endophytic fungi diversity and population structure; (2) Chemical diversity and bioactive secondary metabolites of endophytic fungi; (3) The effects of endophytic fungi on host plants and the correlation between the qualities of TCMs and endophytic fungi. More than 5,000 endophytic fungal strains were isolated from more than 70 medicinal plants. In addition, fermented products of 32 strains of endophytic fungi with bio-activities were separated. More than 200 compounds were identified. Many endophytic fungi could produce secondary metabolites originally-found from host plants, some could promote the growth of host plant. For example the fungus ZJ44 was able to increase the growth of the host plant-adlay *Coix lacryma-jobi* var. *mayuen* and Arobidopis. Interestingly, one endophytic fungus D16 (*Trichoderma atroviride*) isolated from the root of *Salvia miltiorrhiza,* was found out to produce tanshinone and tanshinone A. Moreover, it was discovered that D16 could significantly enhance the biomass of *S. miltiorrhiza* hairy roots and promote the biosynthesis of tanshinones notably, and protein polysaccharide fraction (PSF) from D16 was the main active fraction to promote tanshinone biosynthesis. Furthermore, technologies of metabolomics, transcriptomics and proteomics were used to investigate the molecular mechanism of PSF on the growth and secondary metabolism of *S. miltiorrhiza* hairy roots. The results indicate that the distribution and population structure of endophytes can be considerably affected by factors, such as the genetic background, age, and environmental conditions of their hosts. On the other hand, the endophytic fungi can also confer profound impacts on their host plants by enhancing their growth, increasing their fitness, strengthening their tolerances to abiotic and biotic stresses, and promoting their accumulation of secondary metabolites.

T1-31-06

Phylogenomics and authentication of the resource medicinal plants using complete chloroplast and nuclear genomes simultaneously obtained from low coverage NGS data

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Cytoplasmic chloroplast (cp) genome and 45S nuclear ribosomal DNA gene cluster (nrDNA) regions are the most popular targets for research of plant genetics diversity, barcoding and evolution. We developed a high throughput method to obtain complete whole cp genome and a major unit for 45S nrDNA tandem array as well as characterization of major repeats simultaneously by de novo sequence assembly using low coverage whole genome shotgun sequence (dnaLCW method). The dnaLCW method can be applied for any plant species without advanced genome data and provide a new era for elucidation of genetic diversity and fundamental understanding the tree of life and evolution. Moreover, the genomic information can be utilized for development of practical barcoding markers not only at inter species level but also at intra species level. The genome information produced by dnaLCW method can be applied to improve breeding of the target species and also to develop the practical barcode markers which can be applied to authenticate the target species or the target cultivars from the related. The species-unique or cultivar-specific markers can be applied to prohibit the illegal trade of economically motivated adulteration instead of the target plants in the production of functional foods or traditional oriental medicinal drugs.

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T1-32: Traditional medicinal plants

T1-32-01

Herbal remedies used in the treatment of scorpion stings in Saudi Arabia

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Traditional medicine has a long history of serving people all over the world. In recent years, the use of traditional medicine information on plant research has again received considerable interest. Medicinal plants are an important element of indigenous medical systems in Saudi Arabia as well as elsewhere. Ethno-botanical survey of the plant based folk practices used for treatment of scorpion stings was carried out in two regions in Saudi Arabia; Tabuk in the northwestern region and Jeddah in southwestern region. The people in the study area still use plant therapy to considerable extent although the modern medical facilities are available. From the survey, a total of a total of twelve plant species belonging to eleven families of angiospermae are used for treatment of scorpion stings in the study area. Six plant species are recorded in Tabuk and other six plant species in southern Jeddah. Alliaceae family is common in the two studied regions. *Astragalus spinosus* (Fabaceae) and *Heliotropium bacciferum* (Boraginaceae) are the most commonly used plant species for treatment of scorpion stings in Tabuk region, while *Allium stivum* (Alliaceae) and *Linum usitatissimum* (Linaceae) in southern Jeddah. It is to be noted that wild plant species are the most commonly used in the northwestern region of Saudi Arabia where five species out of six (about 83%) are wild desert plants, while cultivated and imported plant species are the most commonly used (about 67%) in the southwestern region of Saudi Arabia. Generally leaves, bulbs and seeds are the commonest parts of plants used, while decoctions, infusions, pulverization and grinding are the main methods of preparations.

T1-32-02

Studies on large scale tissue culture of medicinal plants

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Large scale tissue culture of medicinal plants is one important way to solve the resource problem of TCM in China. We have carried out experiments in *Panax ginseng, Panax quinquefolium, Panax notoginseng* and *Glycyrrhiza uralensis*. We have optimized culture condition and realized large scale bioreactor culture, constituting 500 l bioreactor scale. In terms of ginseng adventitious roots, we selected three fungi strains (*Aspergillus niger*, *Aspergillus flavus* and *Aspergillus oryzae*) as elicitors prepared from mycelium or fermentation broth to improve ginsenosides production in adventitious roots culture. The results showed that ginsenosides production (29.90 ± 4.67 mg g^{-1}) was significantly enhanced upon elicitation with 200 mg L^{-1} *A. niger* elicitor prepared from mycelium, which was 3.52-fold of untreated group. Furthermore, we found that *A. niger* significantly enhanced accumulation of Nitric oxide (NO), salicylic acid (SA) and jasmonic acid (JA) involved in plant defense response, and significantly up-regulated the expression of the geranyl diphosphate synthase (GPS), farnesyl diphosphate synthase (FPS), squalene synthase (SS), squalene epoxidase (SE), dammarenediol synthase (DS), Two cytochrome P450 (CYP) genes (CYP716A47 and CYP716A53v2) and three UDPglycosyltransferases (UGT) genes (UGTAE2, UGT94Q2 and UGTpg100). In terms of *Panax quinquefolium* adventitious roots, we combine elicitors and transcriptomics to investigate the inducible biosynthesis of the ginsenoside from the *Panax quinquefolium*. Treatment of *P. quinquefolium* adventitious root with methyl jasmonate (MJ) results in an increase in ginsenoside content (43.66 mg/g compared to 8.32 mg/g in control group). Therefore, we sequenced the transcriptome of native and MJ treated adventitious root in order to elucidate the key differentially expressed genes (DEGs) in the ginsenoside biosynthetic pathway. Through DEG analysis, we found that 5,759 unigenes were up-regulated and 6,389 unigenes down-regulated in response to MJ treatment. Several defense-related genes (48) were identified, participating in salicylic acid (SA), jasmonic acid (JA), nitric oxide (NO) and abscisic acid (ABA) signal pathway. Additionally, we mapped 72 unigenes to the ginsenoside biosynthetic pathway. Four cytochrome P450s (CYP450) were likely to catalyze hydroxylation at C-16 (c15743_g1, c39772_g1, c55422_g1) and C-30 (c52011_g1) of the triterpene backbone. UDP-xylose synthases (c52571_g3) was selected as the candidate, which was likely to involve in ginsenoside Rb_3 biosynthesis. In terms of *Glycyrrhiza uralensis* adventitious roots, the protein fragment more than 10 kDa of *Escherichia coli* significantly increased the metabolites contents in *Glycyrrhiza uralensis* adventitious roots. The results showed the highest accumulation of total flavonoids (7.59 $mg \cdot g^{-1}$), glycyrrhizic acid (0.29 $mg \cdot g^{-1}$), glycyrrhetinic acid (0.27 $mg \cdot g^{-1}$) and polysaccharide (93.11 $mg \cdot g^{-1}$) by up to 2.27-fold, 2.64-fold, 2.70-fold and 2.32-fold than control roots, respectively. Besides, the protein fragment more than 10 kDa significantly activated the defense signaling and extremely up-regulated the expression of defense-related genes and functional genes in glycyrrhizic acid and flavonoids biosynthesis.

T1-32-03

Comparative transcriptome of *Gastrodia elata* in response to fungus symbiosis and genomic divergence in saprophytic *Gastrodia*

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Gastrodia elata is a traditional Chinese herb used for treating neural diseases. *Gastrodia*, with 50 to 60 species mostly distributed in the tropics and subtropics, is one of the saprophytic and achlorophyllous genera in the Orchidaceae. Taiwan, with a high species diversity (19 taxa), represents a hot spot of *Gastrodia*. All *Gastrodia* species are mycoheterotrophic, dwelling underground of forests with rich litter and obtaining nutrients from the symbiotic mycorrhizal fungi. Such a unique growth form makes *Gastrodia* species hard to be found in the field except the flowering and fruiting seasons, generally one month after budding. Seed and pollen disperse to a limited distance with the aides of insects, likely resulting in strong genetic differentiation between populations and divergence among species. Using next generation sequencing techniques, transcriptomes of *G. flavilabella* and *G. elata* rhizome were constructed. We investigated 9 *Gastrodia* species, specifically belonging to three subgenera with separate flowering times, for testing the genetic divergence and the mode of speciation. Compared with other angiosperms, *Gastrodia* attained the interspecific reproductive isolation much sooner due to temporal isolation. We also conducted a comparative analysis of transcriptomes obtained from vegetative propagation corms and tubers of *G. elata* and *Armillaria mellea*. We detected two unigenes that encode monooxygenase and glycosyltransferase, with upregulating gene expression from vegetative propagation corms to tubers, providing insights into the molecular basis of gastrodin biosynthesis in *G. elata*.

T1-32-04

How the West was lost: Exploring the disconnect between traditional Chinese medicine and Western medical science

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The World Health Organization (WHO) has stated that the majority of people worldwide rely on some form of traditional medicine to address their medical needs. Some of these traditional practices, including Traditional Chinese Medicine (TCM) for example, have been used, documented and refined over the space of thousands of years. Efficacy is well documented and often undeniable. Nonetheless, many published and unpublished investigations in Western laboratories have failed to detect and/or isolate the chemical principles that are responsible for the observed biological activities in man. The inability of Western science to 'validate' the efficacy of TCM in the laboratory has contributed to the skepticism about traditional medicine that is prevalent throughout much of the "Developed World", severely limiting the acceptance of TCM outside of China. The present study explores some potential reasons for the apparent disconnect between the observed clinical efficacy of TCM and the disappointing results in laboratory studies.

T1-32-05

The quality comparison between saffron from China and Iran by multiple component analysis

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Saffron is the dried stigma of *Crocus sativus* L. As a traditional spice, it is widely used among the world. Meanwhile, saffron is also a traditional herb, which has abilities like reducing bruises, promoting blood circulation, calming the mind and body, providing anti-tumorous effects and reducing the sugar concentration in blood, *etc*. According to the reference, crocetin, safranal and picrocrocin are the material basis of the color, scent and bitterness of saffron respectively, and they are used as indices to estimate the quality of saffron. Saffron primarily distributed in the Mediterranean regions around Iran and Greece. In Tang Dynasty, it was introduced into China from India through Tibet, and now it forms scale plantings in places like Shanghai, Zhejiang, Jiangsu and Henan. As well known, the growing environment is one of the important factors in the quality of medicinal herbs. Will there be any difference between the saffron grown in China and Iran? In the present study, about 100 kinds of saffron samples were purchased or harvested from Iran and China, and they were divided into different quality levels according to the methods specified in ISO-3632. An HPLC fingerprint combined with multiple components analysis method was established to qualitatively and quantitatively analyze the saffron samples. Also, the difference between the quality of saffron samples grown in China and Iran was explored using chemometrics method. It showed that no significant difference was found between saffron samples from China and Iran on the content of crocetin I, but significant differences were found on the contents of crocetin II and safranal. And the samples could be divided and grouped based on whether it's from China or from Iran according to the data from HPLC fingerprint.

T1-32-06

Impacts of cultivation on genetic diversity pattern of medici-

nal plants in China

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There are about 500 species of commonly used in traditional Chinese medicine, among which 250 species are primarily cultivated. The cultivation of medicinal plants began since 2,600 years ago, but large-scale cultivation (about 200 species) was initiated in 1958. Different medicinal plants may have distinct cultivation histories, which could have different genetic consequences for the cultivated populations. However, few studies have been compared the genetic diversity patterns of medicinal plants with different cultivation histories. *Scutellaria baicalensis* Georgi (Huangqin or Chinese Skullcap) is a newly-cultivated species (ca. 60 years of cultivation), but *Angelica sinensis* (Oliv.) Diels (Danggui) was cultivated since 1,000 ago. The two medicinal species may represent an ideal model to evaluate the impacts of different cultivation histories on the genetic diversity pattern of medicinal plants. Investigations of three polymorphic chloroplast fragments and fifteen nuclear microsatelite loci in 28 wild and 22 cultivated populations of *S. baicalensis* found that there was a slight genetic bottleneck during the cultivation and cultivation populations derived from multiple origins. However, short-term cultivation has lead to extensive gene flow among cultivated populations compared to wild populations. In contrast, through surveying the genetic diversity of 57 cultivated populations and 6 wild populations on eighteen microsatellite loci, a severe cultivation/domestication bottleneck was found in *A. sinensis*. Its cultivated populations formed an independent group in STRUCTURE analysis, although their ancestor remains unclear. This study indicates that different cultivation histories have distinct impacts on the genetic diversity and genetic structure of cultivated medicinal plants, which provides powerful insights into the conservation of genetic resources of traditional medicinal plants in China.

T1-33: The megadiverse island of New Guinea: diversity, evolution, and conservation of a tropical wilderness

T1-33-01

Forest dynamics and conservation in New Guinea's lowland forests

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The Center for Tropical Forest Science-Forest Global Earth Observatory includes a network of large-scale research plots across the globe's tropical and temperate forests. Just one of these 50-hectare (ha) plots is located in the Melanesian tropical region, east of Wallace's line. The plot is in the lowland rainforest of northern New Guinea, near the village of Wanang, Madang Province, Papua New Guinea. The New Guinea plot is in a contiguous area of mature, mixed evergreen forest at 100-200 m a.s.l. and encompasses the full elevation range of the region. The initial plot census and full topographic survey were completed in 2012. All 265,771 individual trees with diameter at breast height ⩾ 1 cm were mapped, measured, tagged, and, where possible, identified. The plot includes 584 species with a further 65 taxa that remain either unnamed or undescribed. The first re-census of the New Guinea plot is currently underway. This effort represents a singular opportunity to examine forest dynamics (tree mortality, recruitment, and growth rates) in this unusual biogeographic region and helps place New Guinea's forest into a global context. Many previous studies have investigated forests in the Neotropics and Southeast Asia but these studies do not necessarily allow us to make predictions about New Guinea forests where there is only a minute overlap in species composition with other tropical areas and significant differences in soils. In fact, preliminary analysis of re-census data from the New Guinea plot suggests the island's recently uplifted, steep slopes, and unstable soils combined with high seismic activity make its forests particularly dynamic and prone to natural disturbance. This has lead to higher than average tree mortality and lower carbon stocks per hectare compared to other tropical forests. There is also lower than expected species turnover across environmental gradients in the plot compared to other tropical areas. In addition to the censuses of trees and saplings, 800 small seedling plots spread throughout the 50-ha plot provide an opportunity to investigate dynamics during the critical transition phase between germination and establishment. These plots track nearly 3,000 seedlings representing at least 270 species. There are also fruit traps across the plot to monitor seed production as well as ongoing monitoring of seed eating insects and experiments to document seed distribution. All research in the New Guinea 50-ha plot is overseen and managed by local landowners in collaboration with researchers from the New Guinea Binatang Research Center. This unique relationship has had a direct impact on forest conservation efforts by strengthening the community-lead development of a 10,000 ha conservation area in the center of an active logging concession. Ongoing visits from international scientists provide employment to village assistants and visitor fees support community development including the only elementary school in the area and improved access to medical care. Additionally, the partnership is enriched through the sharing of indigenous forest knowledge and scientific thinking. The relationship has become a role model for indigenous conservation internationally and was awarded the UNDP Equator Prize for innovative conservation in 2015.

T1-33-02

Morphological variation in Australian and Papua New Guinean *Polyosma* Blume (Escalloniaceae)

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The morphological variation within and between species of *Polyosma* Blume from Australia and Papua New Guinea (PNG) was investigated. The taxonomic usefulness of morphological characters was determined. A total of 161 Operational Taxonomic Units (OTU) of *Polyosma*, comprising 35 known species, three undescribed taxa and nine un-identified herbarium specimens were assessed by the use of 30 characters. Of these characters, 20 were qualitative (non-overlapping) and 10 were quantitative (overlapping). The variability within these morphological characters proved to be useful for circumscribing the currently recognised species. In the analysis, two main groups were obtained based on whether the characters were hairy or glabrous, which were further down had resolved into thirteen groups. Twenty four species were recognised from Papua New Guinea. Three un-published names were discovered. Of these, two species grouped well with published names where as one was significantly different to the published named species it was seen grouped with. Australia has six species, with a possible new species from Mt. Spurgeon, Queensland. More materials are required to analysis the Mt. Spurgeon species. The characters and character-states used were consistent within the entire taxon. In future, few more characters will be included and a molecular phylogeny to compliment this morphology study of the taxon in the two study areas.

T1-33-03

A suryey of the origin, diversity, and conservation status of New Guinean tree ferns (Cyatheaceae, Dicksoniaceae)

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Tree ferns are conspicuous elements of tropical and southern temperate vegetation and most diverse in wet mountain rainforests of South America, Madagascar and southeast Asia. New Guinea is assumed to be one of the epicenters of diversity, with an estimated 130 species of scaly tree ferns (Cyatheaceae) and hairy tree ferns (Dicksoniaceae). However, this estimate is based on a comparatively low specimen number from rather sporadic collection efforts. Our understanding of the ecological adaptations and morphological variation of the species is still in its infancy. Field observations indicate that high local and regional diversity of tree ferns may be due to adaptation to different life strategies (pioneers vs. climax species; understory vs. canopy species) and substrate preferences, meaning that some species are more indicative of undisturbed habitats than others. We present the results of the latest taxonomical assessment of New Guinean tree ferns, which forms the basis for modeling the potential distributions and ecological niches of the species. We use these results to assess the degree of threat for each species according to IUCN guidelines and to identify areas with concentration of rare species, which should be prioritized for protection.

T1-33-04

Trees of New Guinea

Timothy Utteridge, *Laura Jennings, Marie Briggs*
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The tropical island of New Guinea is one of the least explored regions and largest remaining wilderness areas on the planet. There are many gaps in our knowledge of the island's biodiversity as there is no complete flora or species checklist for the island, no island-wide vegetation map, and no conservation strategy. The Trees of New Guinea project (TONG) at Kew will publish a generic guide to the trees on the island of New Guinea. The island includes 698 arborescent genera (smaller taxa have been excluded using a height of 4 m as a cut-off) which account for approx. 5,600 species, or which *c*. 5,000 are arborescent. This presentation will outline the guide, some of the key taxa, and detail the final stages and planned production.

T1-33-05

Morphological phylogenetic analysis of the Astronieae (Melastomataceae)

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The Astronieae (Decne.) Triana represent one of the earliest diverging tribes of the pantropical family Melastomataceae Juss. and are endemic to the rainforests of Southeast Asia and the Pacific islands. They comprise about 150 species of trees and woody shrubs in four currently accepted genera: *Astrocalyx* Merr., *Astronia* Blume, *Astronidium* A. Gray, and *Beccarianthus* Cogn. To date, only one published phylogenetic analysis has focused on the Astronieae, but sampling was insufficient to draw conclusions about generic circumscriptions or species-level relationships. Moreover, an ongoing molecular phylogenetic reexamination of the family reveals that the enigmatic *Tessmannianthus* Markgr. from tropical America has closer affinities with the Astronieae than the neotropical Merianieae Triana, where it was originally placed. This study presents the first phylogenetic analysis of the tribe based on morphological data. Maximum parsimony analyses of 57 ingroup taxa using 205 morphological characters were conducted to test support for the monophyly of each constituent genus and to verify the placement of *Tessmannianthus* within the Astronieae. Characters were partitioned into two sets to identify potential areas of incongruence: discrete and continuous, the latter was coded using the differential gap-weighting method. The global analysis yielded two equally most parsimonious trees (L = 4,800, CI = 0.207, RI = 0.484). The strict consensus tree shows that *Astrocalyx*, *Astronia*, and *Astronidium* are each monophyletic. *Beccarianthus* is biphyletic, consisting of one clade with oblong anthers and another with linear anthers, the latter being restricted to eastern New Guinea. Likewise, *Astronia* section Astigmata Maxw. & Veldk. was found to be polyphyletic. Intergeneric relationships within the tribe are well-resolved but these need further confirmation and support. Finally, *Tessmannianthus* forms monophyletic genus that groups with the Merianieae. However, it is noteworthy that analysis of only discrete characters suggests the monophyly of *Astronia* sect. Astigmata with a single reversal and the sister relationship of *Tessmannianthus* and the Astronieae. The phylogeny of the Astronieae sensu stricto supports the evolution of axile placentae into ascending and bilobed basal-axile, and ultimately, into collapsed ones. Pending molecular analyses, the resurrection of *Bamlera* K. Schum. & Lauterb. to accommodate the deviant Papuasian *Beccarianthus* clade is proposed.

T1-34: DNA metabarcoding biodiversity

T1-34-01

Plant distribution patterns and the factors influencing its diversity in the desert region of Northwest China

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Spatial plant diversity patterns underpins our understanding of ecosystem functioning and guides our environmental management decisions on plant resource conservation priority and utilization. Most researches have been focusing on biodiversity rich areas (hotspots) and ignoring biodiversity “poor” areas (coldspots). Most biodiversity coldspots locate on environmentally sensitive and economically undeveloped areas. Although the visible biodiversity is poor in such areas, the invisible biodiversity may not so poor and some species may even have great ecological importance. In order to understand the spatial plant diversity patterns in coldspots, we barcoded the plant species diversity in different parts of deserts in Northwest China to disclose factors that shape the patterns. We sampled 145 plots over the whole regions of deserts in Northwest China. Surface soils were collected and the ecological data were recorded. DNA were extracted from the soil samples usig Powersoil DNA isolation kit. Four chloroplast regions, *rbc*Lb, *mat*K, and *trn*L-F, were used DNA barcodes. These DNA barcodes were sequenced by metabarcoding method and plant species were determined by blast our data base. Different barcodes performed variably. The *rbc*Lb revealed 574 species belonging to 443 genera. The *trn*L-F identified 737 species belonging to 503 genera. The *mat*K uncovered 94 species belonging to 73 genera. A total number of 1,315 plant species was identified, they belong to 863 genus of plant. It is a common notion that a biodiversity coldspot such as the deserts in Northwest China is a plant diversity poor area. Our results showed that the plant species is not so poor as imagined. The species diversity each plot is underway and then association between plant species diversity and ecological factor could be analyzed.

T1-34-02

Unveiling the mask of microbiome in *Panax notoginseng* via DNA metabarcoding

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Microorganisms dominate the whole world as key decomposers. Many microorganic species coexist together and some are fatal pathogens. Understanding the species of pathogens is the first step toward controlling them and curing diseases. *Panax notoginseng*, a very popular traditional Chinese medicinal plant in resolving blood problems, suffers from microorganism-associated diseases which cause severe economic loss. Our current knowledge of the pathogenic microorganisms is still limited depending on identification of cultured strains. High-throughput sequencing approaches have a potential application in pathogen identification by DNA barcoding, we therefore conducted a study for characterizing microbiomes in *P. notoginseng* by DNA metabarcoding. DNA were extracted from both healthy and infected rhizomes, leaves and seeds and nuclear gene 16S (V1-V4) and ITS were used to catalogue prokaryotes and eukaryotes separately. Both genes of *P. notoginseng* were blocked from amplification. In total, eighteen orders of prokaryotic microbes were detected. For eukaryotes, Dikarya taxa of seventeen orders probably account for fungal induced diseases while eukaryotic animals from Metazoa of fourteen families were characterized. DNA metabarcoding shows extremely high sensitivity in recovering microorganism like *Alternaria*, *Botrytis* and so on which were previously reported to be responsible for a series of plant diseases in *P. notoginseng*. This study found both culturable and unculturable microbiomes in *P. notoginseng*, which would illuminate the way to its disease control, even find the obstacles in continuous cropping.

T1-34-03

Using meta-barcoding to investigate bamboo diversity and plant/animal interactions

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Next Generation Sequencing (NGS) methods have facilitated species identification from environmental samples, via meta-barcoding. This not only expands our ability to rapidly generate DNA-based inventories for plants and obtain information on levels of phylogenetic and species diversity in a region but can also to provide insights into the interactions between animals and plants. However, in some taxonomic groups the standard ‘universal’ barcodes provide only limited resolution and multiple group specific barcodes are required. Capture-based approaches provide a means for assessing multiple barcodes and mini-barcodes as well as allowing specific gene regions, which may be of interest or relevant to species identification and/or phylogenetic diversity. These methods can also be used to identify and validate regions for more targeted assays (e.g. qPCR). The relationship between specialist herbivores and the plants they feed on can often be complex than anticipated, as the full diversity of species consumed is overlooked. The giant panda is a key example of this. Whilst feeding almost exclusively on bamboo, there have been over 60 species reportedly consumed. Difficulties in distinguishing bamboo species, due to the infrequent flowering complicates out understanding of this relationship and research typically focus on a much smaller number of species. Standard ‘universal’ plant barcodes have limited species resolution due to low rates of molecular evolution, and even whole chloroplast sequencing shows limited differences. Using existing genomic data from bamboos and other grasses, a capture based approach will allow identification of regions/SNPs useful for distinguishing bamboo species as well as information of specific gene regions of interest (e.g. flowering genes and lignin biosynthesis). This information can subsequently be used to develop assays to provide robust and reliable identifications and hence detailed information of the distribution of bamboo species. In addition, the level of genomics data can provide insights into the bamboo (and non-bamboo) species giant panda predominantly feed on, and why they may be selected.

T1-34-04

Monitoring the Chinese herbal medicine market using metabarcoding method

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China is rich in plant species diversity. About 12800 species are used as medicinal plants. However, only nearly 800 medicinal plants species could probably be found in the medicine markets in China. Medicines made of these species are consumed not only by Chinese, but also by people all over the world, especially those in Korea, Japan, European Union and USA. In 2015,1.79 million tons of herbal medicines were exported, a drop of 8.84% from 2014. The governments committed a general check-up on major local medicinal markets and famous companies to find out fakes, adulteration, involvement of endangered species and many other aspects of herbal medicines. As an example, we scrutinized the Bozhou medicine market at Bozhou City, Anhui Province, China. In total 676 samples were collected from several companies. DNA was extracted, and four DNA barcodes were amplifies. PCR fragments of each sample were labeled by a unique DNA sequence adhered to primers. All PCR products were then mixed together to build a sequencing library for a PE250 run on Illumina HiSeq X platform. The resulting reads were sorted into genes according to the primer sequences and into samples according to the unique label sequences. Reads of different alleles of the same gene of a sample were separated with Usearch by limiting a similarity at 99%. Consensus sequences were created from the aligned contigs. The consensus sequences were identified by blasting them against the reference database. If unexpected species were found in a sample, the sample is considered adulterated; if no expected species was found, the sample is considered fake. The 676 samples belong to 607 medicines, which are supposed to be from 477 species, including six poisonous plants and 24 endangered medicinal plant species under cultivation. Although some samples have been processed, PCR is proved to be successful. The percentages of sequencing successes for ITS, *matK*, *rbcL* and *trnL-F* are 92.9%, 95.6%, 95.9 and 94.8%, respectively. Identification by DNA barcoding indicated that misidentification is not a big problem. However, contamination is common, which is likely to be due to adulteration on purpose. The Bozhou herbal medicine market is generally safe in terms of herbal medicine materials.

T1-34-05

Increasing efficiencies in the inventory of tropical rainforest floras: A case study from the Brazilian Amazon

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The under-documentation of tropical rainforest floras continues to inhibit research on global patterns of plant diversity and endemism and impedes efforts to assess the conservation status of numerous plant species. Carrying out floristic inventories in tropical rainforests presents many challenges, among the most notable are the relatively intensive efforts needed to reach exhaustive sampling of species in hyper-diverse rainforests, and the paucity of resources that are available to identify the plant species that occur in many tropical areas. We are in the midst of assembling floristic lists of the vascular plants of two large protected areas in an under-sampled part of the Brazilian Amazon. We present preliminary data comparing the relative efficiency of various sampling techniques as measured by the number of woody species encountered per time and sampling effort. We also assess the efficacy of DNA barcoding as a tool for expediting identifications of taxa in a study area that lacks a local barcode reference database. Sampling in ten contiguous 2 m x 50 m (= 0.1 ha) transects was found to have higher sampling efficiency than other widely used methods, such as general collecting and quantitative sampling in square plots. Barcoding employing matK sequences obtained using universal primers and a non-local reference database yielded accurate generic identifications for approximately 75% of species. We conclude that the integration of transect-based sampling and identification of sterile voucher specimens using a combination of DNA barcoding and traditional approaches to identification holds promise for increasing the rate and accuracy of plant species inventory in tropical rainforests.

T1-34-06

Use of DNA barcoding to validate identity of living *Angiopteris* Hoffman species in a university fernarium

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The study was focused on the validation of the species of living *Angiopteris* in a university fernarium through DNA barcoding using *rbc*L gene. Extraction of genomic DNA was carried out using modified cetyltrimethylammonium bromide (CTAB) DNA extraction method. A set of primers, *rbc*LaF (5′ATGTCACCACAAACAGAGACTA AAGC-3′; Levin *et al.,* 2003) and *rbc*LaR (5′GTAAAATCAAGTCCACCRCG-3′; Kress and Erickson, 2007) was used in this study for the amplification of *rbc*L gene of the chloroplast. A genomic sequence of 539 base pairs was obtained for 14 collected DNA samples of *Angiopteris*. BLASTn analysis revealed that the unknown species of a previous unpublished work matched at 99% similarity with *Angiopteris evecta.* Previously identified *A. elmeriana* also matched with *A. palmiformis.* All *A. palmiformis* samples were confirmed as the same species through BLASTn. Neighbor joining tree further showed that the unidentified species, *Angiopteris* sp. 1 and 2, were found in the same cluster with bootstrap value of 70%. The previously identified *A. elmeriana,* clustered together with *A. palmiformis* with bootstrap support of 69% and 66%. Five potential barcoding regions were identified through haplotype construction. *Angiopteris* sp. 1 and 2 exhibit a distinguishable haplotype from the other *A. palmiformis* species. *Angiopteris elmeriana* however had same haplotype as *A. palmiformis*. Thus, the two previously unidentified *Angiopteris* species were identified as *A. evecta* and the identified *A. elmeriana* species was re-identified as *A. palmiformis* using the *rbc*L sequence. The *A. palmiformis* samples were however confirmed as the same species. It could be seen that *rbc*L as DNA barcode could be used to discriminate the *Angiopteris* species in this study.

T1-35: Plant conservation, learning from the past!

T1-35-01

The evolution of monsoonal climate and its influence on plant diversity in Southwestern China

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The paleoclimate history played an important role in the distribution and evolution of plants. Southeastern China is dominated by monsoonal climate at the present day, which is characterized by wet summers and dry winters. Quantitatively paleoclimate reconstructions by fossil floras indicate that the monsoon gradually intensified since the Neogene, which means the seasonality of precipitation in the past was less significant the present day. Fossil records indicate that, some plants disappeared in this region because of the intensification of monsoonal climate, such as *Cedrus*. Combined with information on *Cedrus* fossil records and its seed physiology, we concluded that the intensification of a drier climate after the late Pliocene may have prevented the survival of *Cedrus* seedlings, leading to the eventual disappearance of *Cedrus* in western Yunnan. Meanwhile, many other plants, e.g., *Pinus*, and *Quercus* subgenus *Cyclobalanopsis*, survived and diversified successfully here by the morphological plasticity under paleoclimatic changes. These findings suggest that the evolution of monsoonal climate is crucial in shaping the patterns of plant diversity and floristic structures in southwestern China.

T1-35-02

The Miocene forest in western Amazonia and its response to abiotic changes, lessons from the past

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The Amazonian forest is a global treasure and its preservation concerns us all. Climate change and destruction by mining, logging and farming constitutes a real threat. If current rates of destruction and global warming persist within 100 years most of the forest might be lost. It is thus of great importance to properly understand the response of the forest to past environmental change and climatic optima. Based on palynological records from western Amazonia we evaluated two extinct, key components of the Miocene forest that occurred there around 16-17 million years ago. We established the nearest living relatives, past distribution, phylogeny, and closest associates of these taxa. Our data suggests that these fossils represent Malvaceae and that they occurred during the mid Miocene global climatic optimum (MMCO), when regional climatic patterns were quite different from the Present. At the time the Eastern Cordillera (Andes) was not yet fully formed as orographic barrier, and the Orinoco and Amazon drainage basins were still connected. In the course of the middle Miocene this situation changed with the development of a large wetland in western Amazonia and an influx of water and sediments from the Andes. We hypothesize that climate and sedimentary environments changed to such a degree that our Malvaceae perished, while new taxa (e.g. Arecaceae) evolved, as they were better suited to the new environmental conditions. At least one of these extinct Malvaceae was related to a taxon of the Gondwanan flora, which had its past distribution in southern South America, Antarctica, Australia and New Zealand. The other extinct Malvaceae taxon possibly was an immigrant from northern latitudes. Our study suggests that during the MMCO, when climate was warmer and more humid than Present, the forest did not perish. Instead, it was rather diverse albeit of different composition than at Present. Perhaps a better analogue for future scenarios of climate change in Amazonia might be found in late Miocene records, when global climatic cooling (albeit still warmer than at Present) and lower global sea level created generally dryer conditions, and coincides with the development of the savanna biome.

T1-35-03

First fossil flowers from the Danian (earliest Paleocene) of South America reveal complex biogeographic history of Gondwanan flora

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The early Danian (earliest Paleocene) Salamanca Formation in southern Chubut, Patagonian Argentina yields well-preserved plant fossils from within 2.5 million years of the end-Cretaceous extinction event. Flowers attributable to Rhamnaceae and Cunoniaceae were found among the Salamanca fossils. Herein, we describe these flowers, and we discuss the implications of their presence just above the K-Pg boundary of Patagonia. The rhamnaceous flowers are pentamerous with an obconic hypanthium, keeled sepals, stamens opposite cucullate petals, and a floral disk. Today, Rhamnaceae are cosmopolitan with nearly 1,000 species, and the combination of molecular phylogenetic and paleobotanical studies suggests the family originated in tropical forests during the Cretaceous. The presence of Rhamnaceae in the Salamanca Formation indicates that the family spread to subtropical climates at mid-latitudes from the tropical forests by the earliest Paleocene. By contrast, the cunoniaceous flowers are 8-10 merous with notched petals, a pubescent and superior ovary, and two styles. This combination of features indicates an affinity with the tribe Schizomereae, a primarily southern-temperate clade with 36 species previously known only from Australasia and South Africa. The Salamanca fossils are the oldest evidence of the Schizomereae, and they indicate that the evolution of this group was not constrained to Australasia, strengthening the Gondwanan connection between Patagonia, Australia, and South Africa during the early Paleogene.

T1-35-04

Environmental impact on differentiation development of Late Cenozoic floras between East China and Japan

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Floristically, East China and Japan belong to the East China-Japan Forest Floral Subregion. There are 12 seed plant families in the Subregion which do not occur in Japan. Some well represented Tertiary plants in Japan, such as *Keteleeria*, *Pseudolarix*, *Cunninghamia*, *Glyptostrobus*, *Carya, Hemiptelea, Sassafras, Liquidambar* and *Sycopsis*, are currently only growing in China. To explore these floral changes in the geological past, some representative Late Cenozoic floras in Japan are evaluated in comparing with those in North China. Evidences show that some major environment events have exerted a great influence on the development of local floras. Pollen floras from Neogene strata of Northern China and Japan show a detailed changing process prior and after some major Neogene environmental events. Climate reflected by the Neogene pollen floras of both places are mostly similar at the early stage, showing a gradual climatic resuming process in the Early Miocene, followed by a period of a period of climatic optimum in the late Early-early Middle Miocene. Since the Middle Miocene (ca. 14 Ma), the flora in North China was under the control of ever intensified continental condition, while the one in Japan had undergone the impact of prevailing marine climate. The floras in Japan are indicated by high occurrence of conifer and amentiferous elements at the early Early Miocene, followed by a distinctive floral flourishing period at the late Early Miocene-early Middle Miocene. Pollen floras in the Middle-Late Miocene Japan display a monotonous composition with Pinaceae predominated in the early Middle Miocene, and some broad-leaf and Tertiary elements, such as evergreen *Quercus, Carya, etc.* in the late Middle Miocene. A rise in boreal components and a decline in the broad-leaf and Tertiary elements are recognized again in the Late Miocene (since ca. 7 Ma) flora. Early Pliocene flora shows some Tertiary and warm-temperate broad-leaved types partly re-expanded, while in the Late Pliocene flora conifers are prominent again, and broad-leaf trees are minor with the exception of *Fagus*. Cause of the differentiation development of Neogene floras between East China and Japan is suggested to be raised mostly by the opening of the Sea of Japan started at the early stage of the Miocene. It has already been revealed that the initial stage of the opening of the Sea of Japan began 16 Ma ago, and the main stage of the opening was 15 Ma ago. By 14 Ma ago, the opening of the Sea of Japan had been almost completed. Since then, the Japanese Islands has been surrounded by the prevailed marine environment, while the mainland of China dominated by the continental condition, resulting in the differential development of their floras. Stratigraphic distribution of plant macrofossil shows a gradually extinction of Tertiary plant types in the Pliocene-Pleistocene of Central Japan. It is deduced that these extinct events are closely related with the global climate cooling, racial crustal movement, formation of the modern mountain system, and frequent volcano activities, *etc.*

T1-35-05

Impact of the Plio-Pleistocene climate and geomorphological changes on floral diversification and plant extinction in Japan

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Plio-Pleistocene floral changes in central Japan are characterized by extinction of major elements in the Neogene flora, including *Metasequoia* and *Glyptostrobus*, and expansion of plants dominant in modern cool-temperate and subalpine forests. The floral change was stepwise, and the events were concentrated in transition periods of climatic fluctuation and/or in a downward shift of the glacial climate in the late Pliocene (ca. 3.3 – 2.5 Ma) and late Quaternary since 1.4 Ma. The last occurrences are generally recorded in warm stages followed by significantly colder stages. Their extinction is thus attributed to extirpation of refugia during the cold stages in and around the sedimentary basin. Increased dryness and coldness provided by winter monsoon that developed in a stepwise manner would have altered the climate regime and the ability of plants to grow and establish populations. Mountain uplifts became active after the late Pliocene and may have promoted plant extinction by constituting barriers of migration. It is because the decreasing winter temperatures in higher altitudes would have hindered migration to the north and inland over mountain ranges, even as exotic taxa surviving in southern sedimentary basins had the potential to expand their distribution under improving climatic conditions. However, *Metasequoia* and *Glyptostrobus* occurred during the Early Pleistocene glacial stages and it indicates their tolerance for cool-temperate climate condition. Along with mountainous areas surrounding the basins, the landform of the lowland plains in the sedimentary basins was also changed by tectonic movement and sea level changes, which increased in magnitude during the later stages of the Quaternary. This change influenced directly on habitats of wetland plants such as *Metasequoia* and *Glyptostrobus* which were distributed in the lowermost altitude in basins. Pronounced sea level changes after the late Early Pleistocene also transformed alluvial plains. During interglacial stages, basins and coastal plains were submerged during marine transgressions that reached near mountain bases and diminished habitat of wetland plants. Furthermore, alluvial plains that lost water after sea levels decreased became drier uplands with steep and narrow valleys at the lowest sea level stands in glacial stages. Such drastic environmental changes in the lowlands possibly influenced the regeneration and growth of *Metasequoia* and *Glyptostrobus*, which dominated wetland vegetation before the late Early Pleistocene.

T1-35-06

Learning from the past: Relic plants in SE and E Asia and paleoecology of their fossil relatives in Europe

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Relatives of E and SE Asian plants were major constituents in the Paleogene and Neogene European vegetation and thus tend to be important indicators for deep time climate and environments. These extant genera and species are often relics and endemic to SE Asia. Relics have very specialized demands on habitat conditions such as soil nutrients, water availability and temperatures which evoke relatively narrow ecological amplitudes for those plants. This constraint is important for conservation purposes of endemics and relics. In contrast, their fossil relatives in Europe often dominated characteristic plant associations from particular depositional settings. Combined knowledge from phytosociological composition of fossil assemblages, from burial sediments and accompanying fauna provide often evidence that ecological demands of the fossil-taxa remarkably differ from those of the extant relative species and/or genera. This could be caused either by ecological shifts within the respective taxon, or, alternatively, fossil-species had a distinct autecology in comparison to the extant relic species, with or without overlap in the amplitudes. Thus extinction of fossil-species within a certain genus refers to cuts in the ecological amplitude of the genus ending with the narrow ecological amplitude of the extant relic. This contribution gives several examples from the European fossil record and compares their ecological demands to those from their extant relatives in E and SE Asia. Selected fossil examples are *Cathaya bergeri*, *Cyclocarya cyclocarpa*, *Diplopanax limnophilum*, *Eotrigonobalanus furcinervis*, *Eurya stigmosa*, *Fortunearia europaea*, *Gironniera pyriformis*, *Glyptostrobus europaeus*, *Keteleeria hoehnei*, *Platanus neptuni*, *Rhederodendron wiesaense*, *Trigonobalanopsis rhamnoides* and others. This could potentially give valuable hints for conservation policy and protection strategies of relics. Mismatch in ecological demands between fossil and extant taxonomical relatives should be paid particular attention because the extant relative could not serve as a proxy for paleoenvironmental and paleoclimatic reconstructions.

T1-36: Plant diversity and conservation in Southeast Asia

T1-36-01

Patterns of species distribution and diversity along elevational gradients in sparsely documented areas in Northern Myanmar

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The high landscape heterogeneity along gradients of latitude, elevation and geological formations shapes the physical environment and vegetation structure and favours the diversity and richness in Myanmar flora. The northern part of Myanmar is located between eastern Himalaya and the southwestern China Biodiversity Hotspot, but unfortunately the area is highly under-sampled. Most of the floristic surveys were engaged in the late 19th and the early 20th century. The taxonomic knowledge and distribution patterns are thus very poorly known. The assessment of the elevational gradients of species richness is a major issue of biogeography worldwide, but plot-based complete floristic gradients in species rich tropical mountain areas are not yet available. We undertook the task to close the gap of knowledge in this biodiversity hotspot especially for spermatophytes and pteridophytes. In order to make inventories and patterns comparable to respective studies worldwide, we used a standard protocol of plot-based complete floristic sampling. Our floral inventories were conducted in Natma Taung (Mt. Victoria; 400-3062 m) and Hponyin Razi (400-4057 m). Four plots of 400 m^2 were exhaustively sampled at every elevational step of 200 m starting from 400 m above sea level, making up a total of 132 plots. Here we will report on species composition and richness of pteridophytes and compare the results to adjacent mountain regions from Nepal Yunnan (China) and Japan. Species richness showed hump-shaped patterns for both transects, however with different elevations of highest richness. The highest number of species was found at 2200 m in Natma Taung and at 1200 m in Hponyin Razi. The patterns were mainly driven by epiphyte species. Since most of the ferns species are perennial, the rainfall and relative humidity is crucial for their survival. The occurrence of epiphyte species limited with treeline ecotone. Climate is therefore thought to be the most prevailing factor that influence species richness pattern. This finding coincides with results from regional studies of central Himalayas in Nepal and local studies of islands in Japan. Epiphytes are susceptible to the environmental changes, so the region with high epiphytic species number needs to prioritize for documentation and conservation. To have an overview about the flora of Myanmar and understand more about the species distribution pattern with elevation, the transect study should be extended to more north in the Kachin Sate, northeast to the Yunnan border and southwest to Naga hill such as Mt. Saramati.

T1-36-02

Bryology in Borneo: A review from 1996 to 2016

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This paper reviews the progress and challenges of bryology in Borneo for the past 20 years (1996-2016). Data presented is based on all research carried out by the author, research collaborators and students since 1996. The number of mosses recorded in Borneo has increased from 649 to 755 taxa, with several new species. However, little progress is seen in the other two groups of bryophytes, the liverworts and hornworts. The current number of liverworts and hornworts in Borneo has not changed much, which is 623 taxa. Furthermore, most studies in Borneo are biased to Sabah (East Malaysia), with less progress in Sarawak (East Malaysia), Brunei and Kalimantan (Indonesia). Sadly, only a few local students are interested to pursue postgraduate studies in bryology, especially in taxonomy and ecology, due to job prospects. However, interest in applied studies such as on pythochemical contents, antioxidants, antiproliferative activities and novel chemical compounds of Bornean bryophytes have been increasing in recent years.

T1-36-03

Plant community homogenization due to forest fragmentation in South China, Xishuangbanna

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Habitat fragmentation causes a rapid change, in tropical forest, for species composition, biomass, phylogenetic diversity and increase of disturbance-adapted species. Xishuangbanna Prefecture, south of west China, has experienced a severe change in forest cover in the last decades due to the expansion of banana, tea and mainly rubber plantations. We conducted a study over the entire Xishuangbanna Prefecture (~20.000 km²) assessing the tree and herbs diversity related to the forest structure and patches sizes. The inclusion of different forest patches allowed us to gather species diversity and community composition over a large area and a variety of forest structure. The results shows the importance of the study scale and the different ecosystems. The northern part has large and continuous forest surrounded by numerous small patches, whereas the south has few large forest patches surrounded by extensive cultivation. General conclusion underline that plant community changes with fewer and disturbance-adapted species in small patches and slightly richer community in bigger patches with more forest species. Tree diversity, forest structure (number of trees and DBH per plot) varies, as expected, according to the forest patch size, with simpler diversity and structured forest in small patches and more articulated in species and structure in mature forest. These methods allowed us to obtain important field-based data on plant community diversity and forest structure over a large human modified landscape. We underline how rare species, community composition and forest structure highlight the threat for important areas such as lowland forest or karst ecosystems. These methods can be used and expanded in other south China tropical areas where landscape with high level of biodiversity have been recently heavily modified.

T1-36-04

Inventory and assessment of herbaceous flowering plants in Long Term Ecological Research sites (LTER) of Mindanao, Philippines

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The Philippines harbor floral diversity including the herbaceous angiosperms which are prone to threats brought by human and natural causes. It is therefore essential to inventory and assess these herbaceous angiosperms. One-hectare permanent plots with twenty-five 20 m x 20 m subplots were established in the five Mindanao LTER sites, Philippines *viz*., Mt. Apo of North Cotabato, Mt. Hamiguitan of Davao Oriental, Mt. Kitanglad of Bukidnon, Mt. Malindang of Misamis Occidental and Mt. Musuan of Bukidnon. Species richness, diversity and assessment of herbaceous angiosperms were determined. Among the five LTER sites, Mt. Malindang has the highest species richness with 57 species, followed by Mt. Musuan (56), Mt. Kitanglad (53), Mt. Apo (51) and Mt. Hamiguitan (43). In terms of diversity, Mt. Malindang (H' = 1.58) was recorded to be most diverse ecosystem. This is followed by Mt. Apo with H' = 1.39, Mt. Hamiguitan and Mt. Kitanglad both have H' = 1.29 and the least diversity value is Mt. Musuan with H' = 1.18. Further, Mt. Malindang has the greatest frequency (23.92) of species with *Alpinia alpina* as the most frequent species. Mt. Kitanglad revealed to have the highest density value with *Sarcandra glabra* as the densest species. The high frequency and density of the said species could be related to dispersal since the aforementioned species are prone to herbivory by animals that serve as pollinators and dispersers of their pollen and seeds. Mt. Musuan has the lowest diversity value since it is prone to anthropogenic disturbances because the site is situated at the altitude of 388 m asl. which is near to a residential community hence, vulnerable to exploitation. Mt. Malindang is observed in the mid-montane vegetation which serves as a transition zone between lower montane and upper montane forest where species are observed to be rich and diverse. Species with the highest species importance value (SIV) in Mt. Apo and Mt. Hamiguitan is *Freycinetia* sp., in Mt. Kitanglad is *Sarcandra glabra,* in Mt. Malindang is *Impatiens platypetala* and in Mt. Musuan is *Donax canniformis* sp. These herbaceous angiosperms dominate the understory of each site where these species exert a great influence to the forest ecosystem. Assessment of the herbaceous angiosperms in the five sites recorded 2 endangered, 1 vulnerable, 9 economically important species, 1 rare and 1 other wildlife species. These endangered species are *Nepenthes copelandii* and *Paphiopedilum ciliolare* which are recorded only in Mt. Apo and Mt. Hamiguitan respectively. Most of the recorded economically important species belong to the Orchidaceae family which are usually highly priced ornamentals. These species of herbaceous angiosperms should be conserved and protected to prevent the loss of the country's valuable biodiversity.

T1-36-05

History of floristic exploration in Myanmar including modern collections towards a Flora of Myanmar

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Myanmar, located at the western edge of Southeast Asia and the eastern edge of the Indian sub-continent, is a unique place where four floristic regions intersect: the Indian, Sino-Japanese, South-East Asian and Malesiana floristic regions. Myanmar is known for its rich plant diversity due to the combination and interaction of geography, topography, climate, pattern of seasonal rainfall, presence of high mountains and major rivers. The first scientific collections were made by Francis Buchanan-Hamilton in 1795, and many herbarium specimens were gathered by British explorers in the 150 years that followed. However, very little inventory-based botanical fieldwork has been undertaken since then, whilst in surrounding countries field investigations and also preparations and publications of their floras have been actively progressing. Myanmar is a major part of a global biodiversity hotspot, yet remains a knowledge gap in the floristic knowledge of Asia. During the last 15 years several botanical explorations have been undertaken, led by botanists from Japan and Myanmar. Over 26,500 specimens of vascular plants have been collected, housed in the herbarium of Myanmar's Forest Department and in Makino Botanical Garden. The ongoing project aims to publish a Taxonomic Enumeration of Natma Taung, Chin State, and we, with international collaborative team of botanists, have so far identified 1,843 species representing 169 families. The level of new knowledge generated for the country is remarkable, for example, the Asteraceae consists of 138 species, of which 27 are new records for Myanmar and one species of each *Ainsliaea*, *Himalaiella* and *Taraxacum* appear to be new to science based on these recent collections. We continue on field collections and taxonomic research collaborated with international team of botanists, which are the vital first steps towards a Flora of Myanmar.

T1-36-06

Regional patterns of local processes based on the '101° E' forest transect from Southwest China to the Central-Southern Peninsula

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One large forest plot (16-50 ha) can be used as a platform to explore ecological processes underlying tree species coexistence at local scales (<1 km^2). However, little is known about how these local processes vary at regional scale. The '101° E' forest transect from Southwest China to the Central-Southern Peninsula (here after '101 transect') can acts as a platform to explore how ecological processes occurring at local scale change along an environmental gradient at larger scale. This is so-call 'regional patterns of local processes'. The '101 transect' consists of ten large forest plots, from five of which we have collected key functional traits of more than 1,000 tree species. We use trait difference among species pairs which co-occur in the same plot to quantify relative importance of environmental filtering and similarity limitation underlying species coexistence, especially we compare the difference between common and rare species in trait variation to distinguish how common and rare species co-exist at local tree assemblages. Based on above, we want to test a hypothesis at regional scale, namely the strength of environmental filtering increases with habitat harshness, while the strength of similarity limitation decreases with habitat harshness. I will report the advance on construction of the '101 transect' and research based on it in my oral presentation.

T1-37: New insights on the assembly and biodiversity of the flora of North America (two sessions)

T1-37-01

The roles of molecular evolutionary rates and functional traits in the colonization success of maples in North America

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We are using *Acer* L. (maples, Sapindaceae) to investigate the roles of molecular evolutionary rates and functional traits in successful colonization of new habitats by plants in North America. Colonization success is an understudied historical aspect of the North American flora and has important implications for patterns in the spatial organization of biodiversity. Colonization of an available area is dependent upon the regional species pool, and the species pool hypothesis asserts that colonization is logically more probable for larger areas that have persisted for longer times. Thus, area processes drive colonization success. However, colonization from regional species pools is undoubtedly also strongly affected by biological processes such as life history and evolutionary rate, including molecular substitution rate, speciation, and extinction. Our study is one of the first to seek correlations between colonization success and evolutionary rate and life history at a continental scale. To obtain evolutionary rates for all eight North American lineages of *Acer*, we are reconstructing a dated phylogeny from existing molecular sequences and fossils. We are using the dated phylogeny to estimate substitution rates as well as speciation and extinction rates while controlling for time. We are using functional traits as a proxy for life history strategies, and we are obtaining functional trait data from herbarium specimens and the literature. Our method of measuring colonization success is to compare the size of the distributional range of each lineage of *Acer* with the size of its climatically suitable range in North America according to species distribution models (i.e., ecological niche models); that is, how much area it does occupy compared to how much area it could occupy. We are evaluating the relationships between colonization success and evolutionary rate and the states of functional traits using standard statistical methods. Our study provides insights into the biological influence on colonization success of North American maples and has relevance for other woody plant clades. Moreover, the study offers a methodological framework for integrating biological processes into future work on colonization.

T1-37-02

Assembly and biodiversity of the Flora of North America: Areas of endemism of Mexican vascular plants

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From the approximately 25,000 species of native vascular plants of Mexico, nearly 50% are endemic. Moreover a number of lineages are endemic to Mexico, including one family, several genera and clades in different plant families comprising many species. The most remarkable are the monotypic family Setchellanthaceae, and several clades/genera in Asteraceae and Cactaceae, as well as nine monocot genera. With regard to their distribution, the majority of the regions with the highest indices of endemism have dry climates, however areas of endemism have been recorded in tropical humid climates as well. Among objectives of this study are: 1) identify areas of neo- and palaeoendemism, 2) examine variation of phylogenetic diversity in areas of endemism, 3) identify whether areas of endemism are associated with current climate conditions and historic climate stability. Occurrence points for almost 400 species belonging to angiosperm endemic lineages of Mexico were obtained from literature and databases and they were scored taking into account biogeographic provinces. Phylogenies for the endemic groups in Cactaceae and Asteraceae were constructed and molecular dating analyses were used for estimating divergence times. In addition, a megatree was obtained to estimate phylogenetic diversity and different metrics related to this diversity. Current and past ecological niche were modeled. Results suggest that most of the areas are identified by neoendemisms congruent with hypotheses of the assembly of the Mexican flora. The biogeographic regions, the Trans-Mexican Volcanic Belt and the Tehuacán Valley are outstanding for their endemism. Areas of endemism are explained by historic climate rather than current climate.

T1-37-03

Adaptation, competition, and the distributions of North American woody plants

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Distributions of species within regions (the "regional community") are influenced by adaptations to the physical environment and by interactions—particularly competition—among species. These factors can be revealed indirectly by analyzing the distributions of related species with respect to characteristics of the environment and to each other. Comparisons of tree species richness among temperate forests on different continents indicate significant regional influences, in addition to the effects of present-day climate, presumably reflecting the unique history and geography of each region (Qian and Ricklefs, 2000; Ricklefs and He, 2016). Within North America, climate variables explained about 70 per cent of the variance in the ordinated positions of 340 forest plots based on tree species composition. Considering the ordinated positions of species based on their distributions across plots, related (congeneric or confamilial) species tended to be marginally closer than randomly-selected species, indicating a stronger role for phylogenetically shared adaptation compared to competition in shaping distributions (Ricklefs, 2015). Moreover, the abundance of individual species within plots was not related to the number of co-occurring closely related (congeneric) species, further suggesting that competition is not stronger among closely related compared to distantly related species. Comparisons of the distributions of related (i.e., congeneric) herbaceous (but not woody) taxa in eastern Asia and North America suggest a general conservatism of latitudinal distribution and geographic range size over long evolutionary time scales (Ricklefs and Latham, 1992; Qian and Ricklefs, 2004). In contrast, a taxonomically nested ANOVA of tree distributions in eastern North America showed little evolutionary conservatism in population traits (range position, distributional extent, and abundance); nor were local abundance and geographic (ecological) dispersion related (Ricklefs, 2015). These present-day patterns can be compared to the postglacial fossil pollen record of plants in North America, which suggests that the distributions of individual species changed relatively independently during the past 21,000 years, such that the positions of pollen localities in ordination space based on species became uncorrelated over time. Finally, beta diversity (turnover of species with distance) is greater with respect to both geographic distance and climate in the more diverse eastern Asian flora than in the North American flora (Qian *et al.,* 2005), suggesting that diffuse competition constrains ecological distributions more strongly within eastern Asia. These analyses suggest that the distribution and abundance of species in temperate deciduous forests are largely determined by labile adaptations to the physical and biological environments and are independent of competition among closely related species, although diffuse competition within the entire local flora apparently has an important impact. Nonetheless, factors that influence the relative abundance of species and their distributions within large regions, which vary over several orders of magnitude, remain poorly understood.

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T1-37-04

Floristic changes from Paleocene through Miocene time across midlatitude western North America

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Floristic inventories of Tertiary localities in the middle latitudes of western North America document patterns that reflect climatic shifts and biotic interchange with other regions. Most Paleocene floras are dominated by broadleaved deciduous taxa, including Platanaceae (*Macginitiea*, *Platanites*, *Platanus*), Coryloideae (*Palaeocarpinus*), Juglandaceae (*Polyptera*, *Cyclocarya*, *Juglandicarya*), Cercidiphyllaceae, Nyssaceae (*Davidia, Amersinia, Browniea*), plus *Aesculus*, *Cornus* and *Celtis*. *Glyptostrobus*, *Metasequoia*, Taxaceae, *Ginkgo*, and occasional cycads were present, as were palms and Zingiberaceae. Dominant dicots had serrate leaves with long petioles. Ferns included *Onoclea* and *Woodwardia*. Although richness per locality is typically low (< 20 species), many taxa were shared with the Paleocene of Greenland, Scotland and Asia. The Paleocene-Eocene transition, well documented in north central Wyoming, coincides with a peak in mean annual temperature. Most of the usual Paleocene elements are absent, and new taxa appear, including abundant legumes, Sapindales and Hernandiaceae. Early Eocene floras of the same region are relatively low in megafossil diversity (< 10 taxa per locality), commonly including *Alnus*, *Platycarya*, Malvaceae sl., Simaroubaceae (*Ailanthus*, *Chaneya*), and *Salvinia*. Possibly endemic, extinct genera of uncertain affinity, are common in these floras. Early middle Eocene sites of the Rocky Mountain region are dominated by Platanaceae (*Macginitiea*), Salicaceae (*Populus, Pseudosalix*), and Ulmaceae (*Cedrelospermum*), with abundant legumes and Sapindales (Anacardiaceae, Sapindaceae). Climbers included *Lygodium*, Vitaceae, Icacinaceae and Menispermaceae. Long-petioled taxa no longer dominated. Similarly aged floras near the west coast shared some of these taxa (e.g. *Macginitiea*, *Cedrelospermum*, Icacinaceae, *Lygodium*), but had fewer Salicaceae and Leguminosae and a greater diversity of mesic, thermophilic taxa (diverse ferns, Icacinaceae, Lauraceae, Mastixiaceae, Menispermaceae, Sabiaceae, Torricelliaceae, Musaceae, and cycads, along with *Juglans* and *Ulmus*. Many genera are shared with coeval European floras. Rosaceae, Betulaceae, Sapindaceae, Trochodendraceae, and diverse conifers proliferated in parts of Washington and British Columbia, but remained relatively rare in other western floras. *Cedrelospermum* remained common through the late Eocene, as new elements arrived. Late Eocene Rocky Mountain floras show increased occurrence of *Koelreuteria*, *Carya*, *Cercis*, Rosaceae (*Cercocarpus, Crataegus, Rosa*) as well as some extinct genera (*Asterocarpinus*, *Fagopsis*, *Florissantia*). Overall, this inland late Eocene flora has markedly smaller leaves than those of the west coastal Eocene and Oligocene floras. Early Oligocene floras contain *Pinus*, Cupressaceae (*Metasequoia, Se-*

quoia, Tetraclinis), many deciduous angiosperms, e.g., Betulaceae (*Alnus, Asterocarpinus*, *Betula*, *Ostrya*), Aceroideae, Fagaceae, Hamamelidaceae, Juglandaceae, Tilioideae, Ulmaceae, *Cedrela* and only few broadleaved evergreens, e.g. *Mahonia*. Rare Late Oligocene floras indicate lower diversity of arborescent taxa. They included *Juniperus*, *Pinus* and a few angiosperms e.g., *Salix*, *Mahonia*, with smaller leaf size. Phytoliths indicate increasing presence of grasses coinciding with cooling and drying. Middle Miocene floras in Washington, western Idaho and adjacent areas indicate floristically diverse mixed mesophytic assemblages with leaves of moderate size. Many taxa are shared with Miocene floras of Europe and Asia, including those extirpated from western North America and Europe, but surviving today in eastern North America and/or eastern Asia, e.g., diverse conifers (*Abies*, *Cunninghamia*, *Calocedrus*, *Metasequoia*, *Taxodium*), Magnoliaceae (*Liriodendron*, *Magnolia*), *Liquidambar*, Fagaceae (*Castanea*, *Quercus*, *Lithocarpus*), Betulaceae, Juglandaceae, and *Cercidiphyllum*.

T1-37-05

Spatial phylogenetics of the North American flora

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Biodiversity has usually been measured by examining changes in the number of species across a region to identify areas of particularly high diversity and endemism. Beta-diversity, or turn-over on the landscape, is likewise typically measured by comparing proportions of species shared among subareas. However, investigations based on species distributions alone miss the full richness of analyses that can result from taking a phylogenetic approach. Fortunately, advances in digitization of natural history collections, broad-scale DNA sequencing of many taxa increasingly represented in public databases, and rapid scaling-up of methods for building phylogenies have made it possible to apply a “big data” evolutionary approach to assessment of biodiversity and endemism that can be termed spatial phylogenetics. Our research group has been developing a novel suite of phylogenetic tools including two new metrics, relative phylogenetic diversity and relative phylogenetic endemism, and three new methods, categorical analysis of neo- and paleo-endemism (CANAPE), range weighted branch length difference (RWiBaLD), and phylogenetic range-weighted turnover (PhyloRWT). These methods are rank-free since it does not matter what taxonomic levels the terminals represent, as long as they are monophyletic and their geographic distribution can be characterized, and are thus relatively robust to lumping and splitting decisions by taxonomists. CANAPE searches for centers of endemism, and classifies them by the branch lengths of the rare taxa within them, allowing, for the first time, a clear, quantitative distinction between centers of neo- and paleo-endemism across an area. RWiBaLD complements CANAPE and makes a close-up examination of the distribution of branch lengths within a single subarea, allowing a clear understanding of which branches on the tree are responsible for the patterns seen in CANAPE. Both CANAPE and RWiBaLD are both tested statistically using a spatial randomization of terminal taxa on the map. PhyloRWT examines turnover in amount of the phylogeny shared among subareas, while emphasizing the branches that are range-restricted. It serves as a particularly useful measure of phylobetadiversity for purposes of understanding changes in phylogenetic assemblages across the landscape. PhyloRWT can be applied for a variety of purposes including bioregionalization, ecological studies of causes for beta-diversity, and complementarity analyses for applied conservation studies, which are important given the need to prioritize efforts in the face of rapid habitat loss and human-induced climate change. These new phylogenetic methods allow assessments of protected lands and can identify complementary areas of biodiversity that have unique evolutionary histories in need of conservation. The prospects for applying these methods across North America are rapidly improving given advances in herbarium digitization; the time is ripe for planning a continent-wide analysis. The first step is identifying gaps in spatial data and taxon sampling for genetic data. Regional studies have been completed for California and Florida; these will be presented as springboards for the continental study. In addition we will present a very preliminary analysis for North America using existing (albeit incomplete) data in order to look at preliminary patterns as well as to highlight data gaps.

T1-37-06

The role of land bridges, ancient environments, and migrations in the assembly of the North American flora

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North America was connected and separated from adjacent and more distant regions during the Late Cretaceous and Cenozoic (100 Ma to the Present) by five land bridges and through significant alterations in climate. Beringia (connecting NW North America to NE Eurasia) was mostly continuous for much of the time with warm-temperate to tropical climates in the Paleogene trending toward cool to cold temperate in the Neogene, and becoming discontinuous with each of the 18-20 interglacial cycles of the Quaternary. The North Atlantic (Greenland, the Arctic Islands, Iceland, NW Europe) was essentially continuous land until about the early Eocene with disruption (northward promulgation of the mid-Atlantic Ridge) corresponding to a change in climate from hot house (through the Middle Eocene Climatic Optimum) to ice house conditions in the late Cenozoic. The Antilles (NE South America, SE U.S. via the Bahamas, Yucatán region of Mexico) afforded discontinuous stepping stones throughout their history (present consensus, with an alternate interpretation) and muted mean annual temperatures varying by ca. 3-5^{o}C. Central America (connecting South America with Mexico into the southern U.S.) had an open seaway through the Darian region until about 3.5 Ma (present consensus) after which began the Great American Biotic Interchange; climates were comparable to those of the Antilles. The more distant Magellan Land Bridge (southern South America, Antarctica, Australasia and regions beyond) was physically and climatically disrupted by isolation of Antarctica (separation from South America) at about 34 Ma (early Oligocene) but *Nipa* and *Nothfagus* are reported in earlier Eocene deposits all the way to Texas with *Nothofagus* extending further northward. *Podocarpus* also likely moved into North America from the south; it is known from the Eocene/Oligocene of the Mississippi Embayment becoming extinct there with later Cenozoic cooling. Its present northern distribution is represented by *P. matudae* in the cloud forests of Tamaulipas, Mexico. The message emerging from a more precise knowledge of the physical and climatic history of the New World land bridges, and the taxonomic relationships and phylogeny of the organisms utilizing them, is that over time they provided opportunity for migration of a diverse assemblage of organisms and ecosystems ranging from subtropical and warm temperate to cool and cold temperate accounting for the diversity of the North American flora, the vast extent and widespread similarity of the boreal forest in the harsh environments of the far north (ca. nine principle genera of trees), as well as disruptions that better explain disjunct biogeographical affinities like between the dry regions of SE U.S./northern Mexico and the Mediterranean (ca. 15 genera; e.g., *Ephedra*, *Juniperus*, Pinus, Quercus; Madrean-Tethyan vegetation), temperate eastern U.S. and eastern Mexico (ca. 20 genera; e.g., *Acer*, *Alnus*, *Carya*, *Fagus*, *Fraxinus*, *Juglans*, *liquidambar*, *Magnolia*, *Nyssa*, *Tilia*, *Ulmus*), and the ca. 50-60 trees of temperate eastern North America and central China.

T1-37-07

Diversification and assembly of the North American sedges (Cyperaceae): Evolution of geographical place and niche space

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The North America sedge (Cyperaceae) flora contains over 840 species, making it the third most diverse family on the continent. Here, we analyze the evolution of these species in the context of geographical and ecological diversification. Utilizing over 200,000 georeferenced specimens, we characterized the geographical distribution and climatic and edaphic niche space occupied by each species in a continuous manner, using a modified Parsimony Analysis of Endemicity approach in conjunction with ordination statistics. We integrate these data with a supermatrix phylogeny of the North American sedges, and use a multifaceted approach to assess the role of geographical and ecological divergence on lineage diversification. In addition to measuring phylogenetic signal for these traits, we measured pairwise phylogenetic distance of species within floristic regions, calculated rates of speciation, and tested for correlations of speciation rate to tempo of geographical and ecological evolution. Our analyses consistently show that evolutionarily related species tend to be geographically unrelated. Rates of geographical and ecological diversification are closely linked to tempo of speciation, and exploration of geographical place coincides with divergence in ecological niche space. We highlight the benefits of treating geography in a continuous manner, and stress the importance of employing a diverse suite of analytical approaches in testing hypotheses regarding the evolution of range and niche. We then analyze broad patterns of community assembly throughout the continent, and demonstrate a latitudinal shift from phylogenetic overdispersion to phylogenetic conservatism as lineages become filtered at high latitudes.

T1-37-08

Richness and geographic distribution of selected angiosperm groups in Mexico

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México is a megadiverse country. 22,126 species of angiosperms have been registered on its territory and 11,001 are endemic. However, their geographical distribution is far from homogeneous. In addition, Mexico is the center of origin and diversification of several groups. Our analysis focused on such groups. The aims were to identify areas of species richness and endemism. A data matrix with 25,509 geographical records of Tigridieae (Iridaceae); *Solanum*, *Lycianthes* and Physalinae (Solanaceae); *Cosmos*, *Dahlia* (Asteraceae); Poliantheae, *Echeandia* (Asparagaceae); *Calochortus* (Liliaceae); *Bletia* (Orchidaceae); *Hylocereus* (Cactaceae); *Salvia* subgenus Calosphace (Lamiaceae); Crassulaceae and Amaryllidaceae, was analyzed. Using Geographic Information Systems (GIS), we determined the richness and distribution on a 60 km × 60 km cell grid. The areas of emdemism were estimated using the endemicity analysis (EA). The Transmexican Volcanic Belt (TMVB) concentrates the highest number of taxa and endemics. The TMVB has been recognized as a province on the basis of geologic, tectonic, geomorphologic, physiographic and biogeographical observations. It is a 1,000 km long volcanic arc between 80 and 230 km wide, located in central Mexico between 17°30' and 20°25' N and between 96°20' and 105°20' W. The radiation of several plant groups coincides with the formation of the TMVB, which began 19 mya, during the Middle Miocene.

T1-37-09

Origins and diversity of the fern flora of the Pacific Northwest

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The Pacific Northwest (PNW) is a large and geographically complex region in western North America comprising the Yukon Territory and British Columbia of Canada, and the states of Alaska, Washington, Oregon, Idaho, and western Montana in the United States. The region has been developing over the past 200 million years beginning with the western movement of the North American plate following the breakup of the supercontinent Pangea. The subduction of the Pacific Ocean floor beneath the leading edge of the North American plate has added landmass to the Pacific Northwest through volcanism and the accretion of volcanic island chains and ocean-floor rocks through plate collision. Much of the region has been repeatedly covered by glaciers, which may have impacted the ability of the flora to assemble and diversify. Among vascular plants, the ferns (Polypodiopsida) are well represented, with taxa from seven of the 10 orders currently recognized by the Pteridophyte Phylogeny Group occurring within the region and spanning the diversity of the fern tree of life. Fifteen families and 27 genera have been recorded. We may wonder, however, why there isn't more fern diversity in the PNW, given the high degree of topographic complexity and the range of Köppen climate types that are present. The assembly of a fern flora is of particular interest considering that fern spores are able to disperse thousands of kilometers and aren't reliant on other organisms for their migration. However, for ferns to persist both the gametophytes and sporophytes must be able to survive the environment in which the dispersed spore germinates. The gametophyte and sporophyte stages of ferns are independently exposed to the selective pressures of their environment and their fundamental niche may be smaller than we might expect given their reproductive capacity and the spore vagility. The current research is motivated by the following questions: 1) what diversity of ferns is present using various measures (for example, species and genus richness, phylogenetic diversity), 2) what environmental factors correlate with distributional patterns of selected species, 3) what ancestral areas gave rise to the extant fern flora, and 4) how may we expect the fern flora to respond to predicted climate change? To address these questions, we incorporated data from phylogenetics and ecological modeling. Presence-only distribution data were obtained from the Consortium of Pacific Northwest Herbaria and the Oregon Flora Project. Chloroplast DNA sequence data were obtained from GenBank and used in phylogenetic reconstruction to infer ancestral areas and assess phylogenetic diversity. Bioclimatic data were downloaded from WorldClim.org and maximum-entropy approaches were used to determine environmental predictors for select taxa, including *Equisetum*, *Botrychium*, and *Polystichum*. We will share results of our ancestral area reconstructions and present hypotheses regarding the factors that have shaped the fern assemblage of the Pacific Northwest.

T1-37-10

The role of Pleistocene climate fluctuations in the assembly of boreal steppe communities of Northwest North America

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Boreal or subarctic steppe is an azonal vegetation type dominated by perennial grasses and forbs, plus sagebrush, which occurs as disjunct communities on south-facing slopes within the boreal zone. It is restricted to areas of continental climate in Northwest North America, as well as Northeast Siberia and the Russian Far East. In order to gain insight into the timing and routes of dispersal of species occupying islands of steppe in boreal western North America, we examined elevational distributions of species on the modern landscape. Our study areas were open, south-facing mountain slopes in Alaska, Yukon, and the Russian Far East. We assumed that Asian migrants crossed the Bering Land Bridge under climates cooler and drier than today, and we therefore predicted that these species should have broad elevational ranges extending upward to tundra. In contrast, we assumed North American species migrated during interglaciations or interstadial periods, including the Holocene, as during cold periods they would have been excluded by ice sheets. We predicted they would be restricted to warmer sites. We found that steppe species that are Asian disjuncts indeed tended to have large altitudinal ranges, reaching cold steppe and tundra. North American disjuncts were largely restricted to the warmest, lower-slope locations. Fossil evidence nevertheless indicates at least one North American species survived full-glacial conditions in Beringia. Invertebrates associated with the boreal steppe environment show similar patterns, supporting the concept that the Bering Land Bridge acted a cold filter to species migrating from Asia. The boreal steppe flora of Northwest North America likely comprises species that have migrated during different Pleistocene climate cycles, reflecting assembly over an extended period of time from multiple source regions.

T1-37-11

Isoetes diversity in Eastern North America

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The early lineage of vascular plants, the genus *Isöetes* (quillworts), is found throughout North America, with the highest diversity in the eastern United States. Species of *Isöetes* are all morphologically very similar, causing considerable confusion for both taxonomists and field biologists. Additionally, taxonomy of these closely related species of *Isoëtes,* likely the result of a relatively recent radiation, has been difficult to infer, and is further complicated by the occurrence of many, and often complex, polyploid species. With the recent developments in molecular methods, DNA sequencing is currently the predominant tool for estimating species boundaries and phylogeny in *Isöetes*. To date, we have used both a nuclear gene intron and whole chloroplast genome (plastome) sequences to infer a phylogeny of over fifteen diploid taxa and to investigate the origins of several polyploid taxa. Among our results, we have found that the phylogeny of the entire plastome for diploids was well supported across various models of evolution, with a deep divergence representing a geographic split between the Atlantic seaboard and the Gulf Coast/Midwest being inferred. In an initial study of polyploid *Isöetes*, we have identified different diploids as nuclear genome donors to species originally identified as monophyletic, and used the plastome sequences to identify their chloroplast donor (likely maternal) species. The molecular data also has the potential to generate biogeographic hypotheses regarding range shifts of species of *Isöetes* in the recent geologic past. Finally, mapping spore characters and phenology to our phylogeny indicates white, tuberculate megaspores and white, papillose microspores that mature in the spring are plesiomorphic to this eastern American clade, with some other character states subsequently evolving several times.

T1-37-12

Origins and diversification of conifers in North America and implications for the assembly of the conifer flora of the Northern Hemisphere: An example using *Abies* (Pinaceae)*

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The relationships and diversification histories of the floras of North America and eastern Asia are widely studied. Studies using phylogeny and historical biogeographic reconstructions, often infer that Eastern Asia is the origin of angiosperm groups. In contrast, these studies frequently find that conifers originated and migrated out of North America. The ‘out-of-North America’ hypothesis in conifers is also congruent with the fossil record of many angiosperm lineages. However, it remains challenging to integrate fossils into historical biogeographic reconstructions across phylogenies. Thus, insights into conifer origins and geographic radiation may provide key penetration into patterns and processes in angiosperms. In the conifer genus, *Abies*, two East Asia-North American disjunct sections were formerly recognized based on morphological characters: sect. *Balsamea* and sect. *Amabilis*. In order to examine the geographic origins of these two sections, we inferred a dated phylogeny using nearly complete species sampling of the genus, fossil evidence, and DNA data from three genomes: nrITS, low copy nuclear gene, chloroplast DNA intergenic spacers, and mitochondrial intergenic spacers. We used the dated phylogeny to perform historical biogeographic reconstruction. Our biogeographic reconstruction supported ‘out-of-North America’ dispersal in section *Amabilis* via the Bering Land Bridge from western North America to Japan during the middle Miocene. The situation in sect. *Balsamea* is unclear because the hybridization, with parent-ship from eastern Asia and North America, was probably involved in its origination. Our present provides insight on the assembly of the conifer flora in the Northern Hemisphere, and we expect that future work may improve understanding of how diverse, early conifer lineages in North America impacted the assembly of the conifer flora on other continents. In the future, we intend to use next generation sequencing methods, including RAD-Seq, to better resolve the phylogeny of *Abies*. Moreover, we expect to use resolved phylogenies of *Abies* and increasingly available, stable phylogenies of other conifer genera to further test the out-of-North America biogeographic hypothesis.

T1-38: Biogeographic patterns and adaptive radiations in the Pacific

T1-38-01

The biogeographic and evolutionary origins of the Hawaiian peppers (Piperaceae: Peperomia)

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Peperomia represents one of the most species-rich genera globally (over 1,000 species), and is widely distributed across the world’s tropics. In addition, owing to their high dispersal ability, *Peperomia* have also colonized and diversified across the islands of the Pacific, which collectively harbor a tenth of its global diversity. On Hawaii, *Peperomia* represents one of the most species-rich genera among the flowering plants of Hawaii, comprising 25 native species, 22 of which are unique to the archipelago. Taxa of *Peperomia* on Hawaii are also highly diverse morphologically and exhibit a range of ecologies. However, it remains unclear whether the endemic taxa on Hawaii originated through diversification from a single successful colonist, or if they were derived from multiple colonization events and thus, parallel radiations. Furthermore, the islands of Hawaii may also represent a “stepping-stone” for dispersal and hence act as a biogeographic source of colonists for the rest of the Pacific. Thus, a greater understanding of patterns of dispersal and diversification on Hawaii will provide insights to their evolutionary success across the wider Pacific region. Here, we constructed a multi-loci molecular phylogeny using a multiplex high-throughput sequencing approach for 45 taxa of *Peperomia* across the Pacific, with particular focus on Hawaiian taxa. Using this phylogeny, we then inferred patterns of dispersal in this group across the Pacific and within the Hawaiian archipelago using probabilistic historical biogeographic models. Our results shed light on the evolutionary history and extraordinary radiation of this diverse group across the Pacific, and provide insight into the evolutionary assembly of island floras.

T1-38-02

Applying RADseq to resolve the phylogeny of the Hawaiian radiation of *Myrsine* (Primulaceae, Myrsinoideae)

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Myrsine ranks among the largest genera of the primrose family (Primulaceae s.l.) and its +/- 200 species are distributed in subtropical and tropical areas worldwide except Europe. A particularly high number of species are found in the Pacific area. The largest of these pacific radiations is that of the Hawaiian Archipelago which consists of 20 endemic species. *Myrsine* has colonized all major Hawaiian Islands except Kaho’olawe and Ni’ihau and is most diverse on the oldest islands Kaua’i (14 species of which 10 are endemic to the island) and O’ahu (8 species, 2 endemic). Hawaiian *Myrsine* are woody plants and range from small and many-branched shrubs to trees of up to 8 m in height. While many species have a very narrow distribution and show little morphological variability, the species *M. lessertiana* is extremely variable. Several subspecies and varieties have been described for this species, and populations that show intermediate characters with *M. emarginata*, *M. pukooensis* and *M. sandwicensis* have been observed. The genus *Myrsine* and especially the Hawaiian taxa have been largely neglected by systematists and virtually nothing is known about their relationships among each other. Not a single DNA sequence of the Hawaiian species has been deposited at Genbank so far. Our study aims at resolving the phylogenetic history and the inter-island colonization pattern of Hawaiian *Myrsine*. Since other examples of radiations from the Hawaiian Islands have shown that Sanger sequencing does mostly not deliver enough information to untangle phylogenetic relationships at the species level, we decided to use the NGS method RADseq (restriction-site associated DNA sequencing) in our study. Our taxon sampling includes all but one species of Hawaiian *Myrsine* as well as *M. africana* and two species of the closely related genus *Ardisia* as outgroups. Seven specimens of the variable *M. lessertiana* were sampled from all major islands.

T1-38-03

Assembling a colonisation timeline for the endemic tree flora of New Zealand

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The extant flora of New Zealand contains 271 species of endemic forest trees, spread across a broad spectrum of plant diversity (147 asterids, 83 rosids, 20 conifers, 10 magnoliids, 6 ferns, and 5 monocots). Here, we set out to characterise the colonisation history of this endemicity. Bayesian relaxed molecular clocks were used to date the NZ vs. overseas floristic disjunctions, and place the NZ immigrations along an evolutionary timeline. The primary aims were: (1) to contrast the historical arrivals with significant geological and climatological events, (2) build a time-calibrated framework for tree diversification and biogeography research, and (3) assemble a database useful for community assembly questions, focused on the role of deep-time priority effects in shaping plant community structure. Towards this an assessment of the molecular dating literature was carried out (focusing exclusively on studies employing relaxed clock models in BEAST), resulting in immigration ages for 39 tree genera. For the remaining 46 genera that were undated in the literature, molecular dating analyses were conducted using a combination of publically available and newly generated DNA sequence data. The results suggest that although the Zealandia continental fragment has been geographically isolated for *ca*. 80 million years, over 85% of the endemic tree colonisations can be attributed to dispersal events in the Early Miocene or later (mean ages). Broadly, most of these dates are in line with the ‘fly-paper of the Pacific’ hypothesis of NZ biogeography, although some lineages represent possible examples of continuous post-Gondwanan presence.

T1-38-04

Biogeography and evolution of the largest adaptive radiation of woody plants (Melicope, Rutaceae) on the Hawaiian Islands

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The Hawaiian Islands are a perfect natural laboratory to study recent and species-rich radiations. With an age of approximately 5 millions of years Kauai and Niihau are the oldest of the current main islands and the archipelago ranks among the most isolated landmasses on the planet. Due to their recent age and their isolated geography, only relatively few organismal lineages successfully colonized the archipelago and some of them underwent adaptive radiations that lead to great numbers of species. There are often vast morphological differences between those species, which is usually contrasted by very low genetic variation. The Hawaiian Melicope lineage is a very good example for this phenomenon as it shows a wide array of morphological variation (e.g., growth form, connation of carpels, leaf shape, indumentum), but low genetic variation. Melicope constitutes one of the most species-rich plant lineages on the Hawaiian archipelago and, with a total number of 54 species, it accounts for the largest lineage of woody plants. The bird-dispersed genus Melicope also occurs on other Pacific Islands, Australasia, Malesia, Southern Asia, Madagascar and the Mascarene Islands. Altogether, with about 230 species, Melicope is the largest genus of Rutaceae. Phylogenetic reconstructions of Melicope based on Sanger sequencing datasets were highly supported and well resolved for most branches, but the Hawaiian clade showed relatively poor resolution. Similar results have been reported for other Hawaiian plant lineages. We here present the first attempt to investigate the phylogeny and biogeography of Hawaiian Melicope species using Next-Generation Sequencing. We chose RADseq (restriction site associated DNA sequencing) since it is perfectly suited for evolutionary young groups and does not require a reference genome. Out of the 54 known species, five are believed to be extinct and 39 species from all major islands have been sampled in our study. Our results clearly demonstrate the suitability of the RADseq method in resolving rapid radiations. More than 70,000 RAD loci proved to be sufficient to reconstruct a well-resolved and supported phylogeny. Our results show that the traditional division of Hawaiian Melicope into four sections is artificial since only one of the sections proved to be monophyletic. Our molecular dating results suggest that the diversification of Hawaiian Melicope (crown group) began in the Late Miocene or Early Pliocene (7.5Ma; 4.7-11.7Ma), so that the lineage might be older than the current main islands. Other islands such as Necker and Nihoa, which are now shallow islands without forests, might therefore have been the first habitat of the ancestor of the Hawaiian lineage. From these islands, Melicope colonized the other islands mainly following the rule of progression (i.e. directional from the older to the emerging islands).

T1-38-05

Integrating biogeography, threat, and evolutionary data to explore extinction crisis in the taxonomic group of cycads.

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Will the ongoing extinction crisis cause a severe loss of evolutionary information accumulated over millions of years on the tree of life? This question has been largely explored, particularly for ver-

tebrate and angiosperms. However no equivalent effort has been devoted to gymnosperm. Here we address this question focusing on cycads, the gymnosperm group exhibiting the highest proportion of threatened species in the plant kingdom. We assembled the first complete phylogeny of cycads, and assessed how species loss under three scenarios would impact the cycad tree of life. These scenarios are: i) all top 50% of Evolutionarily Distinct (ED) species are lost; ii) all threatened species are lost, and iii) only all threatened species in each IUCN category are lost. Finally we analysed the biogeographical pattern of cycad diversity hotspots and tested for gaps in the current global conservation network. First, we showed that threatened species are not significantly clustered on the cycad tree of life. Second, we showed that the loss of all vulnerable or endangered species do not depart significantly from random loss. In contrast the loss of all top 50% ED, all threatened or all critically endangered species would result in a greater loss of PD than expected. To inform conservation decisions, we defined five hotpots of diversity, and depending on the diversity metric used, these hotspots are located in southern Africa, Australia, Indo-Pacific and Mexico and all are found within Protected Areas. We conclude that the phylogenetic diversity accumulated over million years in the cycad tree of life would not survive the current extinction crisis. As such, prioritizing efforts based on ED or concentrating efforts on critically endangered species particularly in southern Africa, Australia, Indo-Pacific and Mexico are required to safeguarding the evolutionary diversity in the cycad tree of life.

T1-38-06

Revisiting *Astrocalyx* Merr. (Astronieae: Melastomataceae): New morphological observations on the Philippine endemic, enigmatic, endangered, and monotypic genus

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The monotypic *Astrocalyx* Merr. (Astronieae: Melastomataceae) was collected in 2015 from the Mount Kitanglad Range Natural Park (Mindanao Island, the Philippines) during a joint expedition conducted by the Central Mindanao University (CMUH) and the California Academy of Sciences (CAS). Since the original publication of the genus, no other detailed account of its enigmatic morphology has been published. Examination of herbarium vouchers revealed discrete variations in morphology, which may be indicative of reproductive isolation or presence of emerging selection pressures among populations of the species across the archipelago. This documentation aims to rekindle interest and initiate approaches toward conservation of this poorly known endemic taxon, already listed as endangered in the Philippine Islands.

THEME II: TAXONOMY, PHYLOGENETICS & EVOLUTION

T2-01: Evolution, ecology, and physiology of carnivorous plants - Part I and Part II (two sessions)

T2-01-01

Evolution of carnivory in angiosperms and diversification of trap types

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Approximately 800 species of carnivorous plants are currently recognized, which have evolved at least ten times from non-carnivorous ancestors in five angiosperm orders (Poales: 4 species in 3 genera, Caryophyllales: ca. 370 spp./6 genera, Oxalidales: 1 monotypic genus, Ericales: 33 spp./4 genera, Lamiales: ca. 390 spp./5 genera). In the 19 genera of carnivorous plants, a variety of trap types have evolved to attract, catch and digest small animals – ranging from simple pitfall traps made up by the entire plant body in the tank-forming carnivorous bromeliads, leaf-derived epiascidiate pitcher leaves of various pitcher plant genera, active and passive adhesive (flypaper) traps, eel traps as well as sophisticated, fast-moving snap traps and suction traps. Putative steps towards the evolution of the carnivorous syndrome in flowering plants, and the diversification of trap types within certain lineages are discussed in the light of dated phylogenetic reconstructions.

T2-01-02

What conditions favor carnivorous plants? Cost/benefit analysis and the context-specific advantages of botanical carnivory

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The cost/benefit model for the evolution of carnivorous plants advanced in 1984 addressed the ecological value of carnivory in detail for the first time. It provided a potential explanation for why carnivorous plants are common in habitats that are not only nutrient-poor but sunny and moist as well, and for how plants should adjust their allocation to carnivory as a function of environmental conditions. Over the past 32 years, this model has stimulated a large amount of research, and provided a conceptual framework for many studies of photosynthesis, respiration, carbon balance, and relative growth rates in carnivorous plants, of the causes of their ecological distributions and variation in allocation to carnivory under different conditions, and of the evolutionary pathways to carnivory. Here we re-assess this model 32 years after its proposal, discuss its predictions and how the model and its predictions can be extended in a number of important ways, and evaluate the extent to which the model's assumptions and predictions have proven correct. We briefly review what is known about photosynthesis, respiration, relative growth rate, and resource allocation in carnivorous vs. non-carnivorous plants, the degree to which various resources are known to limit their growth and stoichiometry, adaptation to different kinds of prey, and what this means about the model's predictions. We conclude by assessing how future studies might test the cost/benefit model for carnivory in more rigorous fashion.

T2-01-03

Floral features, gland distribution and architecture in flowers of Lentibulariaceae with different pollination syndromes

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The aim of our study is to compare glands and other floral features in diverse species from the genera *Pinguicula*, *Genlisea*, and *Utricularia* that are known to be pollinated by different vectors. We analysed four groups of species including potential ornithophilous species such as *Utricularia menziesii, Utricularia quelchii* and several Mexican *Pinguicula* species, entomophilous species, suspected sexually deceptive species (*Utricularia capilliflora* complex), and also sterile species (e.g. *Utricularia bremii*, *Utricularia australis*), that do not produce seeds and therefore don't need pollinators. In entomophilous species from genera *Genlisea* and *Utricularia* the palate possesses diverse micro-morphology comprising unicellular, papillae (conical to villiform) and/or multicellular, uniseriate glandular trichomes. In some entomophilous species, the palate functions as an unguentarius. Nectariferous trichomes are observed in floral spurs. In the sexually deceptive species the nectary spur is reduced, and pollinators are probably attracted largely by the insectiform configuration of the flower and volatilisation of fragrance produced by glandular trichomes distributed upon the modified floral appendages. The flowers of ornithophilous species possess colours that may attract birds, along with well developed spurs that reward pollinators with nectar. Acknowledgements: This study was funded by the National Science Centre, Poland. Contract grant number: DEC-2013/09/B/NZ8/03308.

T2-01-04

Sarracenia purpurea microecosystem as a model system for understanding and predicting aquatic ecosystem dynamics and tipping points

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The northern pitcher plant, *Sarracenia purpurea*, has long been used as a model system for understanding ecological processes such as community assembly and food web interactions. Recently, we have investigated the utility of the *S. purpurea* system to serve as a model system for understanding state change dynamics and identifying early warning signals of tipping points in aquatic ecosystems. Using field and greenhouse experiments, we have shown that a tipping point in the *S. purpurea* pitcher ecosystem can be induced by enriching pitchers with organic matter. The microecosystem undergoes a change from an oxic state to a hypoxic state, characterized by an increase in microbial productivity and resulting rapid decline in dissolved oxygen. This change is similar to changes in larger aquatic ecosystems in which enrichment leads to a shift from a green food web to that of a brown food web, driven by microbial decomposition of organic matter, ultimately causing anoxia or hypoxia in bottom waters. Using metagenomics and metaproteomics, we have identified differences in the composition and function of functionally active bacteria in organic-mater enriched and non-enriched pitchers. Functionally active bacteria in enriched pitchers were dominated by Betaproteobacteria in the family Neisseriaceae and were predominantly facultative anaerobes. In non-enriched pitchers, aerobic bacteria, especially those associated with the family Comamonadaceae, comprised the majority of bacteria contributing to protein expression. Species evenness of functionally active bacteria was higher in non-enriched pitchers. There was more variation among non-enriched replicates in taxa contributing to protein expression, protein identifications, and functional pathway representation than in enriched pitchers. A time series metaproteomic study identified 50S ribosomal proteins (L2 and L5) and molecular chaperone GroEL as potential early warning protein biomarkers of an impending change to a hypoxic state in the *S. purpurea* pitcher microecosystem. The relationship between organic matter concentration and dissolved oxygen concentration in enriched pitchers was nonlinear, displaying hysteretic behavior similar to that between driver and response variables in larger aquatic ecosystems. To date, our results imply that the *S. purpurea* microecosystem can be experimentally manipulated to undergo a state change, that such states are distinguishable from one another using environmental proteomics methods, and that results from experiments using the *S. purpurea* model microecosystem may be scalable to larger aquatic ecosystems.

T2-01-05

Carnivorous plants are ideal model systems for experimental research

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The more than 600 carnivorous plants have evolved in at least six independent radiations within five angiosperm orders. Despite these independent origins, there is a remarkable morphological convergence of carnivorous plant traps, physiological convergence of mechanisms for digesting and assimilating prey, and functional convergence in interspecific interactions between the plants and their associated microbial and macrobial fauna. These convergent traits have made carnivorous plants model systems for addressing questions in plant molecular genetics, physiology, autecology and community ecology, and evolutionary biology. For example, the observation that carnivorous plant genera with morphologically complex traps have higher relative rates of gene substitutions than do those with simple sticky traps has suggested two alternative mechanisms for the evolution and diversification of carnivorous plant lineages. Comparative studies of carnivorous plant physiology and morphology have revealed new types of sticky and wettable surfaces that facilitate prey capture and have suggested new types of biomaterials. Experimental studies of pitcher-plants and their associated infauna have provided compelling examples of convergent interactions that are being used to identify new symbioses in previously unexplored habitats. These microecosystems also have been developed as scalable models for identifying and manipulating rapid state changes in ecological systems. Finally, the widespread popularity of carnivorous plants among people of all ages and nationalities has led to the development of educational curricula and citizen-science initiatives that are advancing both botanical literacy and scientific discovery. Nearly 150 years after Charles Darwin first experimentally demonstrated that plants could attract, trap, eat, and digest insects, we continue to be surprised and fascinated by the knowledge gained from studying these "most wonderful plants in the world."

T2-01-06

Ecological and evolutionary genomics of carnivorous *Nepenthes* pitcher plants

Mathias Scharmann

ETH Zürich

Carnivorous plants of the genus *Nepenthes* are widely known for their bizarrely modified pitcher leaves that can trap and digest animals. While recently much has been learned about the physiology, mechanics and ecology of these enigmatic organs, little is known about the vast variation in the carnivorous syndrome among the 150+ species of the genus. Is divergence within *Nepenthes* dominated by adaptive or neutral processes? Could it be that the carnivorous syndrome is directly involved in adaptative divergence, or even play a key role in speciation? Population genomics (ddRAD-seq, coalescent modelling) of natural sympatric *Nepenthes* communities in Southeast Asia reveal that reproductive isolation is far from complete, and multiple species engage in divergence with gene flow simultaneously. In theory, gene flow between lineages should lead to the erosion of neutral divergence. Maintenance of genetic and phenotypic divergence in the presence of gene flow therefore indicates adaptive processes, presumably through ecological selection against certain hybrids. Thus, many of the differences that are observed among long-term introgressing sympatric species may be adaptive, such as trap morphologies. A transcriptomic approach elucidates for the first time the molecular reaction of *Nepenthes* traps to feeding, and in combination with proteomics identifies genes underlying the digestive process. In a pair of recently diverged sister species, expression divergence across all expressed genes is higher in pitchers than in leaves. Branch-site tests over the transcriptomes of multiple species at phylogenetic and phenotypic extremes reveal that candidate carnivory genes are more commonly under positive (diversifying) selection than expected by chance. Overall, it appears that aspects of the carnivorous syndrome may be under divergent selection in introgressing sympatrics, and are among the most important targets of selection within the *Nepenthes* radiation, at both shallow and deep time scales.

T2-01-07

From prey to predator – Nutrient flows from animals to temperate carnivorous plants (terrestrial Pinguicula, aquatic Utricularia) elucidated with a stable isotope natural abundance approach (^{15}N, ^{13}C)

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Carnivorous plants fascinate humans all over the world due to their habit of trapping animals by creating diverse, extraordinary trap structures. Many studies were performed about carnivorous plants of hotspot tropical and subtropical regions. With exception of *Drosera* L. species knowledge on nutrient-translocation from prey to plant in temperate occasions is rather limited. The utilization of stable isotope natural abundance approaches is an elegant minimal destructive method to analyse nutrient flows from prey to plant as well as within the plant species. Thereby, benefits of a carnivorous life strategy can be elucidated using the stable nitrogen isotope (^{15}N), while the stable carbon isotope (^{13}C) mirrors cost and physiological adaptions to environmental conditions. Terrestrial carnivorous butterworts (*Pinguicula alpina* L. and *P. vulgaris* L.) as well as aquatic bladderworts (*Utricularia australis* R.Br. and *U. minor* L.) from Germany and Austria were analysed for their efficiency in gaining prey-derived nutrients. A stable isotope natural abundance approach with ^{13}C and ^{15}N was applied. (^{13}C) and benefits (^{15}N) of the carnivorous life strategy of the temperate plants (plant-family Lentibulariaceae) were investigated. To calculate the nutrient-uptake efficiency non-carnivorous references (terrestrial autotrophic plants, aquatic phytoplankton) and prey animals (insects, spiders, zooplankton) were used as end-members for the linear mixing model. Prey animals were graded by trophic level to identify trophic-level-dependent influences of plants' enrichment. The temperate carnivorous plants were successful in translocating prey-derived nitrogen to their tissue (nitrogen-uptake-efficiency: *Pinguicula alpina* 63%, *Utricularia australis* 100% and *U. minor* 21%). Main prey-source for *P. alpina* and *U. minor* was phytophagous prey, while zooplankton and prey of higher trophic levels were main sources for *U. australis*. While *P. vulgaris* was shown to be successful in nitrogen gaining from prey in a nitrogen-poor alpine habitat (54%), efficiency-calculation for *P. vulgaris* inhabiting a lowland forest side failed due to an unsuited data structure. Apart from nitrogen the carnivorous *Pinguicula* species utilize a proportion of carbon. The efficiency of terrestrial *Pinguicula* species in gaining prey-derived nutrients is similar to other sticky leaf plants (e.g. *Drosera*). The calculation of the efficiency using the linear mixing model depends on suitable data (difference in isotopic values between references, target plant and prey), which can be influenced by habitat properties (e.g. N-deposition). A certain proportion of carbon is taken up from prey, but habitat properties seem to influence carnivores' ^{13}C values additionally (e.g. stomatal behaviour). Aquatic *Utricularia* species turned out to be equally effective as terrestrial carnivorous plants. The utilization of plankton as end-members of the linear mixing model states itself as elegant method to circumvent issues with a lack of suitable aquatic reference plants. The position of carnivorous plants as indicators for climate change and environmental pollution makes these plants key-species for further studies to better understand influences of anthropogenic hazards and find solutions to preserve unique habitats.

T2-01-08

Phylogenetic analysis of carnivorous plants *Nepenthes* with their phytogeography in southeast Asia and evolutionary trends of the peristom

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Nepenthes is a genus of carnivorous plant that belongs to the monotypic family Nepenthaceae. The genus currently contains 120 species, which are primarily distributed across Southeast Asia. Molecular phylogenetic analysis of this genus had been performed based on the DNA sequence of the plastid *trn*K intron. But further study revealed that the phylogeny of the *trn*K intron did not represent the true phylogeny of *Nepenthes*. Therefore, further phylogenetic study of this genus is required to clarify its evolutionary trends and to establish its infra-generic taxonomy. In total, 57 ITS sequences from 56 *Nepenthes* species were analyzed. Phylogenetic analysis were conducted using 4 different methods: Maximum parsimony, Bayesian analysis, Neighbor Joining, and Maximum Likelihood. Bootstrap values were calculated from 1000 replicates. Character states for distribution areas and the peristome of upper pitchers were mapped onto 1 of the MPTs by using the MacClade program (v4.06), with ACCTRAN optimization, to study the phytogeography of *Nepenthes* in Southeast Asia and the evolutionary trends of their morphological characteristics. Using *A. robertsoniorum* and *D. muscipula* as outgroups, 2 basal branches and 8 subclades were recognized in the phylogenetic tree. The 2 basal taxa were *N. pervillei* from Seychelles and *N. madagascariensis* from Madagascar. Subclade I comprised species distributed in New Guinea and outlying areas; and a widely distributed species. Subclade II contained a species from New Caledonia, a species from Misool Island, and 2 widely distributed species. Subclade III consisted of a species endemic to Sulawesi and a species distributed in Sulawesi and Borneo. Subclade IV and V, both contained species that were restricted to the Philippines and a species endemic to Borneo at the basal position. Subclade VI contained only species from Borneo. Subclade VIIA comprised exclusively 4 species from Peninsular Malaysia and Indochina, a species endemic to Borneo, and a species distributed in Borneo and Sumatra at the basal position. Subclade VIIB comprised 13 *Nepenthes* species from Sumatra with 1 species that was also distributed in Java. Judging from the distribution patterns and phylogenetic relationships, it is possible that *Nepenthes* have expanded from the eastern region to the western region of Southeast Asia. The tree topology suggested that *Nepenthes* had diverged in Borneo, resulting in the evolution of many local species and could have also migrated to adjacent areas. Therefore, Borneo could be a secondary center of diversification for *Nepenthes* in the Sunda Shelf of Southeast Asia. The evolutionary trends of the peristomes in this genus with 3 character states: narrow (<10 mm), intermediate (10-20 mm) and broad (>20 mm), suggested that the narrow peristome is the plesiomorphic state in this genus. The intermediate and broad peristomes likely evolved at least 7 and 8 times, respectively, in this genus, whereas the narrow peristome evolved again at least 4 times after the widening of the peristome. These results suggested that repeated evolution of these 3 peristome characteristics occurred in this genus. Judging from the tree topology, the evolutionary trends of the peristomes revealed the limitedness of Danser's system of classification for *Nepenthes*.

T2-01-09

Convergent evolution of two Mediterranean-climate carnivorous plants: investigating the relationship between investment and reward for *Byblis gigantea* and *Drosophyllum lusitanicum*

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Convergent trait evolution is apparent amongst many of the world's recognised carnivorous plant groups. Adhesive traps, for example, have evolved independently within Caryophyllales (*Drosera* from Droseraceae, *Drosophyllum* from Drosophyllaceae, and *Triphyophyllum* from Dioncophyllaceae), Ericales (*Roridula* from Roridulaceae), and Lamiales (*Byblis* from Byblidaceae, and *Pinguicula* from Lentibulariaceae). With some structural differences, all of these groups possess species with mucilage-producing, glandular leaves capable of insect capture. In this study, we compare two morphologically and ecologically similar yet phylogenetically and geographically distinct carnivorous plant species with adhesive traps: *Byblis gigantea* Lindl. from southwest Australia, and *Drosophyllum lusitanicum* (L) Link. from the western end of the Mediterranean Basin. Both species occur in nutrient-poor and fire-prone environments, within globally significant biodiversity hotspots with Mediterranean climates. Despite their similarities, these two species seem to exist at opposite ends of the spectrum of investment in carnivory: *D. lusitanicum* actively attracts and captures prey with fully vascularised sticky-glands, while *B. gigantea* passively captures prey with simple sticky-glands. To better understand the ecological relationship between degree of investment in carnivory and the degree of success in gaining prey-derived nutrients, we applied stable isotope methodology. Samples of each carnivorous plant, along with neighbouring non-carnivorous plants, prey insects, and soil were collected *in situ*. Leaf and insect tissue samples were analysed to determine d^{15}N, using an elemental analyser coupled to an isotope ratio mass spectrometer. Percentage of prey-derived nitrogen was calculated using a two-source linear mixing model. This study provides empirical evidence for the different reliance on carnivory of *B. gigantea* and *D. lusitanicum*. These differences in nutritional gain link with the differences in gland morphology, providing a framework for the positive relationship between investment in carnivorous traits and success in gaining prey-derived nutrients. Determining how other adhesive-trap carnivorous plants fit into this model will enhance our understanding of convergent trait evolution amongst carnivorous plants globally.

T2-01-10

A possible new carnivorous plant in the monocots, and the molecular evolution of its plastid *ndh genes*

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At least nine independent origins of carnivory are known in five angiosperms orders. Most of these are in the eudicots: there are only four species known to be carnivorous in monocots, all in the order Poales. Here we report a possible new instance of carnivory in the monocot order Alismatales (*Triantha*, Tofieldiaceae), the first report in this order. *Triantha* has four recognized species, three of which occur in North America (*T. glutinosa, T. occidentalis and T.racemosa*), with a fourth in Japan (*T. japonica*). In Canada we found plants in this genus growing in bogs with other carnivorous plants, such as sundew and butterwort. Plants appear to capture insects using sticky glandular hairs on infloresence stalks. Enzymatic tests of phosphatase activity are positive for their trichomes, supporting the possibility that they are carnivorous. We sequenced the plastid genomes of two species (*T. glutinosa* and *T. occidentalis*) and found that one or more of 11 plastid *ndh* genes (which code for subunits of the plastid NADH dehydrogenase-like complex, NDH-1) have been lost or pseudogenized. A similar phenomenon has been observed in some members of carnivorous Lentibulariaceae. We also performed a population-level survey of *ndh* genes in *Triantha* across North America, and find that the degree of gene loss varies among different populations in a manner that is inconsistent with current species boundaries.

T2-01-11

Molecular evolution of the key digestive enzyme nepenthesin II in the genus *Nepenthes*

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Nepenthes is a genus of carnivorous plant that specifically evolved to inhabit marginal environments with nutrient deficiency. *Nepenthes* have adapted the trapping process to live and grow in rainy tropical habitats. They augment their nutrient uptake by carnivory with their pitchers. Morphological adaptations, including wetness-dependent peristome, encourage the prey to fall into the pitcher. Then the body of the prey is drowned by the pitcher fluid and subsequently digested by enzymes within the pitcher fluid. Nepenthesins are major constituents of *Nepenthes* digestive enzymes. Nepenthesin I and II had been purified from *N. distillatoria*, and their partial internal sequences had been determined. However, the molecular genomic study of nepenthesin-encoded genes, in addition to their structure-function relationships, have never been conducted. The structure of nepenthesin-encoded genes might be related to the functioning of genes in response to environmental stress, as the habitat characteristics where *Nepenthes* live. Therefore, the objectives of this study are to examine the molecular evolution of the *Nep2* gene, which expresses the nepethesin II digestive enzyme in pitcher fluid. In the present study, 29 DNA sequences of *Nep2* gene have been succeeded amplified for the first time. The *Nep2* genes from 29 *Nepenthes* species varied in length between 1314-1317 bps, but mostly 1317 bp. All of the DNA sequences of the *Nep2* genes show no introns. Intron-less *Nep2* gene will produce its protein product rapidly for digesting the trapped prey. This rapid production of nepenthesin II enzyme may help to avoid putrefaction of trapped prey, which resulted in an accumulation of ammonium that may harm the pitcher to die. Thus, *Nep2* gene is supposed to have adapted specifically to produce nepenthesin II digestive enzymes rapidly by removing its introns during the course of molecular evolution. All of the enzyme of nepenthesin II examined contained 12 cysteine residues which would form 6 disulphide bonds expected to contribute greatly to the stability of the enzyme and allows the protein to be resistant to protease degradation, thus can remains in the pitcher fluid without digestion. In this study, *Nep2* gene suggestively experienced significant positive selection on *N. sumatrana*, a species inhabits the lowest altitude habitats (0-800 m) amongst Sumatran endemic species, which most of them are highland species. Adaptation to lowland habitats in Sumatra would be influenced by multiple physiological factors. For instance, at the physiological level, an obvious adaptive phenotype is the development of unusual 2 types of lower pitchers of *N. sumatrana*. The functions of both types of lower pitchers are to trap creeping insects. Therefore, the 2 distinct types of lower pitchers of *N. sumatrana* are mirrored by the strategy employed to obtain nutrients from the trapped prey in the lowland habitats. *Nepenthes* are highly adaptable plants and actively respond to the environmental conditions and availability of the prey in their habitats, by changes in phenotypic and genotypic characteristics. These changes are correlated to the fitness of the plants and are supposed under strong selective pressure of nutrient and environment that characterize the habitats of *Nepenthes*.

T2-02: The moss tree of life: phylogenomic approaches to reconstruct moss evolution, diversification, biogeography and biotic interactions

T2-02-01

Phylotranscriptomics reveals widespread gene duplication associated with the diversification of pleurocarpous mosses

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The pleurocarpous mosses (Hypnanae) are the largest group of extant mosses, and share a common ancestor less than 100 million years ago. The most speciose families within the Hypnanae experienced a rapid early burst of diversification, associated with a change in basic growth form (pleurocarpy). In addition to making phylogenetic inference among pleurocarpous moss groups difficult, the radiation likely left signatures of genome evolution observed in other plant radiations, including evidence of whole genome duplication, gene family expansion, and changing evolutionary pressures. Using transcriptomes assembled from over 90 moss species, including proto-pleurocarps and acrocarpous mosses, we constructed more than 27,000 orthologous gene families. Using a species phylogeny constructed from over 600 single-copy loci, we mapped the expansion and contraction of gene families. Gene family expansions greatly outnumbered gene family contractions, and one-sixth of all gene families expanded in size in the ancestors of pleurocarpous mosses. We test whether the proliferation of gene families is associated with paleopolyploidy, discuss the enrichment of functional annotations in expanded gene families, and examine the possible origin of pleurocarpous moss gene families with no homology in currently published genomes.

T2-02-02

Do moss host phylogenetics shape microbiome composition and function?

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Bryophytes and their associated microbes are critical components of carbon (C) and nitrogen (N) cycling in arctic tundra and boreal forest ecosystems. Mosses account for approximately half of the net primary productivity in these biomes, and moss-associated microbes are the major source of biologically fixed N into these nitrogen-limited ecosystems. The extent to which climate change will perturb the ecosystem C and N dynamics critically depends upon how the moss-microbiome assemblages respond to new temperature and precipitation regimes. Here we evaluate the roles of environmental factors as opposed to moss host genotype in shaping moss-microbiome community assembly and function. We collected ten moss species from multiple sites near Fairbanks and Toolik Lake, Alaska. We used stable isotope incubations to estimate the rates of nitrogen and methane fixation in individual samples, 16S amplicon sequencing and shotgun metagenomic analyses to survey the community of bacteria hosted on each sample, and sequence capture to generate a moss phylogeny. Microbiomes from different moss species and sites fixed variable quantities of N and C. We found high relative abundances of members of novel bacterial phyla as well as a high diversity of putative N_2-fixing bacteria. Furthermore, we identified large numbers of nifH sequences from these metagenomes, including many from poorly described lineages. We are currently developing approaches to link biogeochemical processes to specific bacterial communities to understand the role of moss phylogeny in shaping microbiome function, and the potential for climate change to influence the function of the moss-microbiome.

T2-02-03

The Carboniferous set the stage for the major moss lineages which later experienced multiple independent rate shifts

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Uncovering the branching order of major moss lineages is central to reconstruct the transformation of morphological traits during their evolution. This is particularly critical for understanding the order in which major peristome types arose. Current reconstructions mainly based on multi gene approaches either using chondrome data and/or coding regions from the plastome are inconclusive, as the short deep backbone nodes splitting the major clades receive inconsistent support. We generated a multi loci data set using mainly fast evolving regions from both organellar compartments. This data set comprised 15,759 characters (plastome: 8,576 char; chondrome 7,183 char.) plus 1,719 indels generated by simple indel coding. Phylogenetic analyses (Bayes & RAxML) yielded a highly supported backbone phylogeny of mosses that revealed the nematodontous lineages (Polytrichopsida & Tetraphidopsida) as a grade leading towards the arthrodontous mosses (Bryopsida). Among arthrodontous mosses the two first branching lineages contain mosses characterized by an unique endostome architecture (Buxbaumiidae & Diphysciidae) followed by the gymnostomous or cleistocarpous Gigaspermidae. Gigaspermidae are followed by the Funariidae indicating the diplolepideous-opposite peristome as the ancestral peristome type of which the haplolepideous (Dicranidae) and diplolepideous-alternate (Bryidae) peristome as well as the *Timmia*-type was derived. According to the BEAST analyses these major peristomes types were already established 300-350 MYA, indicating that the Carboniferous set the stage for the split of the major moss lineages (subclass) known today. Overall four independent rate shifts (BAMM) were detected, of which two affect the two biggest moss lineages, i.e. the haplolepideous (~ 4000 species) and the pleurocarpous mosses (~ 5000 species), that experience rate shifts at different stages of earth history. Both lineages are not only characterized by different peristome types (haplolepideous versus diplolepideous-alternate) but also by their different growth form (i.e. acrocarpy versus pleurocapy). The BEAST analyses based on an extended data set including a representative sampling of all other major land plant lineages indicate that the rate shift at the pleurocarpous branch in the Cretaceous can be associated with the radiation of angiosperms, i.e. new habitats, especially epiphytic habitats. Most likely the exploitation of the new habitats was aided by the invention of pleurocarpy. In contrast, the haplolepideous lineages represent a much older radiation experiencing a rate shift already in the middle/upper Jurassic that leads to a diversification in the Cretaceous. This finding goes in line with the increase in origination of spore bearing plants in the recovery phase of the late Permian mass extinction. Whether the evolution of the haplolepideous peristome that facilitates spore distribution enabled a higher colonization and origination rate needs to be shown.

T2-02-04

Sex-specific gene expression in the pleurocarpous moss species *Brachythecium rivulare*

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Molecular data on structure and evolution on sex chromosomes on early land plant lineages including mosses, liverworts and hornworts with ca. 20 000 species is currently based only on five species. Among mosses presence of sex chromosomes has been confirmed only in one species, but otherwise genetic regulation of sexuality remains unstudied. Based on scattered phylogenetic position of dioecious species, shifts to separate sexes have taken place several times independently among mosses and even within families, such as Brachytheciaceae. Evolution of characters that are directly linked to reproduction, such as position of female gametangia and sporophytes on moss shoot have also had important consequences in diversification of mosses. Evolution of pleurocarpy, a condition where female gametangia develop on reduced lateral branches may have resulted in diversification one of the most specious group of bryophytes, pleurocarps. The aims of the project were to 1) Identify differentially expressed (DE) genes between female and male samples in *B. rivulare*. 2) Find out whether DE genes are unique to pleurocarpous and/or dioicous mosses. We identified DE genes that are present in pleurocarps but are absent in Physcomitrella patens, the hermaphrodite acrocarpous model species. 3) Investigate if the DE genes are expressed both in autoicous and dioicous species by comparison to several Brachytheciaceae transcriptomes. We sequenced the transcriptomes of male and female samples of the dioicous, pleurocarpous moss species *Brachythecium rivulare* (Brachytheciaceae) using Illumina short read sequencing platform. The high-throughput sequencing was followed by *de novo* transcriptome assembly. The transcriptome data was compared with the transcriptomes of six Brachytheciaceae species, one other pleurocarp, Anomodon rostratus, and Physcomitrella patens. Differential expression between the sexes was detected in 820 expressed transcripts, representing 770 open reading frames. A search for orthologous sequences revealed 102 of them to be present in at least four other pleurocarpous mosses but not in Physcomitrella. Most of them were present in both autoicous and dioucous mosses. These genes will be analyzed in detail to learn more about their function and evolutionary history. BLAST searches against land plant, bacteria, fungi and other eukaryote data bases will show if they are ancient but lost in Physcomitrella, or synapomorphous in pleurocarps.

T2-02-05

The origin of the model taxon *Physcomitrella patens*: Resolving a rapid diversification using a phylogenomic approach based on enriched genomic libraries

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The Funariaceae comprise about 250 species of mosses, mostly specialist of ephemeral habitats, including *Physcomitrella patens* and *Funaria hygrometrica*, two taxa frequently used as model organisms. From the morphological point of view, the Funariaceae combine a rather uniform gametophytic generation with a sporophyte that greatly varies across taxa, displaying a diverse range of architectures. The evolutionary history underlying this morphological diversity is obscure. Historically, relationships among species were drawn from shared sporophytic traits but inferences from DNA data revealed that the transformations of the sporophyte were homoplasious, and hence poor indicators of shared ancestry. Furthermore, the reconstruction of ancestral traits, and hence of the polarity of the transformations was hampered by the much of the diversity from a rapid diversification. To resolve the relationships among lineages within the Funariaceae, we combined high throughput sequencing and enriched genomic libraries to obtain the full organellar exome plus a selection of about 600 single copy nuclear genes from a representative selection of 91 Funariaceae. The analysis of these datasets provides a new timeframe for this diversification plus an increased resolution among taxa. As a result, we confirm the impact of sporophytic homoplasy across the family and making possible a comparison among morphological and genomic divergences. Our results also provide a genetic framework for the model organism *P. patens* concluding that extreme sporophytic reductions are not linked to significant genetic divergence in the studied datasets.

T2-02-06

Resolving the backbone phylogeny of mosses, using targeted NGS data from plastid, mitochondrial and nuclear genomes

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Mosses compose a diverse lineage of land plants that arose soon after the conquest of land. The evolution of mosses is marked by transformations of the sporophyte and gametophyte generation, with ontogenetic changes of the spore-bearing generation defining the major lineages (i.e., classes) of mosses. However, homology among characters and their states remains ambiguous, and hence the relationships among these lineages are unresolved. Further, inferences from DNA sequences highlighted several lineages lacking specific apomorphies (e.g., Oedipodiales, Gigaspermales) in likely critical position along the backbone of moss tree of life, adding to the difficulty of reconstructing the transformation of the sporophyte. Finally, phylogenetic inferences have been drawn from few discrete loci that often lack the power to resolve the relationships along the tree. Here we present the first inferences based on variation in all organellar and close to 150 nuclear protein-coding genes sampled based on a liquid phase enrichment strategy for 134 exemplars of all orders of mosses. The conserved nature of the targeted loci avoids, following critical bioinformatics processing and manual inspection, uncertainty or conflict arising from alignment issues and hence the exclusion of data. Furthermore, the gene sequences can be translated and the signal of the nucleotides and amino acids compared, to assess artifacts due to saturation. We explored the homogeneity of the signal across loci and genomes, the congruence among topologies inferred from each of the three genomic compartments, and finally the distribution of the support among nuclear loci for individual nodes along the backbone. The moss tree of life is well supported at most nodes, but ambiguity remains in particular regarding the monophyly of mosses with nematodontous peristomes (i.e., Tetraphidales and Polytrichales), and their shared ancestry with the Oedipodiales, the sister-group to the Funariales, and the lineages emerging from the diversification of mosses with a diplolepideous alternate peristome. The monophyly of the Hypnanae (pleurocarpous mosses) is robust and their sister-group consistently consists of the Aulacomniaceae only. The Takakiales are resolved as sister to the Sphagnales based on chloroplast data but mitochondrial and nuclear data support them being sister to all mosses. The origin and evolution of the arthrodontous peristome and its derivation from a nematodontous one remain uncertain and depend on the resolution of relationships among the Nematodonteae, but also further ontogenetic studies to establish homology. However, with the arthrodontous mosses, those with an opposite arrangement of exostomial teeth and endostomial segments compose a grade leading to the lineages wherein teeth and segments alternate. Our study highlights the benefits of a broad genomic sampling and the advantages of using coding regions for resolving ancient evolutionary events (e.g., older than 150 my) but also draws attention to the need to investigate the nature of the support, as robust support can be drawn from few, or at least a minority of loci, and that the majority of the loci may not be congruent in the signal that they carry for a particular node.

T2-03: Ericaceae – phylogeny, biogeography and evolution

T2-03-01

Evolution and diversification of Ericaceae - Insights from whole plastome sequences

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The Ericaceae clade is remarkable for its diversity of growth form, physiology, anatomy and morphology and includes a diversity of mycorrhizal associations greater than any other angiosperm family. To date, most of the major clades within Ericaceae have been identified and the relationships among these groups has been resolved to some extent from phylogenetic analyses of selected regions within the chloroplast and nuclear genomes. The results of these analyses agree with most of the previous studies, but the relationships at the deeper nodes of Ericaceae are still not strongly supported. Previously published complete chloroplast genomes of *Arbutus unedo* (strawberry tree) and *Vaccinium macrocarpon* (cranberry) showed unexpected levels of diversity in gene order and content and suggest that plastome structure and content within the Ericaceae may vary significantly reflecting the already documented variation in habit, mycorrhizal association and morphology. We sequenced the plastome of more than 40 representatives from the eight currently recognized subfamilies (Enkianthoideae, Arbutoideae, Monotropoideae, Cassiopoideae, Ericoideae, Harrimanelloideae, Epacridoideae, Vaccinioideae) and 20 named tribes. Among these we obtained plastomes that included representatives of different growth forms and functionalities: e.g., green and non-green (mycoheterophic); different mycorrhizal associations (vesicular-arbuscular, arbutoid, ericoid, monotropoid, cavendishioid); tropical cloud forest, open heathlands, boreal habitats; woody, herbaceous. Preliminary results indicate that ericaceous plastomes are much more variable in gene order, content and size than expected. In addition, some plastomes showed organizational shifts/changes that were not obviously correlated with, e.g., physiology, but were coincident with previously identified major clades within Ericaceae. Further analyses of plastome variation across the ericads indicates that even within closely related groups, e.g., Vaccinieae (blueberries), the plastome can significantly vary in size and gene content. These new findings provide important insight into the evolution and diversification of the Ericaceae and its biogeography, while also indicating that there is likely a more diverse and dynamic pattern of plastome evolution in general than previously thought.

T2-03-02

Historical biogeography of the Australasian Ericaceae: Evaluating competing vicariance and dispersal scenarios

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The subfamily Epacridoideae, Ericaceae (commonly known as the epacrids) comprises 37 genera and ca. 640 species. This clade includes mostly perennial woody shrubs that occur in heathlands, sandplains, and tropical forests. Their distribution is mostly restricted to Gondwanan fragments (Australia, New Zealand, New Caledonia, and South America) with outliers in SE Asia and Hawaii. The epacrids are however most diverse in South-Western (WA) and South-Eastern Australia (EA). The Epacridoideae also typify many of the controversies in the historical biogeography of Australasia and the contributions of long distance dispersal versus Gondwanan vicariance. Such hypotheses can be tested with molecular dating and ancestral area reconstruction analyses. While a vicariance scenario predicts patterns of near-simultaneous divergences in multiple lineages, long-distance dispersal predicts independent, asynchronous divergences. We present the most densely sampled phylogeny to date that includes representatives from all genera and ca. 65% of the species in the Epacridoideae. Based on a 5-gene dataset (the nuclear ribosomal ITS region, and the chloroplast regions *rbc*L, *mat*K, *psb*A-*trn*H, *atp*B-*rbc*L) we estimated the phylogenetic relationships and tempo of evolution within the subfamily with a focus on the relationships within the Australian clades, and their relationships with the New Zealand and New Caledonian clades. Furthermore, we used the resulting phylogeny to evaluate multiple calibration schemes, molecular clock models, and speciation priors, and estimated the ages of the disjunctions between these clades. Our results under different schemes consistently suggest that the multiple disjunctions between the Australian, New Zealand, and New Caledonian clades are independent events and not older than ca. 10 Ma. They also indicate that the WA and EA clades diverged approximately over the same period of time during the Miocene (10 – 20 Ma), after the emergence of the Nullarbor Plain. We conclude that the current distributional patterns of the epacrids can be explained by both vicariance and long distance dispersal events.

T2-03-03

Ericaceae phylogenetics revisited: Developments and prospects

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The Ericaceae, with ca. 4200 species and 125 genera, has a near-global distribution and many taxa of great economic (e.g. blueberries, cranberries, and rhododendrons) and ecological (e.g. northern heathlands, montane-heath shrubberies, and Cape heaths) importance. Here, we review the current state of systematics of the family, highlight where major problems exist, and evaluate how close we are to a well-resolved comprehensive phylogenetic estimate for all taxa. Towards this we briefly discuss the recent literature, and present new phylogenies based on a DNA sequence matrix sampling 22 regions and 2027 species (ca. 50% species-level coverage, with 5-96% coverage per marker). The resulting phylogenies are then compared to the prevailing classification system of Kron *et al.,* (2002). Additional studies on the systematics and historical biogeography of the family will be outlined and discussed, as well as directions for future research.

T2-03-04

Biogeography, classification, breeding system evolution, and new species discovery in the Gaultherieae (Ericaceae)

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The tribe Gaultherieae (Ericaceae: Vaccinioideae) contains ca. 280 species with a circum-Pacific distribution that encompasses a wide range of bioclimatic areas. We employed an eight-gene phylogeny based on three nuclear and five plastid gene regions to reconstruct the biogeographic history of the tribe, reassess subdivisional classification, and trace breeding systems evolution. Results of combined Bayesian divergence time and ancestral area analyses recovered an origin for crown clade Gaultherieae of ca. 33 Ma in temperate North America. Of the 15 dispersals inferred from the analysis, five occur across the Northern Hemisphere, and seven occur from north to south. Two of the latter are from temperate North America to tropical America, and three from temperate East Asia to tropical East Asia, consistent with global drying and cooling during this time. In addition, one dispersal is inferred from tropical America to temperate South America, one from temperate South America to New Zealand, and one from New Zealand to Australia. All 15 dispersals occurred later than 23 Ma, and so the dispersal from South America to Australasia must have been over-water, likely by birds because an analysis of fruit type and color reconstructed the ancestor of the Australasian lineage as fleshy and red. *Gaultheria* exhibits variation in breeding systems, with hermaphroditic, gynodioecious, and dioecious species. Tracing the evolution of breeding system onto the phylogeny recovered the evolution of gynodioecy from hermaphroditism in two major clades, i.e., Himalaya-Hengduan Shan species and the clade of temperate South American and Australasian species. In the latter clade, full gynodioecy evolved in temperate South America. Gynodioecy had already evolved in the ancestral lineage that migrated from South America to Australasia, and then descendants largely retained gynodioecy in New Zealand, confirming the allochthonous origin of dicliny in New Zealand for the genus as previously hypothesized. Because *Diplycosia* and *Tepuia* are nested within *Gaultheria*, these three genera are best treated either as a single genus (*Gaultheria*) or as six genera, i.e., with the former two genera retained as is and *Gaultheria* divided into four genera. We prefer the former because one of the large clades that would require its recognition as a genus is not diagnosable with morphology. The infrageneric classification of *Gaultheria* requires revision at the sectional level. Through the combination of field, herbarium, and molecular studies we have discovered 14 new species in the tribe from China and SE Asia. Because most species grow in high elevation zones with difficult access, further exploration in these areas are likely to uncover many more.

T2-03-05

Fruit color variation and diversification of a circum-Pacific clade of plants, the tribe Gaultherieae (Ericaceae)

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Fleshy fruit is generally thought to have evolved for effective seed dispersal by frugivores. However, the variation of fleshy fruit colors and its adaptive significance in plant diversity has received little attention in an evolutionary context. Here we investigate fruit evolution in the circum-Pacific ericoid tribe Gaultherieae, a clade with a wide range of fruit types and colors, with diversification and correlated evolution tests in a phylogenetic context. We found evidence for independent diversification rate accelerations facilitated by a switch from red to the colors in the violet part of the spectrum (blue, purple, or black), suggesting that violet fruit is a key innovation in the tribe. We show that violet fruits predominate in either low-latitude or high-elevation environments. With phytochemical analysis, we isolated cyanidin 3-O-*β*-D-xylopyranosyl- (1→2)-β-D-glucoside and delphinidin 3-O-β-D-xylopyranosyl- (1→2)-β-D–xylopyranoside from red and violet fruits of the Gaultherieae, respectively, and detected no pigments in white-fruited species. Dephinidin and its derivatives have been found to have greater absorption in the UVB portion of the spectrum than cyanidin. Because UV intensity increases with elevation and decreases with latitude, the spatial distribution of fruit color suggests that the the delphinidin derivative in violet fruits functions as a stronger UV protectant for progeny (seeds) than does the cyanidin derivative of red fruits. This can be tested more directly by experiments involving the insertion of UV-detecting microprobes into Gaultherieae fruits of various colors.

T2-03-06

Optimizing RADseq methods and inferring histories of diversification of rhododendrons in mountain ranges of Asia

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The genus *Rhododendron* consists of about 1000 species with centers of diversification in the Himalayas and the Malesian islands. The Himalayan diversification is predominantly represented by subgenus *Hymenanthes* with 225 species historically classified into 24 subsections (Chamberlain 1982). The Malesian diversification is primarily represented by subgenus *Rhododendron* section *Schistanthe* (formerly sect. *Vireya*) with 320 species currently classified into 4 subsections (Craven *et al.*, 2011). Previous molecular phylogenetic studies on these two groups using several loci showed conflicting data or lack of resolution in phylogenetic reconstructions. Therefore, in this study, we employed genomic sampling of loci using single-digest restriction site associated DNA sequencing (RADseq) with *PstI* to infer biogeographic histories for subg. *Hymenanthes* and sect. *Schistanthe*. We sampled 90 species from all subsections of subgenus *Hymenanthes* and 108 species from all subsections of sect. *Schistanthe*, as well as 31 outgroups sampled from all subgenera of *Rhododendron* and several closely related genera. We compared results from denovo versus reference guided assemblies of our RADseq data. We also compared results using single-end RAD sequences (100 bp) versus paired-end RAD contigs (mean 450 bp). Resulting phylogenetic analyses based on entire RAD loci were calibrated with several primary and secondary calibration points using penalized likelihood for ancestral area reconstructions. Our preliminary results infer an origin of subg. *Hymenanthes* in northeast China during the Oligocene with subsequent radiation into the Himalayas during the Miocene. For sect. *Schistanthe*, we infer an origin in Indochina during the Miocene with subsequent radiation into the mountains of Borneo beginning in the Miocene and New Guinea beginning in the Pleistocene.

T2-04: Phylogenomic perspectives on flagellate plant evolution

T2-04-01

Can ancestral state reconstructions of dioecy in mosses be Independently confirmed using coalescence of sex linked genes?

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A major goal of many phylogenetic analyses is to reconstruct the evolution of key organismal features. Although sophisticated methods exist to reconstruct ancestral states, in general, there is much uncertainty in the reconstruction of evolutionarily labile traits, even in groups with a rich fossil history. Here we take advantage of unusual features of the moss sex determining system to evaluate our previous reconstruction of sexuality across the mosses. Sex in dioecious mosses is often determined by a UV sex chromosomal system. In this system, each sex has a non-recombining chromosome (U for females, and V for males) that pair at meiosis in the monomorphic sporophyte, and segregate to the male and female haploid gametophytes. Because the sex chromosomes are transcriptionally active in the haploid stage, and therefore subject to purifying selection, we expect many homologous genes will be retained between the U and V chromosomes. Thus, we expect that the most ancient sex-linked loci should coalesce at or below nodes where the common ancestor was reconstructed as dioecious. To find sex-linked genes, we first sequenced transcriptomes from ten male-female sibling pairs of the moss *Ceratodon purpureus*. Next we assembled the transcriptomes using standard genome-guided RNAseq approaches. Since the reference genome was female, we de novo assembled the unmapped male reads and used BLAST to identify female homologs for these transcripts. We used a genetic map to confirm the sex linkage of many of these loci. We estimated Ks between the male and female homologs, and retained those with Ks > 0.1 for further study. We identified additional homologs using the 1KP data, and we built phylogenetic trees using RAxML to estimate the placement of the coalescence between the *C. purpureus* male and female homologs. We are currently increasing taxon sampling to test the accuracy of multiple ancestral reconstruction methods.

T2-04-02

GoFlag: A next generation sequencing perpective on flagellate plant phylogeny

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GoFlag is an NSF-funded project to construct a species-level phylogenetic tree of thousands of flagellate plant species, including bryophytes, lycophytes, ferns, and gymnosperms, and link these trees to relevant phenotypic, environmental, and geographic data. We report on a pilot study to use a sequence capture approach to generate a common set of ~500 low-copy nuclear loci and well as chloroplast data for phylogenetic inference across all flagellate plant groups. The pilot study is based on 192 phylogenetically diverse flagellate plant species. We discuss the methodological challenges of our approach as well as analytical issues of analyzing this next-generation sequence data. We also discuss new approaches for linking metadata to the phylogenetic tree for evolutionary and ecological analyses. Although constructing a well-resolved phylogenetic tree for flagellate plants is a difficult and complicated endeavor, new sequencing technologies and analytical methods provide much promise for revealing the rich history of flagellate plants, and we describe how the GoFlag project will expand upon this pilot study to build a comprehensive flagellate plant tree.

T2-04-03

A phylogenomic analysis of the cycad genus *Cycas* using RADseq

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Relationships among cycad species have long been difficult to infer, principally due to their recent rapid radiation and the reliance on data from few, relatively slowly evolving, uninformative markers. Next Generation Sequencing (NGS) data offer promise for resolving these relationships through the sheer vast number of rapidly evolving nuclear markers. Since cycads have large genomes (~14,500 Mb), we used a reduced-representation sequencing approach in the form of RADseq (Restriction-site Associated DNA sequencing). Here we focus on *Cycas*, the largest cycad genus, which has over 100 species and is the sole member of Cycadaceae. We generate a phylogeny for three-quarters of species, covering the range of *Cycas* in Asia, Australia, Madagascar, India and the southwestern Pacific Ocean islands. Using the RAD phylogeny, we infer species relationships, assess biogeographic patterns, and evaluate the current classification of the genus.

T2-04-04

A phylogenetic approach to assessing the evolution of genomic repetitive elements across the fern family pteridaceae

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Due to their large genome sizes and high chromosome numbers, ferns have been sidelined from most phylogenomic and comparative genomic analyses. While published fern genomes are still forthcoming, low-coverage genome skimming has provided initial glimpses into the broad-scale genomic composition of these plants, particularly with respect to their abundance of non-coding repetitive elements relative to seed plants. That said, the relative contributions of transposable elements and other repeat types within major fern lineages remain unexplored. Here, we present the results of a comparative study of repeat content, in a phylogenetic context, by analyzing genome skimming data for 32 species across the polypod fern family Pteridaceace. This family accounts for approximately 10% of extant fern diversity and comprises five monophyletic clades spanning diverse ecologies and morphologies. We assess whether the abundance of various repetitive elements are correlated with phylogenetic distance and genome size. This work lays the groundwork for exploring the role of repetitive elements in shaping genome structure, content, and size across fern family, and greatly expands genomic resources for these plants.

T2-04-05

Evolutionary divergence in the coralloid root microbiome of cycads

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Cycads are the only gymnosperms and seed plants that have evolved a specialized coralloid root to host endophytic bacteria. There is no precedent of exploring the taxonomic, phylogenetic or functional diversity of the microbiome of this million-year old association. We provide a genomic characterization of the cycad coralloid root microbiome of the Mexican *Dioon* species collected from natural populations, and yield insights on its evolutionary history. We explored the microbiome of the coralloid root employing a co-culture-based experimental strategy to unveil rare bacteria, in par with phylogenomic analyses to capture the biological diversity and the presence of Bacterial Genetic Clusters (BGCs) associated to specialized metabolites. Most taxa were identified as diazotroph plant endophytes and included known and previously undescribed Cyanobacteria OTUs. We sequenced three of their genomes, which formed a monophyletic group that suggests a level of specialization typical of obligate or closely co-evolved symbiotic relationships. This contrasted with our finding of their large genome sizes, typical of facultative endosymbionts of complex alternative lifestyles. Nine out of 23 novel BGCs are specific to these coralloid root endophytes. Testing of vertical transfer of the root endophytes via the seeds, gene expression profiles of the coralloid roots, and population genetics of the genus is ongoing. Combined, our results thus far show that the highly diverse taxonomic, phylogenetic composition of the coralloid root, and a specialized biosynthetic repertoire, correlate more with specificity to the *Dioon* plant host than other closely related plant endosymbionts, or to the environment. We add to the growing notion that symbiotic relations occur under heavy influence of chemical and genomic interactions, providing a rich source of novelty for drug discovery and understanding of the evolution of gymnosperm-bacteria symbiosis.

T2-04-06

Analyses of structure-function relationships in the evolution of embryophyte shoot meristems: A macroevolutionary transcriptomic approach

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Shoot apical meristems (SAMs) have increased in complexity over the course of embryophyte evolution from single apical initials that generate the gametophytic body plans in bryophytes, to the histologically stratified, multicellular SAMs that give rise to the entire sporophytic shoot in angiosperms. Despite this diversity in shoot apical structure, our understanding of the genes involved in SAM function is largely limited to the angiosperms. We used laser microdissection paired with RNA-sequencing (LM-RNA-seq) to generate shoot meristem molecular profiles for species from anciently evolved land plant lineages. This data was used to identify genetic networks that correlate with three major advances in embryophyte SAM structure: (1) the transition to multidimensionally growth; (2) the rise of indeterminate apical growth in the sporophyte generation; and (3) structural diversification of the sporophytic SAM in seedless vascular plant lineages. Our comparative transcriptomic analyses contribute to new molecular models for these pivotal events in embryophyte evolution. Specifically, we propose that the combination of asymmetric cell division and shoot meristem maintenance programs is associated with multidimensional shoot meristem function in the moss *Physcomitrella patens*. In addition, we suggest that pleiotropic gene expression in both the gametophytic meristem and the non-meristematic sporophyte of gametophyte-dominant plants contributed to the rise of indeterminate SAM growth in the sporophyte generation of early polysporangiophytes. Finally, we present transcriptomic evidence for the convergent evolution of meristem structures in the seedless vascular plant lineages containing *Selaginella* and *Equisetum*. The comprehensive nature of the data leads to testable, functional models for developmental genetic programs that contributed to fundamental strategies in embryophyte evolution.

T2-05: Hybridization and phylogenetic reconstruction

T2-05-01

Phylogenetics and the discovery of plant hybrid lineages

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Reticulation is a pervasive issue in phylogenetic inference. Because hybridization is not normally something you look for but something you run into, you may face it under two perspectives. By far the first, and most common one, is trying to account for hybridization avoiding the disturbing effects that it introduces in the reconstruction of phylogenetic relationships of group of organisms in which we assume a predominance of divergence processes. Under this focus, hybridization events are hurdles that one has to overcome mainly by telling apart such events from incomplete lineage sorting (ILS). Different approaches have been developed for this task, which is particularly challenging when tracing both old events and those lying in the so-called 'coalescent stochasticity zone' in which attributing gene conflict to hybridization is hampered by the high frequency of non-coalesced genes. The second perspective is to focus on the process itself, i.e., on hybridization events associated to 'discordant' lineages. The main contribution of phylogenetic studies to this aim is unveiling unsuspected hybrid lineages. However, compared to studies of speciation that usually focus on reproductive isolation, phylogenetic studies also provide a systematic and evolutionary frame, including a temporal component, that represent a basis for understanding the evolutionary consequences of the hybridization event (s). Whether the merging of two genomes contributes positively to evolution depends on a number of factors that need to be explored in each case. Phylogenetic approaches may provide assessments of factors such as how closely related are the hybridizing species, how old are their speciation events, how old is the hybridization event, or how close is the hybrid lineage from the progenitors from a genetic standpoint. A quick look at cases of reticulation in phylogenetically oriented studies suggests a wide range of situations in which hybridization is involved and also shows how such studies can throw light on the circumstances surrounding hybridization events, making phylogenetics a relevant complementary approach to the field of hybrid speciation.

T2-05-02

Genomic relationships of recent species radiations

Alex Twyford
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Phylogenetic reconstructions with next generation sequencing data may resolve challenging species relationships where Sanger Sequence data have failed. Restriction site associated DNA sequencing (RAD-seq) and related approaches are particularly promising for phylogenies of non-model taxa due to their scalable recovery of many homologous regions. However, this wealth of sequence data may still fail to resolve relationships in recent species radiations where hybridisation and incomplete lineage sorting are common. I present genome-scale phylogenies of diverse plant taxa that demonstrate how geography rather than current taxonomy frequently explain genome-wide clustering in closely related species complexes. In contrast, taxonomy may be supported by a subset of causal loci underlying traits of interest. These results caution against using concatenated data matrices to delimit taxa in recent radiations. This also highlights the need for careful analysis of gene genealogies for regions that may promote species divergence.

T2-05-03

Inferring species trees given coalescence and reticulation

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Hybridisation is an important process in plant evolution, its impact apparent at all levels from the genome duplications shared by all angiosperms through to recent speciation events. Patterns of inheritance caused by hybridisation (and other reticulate processes) appear to be incompatible with one of the fundamental tools of evolutionary biologists and ecologists seeking to understand the evolution of biological diversity: the phylogenetic tree. The current paradigm for inferring relatedness of organisms – the 'species tree' – can be summed up in one word: coalescence. Current analytical approaches serve to bias against inferring reticulate processes, even though they may be common and of direct importance both for the evolutionary process itself and for the performance of methods used to infer it. I present a brief account of current methodologies and draw on examples from both plant and virus datasets to illustrate the importance of reticulate processes in evolutionary inference. An example from danthonioid grasses shows how our inference of the direction and frequency of long distance dispersal events can be impacted by post-dispersal hybridisation; one from the northern heathers (genus *Erica*) shows the impact on interpretations of morphological evolution. In viruses, recombination can lead to pathogenic strains, and our ability to infer this process, even on human timescales, is dependent on correctly interpreting differences between gene trees. I illustrate an easily implemented approach that may be used to infer the sequence and timing of gene and genome divergences given conflict between individual gene trees, even when the processes underlying that conflict cannot be distinguished. It can in principle be applied to any group of organisms and can be extended to explicitly model both reticulation and coalescence without prior knowledge of the species tree topology.

T2-05-04

Assessing ancestral niche suitability and geographic range dynamics as drivers of hybridization in *Heuchera* (Saxifragaceae)

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Hybridization has received a resurgence of attention from evolutionary biologists, owing to powerful approaches using phylogenomics and the coalescent model that enable their robust detection with molecular data. Sometimes, ancient hybridization has been detected among lineages that are not (currently) extant, consistent with our knowledge of the historically dynamic geographic ranges of taxa. Recent developments in the exploration of niche space using phylogenies have enabled explicit tests of these kinds of hypotheses. As an empirical case study, we demonstrate how the integration of a recently developed ancestral reconstruction approach, using ecological niche models of extant species and a robust phylogeny, can test whether ancestral co-occurrence could have enabled historical hybridization among widely disjunct taxa. Extremely high incongruence between nuclear and chloroplast data has previously been demonstrated in *Heuchera*; coalescent simulations favored a hypothesis of hybridization over incomplete lineage sorting to explain this incongruence. Taxa inferred to have hybridized are as much as hundreds of kilometers disjunct. We show that ancestral parapatry under a Pleistocene glacial scenario likely explains gene flow, and how novel Bayesian applications of ancestral niche reconstruction allow projection of ancestral suitability into past climate models to produce geographic maps of suitable range.

T2-05-05

Population genetics, phylogenomics and hybrid speciation of Chinese *Juglans* (Juglandaceae) determined via whole chloroplast genomes, transcriptomes, and Genotyping-By-Sequencing (GBS)

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Genomic data provide powerful tools for elucidating the processes involved in the evolution and divergence of species, the fundamental units in the fields of ecology, population genetics, and biogeography. We used phylogenomic and population genetic analyses of data from transcriptomics, Genotyping-By-Sequencing (GBS), and whole chloroplast genomes (Cpgs) to infer processes of lineage formation among the five native Chinese species of the walnut genus (*Juglans,* Juglandaceae), a widespread, economically important group. We also used ecological niche modeling and morphological analysis to verify ecological speciation. Our analyses supported the genetic distinctiveness of *Juglans regia*, *J. cathayensis*, *J. mandshurica*, and *J. sigillata*. Comparisons among whole chloroplast genomes and nuclear transcriptome analyses provided strong evidence that *J. hopeiensis*, a species considered threatened, arose through relatively recent hybridization between *J. mandshurica* (female parent) and *J. regia* (male parent). The genetic structure of *J. cathayensis* and *J. mandshurica*, two closely related taxa, indicate they are poorly differentiated. GBS data revealed clear introgression/gene flow between *J. regia* and *J. sigillata*, while population genetic analysis detected previously unrecognized deep lineages within *J. regia*, and the presence of individuals with highly differentiated genomes within otherwise geographically delimited populations. Using trees based on chloroplast and GBS markers, we reconstructed the evolutionary history and estimated divergence times of the five Chinese *Juglans.* The divergence time between sect. *Juglans/Dioscaryon* and sect. *Cardiocaryon* was estimated at 62.93 Myr, while *J. regia* and *J. sigillata* diverged much more recently (0.01 Myr). The current spatial distribution of genetic variability among the five Chinese walnut species likely results from ecological speciation based on local adaptation resulting in morphological and floral differentiation, even in the presence of gene flow and introgression. Together our results highlight the outstanding capacity of NGS sequencing data (GBS, transcriptomes, and Cpgs) for elucidating the complex processes of phylogeny, biogeography, and hybrid speciation, particularly among relatively closely related species.

T2-05-06

Natural hybridization and repeated hybrid speciation in Actinidia through genome resampling from rapidly radiated backbone lineages

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Reticulate speciation caused by interspecific hybridization is now recognized as an important mechanism in creating biological diversification. However, depicting the pattern of phylogenetic network for lineages that have undergone inter-specific gene flow is challenging, and the difficulty can be further compounded by the presence of ancestral polymorphisms owing to incomplete lineage sorting (ILS). We sequenced 25 taxa representing the high diversity deposited in the genus *Actinidia* with an average mapping depth of 26× on reference to reconstruct their reticulate history. Using genome-wide data, we found evidence, including significant gene tree discordance, cytonuclear conflicts, and changes in genome-wide heterozygosity across taxa, collectively supporting extensive reticulation in the genus. Further examinations indicated that at least two separately parental species pairs were involved in the repeated origin of the hybrid lineages, in which some triggered a further phase of syngameon. On the basis of the elucidated hybridization relationships, we finally obtained a highly resolved backbone phylogeny constructed by taxa exhibiting no evidence of hybrid origin. The backbone taxa have distinct demographic histories and are the product of recent rounds of adaptive radiations by sorting of ancestral variation under variable climatic and ecological conditions. They commonly serve as a genomic base contributing raw parental material for hybrid speciation of other *Actinidia* taxa. Our results suggest a potentially universal model for consecutive plant diversification through two layers of adaptive radiations, including the rapid evolution of backbone lineages and further hybrid swarm derived from them.

T2-06: Causes and consequences of the Cretaceous Terrestrial Revolution to the tree of life with focus on seed-free land plants and trophic interactions

T2-06-01

Angiosperms as engineers of ecology and climate

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Ecosystems changed dramatically over the Cretaceous and on into the Cenozoic. Flowering plant evolution is an obvious consequence, but can also have been a key driver of these changes. Angiosperms have dramatically higher transpiration capacities than other plants. The recycling of precipitation via transpiration is important for the maintenance and expansion of everwet environments. Flowering plants themselves are diverse in the everwet tropics, but seedless plants have also been beneficiaries of this climate engineering. The elevated transpiration capacities of angiosperms correspond to higher productivity potential as well and the combination of increasing productivity with the environmental stability afforded by angiosperm buffering of climate may also have led to increased trophic complexity.

T2-06-02

Burmese amber provide insights into the phylogenetic composition of terrestrial habitats in the KTR

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The Cretaceous Terrestrial Revolution is a period of profound changes in the ecology of terrestrial environment which are marked not only by the rise of angiosperms. This ecological revolution is not only marked by diversification of some animal lineages dominated extent terrestrial forest habitats such as various lineages of insects such as ants, rise of birds and mammals but also by the the diversification of non-angiosperm land plant lineages such as liverworts and ferns. In turn, some lineages of ecological importance in the pre-KTR forests, declined or were replaced by phylogenetically related lineages. Burmese amber besides other Cretaceous amber lagerstaetten, provide unique insights in the taxonomic and phylogenetic composition of forests habitats during the KTR. The amber inclusions of these localities include often the first appearances of lineages of great ecological importance to extant forest habits or the latest record of lineages in decline. These fossils provide also information on the phylogenetic composition and in turn provide insights to the phylogenetic context of the trophic networks during the KTR. As a result, the Burmese amber fossils are not only providing evidence to improve efforts to date phylogenetic hypotheses but also provide a unique window to study the dynamics of changes in trophic networks during major ecological transitions in the history of land plants. This patterns are explore here by placing recently describe Burmse amber fossils of bryophytes, ferns, and insects into their phylogenetic context integrating both diversification time estimates and niche reconstructions. This innovative approach is discussed and outlined here for the first time.

T2-06-03

Mesozoic fern floras from China: anatomy, diversity and evolutionary implications

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Ferns are very abundant and well developed in the Mesozoic floras in both northern and southern China, mainly ranging from Late Triassic to Early Cretaceous intervals. During last decade, more than 45 species referred to 16 genera of Early Mesozoic fern fossils have been investigated in China by our research group, including six families such as Marattiaceae, Osmundaceae, Gleicheniaceae, Matoniaceae, Dipteridaceae and Dicksoniaceae. These fossils are mainly recovered from the Late Triassic, Early and Middle Jurassic deposits (a few taxa are from the Late Permian) in a variety of plant fossil localities, including Hubei, Sichuan, Anhui and Jiangsu of southern China phyoprovince as well as Hebei, Liaoning, Nei Mongol and Xinjiang regions in northern China phytoprovince. In our series studies, we mainly focus on the anatomy of reproductive structures, such as sori or synangia, sporangia, annuli, spore output and spores in situ using both LM and SEM approaches, such as *Marattia*, *Asterotheca* and *Daeneopsis* of Marattiaceae, *Oligocarpia* of Gleicheniaceae, *Phlebopteris* of Matoniaceae, *Dictyophyllum, Clathropteris* and *Hausmannia* of Dipteridaceae, as well as *Coniopteris* and *Eboracia* of Dicksoniaceae. In particular, we have investigated fossil spore ultrastructurs of several exceptionally well preserved fern sporangia, including *Marattia asiatica* (Marattiaceae), *Oligocarpia kepingensis* (Gleicheniaceae) and *Dictyophyllum nilssonii* (Dipteridaceae) from Late Permian of Xinjiang and Early Jurassic of western Hubei using both SEM and TEM approaches. These results demonstrate that fossil and living spore ultrastructures are a stable character that endured a long history, providing unequivocal evidence for the evolutionary relationships of fern lineages. The anatomy of fossil osmundaceous rhizomes have been studied from the Jurassic deposits in Northern Hebei and western Liaoning provinces. There are about 20 species ascribed to two genera such as *Ashicaulis* and *Millercaulis* in these localities, including about 12 new species. The development and phylogenetic evolution of these plants are analyzed based on both morphology and anatomical features. Their forming palaeonenvironmental background are discussed based on sedimentary and palaeoecological analyses of the Middle Jurassic deposits in western Liaoning. The fossil record, diversity variation and tempo-spatial distribution pattern of some fern groups, including three families, such as Marattiaceae, Gleicheniaceae, Dipteridaceae and Osmundaceae are investigated with emphasizes on evolutionary implidations.

T2-06-04

Re-evaluation on the systematic position of the Mesozoic fern genus *Coniopteris*

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The extinct fern genus *Coniopteris* was a typical component in the Mesozoic flora with a wide distribution in both Northern and Southern Hemispheres from Early Jurassic to Early Cretaceous, and played a very important role in indicating strata. At the very beginning of the discovery of *Coniopteris,* it was placed in the family Dicksoniaceae based on its cup-shaped indusia (bivalvate only in *C. venusta*), resembling that of *Thyrsopteris elegans* in Dicksoniaceae. However, based on molecular phylogeny *T. elegans* has been moved from Dicksoniaceae to its own family Thyrsopteridaceae, leaving the relationships of *Coniopteris* more unresolved. In this study, we analyzed all phylogenetic and morphological data to re-evaluate the systematic position of *Coniopteris.* Our major results include: 1) All extant genera in Dicksoniaceae have bivalvate indusia, while species of *Coniopteris* have cup-shaped indusia; 2) Most Dicksoniaceae occur in the tropical zone, different from the temperate habitats we thought for *Coniopteris*; 3) *Coniopteris* is herbaceous, very small and slender, less than 20 cm tall, whereas extant Dicksoniaceae are mostly tree-like ferns; 4) Dicksoniaceae and Thyrsopteridaceae all have oblique annuli, while *Coniopteris* has vertical ones. Based on these differences, *Coniopteris* should not be kept in Dicksoniaceae. Instead we found that *Coniopteris* is most similar to Dennstaedtiaceae in having bowl- or cup-shaped indusia, herbaceous habit, and vertical annulus, and living in temperate habitats). On the other hand, molecular dating indicates that the stem groups of Dicksoniaceae, Thyrsopteridaceae, and Dennstaedtiaceae all originated from Lower Jurassic when *Coniopteris* made its first appearance. Our study leads us to propose that *Coniopteris* may be more closely related with Dennstaedtiaceae (Polypodiales) than with Dicksoniaceae.

T2-07: Mimicry in plants

T2-07-01

Key challenges for understanding floral mimicry

Steven Johnson
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Mimicry is considered a classical example of adaptation through natural selection, yet many aspects of the evolution of mimicry are still shrouded in uncertainty. The purpose of this presentation is to identify a broad conceptual framework for mimicry research across the kingdoms of living organisms and then to identify particular challenges that are encountered in the study of floral mimicry. Floral mimics are usually non-rewarding, but rewarding plants can also use mimicry to attract pollinators, and not all non-rewarding plants are mimics. Floral mimics imitate the cues that animals use to locate their food-sources, mating partners and oviposition sites, and thereby exploit the behaviour of these animals for the purposes of pollination. The evolution of mimicry therefore involves complex ecological interactions among multiple species in communities. Mimicry theory was originally developed around ideas of conditioning in signal receivers, but pre-existing sensory bias by pollinators may explain most cases of floral mimicry and also the evolution of imperfect mimicry. Selection for traits that confer mimicry has driven phenotypic diversification in many different plant lineages and this highlights the general importance of community interactions for explaining evolutionary processes and patterns.

T2-07-02

Cryptic coloration in alpine plants: Function and divergence

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Camouflage is a common anti-predator strategy utilized by animals, which may also occurs in plants, given that they face a world with herbivores. Although there is anecdotal evidence of camouflage in plants, it receives few rigorously experimental studies, possibly because a lack of ideal study system. In the alpine area of Qinghai-Tibet Plateau and the nearby region, several plants from far related taxa exhibit typical cryptic coloration. Studying on a host plant - herbivore system (*Corydalis – Parnassius*) in the alpine region of SW China, we investigated the evolution of camouflage in plants. Take advantage of the leaf color dimorphism (green and gray) in *Corydalis*, we first tested the premise that whether herbivores (*Parnassius* butterflies) can distinguish gray leaves from their scree backgrounds, using a butterfly's color vision model. Then we investigated the adaptive significance of cryptic leaf color under the context of cost-benefit analysis. Potential costs of inconspicuousness were estimated by comparing the photosynthetic performance and visual attractiveness to flower visitors of the two color morphs. Potential benefits were examined by comparing damage, survivorship and female reproductive success. Given that the efficacy of camouflage through background matching depends on the specific visual environment that organism lives, and the background color of plants varies between populations, we tested the hypothesis that *Corydalis* may exhibit population-specific leaf color to get optimal local camouflage in different visual environments. We show that it's difficult for butterfly herbivore to distinguish gray leaves against the background. This cryptic leaf color originates in a specific combination of anthocyanins and chlorophylls. The two color morphs had similar photosynthetic performance, visual attractiveness and female reproductive success. However, gray morphs had significantly lower herbivore damage and higher survivorship. And as we expected, there is a remarkable divergence in leaf colors of *Corydalis*. Leaves are better matched against their native backgrounds than against foreign ones, providing local camouflage. In conclusion, the cryptic leaf color benefit alpine *Corydalis* by reducing herbivores' attack with limited cost. The divergence of cryptic leaf color may have evolved in response to local herbivore selection in various visual environments.

T2-07-03

How flower colours and floral colour patterns trigger pollinator behaviour

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The innate colour preferences of bees reflect the evolution of flower colours and floral colour pattern. To discuss this hypothesis it is argued that the yellow and UV-absorbing colour of pollen and anthers, caused by protective flavonoid pigments, was the primary visual signal of flowers predating the evolution of corollas and other secondarily attractive structures of insect-pollinated angiosperm flowers. Many recent bee-pollinated flowers still visually display pollen, anthers or pollen-mimicking floral guides contrasting against the corolla. Hoverflies and bees actively eat and/or collect pollen grains and respond to visual pollen signals. Naïve hoverflies innately respond to this colour pattern by extending their proboscis exclusively towards yellow and UV-absorbing colours. In contrast, bees target at the yellow and UV-absorbing colours of pollen and pollen-mimicking floral guides for the first physical contact with a flower by means of their antennae preceding landing. Of note, not only the behavioural reaction differs among bees and flies, also the colour parameter triggering the response is different. Hoverflies respond to the colour hue, whereas bees respond to the colour saturation of these signalling structures. Since pollen that is eaten or collected by flower visitors is no longer available for pollen transfer to conspecific flowers bee-and hoverfly-pollinated plants benefit both from hiding pollen from their pollinators and from displaying pollen-mimicking structures replacing signalling real pollen. Bee-pollinated angiosperms that either visually display yellow-UV-absorbing pollen or anthers or pollen-mimicking floral guides and their pollinators constitute the most speciose mimicry system of the world. It is discussed that, besides mimicry, sensory exploitation of innate responses and signal standardization are important features of the most common floral signalling system. In addition it will be demonstrated that even hummingbird-pollinated flowers are affected by this signalling system in order to avoid the attraction of nectar- and pollen-robbing bees. In this respect it is important to state, that even experienced bees and hoverflies use their innate preferences when visiting flowers. Hoverflies cannot be conditioned to exhibit the proboscis reaction towards other than UV-absorbing yellow colours. Bees can easily be trained to colours of slightly deviant wavelength, but continue to prefer more saturated colour over trained colours in learning experiments.

T2-07-04

When flowers play dead: Linking the flower microbiome to brood-site deceptive pollination in the common pawpaw *Asimina triloba*

Kyle Martin*, Spencer Debenport, Daniel Buckley, Robert Raguso*

Cornell University

Nearly all eukaryotes form close relationships and interactions with microbes that are essential for their fitness. The presence of microbes on and within plant tissues is ubiquitous and accordingly, the phenotype of most plants is a combination of both plant and microbial-expressed genes. In this respect, the plant-microbe entity can be considered a holobiontic organism expressing plant derived traits interspersed with microbial derived traits that can be overlooked due to the limits and biases of human perception and investigation. In particular, some aspects of floral mimicry, including brood-site deceptive floral scents that mimic fermenting fruit, carrion, or feces, show the chemical signatures of microbial metabolism but have not been investigated to determine whether they are of plant or microbial origin. A particularly striking example is the common pawpaw *Asimina triloba* (Annonaceae), a small understory tree native to the deciduous forests of eastern North America. The clonal patches of trees produce an early spring bloom of small (3cm), maroon colored flowers with a floral scent that is strongly reminiscent of fermenting fruit or bread dough. Not surprisingly, the floral scent of *Asimina triloba* is dominated by aliphatic fermentation products including ethanol, ethyl acetate, 3-methyl-1 butanol, acetoin, and acetic acid and consequently attracts sap beetles (Coleoptera; Nitidulidae) and Drosophilid flies that are deceived while searching for oviposition/mating sites. It remains unclear how these floral traits have evolved, or whether the flowers have outsourced this function to third-party agents (microbes) to circumvent the long process of evolution by natural selection. In this study we investigated the microbiome of *Asimina triloba* whole flowers, as well as the change in microbial community during protogynous floral ontogeny and between distinct spatial units of the flower, using both culture independent and culture dependent methods. In addition, we performed manipulative experiments including flower sterilization, ^{13}C tracing, and the exclusion of biotic microbial vectors to test the hypothesis of microbial signaling within the flowers. Our results document unexpected diversity and abundance of yeasts and bacteria living on the flowers, as well as spatio-temporal patterns of microbial community composition during the flowers' short lifespan. Further, our manipulative experiments have revealed candidate microbially produced volatiles and suggest opportunities for further investigation with bioassays that promise to shed light on the potential for microbial-mediated plant-pollinator interactions.

T2-07-05

Floral mimicry of fruit within Annonaceae

Katherine Goodrich

Widener University

Pollination strategies involving floral mimicry of non-floral resources have long fascinated researchers. These pollination strategies are scattered across many angiosperm families, with documented floral mimicry of models including carrion, dung, insects, fungi, and fruit. Floral mimicry of ripe or rotting fruit is infrequently documented in existing literature, yet may represent a common mimicry type in some plant groups. Within the family

Annonaceae, many species release floral scents similar to ripe or rotting fruits and have fleshy, dull-colored petals. These floral features attract beetles thought to be in search of fruit substrates as food or brood sites for oviposition, yet the similarity of olfactory or other cues between putative mimics (flowers) and models (locally-available fruits) has yet to be tested. The North American species *Asimina triloba* and *Asimina parviflora* (Annonaceae) produce yeasty floral scents similar to fermenting fruit and are known to be pollinated by flies and beetles. I hypothesize that their flowers may mimic locally available fruits. I have analyzed the floral scents of these two species along with volatiles generated by fruit pulp from several species inoculated with *Saccharomyces cerevisiae*, and from fruits of *Asimina triloba* at different stages of ripeness and decay within the understory of a natural habitat in order to distinguish between "general food mimicry" and a match to an ecologically-relevant model. While the odor of fresh fruit pulp for *A. triloba* is dominated by methyl and ethyl esters of hexanoic and octanoic acids, fermentation volatiles including ethyl acetate, ethanol, acetoin, and 2,3-butanediol begin to appear several days after the fruit drops to the ground. Floral scents of *A. triloba* and *A. parviflora* share these fermentation volatiles but lack the esters seen in fresh fruit of *A. triloba*. Floral scent of *A. parviflora* also contains isoamyl acetate, isobutyl acetate, and 2-heptanone, all compounds associated with some ripe fruits but not seen in the fruit odor of *A. triloba*. I use non-metric multidimensional scaling to compare the scent blends from these different sources. The comparison of floral scent chemistry and other floral cues to ecologically-relevant potential models is an important first step to distinguishing Batesian floral mimicry from generalized food mimicry. Furthermore, knowledge of the ecology and behavior of pollinating taxa and the evolutionary history of floral mimics are important to establishing the degree and efficacy of floral mimicry. Future work in these areas is underway for this particular system.

T2-07-06

Inaccurate color discrimination by pollinators promotes evolution of discrete color polymorphism in food-deceptive flowers

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2. The University of Tokyo

Many plant species employing food-deceptive pollination strategy show discrete or continuous floral polymorphism within their populations. Previous studies have suggested that negative frequency-dependent selection (NFDS) caused by learning behavior of pollinators is responsible for maintenance of floral polymorphism. However, NFDS alone does not explain why and under what conditions discrete or continuous polymorphism evolves. In this study, we use an evolutionary simulation model to propose that inaccurate discrimination of flower colors by pollinators leads to evolution of discrete flower color polymorphism. Simulation results showed that associative learning based on inaccurate color discrimination by pollinators caused disruptive selection on flower colors where intermediate color variants in the flower color distribution suffered fitness disadvantage. This was because intermediate colors between other common color variants received twofold collateral unpopularity from pollinators who had learnt to avoid these neighboring colors. The degree of inaccuracy determined the number of discrete flower colors that evolved. Our results suggest that animal behavior based on inaccurate discrimination may be a general cause of disruptive selection that promotes discrete trait polymorphism.

T2-08: Cyperaceae I: early diverging lineages

T2-08-01

A new infrageneric classification for the genus *Scleria* (Cyperaceae) based on molecular phylogenetic and morphological data

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The genus *Scleria* is placed in the monotypic tribe Sclerieae. Its distribution is primarily pantropical and currently ca. 250 species are recognized. Previously, infrageneric classifications have been proposed but none has been based on molecular phylogenetic evidence and most studies have treated the genus from only one geographical region. DNA sequence data from more than 100 species of *Scleria* from across its distributional range and all infrageneric groups was generated for three molecular markers. Phylogenetic hypotheses were constructed using Bayesian inference and maximum likelihood approaches. Additionally, ancestral states of four morphological characters were reconstructed at important nodes using the program BayesTraits. A new infrageneric classification based on molecular evidence and supported by morphology is presented. *Scleria* is now confirmed as monophyletic and sister to tribe Bisboeckelereae. A subdivision of *Scleria* into four subgenera (*Scleria* subgenus *Browniae*, subgenus *Hypoporum*, subgenus *Scleria* and subgenus *Trachylomia*) is supported in our results. Twelve major clades are recovered within *Scleria* subgenus *Scleria*. Clear morphological diagnostic features match these clades. Ancestral state reconstruction indicates that androgynous spikelets, a zoniform hypogynium, a paniculate inflorescence and normal linear leaves are ancestral in *Scleria*. A more detailed molecular study of *Scleria* subgenus *Hypoporum* revealed several new species and resolved some of the more complex synonymy questions in this group. Species from this subgenus have few identifying morphological characters, and this led to many erroneous interpretations of species relations in the past. In our study, we clarify those relationships, the synonymy and typification of species belonging to *Scleria* subgenus *Hypoporum.*

T2-08-02

Biogeography and diversification in the megadiverse genus *Carex* (Cyperaceae)

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6. McGill University

Carex, with about 2,000 species is one of the largest angiosperm genera in the World, the second largest in the temperate zones, and the largest among monocots. It has a cosmopolitan distribution, with high species richness in temperate and cold areas of the northern hemispheres and a presumed origin in SE Asia. *Carex* may be found in a wide range of habitats, from Arctic swamps to semi-desert areas, and is sometimes ecologically important. As a result of a 4-years project we have compiled a 3-markers megaphylogeny of *Carex* based on an extensive sampling of about 70% of the species diversity of the genus and representing all the main lineages. This increased amount of data has allowed us to perform the first studies on the evolution of the genus as a whole. Using a time-calibrated tree, with several *Carex* fossils as calibration points, we performed biogeographic and diversification analyses on a spatial and temporal framework. We performed an ancestral area reconstruction to test the hypotheses of the different centers of origin and to assess colonization, dispersal and extinction processes underlying possible range shifts. We also studied the patterns of diversification rates between different lineages, looking for shifts in speciation rates and its possible correlation with different morphological and biogeographic traits. Overall, our objective was to investigate the different evolutionary processes that have shaped the present day distribution of the genus, its impressive species diversity, and the contrasting patterns in species richness between regions.

T2-08-03

Tribe Schoeneae (Cyperaceae): The taxonomic iceberg of sedges

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6. National Herbarium of New South Wales

The tribe Schoeneae is not monophyletic, yet even in a strict sense, it will include more genera than other tribes in the Cyperaceae. We present a synthesis of recent phylogenetic studies and an outline of proposed taxonomic changes that are needed at the tribal and generic levels. We use several genera including *Costularia*, *Lepidosperma* and *Tetraria* to demonstrate recent findings and continuing challenges to understanding the systematics, biology (including Honey Bees collecting resins from sedges) and ecology (including myrmecochory) of these fascinating plants.

T2-08-04

The *Schoenus* clade of the tribe Schoeneae (Cyperaceae)—generic circumscriptions, taxonomic complexity and polyploidy

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The tribe Schoeneae is unique among Cyperaceae, as it is predominantly restricted to nutrient-poor sandstone habitats in the temperate continents of the southern hemisphere. Recent evidence suggests that the tribe originated in Australia during the Paleogene and dispersed to southern Africa in five independent events. Two of these lineages are currently included in *Tetraria* P. Beauv., which has an estimated 46 of its 54 species in the Cape Floristic Region (CFR) of South Africa and is considered a 'Cape floral clade' because of its evolutionary history. Molecular and morphological evidence suggests this morphologically-complex genus is polyphyletic with two distinct clades, one of which includes *Epischoenus* C.B. Clarke—a small genus of eight species endemic to the CFR. The clade of *Tetraria* that includes *Epischoenus* is currently referred to as the *Schoenus* clade of *Tetraria*, since recent phylogenetic evidences places the generic type of *Schoenus* (*Schoenus nigricans* L.) within the clade. Relatively little taxonomic attention has been given to the *Schoenus* clade of *Tetraria* compared to other 'Cape floral clades', most likely because of character similarities in taxa, possible hybridization and a paucity of collections, which has led to confusion in species boundaries. In addition, preliminary data indicate that genome sizes within the *Schoenus* clade are substantially larger than the genomes of other Schoeneae species, suggesting that polyploidy and possibly hybridization could play an important role in speciation within the clade. We will present results from recent taxonomic work within the *Schoenus* clade of *Tetraria*, including a taxonomic realignment of the genus, changes in species boundaries and the description of several new species. In addition, we will discuss diversification in the *Schoenus* clade in the context of speciation patterns among the Cyperaceae and Cape lineages and present future research questions related to genomes sizes and evolutionary relationships within the clade.

T2-08-05

Phylogenomics of Cyperaceae subfam. Mapanioideae

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Previous phylogenetic studies, combining *trnL-F*, *rps16* and pollen data, more-or-less resolved the Mapanioideae into two distinct well supported lineages, the Hypolytreae and the Chrysitricheae, providing a backbone for further taxonomic reorganisation and evolutionary study of this group. Although bootstrap values were mostly high, *Hypolytrum*, *Mapania* and *Scirpodendron* were not recovered as monophyletic. In the present study fresh, silica dried and herbarium samples, including collections from New Caledonia, have been used to expand on the existing analyses. *Hypolytrum* in particular was densely surveyed, with 70 additional samples. This has resulted in a unique mapaniid dataset of 140 terminals, consisting of sequences for six plastid and ribosomal gene regions (*trnL-F, rps16, rbcL*, *matK*, ETS, ITS), yielding a highly resolved dated phylogenetic tree. The talk provides a summary of the molecular data, as well as comparative morphological and evolutionary analyses towards the goal of unravelling the mechanisms involved in the emergence and evolution of mapaniids.

T2-08-06

Geographical and ecological drivers of diversification in the Mediterranean basin: The evolutionary history of the *Carex panormitana-C. reuteriana* complex (Cyperaceae)

Santiago Martín-Bravo[1], *Carmen Benítez-Benítez*[1], *Marcial Escudero*[2], *Pedro Jiménez-Mejías*[3]
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The Mediterranean basin is one of the most important hotspots of plant biodiversity, characterised by the high species richness and endemicity (ca. 50%) of its flora. *Carex*, with ca. 150 species, is one of the richest genera in this region. Within the mainly circumboreal *Carex* section *Phacocystis*, *C. reuteriana* and *C. panormitana* are two sister, disjunctly distributed species inhabiting margins of Mediterranean creeks and brooks. *Carex reuteriana* is endemic to the Iberian Peninsula and North West Africa, while *C. panormitana* is found in Sardinia and in two single isolated populations in Sicily and Tunisia. A molecular study based on DNA sequencing and fingerprinting data, with population sampling covering both species' range, as well as bioclimatic niche modeling, were carried out to elucidate the geographical and ecological drivers of diversification in this group. Phylogenetic and divergence time analyses revealed that the two species form a monophyletic group originated in the late Pliocene-early Pleistocene. A clear genetic differentiation was detected between both species, suggesting long term reproductive isolation. Moreover, the populations of each taxon formed genetic clusters according to disjunct areas, indicating restricted gene flow among them and the role of geographic barriers in shaping the observed genetic variation. Bioclimatic modeling detected an important niche overlapping of between the two species, although also certain degree of ecological differentiation. The projection to the Pliocene showed that Central Europe was a suitable area for *C. panormitana-C. reuteriana*, which was validated by the occurrence of fossils assignable to this group in this area. This suggests bioclimatic niche conservation through time rather than adaptation to new habitats as the ecological strategy of these plants. In conclusion, allopatric divergence and niche conservatism seem to be the main evolutionary features of the *C. panormitana-C. reuteriana* group. Thus, the observed phylogeographic structure appears to be consequence of the splitting of a larger range into allopatric sets of populations established in ecologically suitable areas.

T2-09: Electronic Floras–integrating the best of the past with the possibilities of the future

T2-09-01

The New Zealand eFlora - delivering what's needed in the electronic age to the people of New Zealand

Peter De Lange
Department of Conservation

The Flora of New Zealand has been traditionally served to the tax payer through a series of technical hard copy publications. The most recent of these, the New Zealand Flora Series, was initiated in the 1940s with the first volume published in 1961 and the last (as a second edition) in 2010. Although excellent for their time they served as static accounts, often dated when they went to print, and because of this sometimes causing confusion, or in the case of biosecurity matters a false impression of priorities. The move to an eFlora model for New Zealand has been relatively recent, the first treatment - The Hypericaceae was published in 2010. Since then 49 treatments have been published online covering 30 Moss, 16 Pteridophyte and nine flowering plant families (values as of 9 November 2016). In this presentation I explore the eFlora of New Zealand as seen by a major end user - the New Zealand Department of Conservation.

T2-09-02

eFlora of New Zealand – integrating the best of the past with the possibilities of the future

Rob Smissen, *Aaron Wilton, Ilse Breitwieser*
Allan Herbarium, Landcare Research

Floras are an authoritative summation of taxonomic knowledge for a given region at a given time. Traditionally Floras are books, but because of the considerable time and resources required to compile and produce a Flora, these books are often out of date as soon as the Flora is printed. Developments in biodiversity informatics make it now possible to use different approaches to create Floras, and worldwide there are numerous "next-generation Flora" projects underway. These range from digitisation of existing Floras to development of novel information systems that replace or extend traditional Floras. Such systems variously include tools to address different parts of the Flora writing process from accumulating and synthesising data through to generating products to meet specific audience needs. These new approaches substantially change the way in which we can both construct and manage Floras, and make our knowledge widely available and accessed. It may also, at least potentially, substantially change the way we do our work, and think about our information, its management, and the ways in which we can help our users. This talk is an introduction to the symposium on electronic Floras that will explore some of the implications of the eFlora platform for taxonomy, systematics, and the world by using the eFlora of New Zealand as an example. We have started to deliver the next-generation of New Zealand Floras as a dynamic resource: a resource that is both up-to-date and engages a wide range of users. We will present our experiences in developing this next-generation of New Zealand Floras, our planning processes, our work processes, some of the difficulties we encountered, the lessons we learned, what we have achieved so far, and some of our plans.

T2-09-03

Building the Australasian e-flora collaboratively - It's more than science and technology

Judy West[1], ***Zoe Knapp***[2]
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2. Australian Biological Resources Study

Creating descriptive content for a flora, whether it is a book series or a web based e-flora platform, follows a familiar pathway. The Australian Biological Resources Study has been working with Australian botanists to develop standardised taxonomic treatments within its publications for over forty years. The shift of the *Flora of Australia* from a hard-copy book series to a truly interactive e-flora has not only involved the careful transfer, review and update of existing floral profiles, it has also seen the need to take a much more challenging journey. This presentation will outline some of the key governance considerations and cultural changes that need to be taken into account when building a national or regional scale e-flora. The *Australasian e-flora* platform, built by the Atlas of Living Australia and project managed by the ABRS and the Council of Heads of Australasian Herbaria can, after addressing many technical challenges, effectively accommodate a continental scale flora. As significant as this technical achievement has been, project partners have also needed to work closely with the Australian and New Zealand botanical communities - to build a consensus view of accepted taxonomic concepts for all forms of the kingdom Plantae - as part of the developing [Australian] National Species List and the established New Zealand Organisms Register. Developing accepted taxonomies across multiple-jurisdictions has proven to be one of the more challenging but critically important elements in building a regional e-flora, its taxonomic hierarchy and related diagnostic keys. The mix of science, technology and ability for people to work together are all important factors in successfully creating an e-flora.

T2-09-04

VicFlora: Turning hardcopy digital

David Cantrill

Royal Botanic Gardens Victoria

VicFlora is a innovative digital system that presents up to date knowledge on the Flora of Victoria (https://vicflora.rbg.vic.gov.au/). The system was developed through the ingestion of a hardcopy (printed) flora and then has been designed so that species profiles can be easily added (or deleted) including adding taxa to the identification tools (keys). Distribution information is not only generated from MEL herbarium records but harvested from web resources. Checklists for regions can be generated by users is geographic polygons that are overlaid on the distribution data. This talk focuses on the key lessons learnt in developing a digital flora from hardcopy.

T2-09-06

Thinking about the e- in e-Floras; or, Floras old, new, and not-yet

Kevin Thiele

University of Western Australia

When faced with a disruptive new technology such as the World Wide Web, it often takes a lot of effort, time and creativity, to escape conventional ways of thinking through a problem and to fully realise the implications of the new. So-called e-Floras are no exception. How much does the e- in the name really reflect a new way of approaching a Flora's core task (to communicate authoritative, synthesised taxonomic knowledge to as wide an audience as possible in the best way achievable)? If modern taxonomy and systematics were invented, or re-invented, now (in the age of the internet, social media, citizen science and the block chain), rather than in the 18th Century, would we do it all differently? And, what near-future disruptive technologies and paradigms do we need to be aware of when thinking about, or planning, an e-Flora project? Taking the new e-Floras of Australia and New Zealand as starting points, this talk will explore current and future thinking about e-Floras, and map out some ideas for, perhaps, the next wave.

T2-10: Polyploidy: evolution, function, and diversification

T2-10-01

Of dups and dinos: Evolution at the K/Pg boundary

Yves Van De Peer[1,2,3], *Rolf Lohaus*[1,2]

1. Ghent University (UGent)
2. VIB
3. University of Pretoria

Fifteen years into sequencing entire plant genomes, more than 30 paleopolyploidy events could be mapped on the tree of flowering plants (and many more when also transcriptome data sets are considered). While some genome duplications are very old and have occurred early in the evolution of dicots and monocots, or even before, others are more recent and seem to have occurred independently in many different plant lineages. Strikingly, a majority of these duplications date somewhere between 55 and 75 million years ago (mya), and thus likely correlate with the K/Pg boundary. If true, this would suggest that plants that had their genome duplicated at that time, had an increased chance to survive the most recent mass extinction event, at 66 mya, which wiped out a majority of plant and animal life, including all non-avian dinosaurs. I will review several processes, both neutral and adaptive, that might explain the establishment of polyploid plants, following the K/Pg mass extinction.

T2-10-02

Higher frequency of polyploidy associated with the colonization of land

Zheng Li, *Thomas I. Kidder, Chris R. Reardon, Stacy A. Jorgensen, Anthony E. Baniaga, Hannah E. Marx, Xinshuai Qi, Michael S. Barker*

Dept. of Ecology & Evolutionary Biology, University of Arizona

Increased rounds of whole genome duplication (WGD) in the ancestry of the most derived lineages of green plants. Here we provide the first summary of paleopolyploidy across the entire evolutionary history of the viridiplantae. Our analyses of genomic data from more than 1600 species revealed that the incidence of ancient polyploidy increased nearly 500% from the algae to flowering plants. We identified 235 WGDs in the history of the viridiplantae. Nearly 150 WGDs were newly discovered in this study. We also found that nearly every lineage of land plants has experienced at least one round of ancient polyploidy. In contrast, most algae have no evidence of paleopolyploidy in their genomes. Intriguingly, the green algal clade with a land plant like frequency of paleopolyploidy was the Zygnematophyceae. This clade has relatively recently been recognized as the most likely sister lineage to land plants and was confirmed by our present phylogenomic analyses. These results suggests that perhaps the proclivity for polyploidy found among the land plants, and especially the flowering plants, may have its roots deep in the history of the green plant phylogeny. Overall, our study demonstrates that paleopolyploidy has been an increasingly important component of green plant evolution, rising from zero rounds among the algae to more than five rounds of WGD within the flowering plants.

T2-10-03

Subgenome dominance in an interspecific hybrid, synthetic allopolyploid, and a 140 year old naturally established neo-allopolyploid monkeyflower.

Patrick Edger

Michigan State University

The importance and applications of polyploidy have long been recognized, from shaping the evolutionary success of flowering plants to improving agricultural productivity. Recent studies have shown that one of the parental subgenomes in ancient polyploids is generally more dominant - having both retained more genes and being more highly expressed - a phenomenon termed subgenome dominance. How quickly one subgenome dominates within a newly formed polyploid, if immediate or after millions of years, and the genomic features that determine which genome dominates remain poorly understood. To investigate the rate of subgenome dominance emergence, we examined gene expression, gene methylation, and transposable element (TE) methylation in a natural less than 140 year old allopolyploid *(Mimulus peregrinus)*, a resynthesized interspecies triploid hybrid *(M. robertsii)*, a resynthesized allopolyploid *(M. peregrinus)*, and diploid progenitors *(M. guttatus* and *M. luteus)*. We show that subgenome expression dominance occurs instantly following the hybridization of two divergent genomes and that subgenome expression dominance significantly increases over generations. Additionally, CHH methylation levels are significantly reduced in regions near genes and within transposons in the first generation hybrid, intermediate in the resynthesized allopolyploid, and are repatterned differently between the dominant and submissive subgenomes in the natural allopolyploid. Our analyses reveal that the subgenome differences in levels of TE methylation mirror the increase in expression bias observed over the generations following the hybridization. These findings not only provide important insights into genomic and epigenomic shock that occurs following hybridization and polyploid events, but may also contribute to uncovering the mechanistic basis of heterosis and subgenomic dominance.

T2-10-04

The allotetraploid grass *Brachypodium hybridum*: An evolutionary dead-end or a newly emerging lineage?

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2. Estación Experimental de Aula Dei-Consejo Superior de Investigaciones Científicas

3. DOE Joint Genome Institute

Hybrid allopolyploids have been alternatively viewed as drivers of biodiversity or evolutionary dead-ends; the ‘dead-end’ case reflecting either a non-significant contribution to evolution or a higher probability of extinction compared to those of parental diploids. Defendants of the drivers-of-diversity-and-evolution hypothesis argue that common recurrent polyploidization has led to polyploid species showing higher genetic variation from multiple populations of diploid progenitors and highly dynamic genomes generating novel genetic variation. By contrast, oponents interpret polyploids as blind alleys unable to accumulate new variation or to give rise to newly divergent lineages. We compare the two competing hypotheses in *Brachypodium hybridum*, a model species for polyploid speciation in grasses [allotetraploid *B. hybridum* (2n=4x=30) derived from the cross of diploids *B. distachyon* (2n=2x=10) and *B. stacei* (2n=2x=20), followed by WGD]. Comparative genomics between the reference genomes of parental *B. distachyon* (Bd21 line) and *B. stacei* (ABR114) species, and the respective reference subgenomes of *B. hybridum* (ABR113; D=distachyon-type, S=stacei-type) show both a high colinearity between the *B. distachyon*/*B. hybridum*-D and *B.stacei*/*B.hybridum*-S genomes, and a similar syntheny between the two progenitor genomes (*B. distachyon*/ *B. stacei*) and the two *B. hybridum* subgenomes (D/S), suggesting that few genomic variation has been incorporated into the *B. hybridum* subgenomes since the hybridization took place. However, phylogenomic analysis of a genome-mapped SNP data set (>1800 kbp nDNA) including several samples of *B. hybridum*, *B. distachyon* and *B. stacei* reconstructs distinct evolutionary placements for the differently inherited D and S subgenomes. Phylogenomics of plastome data confirm the bidirectional crossing origin of *B. hybridum*. Barcoding data from nuclear genes and SSR data further demonstrate the existence of recombinant or new allelic variants in *B. hybridum*. Metabolomic and phenotypic data also detect new characters and significant differences in other traits in *B. hybridum* with respect to its progenitors. All these evidences suggest that *B. hybridum* is not an evolutionary dead-end species but a newly emerging lineage. It still maintains relatively intact parental genomes, probably due to the short time elapsed since its estimated first origin (~1 Mya), though it is reproductively isolated and genomically divergent from its parents. *B. hybridum* ocuppies a native circum-Mediterranean distribution area broader than those of its progenitor species and has also successfully colonized other continents. *B. hybridum* might also have potential evolutionary future as other high-ploidy level polyploid *Brachypodium* species have apparently originated from low-ploidy level polyploids.

T2-10-05

Polyploidy and the origins of novelty: Impact of duplication on genomes and network evolution

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Ancient whole genome duplications (WGDs) are ubiquitous throughout the evolutionary history of higher eukaryotic lineages. These events have been hypothesized to be the basis for major evolutionary transitions, including the origin of novel traits in large species radiations across plants, fungi, protozoa, and animals. Repeated rounds of WGDs, or polyploid events, have been best documented among the flowering plants. We have analyzed the impact of WGDs in *Arabidopsis*, *Brassica* and other members of the mustard family (Brassicaceae). Phylogenomic analyses of these WGDs show striking correlations of duplicate gene retention and novel traits. For example, the origin of two novel classes of chemical defenses, indole and met- derived glucosinolates (i.e. mustard oils), are associated with duplicated regulatory and biosynthetic pathways that arose via WGDs. Our analyses suggest that the origin of these novel defense compounds spurred an evolutionary arms-race with insect herbivores, resulting in massive co-radiations of both the host plant and predatory insects. The use of new systems biology and network approaches to discover co-evolved modules will be discussed.

T2-10-06

Asymmetrical evolution of polyploid genomes: The role of recombination linking genomic variation to traits in *Brassica napus*

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The process of genome polyploidization and subsequent rapid diploidization has provided significant sources of genetic variation for plant adaptive evolution and new species formation. However, the way in which molecular evolution of polyploid genomes builds up genetic architecture underlying speciation is unclear and its impact on trait formation related to duplicate genes is unknown. Based on our previous findings that polyploid subgenomes have undertaken an asymmetrical evolution, we have further been using *Brassica napus* (AACC) as a model to present the role of recombination in the process. First we demonstrated recombination of the subgenome A is dominant over C by using diverse segregation populations. Further we re-sequenced 420 core germplasm collections and confirmed asymmetrical recombination between A and C. Recombination links to selection and further link DNA and epigenetic variation to traits. By analysis of SNP and DNA deletion and their association with traits, we showed that there are high correlations between recombination rate and either of abundance of SNP, DNA deletion and QTLs. By genome-wide analysis of gene expression, small RNA and DNA methylation in representative collections, we showed that these three genomic parameters are highly correlated (positively or negatively) with recombination rate and all other parameters of genome characteristics mentioned above. The above relationships also exist in many blocks of meso-subgenomes (each A or C contains three subgenomes originated from meso-polyploidization ca.15MYA). These results suggested a mode of molecular evolution of plant polyploid genomes.

T2-11: Next-generation botany: Outcrossing evidence, integrating knowledge

T2-11-01

Integrating morphology, molecules, and fossil flowers: First results from the Paleo-eFLOWER initiative

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The fossil record of angiosperms has increased substantially both in size and quality in recent decades. This is mainly due to the discovery of numerous Cretaceous localities yielding 3-dimensionally preserved, charcoalified mesofossils comprising a great diversity of angiosperm reproductive structures. In addition, novel technical applications such as high resolution computed tomography have recently boosted our abilities to retrieve high-quality structural data from fossil specimens. This rapid increase in knowledge of the fossil record has provided important information about the antiquity of different floral morphologies and of different phylogenetic lineages of angiosperms. However, what has not kept pace with these recent developments, are our abilities to test the systematic placement of fossils based on explicit phylogenetic analyses across the tree of angiosperms, mainly due to the lack of adequate morphological data sets for extant taxa. Here, we propose a new approach, centered on a multi-user database (PROTEUS) and an angiosperm-wide floral data set for 792 extant species (exemplar approach) representing all orders and 367 families (87%) of angiosperms. In the framework of the eFLOWER initiative, we have recently used this data set to infer ancestral flowers across the angiosperm tree. For the present study, we have supplemented the original data set with additional floral and pollen traits, now adding up to a total of 30 characters. To demonstrate the potential of the present paleo-eFLOWER initiative, we scored 30 published Cretaceous flowers for the same set of characters. Our main long term goals with the inclusion of fossil taxa into our data set are: (i) to develop an online tool for the paleobotanical community to systematically test the phylogenetic position of fossil flowers in an angiosperm-wide context, (ii) to test the impact of fossils on phylogenetic relationships (of extant taxa), and (iii) to incorporate the fossil record into the reconstruction of trait evolution. For this presentation, we focus on our first goal. We use a molecular backbone approach and apply parsimony as well as model-based analyses to evaluate the phylogenetic position of selected fossils. We find that some are placed congruently with earlier suggestions, while others are found in various equivocal positions, including the stem branches of very large clades. Possible reasons for equivocal results include the low number of characters for some fossils, or the lack of distinct combinations of apomorphic characters for others as well as the still limited sampling of extant species in our current data set. We also report on various sensitivity analyses (e.g., random resampling of taxa), which inform us about the consistency of our approach. Although further work is needed, including total evidence analyses, these first results are promising. While such angiosperm-wide analyses will in many cases only provide a coarse estimate of the phylogenetic position of a fossil taxon and will need to be followed by finer-scale analyses focusing on a particular clade with more and different characters, we envision to develop future automated 'phylo-scanning' community tools so that such broad-scale analyses become commonplace for the initial, exploratory testing of angiosperm fossil relationships.

T2-11-02

Fitting models to the real world: The use of external validation of spatial and temporal parameters in phylogeographic studies on oceanic islands

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The accumulation of phylogeographic studies on certain geographic regions has stirred the interest for linking the estimated genetic patterns to the species' natural history traits, its interactions with other species within ecological networks, or the species evolutionary history. Trying to fit fine-scale phylogeographic studies and associated genetic data in plants to historical events and the dynamic structure of ecosystems involves a series of methodological hurdles that often makes this task frustrating and leads to inconclusive results if only reliant on genetic data. Oceanic island species offer the advantage of providing a finite spatial framework for ecological and ecological evolutionary studies. We present a study on the bird-pollinated Macaronesian paleoendemic *Navaea phoenicea* (Malvaceae), a monotypic genus exclusive to Tenerife (Canary Islands) with a disjunct distribution in two geographically isolated areas. To understand the origin of this phylogeographic pattern, we estimated population genetic parameters and compared them with the geological history of the island and present biological data. The latter were used as external validators of evolutionary traits. First, we examined the influence of the complex geological history of Tenerife in the disagreement/discordance between the potential *versus* the realized niche of the species. In plants, the bioclimatic niche is an important predictor of the species spatial distribution. We constructed ecological niche models (ENMs) under high-resolution bioclimatic layers to examine whether the species potential niche corresponds to the realized niche, and how present populations distribute across them. The gaps observed in the potential ENM prediction corresponded to areas in the island that have undergone a series of catastrophic events. Second, we used demographic coalescent models (IMa2) to test whether the temporal scale of these events matches the divergence time estimation for the two lineages present at Tenerife. To determine empirically the generation times for the species (a critical parameter in coalescence models), we performed a demographic study of the natural populations. Using these generation times in IMa2, we obtained absolute time estimates for the genetic isolation between populations, which compared with the geological dating of the large-scale landslides and eruptions in Tenerife helped to set a temporal window for the influence of these events in the observed disjunct pattern. Finally, we carried out a third comparative analysis to try to explain the role of pollinators in the present genetic structure of populations. Estimates of intra and inter-population paternal gene flow obtained from nuclear hypervariable genetic markers were compared with empirical values of maximum distance in pollen dispersal recorded for three bird species, which are here confirmed as natural pollinators. Interestingly, the observed spatial genetic structure matched the one expected from flower visitation data by present pollinator. A second validation test via paternity analysis of plant individuals confirmed these results and identified the limits of current inter-population gene flow. In sum, our results showcase the potential of using multiple sources of information as external validators in phylogeographic studies, such as species natural history traits (reproductive biology), demographic studies, and a region's geological history to obtain reliable inferences on species evolutionary responses and distribution patterns.

T2-11-03

Bayesian biogeographic models and their application in macroevolution and macroecology: Integration and partitioning of multiple sources of evidence

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Bayesian hierarchical biogeographic (BHB) models allow joint estimation of the posterior distribution of tree topology, divergence times, and ancestral ranges given molecular data and the geographic location of sequences. Because of their relatively simple underlying biogeographic model, based on a continuous-time Markov chain (CTMC) process with single discrete states, and the use of Bayesian inference to ease computational tractability, these models have become very popular in phylogeography to answer a wide range of questions, from the transmission of diseases to routes of viral spread or historical patterns of gene flow across populations. In 2008, we first proposed the use of these models in biogeography to estimate dispersal rates and area carrying capacities (equilibrium frequencies) from DNA sequences and species geographical distributions. Here, I focus on the use of these models to test general hypotheses in ecology and evolution by using datasets of multiple lineages that inhabit the same geographic region. An advantage of BHB models is their ability to integrate external sources of evidence (geological connectivity, paleoclimatic data, fossil record, species biological traits..) in the form of new variables, scaling factors, or prior distributions informing the posterior estimates of parameters. The use of a Bayesian hierarchical approach allows estimation of global biogeographic parameters over multiple clades – thus decreasing the uncertainty associated to a single character set – while integrating over clade-specific biological traits such as the rate of molecular evolution, age, or ability to disperse, as nuisance parameters. These models have been used to partition the contribution of different abiotic (geographic distance, area) and biotic factors (habitat specialization) in migration rates and area carrying capacities in animals versus plant lineages. The original CTMC process implemented in BHB was a time-homogenous model, assuming constancy of rates over time. I describe here extensions of the BHB model to relax the time homogeneity of the Markov process by allowing dispersal rates to vary over time and to test for the existence of temporal barriers to colonization. Non-equilibrium models can be employed to detect the signal of mass extinction events, evidenced as a sudden decrease in area carrying capacities, due to past catastrophic (geologic, climatic) events that partly wipe out the biota of a region. By allowing the intensity of these events to vary depending on clade-specific traits, these models could be used to "predict" the fate of lineages, for example, did some island guilds react more severely to catastrophic events (environmental perturbation) than others? Further work focuses on: a) extending these models to their use with phylogenomic “big” data (hundreds of sequences and genes) and to account for intraspecific processes (gene flow, incomplete lineage sorting, introgression); b) modeling the interaction among species (competition, facilitation) and among processes (trait- and geographic-dependent models). I will illustrate the use of these models with empirical studies on island and continental floras.

T2-11-04

Recent advances in biogeography: Integrating genomic and ecological data

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Some of the key questions that have fascinated naturalists and evolutionary biologists ever since Darwin and Wallace include: Why do species occur where they do? How did species attain their current distribution? Why do some species have very broad ranges of distribution, and others very narrow ones? How do ecological preferences, geologic/climatic events, and evolutionary processes shape species distributions? What are the relative roles of geographic barriers and adaptation in driving speciation? The recent advent of new methodological and analytical approaches opens unprecedented opportunities to gain novel insights into these classic biogeographic questions. I will illustrate how the integration of high-resolution molecular, distributional, geologic and climatic data with powerful analytical tools in phylogenomics and population genomics, ancestral area inference, species distribution modeling, and demographic modeling enabled us to answer long-standing questions on the biogeography of alpine, Mediterranean, and island plants, with emphasis on the first. By employing the integrative approach described above, we tested two competing hypotheses on the distributional responses of cold-adapted, alpine plants to past climatic oscillations. Specifically, we asked whether *Primula farinosa* contracted or expanded its range during interglacials of the Late Quaternary. At variance with prevailing notions that explain the currently fragmented distribution of cold-adapted species in terms of postglacial contraction, we found that *P. farinosa* expanded its range during interglacials. Using a combination of high-throughput genomic data and forecasting models of species distribution, we predict that lineages within the same species harboring sufficient ecological and genomic diversity are resilient to climate warming, while less diverse infra-specific lineages are threatened with extinction. Comparisons of species distribution models also allowed us to test the hypothesis that the ecological preferences of newly arisen polyploids should be both distinct and broader than those of their diploid relatives. As expected, we discovered that the ecological niches of species in *Primula* sect. *Aleuritia* at different ploidy levels differ significantly in both climatic and geographic space. However, contrary to expectations, we found that the ecological niches of polyploid species are narrower than those of their diploid relatives. The integration of ecological niche modeling with quantitative analyses of floral traits allowed us to ask whether niche and/or floral shifts favor the establishment and persistence of newly arisen polyploid populations within *Primula marginata*. In this case, we found that neither the ecological niches nor the floral traits of populations at different ploidy levels within the same species differ significantly, hence the disjunct distribution of dodecaploids in *P. marginata* is probably maintained by dispersal limitation and minority-cytotype exclusion. Expanding the focus of our analyses to the entire family Primulaceae, we used sister-species comparisons of distributional ranges and ecological preferences to investigate the relative roles of geographic vs. ecological divergence as major drivers of speciation. Our results confirmed predominant models of allopatric speciation as a main engine of biodiversity in plant evolution, especially in alpine/arctic environments.

T2-11-05

Estimating divergence times and ancestral breeding systems in *Ficus* and Moraceae

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Flowers are the highly variable reproductive structures of angiosperms. Although most flowers are bisexual (hermaphrodite), many species are characterized by other breeding systems (including monoecy, dioecy, polygamodioecy). The relationship between breeding system and diversity is still not clear. Dioecy was previously thought to be an evolutionary dead end, while in the latest decade, this perspective has gradually changed. In some clades, dioecy has been found to evolve into other breeding systems frequently and these transitions could be linked to habitat, pollination strategies and other environmental or ecological factors. Family Moraceae (40 genera, ca. 1100 spp.) harbors four different breeding systems: monoecy, androdioecy, gynodioecy and dioecy. *Ficus* (ca. 750 spp.), which is the largest genus in Moraceae, has two breeding systems: monoecy and gynodioecy. Previous studies using parsimony found monoecy and dioecy to be ancestral in *Ficus* and Moraceae, respectively. Here, we reconstruct ancestral breeding systems of *Ficus* and Moraceae by using both parsimony and model-based approaches and we explore the sensitivity of our results to various parameters of the inference (including phylogeny, age, model, and taxon sampling). To do so, we first estimated a new timescale for diversification of *Ficus* and Moraceae by combining a revised set of 12 fossil calibrations and a densely sampled molecular data set of eight loci and 320 species (representing all families of Rosales and 36 out of 40 genera in Moraceae). We estimated the crown group-ages of *Ficus* and Moraceae to 40.8-56.0 Ma and 73.3-84.9 Ma, respectively. Using this new timescale and both maximum likelihood and Bayesian approaches to reconstruct breeding system evolution, we found the ancestral state of *Ficus* to be particularly sensitive to model selection. Gynodioecy was inferred as ancestral in *Ficus* with strong support with the unequal-rate maximum likelihood model, whereas, the equal-rate maximum likelihood model and parsimony supported ancestral monoecy in *Ficus*. The Bayesian approach instead suggests high uncertainty in this reconstruction. Conversely, all approaches consistently supported dioecy as the ancestral state in Moraceae. Our results show that monoecy and gynodioecy evolved from dioecy in Moraceae, although uncertainty remains on whether gynodioecy was an intermediate step in evolution to monoecy in *Ficus*.

T2-11-06

A phylogenetic framework for the carpel development regulation: Mixing and matching old with new

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A major evolutionary innovation in the plant lineage is the angiosperm carpel, their unifying character and most complex plant organ, composed of many clearly distinct tissue types to ensure reproductive success. However, the origin of the carpel is unknown, but many components of the gene regulatory network (GRN) governing carpel development and their genetic interactions are described in *Arabidopsis thaliana*. To unravel the evolution of the carpel GRN and to discriminate between "early" and "late" steps in carpel evolution we calculated thorough phylogeny reconstructions based on sequenced genomes such that orthologs of the major *A. thaliana* carpel GRN are now placed in their phylogenetic context. We find that the carpel GRN components are of various ages, and identify especially high retention rates for carpel development genes in Brassicaceae leading to Brassicaceae-specific interactions of carpel GRN members. Further, our data indicate that developmental processes present already in the most recent common ancestor of seed plants, such as reproductive meristem termination or adaxial/abaxial polarity specification requires few interacting transcription factors, which are not retained in duplicates after whole genome duplications (WGD). In contrast, developmental processes associated with derived carpel characters, such as the transmitting tract require larger numbers of interacting transcription factors which were retained as duplicates after WGD.

T2-12: Pattern, function, and evolution of biodiversity across the ancient EA-ENA floristic disjunction (two sessions)

T2-12-01

A genomics analysis of the eastern Asia - eastern North America floristic disjunction

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Large disjunctions in taxon distributions provide excellent opportunities to study biogeographic patterns and the processes that shape them. One of the most notable disjunctions is that of eastern Asia and eastern North America (EA-ENA). This disjunction is thought to have been formed when a large temperate forest circling the Northern Hemisphere was fragmented due to by global cooling and the loss of important habitat and land bridges during the Miocene, followed by further fragmentation during the Pleistocene. Sixty-five angiosperm genera exhibiting this disjunct distribution have been identified. In many of these genera, species richness is greater in EA than ENA, and this pattern has been attributed to higher rates of molecular evolution and speciation in EA. We used transcriptomes from species in 10 genera to gain new insights into how this disjunction may have affected gene and genome evolution and to evaluate whether rates of molecular evolution are elevated in EA relative to ENA. Single to low-copy nuclear genes were identified using MarkerMiner and classified into GO categories. We have focused on seven genera (*Campsis, Cotinus, Gelsemium, Nelumbo, Penthorum, Phrymus,* and *Saururus*) for which two species exhibit the disjunction, and three genera with three or more taxa (*Calycanthus*, *Cornus*, and *Hamamelis*). If rates of molecular evolution are indeed elevated in EA, then genetic divergence from the common ancestor of EA-ENA species to EA species would be greater than that to ENA species, leading to greater species richness in EA, as identified previously in numerous genera. We determined rates of evolution for many genes as a means of testing this hypothesis and determined which GO categories of genes have experienced strong natural selection. No statistically significant differences were identified between EA and ENA species pairs, suggesting equal rates of evolution for both species within pairs, despite long isolation. For larger genera, no pattern suggesting EA taxa evolve at faster rates was identified. Our results suggest that many genes across multiple GO categories are under strong purifying selection, with few exceptions, and indicate that greater species richness in EA may be due to factors other than rates of molecular evolution.

T2-12-02

Exploring intercontinental disjunctions in a rare complex system of sumac-gall aphids, host plants and symbiotic bacteria

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We investigated the co-evolutionary and biogeographic relationships among three associated taxa representing three kingdoms of life: sumac-gall aphids, sumac plants, and bacteria. Sumac-gall aphids (Aphididae: Eriosomatinae: Fordini) live obligately on species of sumac plants (Anacardiaceae: *Rhus* subgenus *Rhus*) for part of their life cycle, and show the classical eastern Asian (EA, 5 genera and 11 species) – eastern North American (ENA, 1 monotypic genus) biogeographic disjunction, a common pattern in many plant groups. While sumac-gall aphids are restricted to *Rhus* hosts in EA and ENA, *Rhus* subgenus *Rhus* has a slightly broader intercontinental disjunction, with two species extending to southern Europe and Hawaii. Furthermore, the sumac-gall aphids depend on the highly variable endosymbiont *Buchnera aphidicola* (Gammaproteobacteria: Enterobacteriales), a bacterium, which supplies the animal host with certain essential amino acids while the endosymbionts receive nutrients from the aphids feeding on the host plant's sap. To explore the diversification of this unique complex co-evolutionary system of sumac aphids, sumac summer hosts and the aphid's endosymbiont *Buchnera* across their geographic range and through time, we obtained complete mitochondrial genome sequences for the sumac-gall aphids and complete genome sequences of *Buchnera* bacteria via the shotgun genome skimming of total aphid genomic DNA. For the host plants *Rhus*, we utilized seven plastid and two nuclear markers. We conducted extensive phylogenetic analyses for all three major associated lineages, inferred their biogeoraphic range evolution, and dated the co-evolution and major disjunctions. We tested the hypotheses that (1) such an aphid/sumac host plant relationship dates back to the early Eocene; (2) the coevolution between the aphids and the sumacs has driven species diversification in sumac aphids; and (3) there is biogeographic parallel evolution of the aphids and the endosymbiont *Buchnera aphidicola*.

T2-12-03

Species delimitation, phylogenetics and biogeography of *Stewartia* s.l. (Theaceae) based on plastomic and morphological evidence

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Stewartineae (Airy-Shaw) Chang is the basal-most clade of Theaceae, and comprises *Stewartia s.l.* which includes *Stewartia* L. and *Hartia* Dunn. The former genus exhibits typical eastern Asian and North American disjunction, while the latter ranges from Southern China to Indo-China. The circumscription of the two genera and delimitation of some taxa had been controversial and the phylogenetic relationship among the clades within *Stewartia s.l.* remained unclear. To examine species delimitation, phylogenetics and biogeography of *Stewartia s.l.*, we collected complete plastid genome sequences for 19 of the 24 taxa and morphological traits from both specimens and field observations. Phylogenetic reconstruction suggested that the evergreen *Haria* Dunn was monophyletic and nested in the deciduous *Stewartia* L. *S. malacodendron* L. represented the earliest diverged species. Hartia was sister to the clade consisted of the other North American species, *S. ovata* (Cav.) Weath., and the sub-clade composed of Asian deciduous taxa. Thus, both genera were supported to be merged. The phylogenetic relationships of all studied species were robustly resolved. The earliest split within the Asian *Stewartia* clade resulted in speciation of *S. koreana* Nakai ex Rehder. The ancestral distribution reconstruction and dating suggested that *Stewartia s.l.* was originated in eastern North America in the mid-Miocene (14~20 Mya). The evergreen and deciduous taxa in Asia resulted from two independent biogeographic events in 8~15 Mya and 7~13 Mya, respectively. The within-clade divergence was initiated earlier in *Hartia* Dunn than that in Asian *Stewartia* L. (4.7~9.2 Mya vs. 1.4~3.5 Mya). The latter clade experienced rapid speciation events started in the Mid-Pliocene. The speciation of the China-endemic *Stewartia* L. involved three independent events. Morphological analyses lent further supports for the species delimitation. Both molecular and morphological evidence supported the controversial taxa as species, e.g., *S. shensiensis* Hung T. Chang, *S. acutisepala* P. L. Chiu & G. R. Zhong and *S. koreana* Nakai ex Rehder.

T2-12-04

Phylogeny and biogeography of Podophylloideae (Berberidaceae) with an emphasis on the diversification of *Dysosma endemic* to China

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Podophylloideae Eaton (Berberidaceae) is a small subfamily of 12 species, which contains *Sinopodophyllum* (Royle) Ying (1 sp.), *Dysosma* Woodson (7 spp.), *Podophyllum* L. (1 sp.), and *Diphylleia* Michaux (3 spp.). This subfamily has a disjunct distribution in eastern Asia (EA) and eastern North America (ENA) with ten species in temperate region of EA. Despite great medicinal pharmacogenetic importance, the phylogenetic relationships of the four genera within this subfamily are still controversial due to limited taxon sampling and few genetic markers employed in previous studies. In addition, the monophyly of *Dysosma* has not yet been tested. Here we reconstructed the phylogeny with extensive taxon sampling (including all 12 species and the close allies) using chloroplast genome sequencing and restriction site associated DNA sequencing (RAD-seq) data. We also examined the biogeography and diversification of this subfamily as well as the spatio-temporal processes of divergence within *Dysosma*. *Our* phylogenetic analyses, for the first time, strongly support *the monophyly* of *Dysosma* and a sister-group relationship between *Diphylleia sinensis* from China and *Diphylleia cymosa* (ENA)-*Diphylleia grayi* (Japan). We inferred that the maximum crown age of Podophylloideae is approximately 19 million years ago (Mya) and that the Himalayas is likely to be its ancestral area, with both migration events across Beringian land bridge (BLB) and vicariant events leading to its distribution in ENA. Climatic changes since the Early *Miocene* and the uplift of the Qinghai-Tibetan Plateau (QTP) probably drove the diversification of major lineages of Podophylloideae and speciation of three high-elevation *Dysosma* species from Southwest China and the south-eastern slopes of the QTP. For the *Dysosma versipellis-pleiantha* complex, our phylogeographic results support that the early divergence of *D. versipellis* and *D. pleiantha* proceeded through allo-peripatric speciation, possibly triggered by Early Pleistocene climate change, while subsequent climate-induced cycles of range contractions/expansions enhanced the eco-geographical isolation of both taxa. Furthermore, modelling of population-genetic data indicated that major lineage divergences within *D. versipellis* likely resulted from long-term allopatric population isolation in multiple localized refugia over the last glacial/interglacial periods, and which in turn fostered endemic species formation (*D. difformis*, *D. majoensis*) from within *D. versipellis* in Southwest China.

T2-12-05

Ecological traits and recent population dynamics of eastern Asian-eastern North American disjunct tree species in North America

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The biogeography of plant species with disjunct distributions between eastern Asian (EA) and eastern North American (ENA) suggests evolutionary stasis (because sister taxa on the two continents tend to occur in similar climates) and a tendency towards drought-intolerance (e.g., disappearance from western North America since the late Tertiary due to increased aridity). These observations suggest that the ecological traits and dynamics (e.g., population trends) of EA-ENA disjuncts may be distinct from co-occurring non-disjunct species. We compared the ecological traits and recent population trends of EA-ENA disjunct and non-disjunct tree species in ENA using a database of ecological traits and systematically sampled forest inventory data collected by the U.S. Forest Service in the 1980s and 2000s. Disjuncts tended to be less drought-tolerant and more shade-tolerant than non-disjuncts. A majority of disjunct species were classified as being pyrophobic (negative population response to fire), whereas a majority of non-disjuncts were classified as being pyrophilic (positive population response to fire). These and other ecological/functional traits displayed a moderate degree of phylogenetic conservatism when examined in the context of a phylogeny of ENA tree species. Despite ecological trait differences between disjunct and non-disjunct species, the two groups had similar population trends from the 1980s to 2000s in the north-central, northeastern, and southeastern USA. We suggest that trait differences had counter-acting effects on disjunct and non-disjunct species, so that the two groups had similar population trends despite being ecologically distinct. For example, drying trends over recent decades in the north-central and southeastern USA would be expected to adversely affect drought-intolerant disjunct species, but the expected population declines may have been counter-balanced by fire-suppression, which favors pyrophobic disjuncts over pyrophilic non-disjuncts.

T2-12-06

Macroecology of leaf morphological traits of Chinese woody plants

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Diverse leaf morphologies reflect the adaptation of plants to their habitats, and can directly influence carbon uptake and water loss of plants. The relationship between patterns in leaf morphology and climate has long intrigued botanists. Among all leaf morphological traits, leaf size and shape have been widely observed to vary along climatic gradients, but quantitative description of the variations and the underlying mechanisms are still largely lacking. Here, using distribution maps and leaf morphological data (including leaf margin states, leaf length, leaf width, and length-width product/ratio) of 10480 Chinese woody dicots and dated family-level phylogenies, we evaluated the phylogenetic signals of leaf size and shape, and demonstrated the geographical patterns in leaf size and shape and their relationships with climate across different life-forms (evergreen and deciduous; trees, shrubs and lianas) and species with different family ages. Significant phylogenetic signals were found for leaf margin state of families, but not for mean leaf size and shape. Our results indicated that 1) proportions of species with entire leaves in regional floras were significantly correlated with mean winter temperature. However, their relationship was substantially influenced by precipitation regimes, plant life-form and evolutionary history, which suggests that caution is needed in the usage of the widely-used method (leaf margin analysis, LMA) for paleo-temperature reconstruction. 2) Leaf size (including leaf length, leaf width and length-width product) significantly decreased from the tropics and subtropics towards the high latitudes. The geographical variations in leaf size were most strongly correlated with annual actual evapotranspiration (AET), net primary productivity and gross primary productivity (GPP) among all environmental variables tested and therefore could be used to reconstruct paleo-AET and ecosystem productivity.

T2-12-07

Evolutionary history and Environment drive Global Diversity Patterns of *Quercus* L.

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Evolutionary process, such as speciation, extinction and dispersal, can directly change species diversity. Climate could have influence on species diversity through these evolutionary process. Therefore the species diversity-climate relationship may be originated from evolution history. Early studies mainly focused on species diversity-climate relationship and proposed many hypothesis to explain the spatial variation of species diversity. Recent researches on spatial variation of species richness proposed new hypothesis based on evolutionary history, such as evolutionary rate hypothesis, time-for-speciation hypothesis and niche conservatism hypothesis *etc.* However, what drives the species diversity-climate relationship and the link between evolutionary history and species diversity-climate relationship is still poor studied. By incorporating a phylogenetic perspective, this question may be best studied. Here we will use a phylogenetic approach to address the causes of species diversity-climate relationship in Oaks (*Quercus* L.) by combining data on distribution, leaf habitat, climate, Quaternary climate and phylogeny. Specifically, we are aiming to answer the following three key questions to address the origin of oak species diversity-climate relationship.1) How the historical event or historical climate change influences the evolutionary rate, niche evolution, leaf habitat evolution and species diversification of oaks? Dose the historical event influence species diversification by speed the evolutionary rate, hence force promote the niche and leaf habitat evolution? 2) Does evolution history drive the species diversity-climate relationship? If it is true, which hypothesis plays a key role in the origin of species diversity-climate relationship, the evolutionary rate hypothesis, time-for-speciation hypothesis or niche conservatism? 3) Dose evolutionary history have influence on the relationship between species diversity and Quaternary climate change? Using the clades with different evolutionary history, we will compare the effects of Quaternary climate change on species diversity among clades and identify which clade in which area is easily to be affected by climate change. This project aims to enhance the understanding of mechanisms of the species diversity patterns; especially the influence of evolutionary history on the species diversity-climate relationship. Taking into evolutionary perspective, the achievements of this project could be used in the conservation policy plan under global climate change.

T2-12-08

Microbial biodiversity in the disjunct forests of Eastern Asia

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The mixed deciduous forest in Eastern China represents important proportions of relics of once widespread Tertiary mesophytic forests that spanned the Northern Hemisphere. This forest type shared dozens of genera of seed plants that have closely related species occurring in China and America, which were also referred to as disjunct taxa. In the context of floristics, we sampled soils and leaves strictly corresponding to 347 disjunct tree individuals in five mountain forest across Eastern China, and explored the diversity and community composition of plant-associated fungi by using Illumina sequencing of the ITS1 and 18S region. Preliminary analyses indicated the strong site effect on soil fungal assemblages, and that pH, nutrient contents were the best predictors for soil fungal communities. A significant distance-decay pattern was observed for fungal community across whole area and individual mountains. Partial Mantel test showed the relative effect of geographic distance (Mantel r=0.491) was slightly stronger than that of environmental factors (Mantel r=0.447). We found a very strong effect of tree species identity on fungal assemblages and significant interactive effect with site. A large of unexplained variations may could be explained by the plant phylogenetic distance and functional traits.

T2-12-09

Phylogenetic, ecological, and functional diversity in the disjunct forests of eastern Asia and eastern North America

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Biodiversity is multidimensional, comprising genetic and phenotypic variation above and below the species level and diverse functional roles played by genotypes across space and time. In this project, co-funded by the US and Chinese National Science Foundations, we are addressing phylogenetic, genetic, and functional diversity in the context of plant and microbial communities that span an ancient floristic disjunction to discover new components of biodiversity that arise at the intersection of these three dimensions. The deciduous forests of eastern Asia (EA) and eastern North America (ENA) represent relics of once-widespread mixed mesophytic forests of the Northern Hemisphere. Despite over a century of observation and study, many questions remain regarding the origin, evolution, and assembly of these forests. Disjunct EA and ENA forests provide an excellent natural system to examine the link between species diversity and ecological function within the context of a shared phylogenetic history; our project is enabling novel analyses of plant community diversity, structure, and function in forests that are linked both ecologically and evolutionarily. Moreover, this biogeographic disjunction provides opportunities to assess the relative roles of historical constraint, local adaptation, and time in shaping extant diversity and function of co-distributed plant and microbial communities. Collaborations among plant phylogeneticists, plant and ecosystem ecologists, environmental microbiologists, and microbial geneticists in the USA and China are exploring biodiversity across spatial and temporal scales to discover those factors that shape biodiversity through space and time.

T2-12-10

Microbial biodiversity in the disjunct forests of eastern North America

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Biodiversity is multidimensional, comprising genetic and phenotypic variation above and below the species level and diverse functional roles played by genotypes across space and time. In this project, we are addressing phylogenetic, genetic, and functional diversity in the context of plant and microbial communities that span an ancient floristic disjunction to discover new components of biodiversity that arise at the intersection of these three dimensions. The deciduous forests of eastern Asia (EA) and eastern North America (ENA) represent relics of once-widespread mixed mesophytic forests of the Northern Hemisphere. Despite over a century of observation and study, many questions remain regarding the origin, evolution, and assembly of these forests. Using 16S rRNA and ITS rRNA sequencing of the bacterial and fungal communities associated with soil and leaves from trees in different forests from EA and ENA we investigated the associations in the microbiome that defined each region and the different host tree phylogeny. This revealed differences in the microbial community composition associated with the different disjunct genera. Interestingly, the microbial communities associated with *Acer* sp. and *Nyssa* sp. cluster together in Ordway sites. Archaea are likely driving these similarities (and relative dissimilarities to the other tree genera) with Thermoplasmata (Euryarchaeota phylum) and NRP-J (Crenarchaeota) orders the predominant members of the archaeal fraction. The *Nyssa* and *Acer* associated taxa in Thermoplasmata are similar to methylotrophic methanogens, which could be explained by the presence of methylated root exudates. A site associated difference was observed between Ordway and Talladega sites. However, no tree-species effect was found on the soil microbial communities. We will discuss these details and highlight ongoing results that are currently being generated.

T2-12-11

New insights into phylogenetic and phylogeographic relationships among intercontinental disjunct members in subfamily Orontioideae (Araceae)

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Subfamily Orontioideae includes three north temperate genera (*Symplocarpus*, *Lysichiton*, and *Orontium*) of the primarily tropical Araceae. Orontioideae and its monotypic sister subfamily Gymnostachydoideae are referred to as the proto Araceae and fossil evidence suggests Orontioideae dated back to the late Cretaceous in the temperate Northern Hemisphere. The monotypic genus *Orontium* is restricted to eastern North America (ENA) and *Lysichiton* with two species occurs in eastern Asia (EA; *L. camtschatcensis*) and western North America (WNA; *L. americanus*). Genus *Symplocapus* includes five species and is disjunctly distributed in EA (4 spp.) and ENA (1 sp.). Based on extensive sampling, we newly evaluated phylogenetic relationships among genera and species within Orontioideae and estimated the timing of intercontinental disjunct events in northern hemisphere. Overall phylogenetic relationships among three genera are congruent with the earlier study. However, we found a deep divergence between two major lineages within *Symplocarpus*, which correspond to their ploidy levels: a diploid lineage includes *S. nipponicus*, *S. egorovii*, and *S. renifolius* (Korea), while a tetraploid lineage consists of *S. foetidus*, *S. nabekuraensis*, and *S. renifolius* (Japan and Russia). This lineage divergence event within *Symplocarpus* was estimated to be 34 mya (late Eocene). Two distinct lineages of *S. renifolius* in Korea and Japan show marked cytological and morphological differences. The origin and diversification of these species were estimated to occur during mid Miocene. Two intercontinental disjunct events in *Lysichiton* (between EA and WNA) and *Symplocarpus* (between EA and ENA) are estimated to be 27.7 mya (late Oligocene) and 17.8 mya (early Miocene), respectively. Based on the phylogenetic framework of Orontioideae, we carried out three phylogeographic studies based on the same four highly variable chloroplast noncoding regions (*trn*Q-*rps*16, *psb*J-*pet*A, *trn*S-*trn*G, and *rpl*32-*trn*L). First, for the phylogeographic study of *S. nipponicus* in EA, we sampled a total of 457 individuals (15 populations with 224 individuals in Korea and 21 populations with 233 individuals in Japan). We found no haplotype sharing between populations in Korea and Japan, higher genetic diversity in populations from Korea, two highly divergent haplotype groups from Japan, and no geographical structuring within each country. Second, we assessed phylogeographic patterns of tetraploid intercontinental disjunct species pair, *S. foetidus* in ENA (32 populations; 485 individuals) and *S. renifolius* in EA (Japan) (22 populations; 233 individuals). We found higher haplotype diversity in *S. renifolius*, no haplotype sharing between two taxa, and two divergent haplotype groups in *S. renifolius* in EA. The detailed phylogeographic patterns of ENA *S. foetidus* only will also be presented. Lastly, we conducted phylogeographic study of intercontinental genus *Lysichiton* (EA-WNA disjunct) (*L. camtschatcensis*, 20 populations with 191 individuals and *L. americanus*, 20 populations with 225 individuals) and found that WNA species had twice the haplotype diversity of EA species. We also found diverse haplotypes present within populations in WNA species. Comparing phylogeographic patterns in *Lysichiton* and *Symplocarpus*, we suggest different timing and direction in two intercontinental disjunct genera.

T2-12-12

Divergent morphological and ecological evolution in the bulrushes *(Scirpus)* and cotton-grasses *(Eriophorum)* explains EA-ENA and circumboreal distributions

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The *Scirpus-Eriophorum* (Cyperaceae) complex is an ideal clade to test hypotheses regarding the timing and directionality of migration throughout the Holarctic, the historical processes resulting in either circumboreal (*Eriophorum*) or disjunct, eastern Asian-eastern North American distributions (*Scirpus),* and the evolution of morphologcal and physiological traits associated with arctic climates. Here, we present the most densely sampled phylogeny of *Scirpus* and *Eriophorum* to date, utilizing chloroplast, nuclear ribosomal, and low copy nuclear genes in conjunction with over 60,000 SNPs recovered from nearly 10 billion bases of Next Generation Sequencing data. Our analyses recover unprecedented resolution to the relationships among the bulrushes and cotton grasses. We complement our extensive molecular dataset with both traditional and geometric morphometric analyses. We reconstruct the ancestral biogeography and ancestral climatic tolerances of *Scirpus* and *Eriophorum*, demonstrating that the relictual eastern North America – eastern Asia disjunct pattern characterizing *Scirpus* is the result of both "out of America" and "out of Asia" migrations across the North Atlantic and Beringian land bridges. We highlight the evolutionary shifts and accompanying morphological and ecological adaptations in *Eriophorum* potentially associated with its persistence in boreal and arctic climates throughout the northern hemisphere.

T2-13: Zingiberales: taxonomy, phylogeny and evolution

T2-13-01

Historical introgressions among *Roscoea* species of different elevations within the south slope of the Himalayas

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The Himalayas locates in the southern margin of the Qinghai-Tibetan Plateau (QTP), it is one of the well-known biodiversity hotspots in the world. Although numerous studies have been conducted to reveal the temporal history of organisms on/around the QTP region, mechanism of diversification and *in-situ* speciation within the Himalayas is still sparsely investigated. *Roscoea* shows a disjunct distribution between the Himalayas and the Northern Indochina. Species distribution of the genus within the Himalayas exhibits distinctive altitudinal differences. There is no habitat overlapping among all species. In elevation, *R. bhutanica*, *R. auriculata*, *R. nepalensis* overlap with *R. alpina*, *R. capitata* overlaps with *R. purpurea*. *R. tumjensis* occurs within the elevational range of *R. purpurea*. We collected samples across the entire distribution range of each species. 12587 SNPs were generated using RAD-seq approach. Hierarchical *D*-statistics analyses were employed to test the introgression directions among different species and clades. Ecological niche modeling with estimation of migration vectors were used to simulate habitat shift of *Roscoea* species during the ice cycles. Clear genetic boundaries among species were reconstructed based on a maximum likelihood method. Hierarchical *D*-statistics analyses estimated significant bidirectional introgression between species with close elevational range but no introgression between *R. alpina* and *R. purpurea* and between *R. alpina* and *R. capitata* (distant elevation between species). Ecological niche modeling predicted that high-elevation *Roscoea* within the southern slope of Himalayas moved to low elevation but low-elevation *Roscoea* seems to be stable during ice age. The habitat rose up back to high elevation during warm periods. These results suggested Quaternary climatic fluctuations likely triggered introgression among *Roscoea* species by secondary contact. Divergences among *R. alpina*, *R. purpurea* and *R. capitata* may be caused by pre-Quaternary uplift of the Himalayas. This is likely the first study to explore *in-situ* speciation processes of for a plant lineage within the Himalayas. It also provides a new insight to understand the origin of biodiversity within the Himalayas.

T2-13-02

Floral development reveals the existence of a sixth staminode on the labellum of basal Globbeae

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The flowers of Zingiberaceae are characterized by a complex perianth consisting of sepals, petals, and petaloid staminodes. Compared to other Monocots and more basal Zingiberales with a full androecium of two trimerous whorls, only a single adaxial stamen of the inner whorl is fertile, and the other stamens are variously transformed in a colourful labellum. The labellum is traditionally considered to consist of two staminodes belonging to the inner whorl, in addition to two smaller lateral lobes representing two staminodes of the outer whorl. The original abaxial staminode is thought to be lost. The tribe Globbeae consists of two smaller basal genera *Hemiorchis* and *Gagnepainia*, and the large genus *Globba*, consisting of about 100 species in Tropical Asia. While *Hemiorchis* and *Gagnepainia* have a well developed labellum with a central appendage, flowers of *Globba* are characterized by a small bilobed labellum. To understand the nature of the labellum in the tribe, the floral development was carried out in 10 *Globba* species representing the major subsections, the two species of *Gagnepainia*, and one species of *Hemiorchis*. In most *Globba* species there is no trace of a third staminode on the labellum, even in early development. The only exception is *G. geoffrayi*, which showed the development of a small third central lobe, that was consecutively lost in older buds. *Hemiorchis* also lacked a third staminode as most *Globba* did, but the evidence of a third staminode was clearly observed in the floral development of *Gagnepainia*, where it persists as the small appendage visible on the matured labellum. These observations indicate that the missing staminode is not lost independently of labellum formation, but is integral part of the labellum. The presence of the lost staminode in basal genera of Globbeae indicates that it has become progressively lost within the clade. Further studies in the other tribes of Zingiberaceae could reveal more cases of this ancestrally retained character, and is a clear link to other members of Zingiberales.

T2-13-03

Specific adaxial-abaxial polarity participates in the evolutionary stamen-to-petal conversion in *Canna indica*

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The Zingiberales is an order of tropical monocots comprising eight families (Musaceae, Lowiaceae, Strelitziaceae, Heliconiaceae, Zingiberaceae, Costaceae, Marantaceae and Cannaceae). The main line of the evolution of Zingiberales is the number of fertile stamens decreasing by (6)5 1 1/2, and the number of petaloid organs increasing contrarily. Bearing only a half fertile stamen in the flower, *Canna indica* (Cannaceae) is one of the representative species to investigate the developmental evolution of Zingiberales. In the androecium of *Canna indica*, 3~4 petaloid staminodes develop in place of stamens, and the only fertile stamen consists a one-theca anther and a petaloid appendage. Current evidences concerning ABC homeotic genes are insufficient to explain the stamen-to-petal conversion since C-function gene is expressed in the sterile petaloid androecial members. The one-theca fertile stamen can be seen as an intermediate state organ from a two-thecae stamen to a completely petaloid staminode, thus we examined the differences of molecular basis for the differentiation of anther and petaloid appendage. Class III HD-ZIP family members, which specify adaxial identity, were mostly expressed lower in petaloid appendage than in anther; while KANADIs and YABBYs, which promote abaxial identity, exhibited opposite expression patterns. Additionally, asymmetric expressions of marker genes for adaxial-abaxial polarity were dynamically detected during the bisymmetric-to-asymmetric change process of stamen primordium; at last, the adaxial marker gene was mainly expressed in the region between the two protrusions of the anther, while the abaxial marker was mainly expressed in petaloid appendage. We hypothesize that the fertile theca represents the adaxial part of the stamen, while the sterile petaloid appendage represents the abaxial part, and that the adaxial-abaxial polarity participates in the distinctive anther-petaloid appendage patterning within the stamen of *Canna indica*. These results can be applied in further study of the evolution of Zingiberales.

T2-13-04

Ovary structure and evolution in the Zingiberaceae and Costaceae

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Recent work on visual character description has established methods for extracting character data from otherwise intractable sources, such as developmental sequences. The methods are based on standardized photographs of homologous developmental stages, or homologous positions in complex structures. The photographs are then sorted into hierarchical similarity clusters called "character cladograms." Each photograph represents a character state, while the cladogram as a whole is the character. A character cladogram is a graphical representation of a single character, based on photographs not verbal descriptions. When used to reconstruct phylogeny each character cladogram serves as a hypothesis of phylogeny, and each dichotomy in the character cladogram represents a character state. The similarity groups in character cladograms may be hierarchically arranged, allowing for multiple levels of data extraction from the same set of photographs. This visual character description method avoids many of the problems that arise from the application of verbal labels, problems that include difficulty in representing variation in character states, and the inability to capture information that can be easily seen but that is hard to describe. In this study two methods of morphological character description were applied to the ovaries of the Zingiberaceae and Costaceae. The traditional method of character description, based on the analysis and verbal description of features, was contrasted with visually-based character cladograms. Taxa in the Zingiberaceae and Costaceae were selected based on published phylogenies of these taxa. To describe the characters, we identified similar positions in in the ovary and took standardized photographs of cross-sections. The levels selected for photography were below the locule, ¼ of the way from the bottom to the top of the locule, in the middle of the locule, ¾ of the way from the bottom to the top of the locule, and above the locule in the middle of the tissue that closes the ovary. Four photographs were taken of the following positions at each level: full cross section, one locule, one septa, the epidermal region opposite a locule. The photographs were used to describe 12 traditional characters and create 16 character cladograms: four character cladograms at each of the four levels, corresponding to the four positions described above. Once created, both the traditional characters and the character cladograms were converted to numerical values and plotted on a combined phylogeny of the Zingiberaceae and Costaceae constructed from published phylogenies of these families. Calculation of individual and ensemble consistency indices allowed comparison of the two methods and an assessment of their strengths and weaknesses.

T2-13-05

Pollen transfer and explosive style movement in Marantaceae

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Marantaceae (29/550) have a unique pollen transfer mechanism including secondary pollen presentation and a rapid explosive style movement. As the flowers have only a single chance to get pollinated, floral structures need to be highly synorganised. The fertile part of the androecium is reduced to a single bisporangiate theca; all other elements are sterile, showy (outer staminodes) and functionally specialised. The pollination complex consists of the style and the hooded staminode which holds the style. The style is a compact highly turgescent structure with a head differentiated into a pollen plate at its back, a rather large stigmatic orifice at the upper side and a sticky gland separating the two parts. The hooded staminode is a thin structure bearing a trigger appendage and a basal plate. Already in the bud, the pollen plate is loaded with own pollen. Shortly before flowering, the style elongates more than the hooded staminode enveloping the style. Thereby, tension is set up. The tension is hold by the hooded staminode and released when a pollinator touches the trigger appendage. The mechanical stimulus is transferred via the basal plate to the contact point at the base of the style head where the style presses against the hood of the staminode. Within a spit of a second, the style springs forward. While rapidly rolling in, foreign pollen is scraped from the pollinator into the stigmatic orifice and own pollen is glued onto the same position of the pollinator. The pollination mechanism in Marantaceae is highly derived and much more complex than the pollen transfer process in the sister group Cannaceae (1/10). The high number of species suggests that the explosive style mechanism might be a key innovation for speciation. However, in view of the low number of seed set throughout the family and the vigorous clonal growth of the plants, the question raises how far the pollination mechanism indeed contributes to speciation. Reconstructing reproductive isolation mechanisms throughout the family reveals that geographic isolation and adaptation to different pollinators may have influenced radiation more than the pollination mechanism. The existence of highly diverse and elaborate floral structures should thus be interpreted as inventions optimizing the reproductive system by pollen saving or reducing investment costs.

T2-13-06

The evolutionary history of the Zingiberales: The final Word on a Charismatic Lineage of tropical plants using targeted gene enrichment

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The evolutionary relationships of the eight families of the tropical order Zingiberales has been debated by taxonomists for decades. Nearly every possible topological arrangement for these eight lineages has been proposed and defended with anatomical, morphological, DNA sequence, and genomic data. Well-supported reconstructions have been especially problematic among the basal branches (the "Banana Families") due to an early rapid evolutionary radiation in the lineage. Previous investigations have suffered in part from the lack of adequate generic sampling within families and the use of a limited number of genetic markers, mostly plastid loci. We have employed hybridization-based targeted enrichment to obtain sequences from 4,618 exons (all longer than 120 bp) within 1,180 orthologous nuclear loci (all longer than 960 bp), and 78 coding regions from the plastome. Concatenated data matrices were analyzed using ExaML and supported individual gene trees were merged using a coalescent framework in ASTRAL and a supertree method (MRL). Only one fully supported topology was recovered in all three multi-locus analyses (concatenated, coalescent, and supertree) with no significant conflicts among data sets and/or analytic procedures. The single topology is composed of two primary clades, which diverged early in the evolution of the order. One clade, the "Ginger Families, " which includes (Costaceae + Zingiberaceae) (Marantaceae + Cannaceae), has been well-resolved and well-supported in all previous studies. The other primary clade, the "Banana Families, " has until now never been strongly supported as a monophyletic group. The topology among these four families, Musaceae (Heliconiaceae (Lowiaceae + Strelitziaceae)), is finally fully and unambiguously resolved.

T2-14: Integrated research in Magnoliales

T2-14-01

Which fleshy fruit traits allow for long-distance dispersal? A deep-time perspective from the custard apple family (Annonaceae)

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Tropical rain forests are the most species-rich ecosystems on Earth and this diversity may have resulted from *in-situ* diversification as well as colonization by (megathermal) lineages through long-distance dispersal (LDD). Frugivory (fruit-eating and seed dispersal by vertebrates) is the primary dispersal mode for the majority of tropical plants, but it remains unclear which fleshy fruit traits have allowed these lineages to colonize new continents historically. Here, we test whether certain fruit functional traits are associated with long distance dispersal events during the Cenozoic in the custard apple family (Annonaceae, ca. 2400 species). Annonaceae has a pantropical distribution and shows a wide-range of vertebrate-dispersed fruit types, which differ in size (0.3 to > 40 cm), color and display (e.g. cauliflory). We use a phylogenetic framework to reconstruct the biogeographic history of Annonaceae in relation to the evolution of fifteen fruit functional traits. We test whether ancestral fruit traits during LDD events are significantly distinct from the average ancestral Annonaceae fruit type, and we classify them according to several Cenozoic dispersal routes. We find that LDD events during the Eocene climatic optimum, when Annonaceae may have taken the boreotropical route of dispersal, are associated with fruits consisting of a few large monocarps (the dispersal unit, ca. 3 cm), growing on relatively short stipes (ca. 0.7 cm) and bearing several seeds (ca. 4). These fruits are typically appealing to large-bodied mammals that are expected to contribute disproportionally to long-distance dispersal events. Several oceanic dispersal events may have been associated with typical bird-dispersed fruits, such as moniliform or dehiscent fruits with bright (red, black, yellow) colors. These results suggest that the evolution of fruit syndromes linked to large-bodied mammals and strong-flying birds may have allowed fleshy-fruited rain forest taxa to disperse and colonize new continents during the Cenozoic when climatic conditions were suitable. This may in part explain the disjunct distribution of megathermal plant taxa across the tropics.

T2-14-02

Subito allegro – the pace of diversification in the Annonaceae

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Despite considerable efforts in recent years to elucidate phylogenetic relationships within the Annonaceae, we only have a faint understanding of diversification patterns. The reasons for this are twofold: first, our efforts to infer the ages of Annonaceae are seriously hampered by lineage-specific heterogeneity of substitution rates. The main effect of the disregard of these differences is the underestimation of node ages in the subfamily Malmeoideae, which in turn results in the overestimation of diversification rates. The second cause is the incompleteness of taxon sampling in most family-wide analyses so far. Tree shape of the two main clades of the Annonaceae, the subfamilies Annonoideae and Malmeoideae, is very different. Phylogenetic signal in the Malmeoideae is fairly evenly distributed over the branches from the crown node to the tips, whereas the topological patterns in the Annonoideae are more radiation-like, with long branches subtending abrupt lineage diversifications. A commonly used approach of sampling a small of number of species per genus is likely to leave many patterns of diversification unrevealed. To explore node ages and diversification patterns in the light of these observations, we gathered a data set containing over 1000 species, for which sequence data of multiple plastid markers are available. In our analyses we accommodate for substitution rate heterogeneity among the major lineages, and apply three undescribed and previously unexploited fossils as calibrations. Using Bayesian approaches to the inference of node ages and diversification rates, we demonstrate the evolutionary build-up of species diversity in the family.

T2-14-03

Phylogenetic analyses and the position of Cretaceous fossils in Magnoliales: Implications for character evolution, age of clades, and past diversity

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Phylogenetic analyses have confirmed and refined relationships of several Cretaceous fossil taxa to Magnoliales, providing minimum ages for nodes, insights on the course of character evolution, and surprises concerning the past morphological and ecological diversity of the order. Archaeanthus, based on bilobed leaves and flowers with an elongate receptacle from near the Albian-Cenomanian (Early-Late Cretaceous) boundary in Kansas, is probably a stem relative of Magnoliaceae; a recent suggestion that it was nested in the crown group was based on use of inappropriate outgroups. Our earlier analysis associated an older fossil, Endressinia, known as leafy shoots and flowers from late Aptian lake deposits in northeastern Brazil, with the living clade consisting of Degeneria, Galbulimima, Eupomatia, and Annonaceae, which is united by inner staminodes (retained in Anaxagorea but lost in the remaining Annonaceae). However, a more recent analysis suggests that Endressinia and Schenkeriphyllum, another genus from the same deposits, may also be stem relatives of Magnoliaceae, attached below Archaeanthus. This would imply that inner staminodes were originally present in the ancestors of Magnoliaceae but later lost.

Based on their leaf morphology and associated paleoclimatic indicators, these fossils suggest that some early Magnoliales were able to adapt to more arid tropical conditions than might be inferred from living members. Futabanthus, a flower from the Coniacian (Late Cretaceous) of Japan, may be a near-basal member of the clade comprising all Annonaceae other than Anaxagorea, based on its lack of inner staminodes. This clade is also represented by seeds with lamelliform endosperm ruminations from near the end of the Cretaceous. Cecilanthus, a bisexual flower with numerous whorled parts from the Cenomanian of Maryland, has conflicting suites of characters, some suggesting relationships with Magnoliales, others with Nymphaeales. However, consideration of the most likely states of uncertain characters favors a position in Magnoliales, which would mean that the early radiation of the order produced now-extinct lines with floral morphology quite different from that of any living members. None of these fossils have associated pollen; in some cases, information on their pollen morphology or seed anatomy could resolve uncertainties on their systematic position.

T2-14-04

Gene tree discordance and coalescent methods support ancient intergeneric hybridisation between *Dasymaschalon* and *Friesodielsia* (Annonaceae)

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Although hybridisation between closely related species is common and known to be important in plant evolution, hybridisation at the generic level or above is comparatively rare. We address ancient intergeneric hybridisation in the early-divergent angiosperm family Annonaceae by reconstructing the phylogenetic and divergence history of the genus *Dasymaschalon* using a multi-locus approach based on molecular data from five chloroplast (*matK*, *psbA-trnH*, *ndhF*, *rbcL*, and *trnL-F*) and five nuclear (ITS, ETS, AP3, *PhyA*, and *PhyC*) DNA markers. We demonstrate incongruence among different gene trees: *Dasymaschalon* is retrieved as monophyletic in the nuclear ribosomal tree (based on ITS and ETS), but is non-monophyletic in the chloroplast and Phy-gene trees, with the majority of species assigned to a strongly supported clade but three species (*D. filipes*, *D. longiflorum* and *D. tibetense*) more closely related to the sister genus *Friesodielsia*. Three contrasting approaches—a coalescent method based on molecular dating, incongruence pattern comparison, and a multi-accession phylogenetic reconstruction—are used to assess the patterns of this gene tree incongruence and test hypotheses of ancient hybridisation and incomplete lineage sorting. Our results support a late Miocene intergeneric hybridisation between members of the *Dasymaschalon* and *Friesodielsia* lineages in continental Asia-west Malesia.

T2-14-05

Next generation Annonaceae phylogenetics: The way forward in Annonaceae evolutionary biology

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Annonaceae has been an important model family to address questions about tropical rain forest evolution and biodiversity. The phylogenetic relationships of the family have been inferred mainly at the generic level and based on few plastid markers. Lots of unresolved relationships remain, especially in subfamily Miliuseae. However, with the arrival of Next Generation Sequencing we now have the potential to generate a well resolved species level phylogeny based on hundreds of nuclear genes. Here, we used transcriptomes sequenced for five species from across the family to generate over 100 nuclear markers amplifiable across Annonaceae for inter and intra species evolutionary studies. The markers are generated with MyBaits and can thus be used to sequence hundreds of species at a time as well as degraded herbarium material. We use these new markers and sequence most species of the enigmatic African Piptostigmateae tribe (Miliuseae sub family). We show how useful these markers are at resolving generic, species and infra species relationships. We hope that the generation of these markers will mark a new age of Annonaceae evolutionary biology studies.

T2-14-06

Phylogenomics and historical biogeography of the recalcitrant annonaceae subfamily Malmeoideae

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Annonaceae (ca. 2400 species of trees, shrubs and lianas) are characteristic and ecologically important elements in tropical lowland forests of both the Neotropics and the Old World. There have been substantial advances in resolving the backbone phylogeny of the family in recent years, but there are notable exceptions. Intertribal relationships in subfamily Malmeoideae (7 tribes, 47 genera, >770 species) and intergeneric relationships in its largest tribe, Miliuseae (26 genera, ca. 540 species), remain very poorly understood. This has impeded downstream analyses such as investigations of the historical biogeography and character evolution in the family. We used a phylogenomic approach to resolve the relationships in subfamily Malmeoideae. Data from sequencing of total genomic DNA on the Illumina HiSeq2000 platform was used to assemble near-complete plastome DNA sequences. The dataset comprised 55 newly sequenced accessions including samples of all seven tribes and representatives of 45 of 47 currently accepted genera in the subfamily. These data were analysed separately and by integrating a supermatrix of previously published DNA data including accessions of all currently accepted genera in Malmeoideae using likelihood and Bayesian phylogenetic reconstruction methods. The resulting phylogenetic reconstructions largely resolve intertribal relationship and intergeneric relationships in tribe Malmeeae, and provide various new insights into intergeneric relationships within tribe Miliuseae. Parts of the Miliuseae backbone, however, remain unresolved. Likelihood ancestral range estimations indicate a wide ancestral distribution for the most recent common ancestor of the Malmeoideae crown group including the Neotropics, Africa and Asia. This is inferred as the result of a range expansion from Africa between the stem and the crown node sometime during a time interval ranging roughly from the mid-Cretaceous to the mid-Eocene. In combination with the fossil record, the results indicate that the 'boreotropics', a frost-free and humid climate corridor in the Northern mid-latitudes in the Paleocene-Eocene, likely played an important role facilitating intercontinental dispersal of this group.

T2-15: Phylogeny and evolution of the lamiids

T2-15-01

Early lamiid evolution: Basal relationships, ancestral characters, and ancient polyploidy

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Although our understanding of angiosperm phylogeny has improved tremendously over the past ca. 25 years, several major clades have eluded resolution, despite two decades of study and increasingly large molecular data sets. *Lamiidae*, an asterid clade with ca. 40,000 species (or 15 percent of angiosperm species richness), is a salient example. The clade includes four families previously unplaced at the ordinal level and an unresolved basal topology, hindering efforts to reconstruct ancestral states and early patterns of morphological evolution. However, recent work—employing plastome sequencing across key under-sampled lineages, namely genera of Icacinaceae s.l., Metteniusaceae, and Oncothecaceae—has greatly clarified our understanding of basal lamiid relationships. We summarize recent progress in inferring lamiid phylogeny, focusing on the positions and circumscriptions of previously unplaced taxa (e.g., Icacinaceae and Metteniusaceae). With this improved phylogenetic framework, we reconstruct the evolution of multiple morphological characters considered important in earlier classifications of asterids (e.g., unitegmic vs. bitegmic ovules, crassinucellate vs. tenuinucellate ovules, presence vs. absence of iridoids) as well as additional characters such as habit and fruit type. Finally, we explore instances of ancient polyploidy (or whole-genome duplication, WGD) in *Lamiidae* based on gene-tree reconstructions across a broad sampling of lamiid transcriptomes. Ancient WGDs have been identified in numerous major angiosperm lineages, underscoring the importance of polyploidy in flowering plant evolution. Preliminary data (KS-plots derived from lamiid transcriptomes) suggest the possibility of one or several WGDs early in the evolution of lamiids. Our results will provide important initial insight on the role of ancient polyploidy in the evolution of this major angiosperm clade.

T2-15-02

Phylogenies and proposed classifications of Hydrophyllaceae and Namaceae

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Recent research focusing on the systematics of Boraginales (Lamiidae) has resulted in revised infrafamilial, generic, and species circumscriptions. The clade comprising members of Namaceae have long been treated as a tribe (Namaeae Choisy) within Hydrophyllaceae, but has now been recognized as a distinct lineage, closely related to an emended Hydrophyllaceae. Namaceae includes 75 spp. in North and Central America, western South America, the Caribbean, and Hawaii. Hydrophyllaceae includes 240–260 spp. of North, Central, and western South America. Results of molecular phylogenetic analyses of Hydrophyllaceae and Namaceae found support for clades corresponding to some previously recognized infrafamilial tribes. However, reconciliation of previous classifications is complicated and requires revision to better reflect evolutionary relationships. Results were used to examine evolution of non-molecular characters, and compare diversification rates and patterns across clades. Proposed classifications are discussed.

T2-15-03

Progress in Gentianales systematics: Molecular phylogeny, morphological evolution and divergence times of a cosmopolitan Asterid order

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There has been substantial progress in our understanding of the phylogeny and biogeography of the Gentianales (Lamiidae, Angiospermae) in the last decades. Its five families are well-circumscribed and well-defined with morphological synapomorphies and key characteristics. Rubiaceae is the largest family (ca. 13550 species, 617 genera), Apocynaceae a medium-sized family (ca. 4700 species, 370 genera), the recently reclassified Gentianaceae has ca. 1700 species and 101 genera, and the remaining species belong to the two small families Gelsemiaceae (11 species, three genera), and Loganiaceae (ca. 500 species, 16 genera). Most order-wide phylogenies show Rubiaceae as sister to the other families, but relationships among the remaining four families have been in conflict. Rubiaceae comprise three subfamilies, Cinchonoideae, Ixoroideae, and Rubioideae, with an uncertain position for the genus *Luculia*. Apocynaceae are divided into five subfamilies (three monophyletic) and 23 tribes. Generic delimitation is a work in progress, with many genera, e.g. *Ceropegia, Vincetoxicum,* and *Marsdenia*, still being para- or polyphyletic as historically circumscribed. A new family classification of Gentianaceae will be published in 2017 recognizing seven tribes, including the resurrection of tribe Voyrieae and the acceptance of several new genera segregated from *Gentiana* and *Fagraea*. Gelsemiaceae has been expanded from two to three genera to include the Southeast Asian genus *Pteleocarpa*, one of the few Gentianales taxa with alternate leaves. Within Loganiaceae, the paraphyletic Australian genera Logania and Mitrasacme have been redefined, resulting in several new genera. In terms of distribution and biogeography, the Gentianales is cosmopolitan with most species in the tropics or subtropics, but certain lineages are more temperate or even alpine, particularly in Rubiaceae and Gentianaceae. Pantropical distributions are common in many clades/tribes. The area of origin is currently uncertain, but appears to be the New World tropics for Gentianaceae and the paleotropics for Rubiaceae. In an effort to provide a well-sampled, large-scale phylogeny for classification and a dated, biogeographic analysis of the whole order, we use automated methods implemented in the SUPERSMART pipeline for phylogenetic inference (www.supersmart-project.org). The time calibration is provided by fossils from the order Gentianales. To this end, we compiled a curated set of molecular sequences from all Gentianales species available in GenBank that are suitable for the analysis. Phylogenetic inference is accomplished in a two-step procedure, in which a genus-level backbone phylogeny is combined with species-level trees estimated under the multi-species multi-locus coalescent model. Our method uses Bayesian inference to ensure that uncertainty in the molecular data is reflected in the posterior node probabilities in the final phylogeny. We also outline key prospects for future research in the phylogenetics and biogeography of this diverse plant order.

T2-15-04

Nuclear phylogeny of Lamiids and related taxa: Implications on divergence and character evolution

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Lamiids form a large group of angiosperms and one of two major subdivisions of Asterids, which also include Campanulids (the other major subdivision, with sunflowers, lettuce, carrot and celery) and the orders Cornales and Ericales. Well-known Lamiid species include tomato, potato and tobacco in the Solanaceae, mint, basil, sage, thyme in Lamiaceae, olive, and many ornamental flowers. Current systematics according the APG IV divides Lamiids into eight orders: four of them (Boraginales, Gentianales, Lamiales, Solanales) are relatively large with multiple families; the other four are much smaller (Garryales, Icacinales, Metteniusales, and Vahliales), each with one or two small families, totaling about 50 families. Molecular phylogenetic analyses in the recent past have largely resolved well-supported relationships amongst many of the families and orders, but some relationships and placements remain uncertain. Similarly, in the larger context of Asterids, there are also many unresolved relationships at the level of families and orders. The present study has obtained hundreds of nuclear gene sequences from newly sequenced transcriptomes and other sequences using a set of criteria including length of aligned regions, species coverage, and putative orthology, and performed multiple phylogenetic analyses of Lamiid lineages and their Asterid relatives. The phylogenetic studies resolve strongly supported relationships, most of which are in agreement with previous results; in addition some relationships that were previously uncertain are now resolved. The nuclear gene sequences were used to estimate divergence times of orders and families, and the newly established phylogeny provides a framework for ancestral character reconstruction.

T2-15-05

Dispersal routes in the southern hemisphere and Neotropics: Examples from Lamiales

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Detailed phylogenetic study of Lamiales has permitted the enumeration and directionality of migration paths between southern hemisphere continents and throughout the Neotropics in several families. Dated trees, biogeographic inference, and habitat preferences of contemporary species, suggest that most intercontinental dispersal events involved long distance, over-water dispersal. Families of South American origin with contrasting dispersal patterns include Bignoniaceae with dispersal primarily to Australasia, and Verbenaceae with dispersal primarily to Africa. Scrophulariaceae originated in southern Africa and reached South America at least two times. Within the Neotropics, amphitropical disjuncts pose some of the same questions about dispersal timing and routes with long-distance vs. terrestrial migration routes possible. Several genera in Verbenaceae exhibit a pattern of disjunction between the arid zones of North and South America. With an origin in South America, the direction is clearly south to north. However, evidence seems to suggest that both dispersal paths occur in the history of the family.

T2-15-06

Evolutionary trends in Boraginales

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The phylogeny and systematics of Boraginales (ca. 125 genera and 2700 spp.) have been the subject of several recent, large-scale studies, largely resolving evolutionary relationships within the group. These have been translated into a revised classification both at the order level and at the infrafamilial level for the largest family (Boraginaceae, >80 genera, ca. 1600 spp.). The order is resolved with two major clades, Boraginales I comprising the families Wellstediaceae, Codonaceae and Boraginaceae, Boraginales II comprising the families Cordiaceae, Coldeniaceae, Ehretiaceae, Heliotropiaceae, Hoplestigmataceae, Hydrophyllaceae, Namaceae, and Lennoaceae. The well-resolved relationships now permit the study of character evolution across this order. A range of different morphological traits appears to show interesting evolutionary trends. Fruit morphology, the classical character complex used to classify Boraginales, can be shown to have a relatively complex character evolution, with the one-seeded mericarpids in Boraginales I and Boraginales II derived independently from capsule-fruited ancestors. Across the order, there has been a trend towards the reduction of seed number and segregation of the seeds into one-seeded mericarpids. Seed dispersal (wind-dispersal) seems to be the ancestral condition in Boraginales I and II, more specialized mechanisms of fruit or mericarp dispersal have then arisen in the more derived groups of both clades. In Boraginaceae the ancestral diaspore dispersal mode was epizoochory, probably associated with the presence of glochidiate ornamented nutlets or calyces, and that shifts to other dispersal modes occurred at least twelve times during the evolution of the clade. In Boraginales II there is an overwhelming trend towards endo-zoochorous dispersal, with several transitions to abiotic mechanisms. Detailed studies on floral morphology and function, especially in Hydrophyllaceae, Codonaceae and Boraginaceae, reveal a wealth of diversifications, especially with regard to flower organization and access to nectar, but also with regard to reward and breeding systems. Morphological trends mainly centre around the formation of specialized corolla outgrowths, which are well-developed in Boraginales I and Hydrophyllaceae. Additionally, a trend towards specific modifications of the nectary, gynoecium, style and stigma in the context of increasingly hypocrateriform corollas and flower compartmentalization can be observed in several lineages. There were multiple inventions of polymery and specialized functional syndromes, including dioecy, heterostyly, buzz-pollination and obligate autogamy across the order. Vegetative morphology and growth habits are diversified, as would be expected from any plant group of this size. It has also long been known that many representatives of Boraginales are characterized by a distinct, rough indument. However, detailed studies on the biomineralization of these trichomes have not been carried out in the past, and we present some data revealing complex mineralization patterns and striking trends, including individual, one-celled trichomes with up to three different biominerals in their cell wall. Our data show that trichomes with three different biominerals (calcium carbonate, calcium phosphate and silica) are found in both Boraginales I and Boraginales II. The basally branching families Codonaceae, Namaceae and Hydrophyllaceae are predominantly mineralized with calcium carbonate and phosphate, whereas the more derived Cordiaceae and Boraginaceae appear to be predominantly mineralized with calcium carbonate and silica.

T2-16: Orchid phylogenomics: Diversification, evolution and biogeography

T2-16-01

Orchid phylogenomics, multiple drivers of extraordinary diversification, and historical biogeography

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Orchidaceae is the most diverse family of angiosperms, with over 25,000 species – more than mammals, birds and reptiles combined – and one of the most broadly distributed families as well. Tests of hypotheses to account for orchid diversity have been stymied by the lack of a fully resolved broad-scale phylogeny. Here, my colleagues and I provide such a phylogeny, based on 75 chloroplast genes for 39 species representing all orchid subfamilies and 16 of 17 tribes, time-calibrated against 17 angiosperm fossils. A supermatrix analysis places an additional 144 species based on three plastid genes, including representatives of almost all orchid subtribes. Orchids appear to have arisen roughly 112 million years ago (Mya); subfamilies Orchidoideae and Epidendroideae diverged from each other at the end of the Cretaceous; and the eight tribes and three previously unplaced subtribes of the upper epidendroids diverged rapidly from each other between 37.9 and 30.8 Mya. Orchids appear to have undergone one significant acceleration of net species diversification in the orchidoids, and two accelerations and one deceleration in the upper epidendroids. Consistent with theory, such accelerations were correlated with the evolution of pollinia, the epiphytic habit, CAM photosynthesis, distribution in extensive tropical cordilleras, and pollination via Lepidoptera, euglossine bees, or deceit. Deceit pollination appears to have elevated the number of orchid species by one-half. The highest rate of net species diversification within the orchids (0.382 sp sp^{-1} My^{-1}) is 6.8 times that at the Asparagales crown. We reconstructed orchid historical biogeography using BioGeoBears. Orchids appear to have arisen on the Australian Plate and then spread to the Neotropics overland via Antarctica by 90 Mya, when all three continents were in close contact and Antarctica had a subtropical climate. Ancestors of the vanilloids, cypripedioids, and orchidoids + epidendroids appear to have arisen in the Neotropics 84 to 64 Mya. Repeated long- and short-distance dispersal occurred throughout orchid history. Stochastic mapping identified a mean of 74 long-distance dispersal events, for an average rate of 0.8 long-distance dispersal events every million years. Of the 33 orchid genera having disjunct distributions on continents separated by tropical oceans, Dressler argued that only five might be old enough to reflect vicariance driven by continental drift. Our data, however, indicate that all of these groups are too recent to reflect continental drift and are instead products of long-distance dispersal. We estimate that 15 transoceanic long-distance dispersal events resulted in the origin of orchid tribes or smaller lineages restricted to individual continents, and that all occurred long after Gondwana had begun to break up. The importance of modest amounts of long-distance dispersal for orchid diversification is highlighted by the fact that 97% of all orchid species are restricted to individual continents or subcontinents. Across orchid history, Southeast Asia was the most important source and maximally accelerated net diversification; across epidendroids, the Neotropics maximally accelerate diversification.

T2-16-02

Next generation species delimitation and phylogeography of a recently radiated clade of Australian *Corybas*

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Problems with elucidating species boundaries and evolutionary history abound in groups that have high rates of speciation, such as orchids. In order to reconstruct the phylogenetic and phylogeographic history in a recently radiated, Australian endemic clade (the Corysanthes clade) in the terrestrial orchid genus *Corybas* (Acianthinae, Diurideae), we used both sequencing of standard molecular markers (plastid spacers, nrITS, and the single-copy nuclear gene phytochrome C) as well as a form of reduced representation library sequencing, genotyping-by-sequencing (GBS). Phylogenetic analyses of the GBS data provided an unprecedented degree of resolution within this group, while remaining highly congruent with the results from the standard molecular regions. Based on our findings, we recommend reducing the number of species in this clade from 10-12, to seven monophyletic, strongly supported, morphologically distinct species (*Corybas fimbriatus*, *C. hispidus*, *C. pruinosus*, *C. diemenicus*, *C. incurvus*, *C. recurvus*, and *C. despectans*). *Corybas dentatus* appears to be a hybrid of *C. diemenicus* and *C. incurvus*. *Corybas limpidus* forms a basal grade relative to *C. despectans*, and *C. expansus* appears embedded within *C. despectans*. On the other hand, several morphological forms recognized by local botanists do appear to represent distinct evolutionary lineages and should be protected to preserve infraspecific genetic diversity and evolutionary potential.

T2-16-03

Development of a phylogeny for Australian *Caladenia* orchids to explore the evolution of specialised pollination

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Floral traits associated with the evolution of specialised pollination systems have been hypothesised to represent key innovations that lead to increased rates of speciation. However, the evolution of specialised floral traits may also constrain subsequent floral evolution. In orchids, the evolution of sexual deception from the more generalised food deception strategies may have important consequences for both diversification and long-term evolutionary persistence. Pollination by sexual deception is predicted to increase diversification rates, particularly when reproductive isolation is potentially readily generated by mutations that control the chemistry of pollinator attraction. The evolution of sexual deception may also lead to a specialised pollination system incapable of undergoing reversals to more generalised strategies. Until now, tests of these hypotheses have been impossible due to a lack of well-resolved phylogenies. *Caladenia* is a diverse genus of Australian terrestrial orchids comprising over 360 species. The genus is unusual among sexually deceptive genera in that it also contains species pollinated by food-reward and food-deception, providing a unique opportunity to investigate the evolution of specialised pollination systems. However, like many other orchid groups, existing phylogenies based on just a few chloroplast and nuclear DNA loci are poorly resolved at both the tips and the deep branches. Therefore, we are presently developing and applying an Exome Capture method with Next Generation Sequencing (NGS) for the phylogenetic analysis of *Caladenia* and other Australian terrestrial orchids. The probe design was based on cDNA sequences from the leaf transcriptomes of 11 species spanning the Australia Diurid orchids and outgroups, including two *Caladenia*, plus additional floral transcriptomes of four other *Caladenia* species. We are also targeting a subset of the 315 putative single locus orchid wide loci identified by Deng et al (2015), which will allow sequence sharing with other studies. In a key distinction from typical methods where the capture probes are designed to target short segments (<200 bp) across several thousand coding loci, we are simultaneously targeting longer regions (>1500 bp) consisting of both protein coding sequences and introns for up to 1500 loci. Modifications during the library preparation phase are designed to maximise the sequencing coverage of the predicted, but unknown introns. The downstream bioinformatics analysis will include *de novo* transcript assembly, alignment with the reference sequences, and extraction of coding only, coding and intron, and intron only sequence subsets for the phylogenetic and population genetic level analyses. At the time of writing the abstract, the laboratory work is still in progress. In the talk itself we will reveal the outcomes of our phylogenetic analysis, and discuss the insights revealed by our overlays of chemical and morphological traits onto the phylogeny. We will also provide a critical analysis of the strength and limitations of this NGS based technique and explore its applicability beyond our study system.

T2-16-04

Phylogenomic insights into the evolution of Australia's rich endemic orchid flora

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The Australian flora is characterized by high levels of endemicity, with 92% of its ca. 19,324 native vascular plant species not occurring anywhere else. The three orchid tribes Diurideae, Cranichideae, and Dendrobieae account for nearly 90% of the species diversity of the rich and highly endemic Australian orchid flora, which harbours many threatened species. Several lineages within these tribes underwent extensive diversification on the Australian continent, such as in the subtribes Prasophyllinae, Caladeniinae and Thelymitrinae (Diurideae), Pterostylidinae (Cranichideae), and sections of the epiphytic mega genus *Dendrobium* (Dendrobiinae). Previous phylogenetic studies in these groups were based on few molecular markers resulting in partially unresolved tree topologies with low support, and had gaps in the sampling of critical taxonomic groups. Hence, they provided only limited insights into phylogenetic relationships, morphological character evolution and spatio-temporal evolution. To improve our understanding of the evolution and biogeographic history of Australia's exceptional orchid flora we carried out phylogenomic studies in the Diurideae (nine subtribes), Pterostylidinae, and Dendrobiinae. Altogether, plastome data for 520 samples was generated via shotgun high-throughput sequencing, representing all genera and major intrageneric lineages within the eleven subtribes. 246 samples were included for Diurideae, 107 samples for Pterostylidinae, 143 samples for Dendrobiinae, plus the respective outgroup samples. Maximum likelihood analyses and Bayesian tree inference were carried out with RAxML and MrBayes based on concatenated alignments of ~ 46,000 bp length representing 64 plastid genes. Divergence time estimation was conducted based on fossil and secondary calibration points in BEAST. Key morphological and ecological traits were coded and character state changes traced along the phylogenies using maximum parsimony and maximum likelihood approaches. Distributions were coded based on major disjunctions, and ancestral areas were reconstructed based on the BEAST chronogram using model based inference methods using BioGeoBears. The phylogenetic analyses of our taxonomically comprehensive data sets for these eleven subtribes yielded highly resolved and well supported tree topologies. This is greatly improving our understanding of phylogenetic relationships within Diurideae, Pterostylidinae, and Dendrobiinae, e.g. the relationships between the nine Diurideae subtribes are now fully resolved and well supported. This also allowed for the reconstruction of key morphological and ecological characters, such as important floral traits or pollination syndromes, for these iconic orchid groups. The ancestral area analyses provided novel insights into the spatio-temporal evolution of Australasian orchid lineages and into the assembly of the Australian orchid flora.

T2-16-05

Phylogenomic Insights into the spatio-temporal evolution of Asian and Australasian *Bulbophyllum* (Orchidaceae)

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The mainly epiphytic orchid genus *Bulbophyllum* Thouars (Dendrobieae, Bulbophyllinae) is the second largest genus of flowering plants with about 2,200 species in 97 sections. The genus has a pantropical distribution with main centers of diversity in Australasia (New Guinea) and Asia, which harbour 67 sections and ~ 1,700 species. Previous molecular phylogenetic studies identified four main lineages within the genus: a large Asian/Australasian clade, an African and a Malagasy clade, and a South American clade. Intrageneric relationships within the Asian/Australasian clade are still poorly understood. This talk will present results from three phylogenetic studies with a focus on the Asian and Australasian *Bulbophyllum*. The first study examines evolutionary relationships within the Asian/Australasian clade based on a representative sampling of the sectional diversity within this clade and plastome data of 70 genes. Bayesian and Maximum likelihood analyses were carried out and yielded highly resolved and well-supported phylogenies providing new insights into intersectional relationships. BEAST analysis based on fossil and secondary calibrations and a broader outgroup sampling were run to estimate divergence times within the tribe Bulbophyllinae. Based on the BEAST chronogram ancestral area reconstructions were carried out in BioGeoBears providing insights into the spatio-temporal evolution of the genus. The second study focuses on the Australasian *Bulbophyllum* section *Adelopetalum*, which diversified mainly in tropical Australia. Phylogenetic analyses were conducted on a combined plastid dataset sampling up to three representatives of the every species of the section plus representatives of closely related *Bulbophyllum* sections as identified in our broader phylogenomic study. Reconstruction of phylogenetic relationships based on three genes (ITS, *mat*K and *ycf*1*)* identified main clades and resolved interspecific relationships within the majority of these clades. Nevertheless, relationships between major clades remained largely unclear. Therefore, plastome data of 70 genes was generated for each major lineage within the *Adelopetalum* alliance and combined with the previous plastid data set into a supermatrix which was then analysed using ML and Bayesian inference. Our results reveal section *Adelopetalum* is comprised of three main lineages and is paraphyletic with Australasian representatives of section *Minutissima* nested within it. Divergence age estimations using BEAST and ancestral area analyses using BioGeoBears were carried out to examine the spatio-temporal evolution of the *Adelopetalum* alliance in Australasia. The third study investigates patterns of intraspecific genetic diversity of two *Bulbophyllum* species of section *Adelopetalum* endemic to the mountain tops of Australia's Wet Tropics (*B. lageniforme* and *B. newportii*) to assess the likely impact of future climate change on the genetic diversity of Australia's mountain top orchids. Restriction site associated DNA (ddRAD) genomic data was generated for 8-15 individuals per population and SNP and multi-locus alignments produced using the pyRAD software pipeline. Our phylogeographic analysis reveal phylogeographic structure is present in both species corresponding to major climate refugia of the Plio- and Pleistocene.

T2-16-06

Genome size and phylogenetics of Vanilloideae (Orchidaceae) inferred from nextgen anchored phylogenomics

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Phylogenetic relationships among genera in tribe Vanilleae (Vanilloideae) remain elusive among various analyses that have addressed this tribe specifically or focused at the family level.

One of the reasons why phylogenetic reconstruction of this group has been so difficult is that five of the nine genera of the tribe are achlorophyllous mycoheterotrophs (e.g., unplaced *Lecanorchis*), and hence are poorly or not at all represented in molecular studies that relied heavily on plastid loci. Interestingly, even the relationships among some photosynthetic genera remain unresolved (e.g., the relationship between South American *Epistephium* and the clade of New Caledonian endemic genera *Clematepistephium*+*Eriaxis*) Through the use of Anchored Phylogenomics via Next Generation DNA Sequencing, we have inferred the phylogenetic relationships of most genera in the Vanilleae tribe using data from 23 low copy nuclear markers. Our results indicate that *Epistephium* forms a clade with *Clematepistephium* + *Eriaxis,* as well as *Lecanorchis*. Most earlier studies showed *Epistephium* to be sister to the entire tribe. Although the relationship of *Epistephium* to the New Caledonian taxa has been suggested before, this is the first time such a relationship is recovered with high support. The placement of *Lecanorchis* in the group reflects what would be expected based on the morphology of the flower, as it has a calyculus, an overgrowth of the top of the ovary that is also present in the other three genera of this clade. In addition to phylogenetic reconstruction, we also estimated genome size for most genera of Vanilloideae, since published reports for the subfamily to date have been entirely from Vanilla. Among angiosperms, orchids have the most variable genome size, ranging from 0.33 pg in *Trichocentrum maduroi* to 55.4 pg in *Pogonia ophioglossoides*, a member of Vanilloideae. Not only does the subfamily have the largest known genome, but it also contains the largest chromosome number reported for Orchidaceae: *Epistephium lucidum* (2n= ca. 170). Here we report the genome size for several additional members of Vanilloideae: several species of *Vanilla, Clematepistephium smilacifolium*, *Eriaxis rigida*, *Epistephium duckei*, mycoheterothroph *Erythrorchis cassythoides*, both *Isotria* species, tropical *Cleistes*, and others. Our data shows that there is at least an 18-fold range of genome size in Vanilloideae, from 2.985 pg for *Eriaxis rigida* to 55.4 pg in *Pogonia ophioglossoides*. The implications of these findings will be discussed in the context of the family's phylogeny.

T2-17: Biodiversity and phylogeography of bryophytes

T2-17-01

Combining ecological niche models and dispersal simulations to predict bryophytes dynamic response to climate changes in Europe

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Dispersal is a key evolutionary force that determines the survival, growth and reproduction of individuals, cycles of colonization and extinction of populations and globally drives species dynamic responses to their environment. In the context of extent climate changes, these dynamics take on even greater importance as the survivability of species depends on their ability to shift their distribution or adapt to changes in local climatic conditions. We focus on bryophytes, whose documented long distance dispersal ability, together with their lack of vascular system, position as likely indicator species to monitor the effects of climate changes. We use an approach combining Ecological Niche Models (ENM) and Dispersal Simulations (MigClim) to model the actual niche and simulate the future potential response of bryophytes to environmental changes in Europe. For this purpose we computed specific dispersal kernels using WALD analytical models that integrate both experimentally derived spores settling velocity and continental wind and forest canopy data to infer potential species dispersal capacities. MigClim cellular automaton was then used to simulate atmospheric dispersal over maps of habitat suitability generated from ENMs and enabled us to question European bryophytes ability to colonize suitable areas under actual, and predicted future climatic conditions.

T2-17-02

Biogeographic patterns of Southern South American bryophytes: New evidence based in recent exploration

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Pontificia Universidad Católica de Valparaíso

The southern Andes are isolated from the rest of the South American continent, and can not be classified as part of the Neotropics. The Atacama desert by the north, the high elevation of the central Andes reaching 7,000 m at Mount Aconcagua, and the dry Patagonian steppes by the southeast, constitute a strong barrier that only a few species can overcome. Remarkable bryogeographic patterns are found in the flora of this region, with the southernmost part of the Andes bearing about 50% of endemic taxa, and a large proportion of taxa shared with distant Austral land masses like New Zealand, SE Australia, Tasmania, the Subantarctic islands, and even with Antarctica. There are four times more species that have this latter distribution pattern than the species that can reach the Tropical Andes. By the other hand, central Chile has a very different bryophyte flora, with a rich endemic component and a large number of Mediterranean elements that links it with the bryophyte floras found in Mediterranean Europe, western North America, South Africa and western Australia, with many ephemeral taxa and a remarkable diversity of Pottiaceae, Bryaceae, and thalloid liverworts. The land between central Chile and the southernmost part of the continent show a combination of these two patterns resulting in a very rich and interesting bryophyte flora. Recent intense bryophyte exploration in different parts of Chile and Argentina (Valparaíso Region, Bío-Bío Region, Chiloé, Aisén, south Argentinian Andes, and Cape Horn) have yielded many new records and new species described for the area, improving the knowledge we previously had about both the diversity and the biogeographic relationships of the bryophyte flora of this part of the world. There is still little information about the conservation status of many rare and/or endemic species, and at the same time the human impact on ecosystems increases year by year, especially in the central Chilean regions. This new information gathered in the last years will help addressing these questions, as well as providing a rich source of specimens of all groups of bryophytes available for bryologists world wide to help understanding the diversity and phylogeographic patterns of many taxa.

T2-17-03

Taxonomy and diversity of Lejeuneaceae (Marchantiophyta): Past, present and future

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Lejeuneaaceae, a predominantly pantropical family with richest epiphyllous species, is usually considered the most difficult group of bryophytes owing to the extensive morphological homoplasy and great morphological plasticity, minute plants, and rich species diversity. It is the largest and most diverse family of bryophytes. Since 1820, over 100 genera have been described and 81,95,68, and 71 genera were accepted in 1983,1997,2013, and 2016, respectively. At present, the Lejeuneaceae contains 1873 species in 70 genera, comprising over 1/4 of the extant liverwort and hornwort species. Although recent morphological and molecular phylogenetic studies have substantially altered our understanding of this notoriously difficult family, the classification and phylogenetic relationships within the Lejeuneaceae remain incompletely known owing to the limited taxon sampling and little monographic work. In the present presentation, we review the taxonomic history of this family and report our recent taxonomic and molecular phylogenetic progresses based on a considerable extension of the taxon sampling. Five new genera and a number of new species were found. To reveal the morphological evolution, diversity, and phytogeography of the Lejeuneaceae, the future work should focus on intensive surveys in diversity-rich areas and comprehensive monographs derived from integrative evidence.

T2-17-04

How molecular evidence transformed our understanding of bryophyte diversity and phylogeography in Europe, the bryologically best known continent

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Bryophyte diversity and species' distributions in Europe have been studied intensively at least since the age of Hedwig at the end of the 18th Century. The bryoflora of Europe is therefore likely better known than that of any other continent. However, during the last few decades investigations of intraspecific molecular variation and phylogeographic patterns have revolutionized our understanding of European bryophyte diversity and its distribution. Earlier, bryophyte species were viewed as less variable than vascular plants, and it was thought that they encountered no dispersal limitations due to their small spores (often < 20 µm). Widespread species were assumed to be genetically more or less homogeneous over their entire distribution areas. This is clearly contradicted by the molecular variation unveiled below to just above the traditional morphological species level, as well as by the clear phylogeographic patterns discovered in many species. We now have evidence on how some European species evolved before the Pleistocene and on how other species recolonized Scandinavia after the latest glacial period. We have uncovered many earlier hidden species thanks to integrative taxonomy, have revealed (so far) cryptic species, and have disclosed paraphyletic species and other complicated molecular relationships among closely related species. I will present how our perception of the European bryophyte diversity has gradually changed from the relatively straight-forward species-centered interpretation before the molecular era, to a more complex and far more interesting modern and comprehensive view. Specifically, I will outline the implications of these new data (i) on our understanding of the extant European bryophyte diversity, its distribution, and evolution; (ii) on how to manage bryophytes in order to safeguard their diversity not only at the (morphological) species level, but their total diversity; and (iii) for bryophyte barcoding, in Europe and elsewhere.

T2-17-05

Diversity, biogeography and conservation of the thalloid liverworts and hornworts of Sri Lanka

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Sri Lanka is a tropical island in the Indian Ocean, with a total land area of about 65,610 km^2. The island harbors a diversity of ecosystems, with a varied climate and topography. Together with the Western Ghats of India, Sri Lanka forms a global biodiversity hotspot. The bryophyte flora of Sri Lanka remains poorly researched, and therefore does not contribute much towards national biodiversity statistics. According to literature-based checklists, there are 560 species of mosses (Bryophyta), 327 species of liverworts (Marchantiophyta) and 5 species of hornworts (Anthocerotophyta). Thalloid liverworts recorded include 8 families, 9 genera and 20 species of complex thalloids (Marchantiidae) and 6 families, 8 genera and 19 species of simple thalloids (Pelliidae and Metzgeriidae). There is no Flora of Sri Lankan bryophytes; endemic and threatened taxa have not been identified, a major impediment to carrying out research on these important lineages. Of the three bryophyte phyla, Marchantiophyta and Anthocerotophyta are the least studied. The present study aims to investigate the biodiversity and biogeography of thalloid liverworts and hornworts of Sri Lanka. Field explorations were carried out throughout the country, from the coastline and lowlands to the central highlands, to collect fresh thalli of liverworts and hornworts. Morphological and anatomical characteristics of the collected samples were observed using light and stereo microscopes. Spore morphological characteristics were observed using scanning electron microscopy (SEM). Specimens were identified to generic and specific levels using taxonomic keys and monographs. Taxa identified include 8 families, 9 genera, 13 species of complex thalloid liverworts (Marchantiidae), 4 families, 6 genera, 6 species of simple thalloid liverworts (Pelliidae and Metzgeriidae) and 3 families, 5 genera, 6 species of hornworts. This includes two new records of hornworts for Sri Lanka. The study presents the first phylogenetic framework of Sri Lankan thalloid liverworts and hornworts, based on DNA sequence data from two plastid genes (*psb*A and *rbc*L) and one nuclear gene region (ITS), sequenced for 100 accessions, representing samples collected throughout the geographic range of the island. Phylogeographic patterns of all identified species were traced and mapped using GIS. The highest diversity of thalloid liverworts and hornworts was encountered in the central highlands (>1000 m) of the country, under a cool temperate climate. Their microhabitats include wet soil, rock crevices, rotten wood, mountain slopes and roadside verges. Many of their habitats are under severe threat due to construction and cultivation. Air pollution by vehicle emissions causes changes in the microclimatic conditions and may force out some sensitive bryophytes from their natural habitats. This study emphasizes the importance of protecting these habitats, and thereby conserving the existing diversity of the bryophyte flora of Sri Lanka.

T2-17-06

Inferring species delimitation of the problematic *Riccia fluitans* species complex (Ricciaceae, Marchantiidae) with integrative taxonomy

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Understanding processes by which species diversify is a fundamental problem in ecological and evolutionary genetics. Among thalloid liverworts, thecosmopolitan *Ricciagenus* contains a large number of species and paradoxically a great endemism rate, common pattern among angiosperms but quite rare among bryophytes. Unlike other Marchantiidae, capsules containing the spores are developing inside the thallus itself.*Riccia*'s spores are amongst the largest in liverworts and bear some of the most discriminant characters. Furthermore, their dispersion mode remains unknown. This set of particularities makes*Riccia*a key genus to understand the evolution of Marchantiidae and how lineages diversify through time and space. Substrate's nature has a strong impact on their installation, implying micro-adaptations likely to ease endemism and radiation events. *Riccia fluitans* species complex contains about ten species including *Riccia fluitans*, *R.rhenana, R. canaliculata, R. duplex* and *R. stricta*. Their high phenotypic plasticity hence the lack of vegetative reliable characters and the rare fertile state induce a challenging taxonomy, uneasy characterization and identification. We selected this species complex as a model to answer the following questions: how do taxa isolate and diversify at a large spatiotemporal scale? How to reconcile a great dispersion and endemism inside the same complex? We used integrative taxonomy to determine the fine scale relationship of 70 samples of the*Riccia fluitans* species complex. Species were sampled from a wide range of geographical locations and environmental niches. Both terrestrial and aquatic habitats are represented. The aims are to delimit species and then to produce a phylogenetic frame to study diversification modes, presence/absence of radiations and the origin of endemism. Study of morphological and ecological data will also allow us to test the relationship between diversification, morphological innovations, habitats and therefore adaptative characteristics related to the diversification. Molecular species delimitation was conducted from different analyses based of chloroplastic and nuclear markers: barcode, genetic distances, coalescence and haplowebs. Several species seem to be confirmed by our study when other are not discriminated. Analysis of morpho-anatomical characters allowed for the reconstruction of evolutionary history. Incongruence across some results suggests a complex evolutionary history for this group. Insights on evolution of spore's ornamentation were drawn. Challenges and future directions are discussed; even though morphological characters are inefficient to delineate species, the power of spores' morphometry approach seems promising. The coming study of type specimens will allow to truly clarify the taxonomy of this complex.

T2-18: Plant speciation in the 21st century (two sessions)

T2-18-01

Molecular evolutionary genetics of hybrid lethality between sympatric *Mimulus* species

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University of Georgia

As a common cause of reproductive isolation in diverse taxa, hybrid incompatibilities are fundamentally important to speciation. Recent work has provided insight into the molecular functions of genes involved in hybrid dysfunction, but we still know little about how such genes initially evolve within species. Here we describe a simple genetic incompatibility that causes lethality in hybrids between two closely related species of yellow monkeyflower (*Mimulus guttatus* and *M. nasutus*). This hybrid incompatibility, which causes a fraction of F_2 hybrid seedlings to lack chlorophyll and die shortly after germination, occurs between sympatric populations that are connected by ongoing interspecific gene flow. In a series of genetic crosses between plants collected from throughout the species' ranges, we have discovered that both hybrid lethality alleles are widely distributed and rarely fixed within populations. Moreover, polymorphism at one of the loci appears to be driven by introgression between the two *Mimulus* species. We have now genetically mapped hybrid lethality to two small regions that contain duplicated copies of a promising candidate gene. This polymorphic hybrid lethality system offers a unique chance to directly investigate the evolutionary dynamics of incompatibilities in nature.

T2-18-02

Phylogenomics reveals common features of recent plant radiations in the Solanaceae

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For decades, studies of rapid species radiations have provided insight into the causes and consequences of diversification, including the factors that can accelerate speciation. Recently, these analyses have been revolutionized by the availability of next-generation sequencing technologies which enable a detailed characterization of clade-wide genetic variation across hundreds to millions of genomic sites. In addition to illuminating the phylogenetic relatedness of population and species, these data have begun to reveal complex patterns of genetic differentiation and sharing that appear to characterize radiations in a diverse range of organisms. Using clade-wide comparative transcriptomic data in two closely related, ecologically diverse, genera in the Solanaceae—*Solanum sect. Lycopersicum* (wild tomatoes) and *Jaltomata*—we describe several features of these genome-wide analyses of diversification. First, we find evidence for abundant shared ancestral variation across whole clades. Second, we show that phylogenetic reconstruction is characterized by rampant gene tree discordance due to incomplete lineage sorting (ILS) that arises from rapid successive lineage splitting events. And, third, we detect discrete post-speciation introgression events between lineages, that also contribute to phylogenetic discordance. Despite these complexities, we show that (when carefully handled) phylogenomic data can still be used to identify candidate loci contributing to rapid diversification. This includes loci where lineage-specific adaptive evolution is associated with ecological differentiation in *Solanum*, and rapid floral evolution in *Jaltomata*.

T2-18-03

What can the genomic composition of the *Helianthus* hybrids tell us about hybrid speciation?

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University of British Columbia

How do the genomes of different species merge to form a new homoploid hybrid species? How quickly do new hybrid genomes become stabilized? Do different parts of the genome stabilize at different rates? Is the process largely stochastic or is governed by natural selection? To address these questions, we employed transcriptome sequence data and a newly developed maximum likelihood approach to analyze the genomic composition of three homoploid hybrid species in the sunflower genus, *Helianthus*. These species are particularly suitable for this research because they are known to have arisen from the same parental species. We find that the hybrid species are older than previous estimates based on microsatellites. Genome composition is highly consistent within hybrid species and even has appreciable consistency between species, suggesting common selection during speciation. Parental fragments are overwhelmingly tiny, but larger (>10cM) fragments exist, highlighting regions potentially targeted by selection. Despite the ages of the hybrid species, we find that the genomes are not fully stabilized and residual interspecific heterozygosity remains. Surprisingly, we find evidence that two of the hybrid species, *H. anomalus* and *H. deserticola*, may have originated from a single hybrid speciation event and then subsequently speciated.

T2-18-04

The evolution of reproductive isolation in orchids

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University Federico II of Naples

The type of reproductive barrier predominant in the initial stages of species divergence can affect the nature and rate of emergence of additional barriers that subsequently strengthen reproductive isolation between species. Different groups of Mediterranean deceptive orchids are characterized by different levels of pollinator specificity. Whereas food-deceptive orchids show weak pollinator isolation, the sexually deceptive species display a highly specialized pollination. Comparative analyses revealed that orchids with high pollinator specificity mostly rely on few premating reproductive barriers and have very little postmating isolation. By contrast, orchids with weak pollinator isolation, such as food-deceptive orchids, show strong evidence for multiple, postmating reproductive barriers. These different types of isolating barriers (and related genetic background) are likely to affect not only the first phase of species divergence but also the evolution of other barriers that emerge when species meet in secondary contact zones. Indeed, secondary contact zones between closely related species are the natural arenas where reproductive isolation is exposed to natural selection and additional reproductive barriers may arise between former independent species as a consequence of ecological interactions or due to selection against hybridization. This process acts on prezygotic mechanisms but it is likely to be affected by different levels of pollinator isolation and by the contribution of other pre-existing barriers. In a case study of sexually deceptive orchids, two species coexist despite the common pollinator and the virtual absence of later barriers. Here, sympatric plants overlap in phenology but have more divergent floral traits than allopatric ones in this way shaping a mechanical barrier that impede or strongly limit the exchange of interspecific pollen. In food-deceptive orchids with weak pollinator isolation natural selection on reproductive barriers usually do not affect pollinator interactions but phenological overlap and postmating prezygotic barriers as pollen-stigma incompatibility. Indeed, in a closely related species pair, allopatric individuals do not show the pollen-stigma incompatibility that characterize sympatric individuals. Also, in several cases, this postmating barrier is highly asymmetric among sympatric species and is generally stronger in the crossing direction that is more likely to occur in nature (i.e. from the early flowering to the late flowering species) thus suggesting that it may have evolved as a consequence of a local selective pressure. These findings highlight that the subsequent evolution of later prezygotic barriers may take different avenues depending on the initial level of pollinator isolation evolved in the early stage of species divergence.

T2-18-05

Genome-wide investigation on parallel speciation in *Oryza nivara*

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Parallel speciation is a typical form of parallel evolution in which similar traits associated with reproductive isolation evolve repeatedly in different populations within species and strongly implicates the action of natural selection. Despite numerous documentations in animals, example of parallel speciation with unequivocal evidence has been lacking in plants. Particularly, the genomic basis of parallel speciation is largely unknown. Two wild rice species, the perennial *Oryza rufipogon* and annual *O. nivara*, are evolutionarily young and distinct in ecology and morphology and thus offer a unique opportunity for studying speciation. Here we take the advantage of high-throughput sequencing, in combination with traditional common garden experiment, to investigate the origin model of *O. nivara* as well as the causes and consequences of genomic divergence between the two species. Based on the re-sequencing data for 24 natural populations, in conjunction with population genomics approach and statistic modeling, we tested for alternative models of *O. nivara* origin and revealed that *O. nivara* originated multiple times under similar habitats from different *O. rufipogon* populations. Common garden experiment on eight sympatric pairs of *O. rufipogon* and *O. nivara* populations showed significantly phenotypic divergence between species but not within species. We showed evidence that the shared phenotypes in different *O. nivara* populations have evolved under natural selection. Importantly, we found an effective premating isolation between species but not within species. In addition, genome-wide screening detected both a large proportion of genomic regions with significant divergence between species, including regions with parallel genomic divergence across multiple population pairs and those unique to individual population pairs. These divergent regions contain many genes associated with functional terms involving phenotypic and ecological differentiation between species. Our study suggests that parallel speciation as a consequence of adaptation to alternative environments might be widespread in plants and provides new insights into the genomic basis underlying parallel speciation in plants.

T2-18-06

Does speciation drive biodiversity? A systematic analysis of multiple mangrove species on the Indo-Malayan coasts and a novel mechanism of speciation

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High biodiversity may be a modern “mystery of mysteries”; in particular, speciation mechanisms have not been one of an ensemble of explanations. Indo-Malayan coasts, having continuous geological records and possessing a high level of biodiversity that includes 70% of the living mangrove species, may offer new insights into the possible connections between biodiversity, geography and speciation. Here, we analyze the pattern of species and population divergence on these coasts by collecting DNA sequences from ~1,700 plants of 5 common mangrove species. Good species and geographical populations provide the upper and lower bound estimates of speciation time, respectively. This large dataset, superimposed on biogeographical observations, reveals that mangrove speciation is driven mainly by the isolation at the Strait of Malacca. However, the isolation is periodically punctuated by migration due to fluctuations in sea level leading to the opening/closing of the Strait. As a result, speciation has generally proceeded through cycles of isolation and migration, with each cycle averaging 100,000 years. Theoretical analyses indicated that while gene flow is permitted during divergence, isolation phase is still indispensable for genetic incompatibility to evolve. This mechanism, referred to as the mixing-isolation-mixing (MIM) cycles, extends several proposals that aim at integrating gene flow and geographical isolation in a single framework of speciation and provides the basis for further investigations of adaptive evolution in mangroves. After n cycles, the mechanism could potentially yield m^n (m>1) species and may contribute to the maintenance of high biodiversity.

T2-18-07

Genomic insights into the combination of differentiated alleles that promote diploid hybrid speciation

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Homoploid hybrid speciation is increasingly viewed as a contributor to current species diversity. In addition to the recombination of chromosomal structural differences, the recombination of alleles that differentiate parent species across their genomes may promote reproductive isolation (RI) of a new homoploid hybrid species. We tested this hypothesis by conducting transplant studies and examining allele combinations at multiple loci in the diploid hybrid species, *Ostryopsis intermedia* (Betulaceae), and its two parents, *O. davidiana* and *O. nobilis,* through whole genome sequencing. First, we examined RI between the hybrid and its parents by transplanting *O. davidiana* into sites occupied by one or other of its two parents. We found that RI between *O. intermedia* and *O. nobilis* resulted from both a pre-pollination flowering time difference and a post-zygotic reduction in fruit set. In contrast, RI between *O. intermedia* and *O. davidiana* appeared to involve habitat selection with *O. davidiana* less able to tolerate high levels of soil iron content. Second, our genomic analyses indicated that more than 70% of genes of *O. intermedia* were closely related to those of *O. davidiana*, including those likely to affect flowering and fruiting, while the remainder grouped with those of *O. nobilis* and included key genes responsible for absorption and transport of iron ions in plants. Further functional tests of parental alleles for one of the latter genes confirmed that they greatly affected iron absorption. We conclude that population genomic data support three independently evolving lineages of *Ostryopsis* with one, *O. intermedia*, sharing alleles that distinguish its two parent species. Our findings highlight the importance of combining differentiated alleles at multiple loci in the promotion of RI of a homoploid hybrid species from its parents.

T2-18-08

Parallel speciation during adaptive radiation in an Australian wildflower

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Deterministic forces can create order in nature. For instance, natural selection can lead to organic discontinuity by reducing the homogenizing effects of sexual reproduction between populations, thus freeing traits to evolve along different trajectories. Equally, if numerous environments occur repeatedly across landscapes, evolution of form can echo over time and space, and traits with similar expression can arise independently in multiple populations. This form of repeated evolution is not uncommon in nature, and seems to define the evolution of costal and alpine forms in the species complex *Senecio lautus*. Here, through as series of ecological and genetic experiments we show that environmental dependency is crucial to the origins of organic discontinuity, but also show that natural selection can lead to the irreversible breakdown of sexual reproduction between populations occupying contrasting environments. Repeated trait evolution seems to originate from abundant levels of standing genetic variation, but only a few mutations seem to break down sexual reproduction between populations. Whether the genetic architectures of trait evolution and speciation evolve in step with one another remains unknown, but it is clear that natural selection can create discontinuity at different levels of divergence. These results have implications for our understanding of the origin and also the nature of new species.

T2-18-09

The role of retrotransposons in reproductive isolation among wild wheats

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University of Neuchâtel

Interspecific hybridization leads to new interactions among divergent genomes, revealing the nature of genetic incompatibilities having accumulated after the origin of species. Conflicts associated with misregulation of transposable elements in hybrids expectedly results in their activation and genome-wide changes that may be a key issue to species boundaries. Here, we first used experimental reciprocal F1 hybrids between three *Aegilops* species to assess restructuring and epigenetic repatterning in relation to the strength of reproductive isolation. Asymmetrical reorganization of long terminal repeat retrotransposons (LTR-RTs) predicted to cause conflicting interactions matched differential survival of F1 hybrids. In particular, non-random sequence losses and methylation appeared coherent with the necessary repression of incompatible TE loci in sustaining hybrid viability. We then used natural hybrid zones between *Aegilops geniculata* and *Aegilops triuncialis* to further tackle the consequences of such conflicts for maintaining species boundaries in face of recurrent gene flow. Coherent with insights from experimental populations, conflicting LTR-RTs presented asymmetrical introgression and specific reorganization among natural hybrids. Genomic conflicts involving LTR-RTs are thus highlighted as key incompatibilities supporting reproductive isolation and shaping species boundaries in the long term.

T2-18-10

Repeated speciation and long-term evolution of allopolyploids in *Achillea* (Asteraceae)

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Allopolyploidy is considered to be an important mechanism of speciation in plants. Many polyploidy species, including a number of allopolyploid species, have been known of recurrent formations with strong evidence. Due to the complexity of polyploidy data, the long-term evolution after recurrent polyploid formation, as well as its influence on speciation remain to be less understood and need more studies on polyploid lineages with relatively long evolutionary histories. Previous work demonstrated that two East Asia *Achillea* allotetraploid species, *A. alpina* and *A. wilsoniana*, share diploid progenitors, one *A. acuminata*-like and the other *A. asiatica*-like. Here, we described a new progenitor-specific amplicon sequencing approach that allows us to separately estimate the divergence of two allotetraploid subgenomes from their progenitors within a framework of population demographic theory. We found dramatic differences between *A. alpina* and *A. wilsoniana* based on both progenitor-specific subsets of nuclear genetic markers, suggesting that the two tetraploid species were formed independently from at least two allopolyploid events with the same diploid species but different populations as progenitors. Furthermore, plastid sequence data indicated at least two formations of *A. alpina alone*. In sum, our analyses show recurrent allopolyploid formations, some of which are consistent with the species delimitation or differentiation on morphology, and some are infra-species. Moreover, with this working system, we were able to reconstructed long-term evolution of allopolyploids after their establishment using Approximate Bayesian Computations (ABC). The analytical result suggested potential competitions between new formed tetraploids and their diploid progenitors. Our method with progenitor-specific genetic markers could also be applied to other allopolyploid species with little genomic information, to uncover the allopolyploid speciation process.

T2-18-11

Evolution of the nuclear and plastid pangenome: Divergence vs introgression in the model grass *Brachypodium distachyon*

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A pan-genome is the non-redundant collection of all DNA sequences present in the entire population of a species. It is composed of a “core genome”, containing sequences present in all individuals, and a “conditionally dispensable genome”, that includes sequences not present in all lines. Though “dispensable”, these genes may be of high value under some conditions as they are enriched for functions associated with potential adaptive traits (e.g., stress tolerance, disease resistance and development). We created a robust nuclear pan-genome of *Brachypodium distachyon*, a wild annual grass endemic to the circum-Mediterranean region and a powerful model system for the grasses. We analyzed 54 whole nuclear genome sequences, including both genes and repeatitive elements, for *B. distachyon* lines chosen to represent a large portion of the genomic and phenotypic diversity of this species. We characterized the interplay between core/dispensable genomes, population structure, gene expression, predicted gene function and non-coding sequences. We constructed a phylogenomic tree based on >4 million SNPs, largely congruent with groupings based on median amino acid similarity in pan-genome clusters among lines, as well as copy number of dispensable pan-genome clusters. There was strong population structure in the tree, which showed a main split into an extremely delayed flowering (EDF) clade and a second clade subdivided into two groups defined by geography (East, West). Many flowering time traits and putative flowering time genes showed strong evidence of correlated evolution with flowering class and weak correlation with latitude. Genetic structure analysis showed that some lines were admixed, indicating recent, though infrequent, interbreeding among groups. This was confirmed through the phylogenomic analysis of their plastomes, that reconstructed a plastome tree highly congruent with the nuclear pan-genome tree showing nine additional chloroplast capture and two ptDNA introgression and micro-recombination events.

Estimations from nested dating analysis suggested that the *B. distachyon* ancestor split in Mid-Pleistocene times (~0.9 Mya), and that intra-specific divergences occurred very recently, during the last half million years. Flowering time variation is a main factor driving rapid intra-specific divergence (microspeciation) in *B. distachyon*, though it is counterbalanced by repeated introgression between previously isolated lineages. Swapping of plastomes between the three different genomic groups (EDF, West, East) likely resulted from random backcrossing followed by stabilization through selection pressure.

T2-18-12

Eucalypt systematics: NGS data do not support the Monophyly of *Corymbia* and indicate a complex evolutionary history

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We used Next Generation Sequencing (NGS) to investigate the phylogenetic relationships of *Angophora* Cav., *Corymbia* K.D.Hill & L.A.S.Johnson, and *Eucalyptus* L'Hér. In particular, we addressed the question of monophyly of *Corymbia* (the bloodwoods and ghost gums). Chloroplast and nuclear data were skimmed from NGS data of genomic DNA. The dataset includes 121,017 base pairs for nine species of *Angophora*, 57 species of *Corymbia* (some with two accessions) and 31 species of *Eucalyptus*, with *Allosyncarpia* and *Stockwellia* as outgroups. Maximum Likelihood and Maximum Parsimony methods show that *Corymbia* and *Angophora* are related as one lineage. *Corymbia* is not monophyletic because *Angophora* is nested within it. Relationships among species of *Corymbia* are not all congruent with current infrageneric classification, some previous phylogenies based on nrITS data, or morphological characters. However, our results confirm outcomes from some previous analyses using fewer chloroplast and nuclear markers. Analyses of our chloroplast data show evidence of chloroplast capture within *Corymbia* and a complex evolutionary history of this group. Our results frame a new working hypothesis for the evolutionary relationships of *Angophora*, *Corymbia*, and *Eucalyptus*. Taxonomic changes are likely after the future re-evaluation of morphological characters and analysis of additional nuclear data.

T2-19: Evolution of the mega-diverse genus *Begonia*

T2-19-01

Species-level functional variation in *Begonia*

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What distinguishes the many species of *Begonia* from each other? We have used QTL analysis and Differential Expression analysis to determine the genetic differences between two closely related species of *Begonia*. *B. conchifolia* and *B. plebeja* are Central American species found in contrasting environments. With the aid of a genetic map we have identified 31 QTLS controlling vegetative and reproductive form, physiology, growth and micro morphology. The degree of variance associated with these QTLs suggests that the genetic differences between these two species are not great, despite their very different morphologies and habitats. To identify candidate genes for these QTLs and to investigate interspecific variation in traits not scorable in our mapping populations we generated transcriptomes from 6 tissues for each species for differential gene expression analysis. This has enabled us to identify the key genetic pathways, enzyme classes and classes of genes which differ in gene copy number, sequence and expression between the species. Our data suggest that the different fate of duplicated genes in isolated populationsis a key factor in the evolution of so many *Begonia* species.

T2-19-02

Insights of the genome architecture for the species *Begonia conchifolia*

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Begonia conchifolia A. Dietr. (Begonia section Gireoudia) is a neotropical herb native from Central America that can be found in disturbed areas from Costa Rica, El Salvador and Panama. Although *B. conchifolia* is not a popular ornamental plant, it is an important model to study leaf morphological variation and sex flower ratio across the Begonia genus. Flow cytometry experiments have estimated its genome size in 587 Mb. We have sequenced the *B. conchifolia* genome using a combination of Illumina and PacBio sequencing technologies. The estimation of the genome size based in Illumina read Kmer distribution was comprised between 831 and 910 Mb with a heterozygosity of 0.48%. We have obtained a draft genome assembly of 25,304 scaffolds for a total size of 736 Mb (N50 = 61.3 Kb). The genome annotation reported 31,935 genes. The pairwise analysis of Ks distribution revealed a Whole Genome Duplication (WGD) dated between 13 and 33 MY ago. The terpenoid cyclases gene family showed a notable expansion (78 genes) compared with other plants species such as *Arabidopsis thaliana* (26), *Glycine max* (23), *Populus trichocarpa* (49) and *Vitis vinifera* (45). The development of a reference genome for the Begonia genus will help to elucidate the complex evolutionary history of one of the more diverse genus in the plant kingdom.

T2-19-03

Species radiation by a random walk through niche space in the mega-diverse genus *Begonia* (Begoniaceae)

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Begonia is among the world's largest genera and has radiated rapidly throughout its pantropical distribution. It is widely assumed that the majority of large, rapid radiations are adaptive. Central to the theory of an adaptive radiation is that the majority of speciation events are adaptive, implying speciation driven by adaptation to different environments and resulting in niche shifts. We evaluate whether observed ecological disparity among species in the mega-diverse genus *Begonia*, calculated through species distribution models, best fits the predictions of models of adaptive or non-adaptive radiations. Patterns of ecological diversification in three clades of *Begonia* strongly diverge from those predicted by an adaptive model, and we conclude *Begonia* is not an adaptive radiation with respects to climatic niche. Ecological disparity within *Begonia* clades best fits the predictions of a non-adaptive, geographic model of speciation and we suggest the remarkable diversity of the genus has developed through geographic speciation, and adaptation to local environments. This phenomenon may be widespread among plant radiations in topographically- and environmentally-heterogeneous areas, and a re-evaluation of putative adaptive radiations throughout the world's montane regions may be necessary.

T2-19-04

Systematic and biogeography of the section *Casparya* (*Begonia*, Begoniaceae)

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Section *Casparya* include around 41 species, being one of the most species rich groups of *Begonia* in the North Andean region. This is a monophyletic and morphologically well defined section, characterized by a unique type of fruit among the American begonias. The mostly North Andean distribution of *Casparya* make to this section an appropriate system to study the patterns and process of diversification in this region. We generate a multilocus and well sampled phylogeny of *Casparya*, dated through Bayesian inference to estimate the ancestral ranges of distribution and reconstruct his historical biogeography using the model DEC (dispersal-extinction-cladogenesis). In order to understand the dominant modes of speciation and the changes in climatic niche, we performed niche modeling and polygons of distribution for each species and then we measure the age-range and age-niche correlation using independent comparisons. We show that allopatric speciation has been the dominant mode of speciation in *Casparya*, and that climatic niche overlap has not varied from the null expectation, suggesting niche conservatism. The ancestral area reconstructions shows that *Casparya* originated in the Cordillera Oriental of Colombia and colonized during the Pliocene all the major montane systems of the northern Andes, but was during the Pleistocene when most of the extant species were originated. We concluded that *Casparya* have responded to the climatic variation mostly moving around the optimum conditions in the mesic cloud forest, generating species allopatrically, but also have been able to cross occasionally the climatic tolerance adapting to extreme habitats as the páramos.

T2-19-05

New discoveries of *Begonia* from Sangkulirang Nature Reserve, borneo, indonesia

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An expedition to Sangkulirang Nature Reserve, East Kalimantan, Borneo was organised in order to visit previously uncollected areas of karst limestone, of potentially high conservation importance. We documented and collected seven native *Begonia* species. Of these, six represent new species, highlighting both the previous lack of knowledge of the area and its high endemism. Living plants and seeds were deposited at Bogor Botanic Gardens for cultivation, and to provide fertile material for description. All species belong the highly species rich section Petermannia, and phylogenetic evidence will be presented as to their biogeographic origin. An overview of the distinctive characters of the species and their distribution will be presented. The study highlights the role of living collections in supporting taxonomic research and conservation, and the high conservation value of the Sankulirang Karst.

T2-19-06

Five new species of *Begonia* (section *Baryandra*, Begoniaceae) from Palawan Island, Philippines

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In a joint expedition to the island province of Palawan, botanists from Taiwan, United Kingdom and the Philippines found 5 unknown *Begonia* and compared with potentially allied species. The unknown begonias are similar in their rhizomatous stem, persistent stipules, large olivaceous, asymmetric leaves, four-tepaled flowers, inferior three-locule ovary with bilamellate placentas and pendulous, three-winged capsule. The 5 are clearly assignable to section *Baryandra*. Thorough studies of literature, herbarium specimens, and living plants support the recognition of the five new species: *Begonia elnidoensis, Begonia gironellae, Begonia quinquealata, Begonia tabonensis* and *Begonia tenuibracteata*. This brings the total of *Begonia* species in Palawan to 22, and represents a 40% increase in the number of species in section *Baryandra* from Palawan. Phylogenetic evidence shows the species to have originated during the Pliocene-Pleistocene, and sharing of chloroplast haplotypes between distantly related species according to nuclear DNA data shows evidence of recent hybridisation.

T2-20: The Brassicaceae family – a model system to study evolutionary processes with high resolution in space and time

T2-20-01

Resolution of Brassicaceae phylogeny using nuclear genes uncovers nested radiations and supports convergent morphological evolution

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Brassicaceae is one of the most diverse and economically valuable angiosperm families with widely cultivated vegetable crops and scientifically important model plants, such as *Arabidopsis thaliana*. The evolutionary history, ecological, morphological and genetic diversity and abundant resources and knowledge of Brassicaceae make it an excellent model family for evolutionary studies. Recent phylogenetic analyses of the family revealed three major lineages (I, II, and III), but relationships among and within these lineages remain largely unclear. Here, we present a highly supported phylogeny with six major clades using nuclear markers from newly sequenced transcriptomes of 32 Brassicaceae species and large datasets from additional taxa for a total of 55 species spanning 29 out of 51 tribes. Clade A consisting of Lineage I and *Macropodium nivale* is sister to combined Clade B (with Lineage II and others) and a new Clade C. The ABC clade is sister to Clade D with species previously weakly associated with Lineage II and Clade E (Lineage III) is sister to the ABCD clade. Clade F (the tribe Aethionemeae) is sister to the remainder of the entire family. Molecular clock estimation reveals an early radiation of major clades near or shortly after the Eocene-Oligocene boundary and subsequent nested divergences of several tribes of the previously polytomous Expanded Lineage II. Reconstruction of ancestral morphological states during the Brassicaceae evolution indicates prevalent parallel (convergent) evolution of several traits over deep times across the entire family. Deep analyses with intense taxa samplings covering all tribes and lage genera are ongoing to reveal the complete relationships in this family, which may form a foundation for future evolutionary analyses of structures and functions across Brassicaceae.

T2-20-02

Towards a backbone phylogeny of Brassicaceae and principles of crucifer evolution – unravelling evolutionary patterns and processes

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The Brassicaceae family (mustards or crucifers) comprises nearly 4000 species based on our newly established species check-list for the entire family and includes *Arabidopsis thaliana* as one of the most important model species in plant biology and a number of important crop plants as well, such as the various *Brassica* species (e.g. cabbage, canola and mustard). Moreover, the family comprises an increasing number of species that serve as study systems in many fields of plant science and evolutionary research such as *Arabidopsis*, *Brassica*, *Boechera*, *Capsella*, *Cardamine*, *Thellungiella*, and *Arabis* species. As any evolutionary hypothesis needs to be placed in a phylogenetic and temporal context, reliable and highly resolved phylogenetic hypotheses of the Brassicaceae are essential. However, those are still missing. Herein we aim to unravel a comprehensive and highly-resolving phylogeny for the entire Brassicaceae family based on whole genome sequences of two organellar genomes: namely the mitochondrial and plastid genomes. Family-wide genomic data are analysed within the framework of the entire Superrosid clade, thereby allowing elaboration on a comprehensive time-calibrated framework with divergence time estimates allowing stem and crown group age calculation for any lineage and tribe of Brassicacae. These data will allow testing the hypothesis of generally occurring diversification-lag-phase after ancient polyploidization (mesopolyploidization) giving rise to many of these tribes and lineages and with more than 43% neopolyploid taxa. Furthermore we aim to elaborate on the idea that spatio-temporal diversification patterns may correlate among the various evolutionary lineages, thereby allowing us to draw conclusions about major past-climate/environmental transitions contributing to the radiation of the entire family in concert with extensive polyploidization, which might either predate or follow these environmental transitions throughout the last 35 million years.

T2-20-03

New insights into crucifer genome evolution

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The Brassicaceae family comprises 320 genera and over 3,600 species classified into some 50 bona fide monophyletic tribes clustered in three or five major phylogenetic lineages. The whole-genome sequence projects along with comparative cytomolecular maps for roughly two dozens of crucifer species (e.g., *Arabidopsis* spp., *Arabis alpina*, *Brassica* spp., *Camelina sativa*, *Cardamine* spp., *Schrenkiella parvula*) allowed us to reconstruct ancestral genomes and infer prevalent trends of genome evolution in the *Brassicaceae*. While two major ancestral genomes, ACK (n=8) and PCK (n=7), remained conserved in a number of extant species, in other groups they were reshuffled by inversions and translocations with or without centromere elimination/inactivation. Multiple independent descending dysploidy events dominate the genome evolution in the Brassicaceae. In tribes which experienced recent whole-genome duplication events, the extent and tempo of genome reshuffling towards quasi-diploid genomes exceed that observed in ?true diploid" genomes. While we are revealing the mechanisms of descending dysploidy, we are less clear on the triggers and evolutionary significance of this process. The importance and consequences of the gene linkage, reduction of recombination, and telomere/centromere reduction will be discussed. More detailed examples are provided from the tribes *Alysseae*, *Camelinae* and *Cardamineae* illustrating different approaches in the search for parental species of polyploids, origin of dysploidy and genetic differentiation of polyploid lineages.

T2-20-04

The role of gene and genome duplication in network evolution

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Gene duplications, derived from polyploidy and smaller scale events, are prevalent and recurring throughout the history of higher eukaryotes including angiosperms. Retained duplicate genes, often that functionally diverge, has helped shape the ultimate success of flowering plants. This presentation will focus on the impact of gene duplications on gene networks, and provide examples of both the origin of novel pathways and network rewiring that were and continue to be selected for (naturally and artificially) that encode plant adaptive traits.

T2-20-05

Recruitment of ecologically relevant duplicates in alpine Mustards

Christian Parisod
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Polyploid taxa represent excellent models to address the underpinnings of genome evolution and the building up of new species in heterogeneous environments. Here, I present an overview of recent works in the alpine *Biscutella laevigata* autopolyploid complex (Brassicaceae). Transcriptomics inferred recurrent whole genome duplication (WGD) events specific to clade of species and that were used to infer processes fostering genome evolution across different timescales: (i) After a 7-8 million years old WGD event, intense chromosomal repatterning selected for clusters of retained duplicates enriched in functions associated with responses to abiotic stresses. Low coverage genome sequencing unraveled the dynamics of several retrotransposons, supporting interplay between genome reorganization and environmental opportunities in shaping the evolution of paleopolyploids. (ii) Retrotransposons in autotetraploids having recolonized the Alps after the ice ages showed considerable dynamics going along with ecological radiation following this recent WGD. Ecological genomics involving transplant experiment indeed supported distinct autopolyploid gene pools firmly associated with contrasted habitats despite gene flow. These ecotypes demonstrated adaptive differentiation at loci whose functions match habitat requirements. WGDs thus recurrently fostered genome reorganization and adaptive recruitment of genes responding to environmental factors, indicating that similar proximate and ultimate factors of genome dynamics may consistently act through time.

T2-20-06

Increased genetic variation from mesopolyploidy preferentially selected during domestication of *Brassica* rapa crops

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University of Arizona

Many crops are polyploid or have a polyploid ancestry. Whole genome duplication could provide raw genetic material for later rapid adaptation and diversification of crop species under strong artificial selection. Despite long interest in the connection between polyploid and domestication, this hypothesis has not been empirically tested. Crops in the genus *Brassica* provide a unique system to explore this question because the genus experienced multiple rounds of polyploidization and these crops show extraordinary morphological variation within a few thousand years under artificial selection. Here we analyzed transcriptome data of 102 representative *B. rapa* cultivated accessions and asked to what extent the *Brassica* mesohexaploidy contributes to the diversification and domestication of *B. rapa* crops. We first categorized *Brassica rapa* genes as derived and not derived from the mesohexaploidy (paleologs and non-paleologs). Using three different approaches, we then identified *B. rapa* genes experiencing recent positive selection or significant differential gene expression. Approximately 68% of the *B. rapa* genes were directly derived from the mesohexaploidy. Genes derived from the ancient hexaploidy have four times higher mean nucleotide diversity than other genes. Candidate genes associated with domestication were significantly enriched with these paleologs (10% - 15% higher than expected). Our results for the first time directly demonstrated in what extent polyploidization contributes to the crop diversification at the microevolutionary scale. The observed pattern should also apply to other crops and wild plants which rapidly diversified after polyploidy.

T2-21: Cyperaceae II: later diverging lineages of the Cyperoideae

T2-21-01

Sumatroscirpus: Taxonomic revision and the discovery of a new monotypic Southeast Asian sedge tribe (Cariceae-Dulichieae-Scirpeae Clade, Cyperaceae)

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Our knowledge of phylogenetic relationships within CDS (Cariceae-Dulichieae-Scirpeae Clade, Cyperaceae) has grown dramatically in recent years due to increased efforts to identify the sister-group to *Carex* and to clarify the taxonomy of the paraphyletic tribe Scirpeae. Approximately half of all CDS species (total ~2,050 spp.) have now been included in phylogenetic studies, but a single genus has escaped attention due to its extreme rarity: the putatively monotypic *Sumatroscirpus*, which is believed to be endemic to remote mountains in North and West Sumatra. As part of ongoing tribal- and generic-level revisionary work on CDS, we have reexamined the taxonomy and phylogenetic position of this genus. Its current placement in tribe Dulichieae appears at odds with several unique characteristics such as sheathing spikelet prophylls and inflated tubercles on fruits. Molecular phylogenetic analyses support these observations in showing its isolated position within CDS, and justify the recognition of *Sumatroscirpus* as the sole member of a new Cyperaceae tribe. In addition, field studies combined with the examination of all extant herbarium material indicate that the taxonomic diversity of *Sumatroscirpus* has been dramatically underestimated, with a total of four morphologically-distinct species now recognized, and with important new records extending its range into continental Southeast Asia.

T2-21-02

Ficinia clade (Cypereae, Cyperaceae): Evolving taxonomy, diversification patterns and biogeography

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Cypereae is the second largest tribe in the Cyperaceae with over 1200 species which have a predominantly tropical and subtropical distribution. The tribe has a crown age in late Eocene (36 mya) and comprises two lineages, the Cyperus and Ficinia clades, with bulk of the diversity in the predominantly tropical Cyperus clade. The Cyperus clade has recently been a focus of taxonomic and phylogenetic studies, showing rapid diversification in the C_4 clade and complex taxonomy where genera were previously described based on autapomophic (and frequently homoplasious) characters. This presentation will focus on the Ficinia clade, comprising about 160 species in eight genera (*Afroscirpoides, Dracoscirpoides, Erioscirpus, Ficinia, Hellmuthia, Isolepis, Karinia, Scirpoides*) and distributed in cool tropics and temperate areas. The Ficinia clade has a crown age in early Miocene, with earlier emerging lineages now restricted to Mediterranean regions in Central America (Mexico, *Karinia*), Eurasia (*Scirpoides, Erioscirpus*) and Southern Africa (*Afroscirpoides, Dracoscirpoides, Hellmuthia, Scirpoides*), whereas *Isolepis* and *Ficinia* have a centre of diversity in Mediterranean South Africa and distributed into Australasia and elsewhere. Unlike the Cyperus clade that has experienced taxonomic mergers of genera, the Ficinia clade has newly described genera (*Afroscirpoides, Dracoscirpoides*) and now includes two genera previously thought to belong to Scirpeae (*Erioscirpus, Karinia*). Furthermore, *Isolepis* and *Ficinia* show Miocene diversification into emerging niches in the South African Fynbos biome and multiple dispersal events out of South Africa to Australia as well as towards emerging temperate island (volcanic mountains) habitats in tropical Africa.

T2-21-03

Sedges of Vietnam: Species discoveries and insights into the evolution of *Carex* (Cyperaceae)

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Although Vietnamese *Carex* species represent a small fraction of global sedge diversity (ca. 85 of 1966 spp.), their inclusion in systematic studies has led to unprecedented insights into the evolution and diversification of this genus. Most recently our investigations have shown that *Carex* is comprised of two major evolutionary lineages, with over 98% of all species (1936 spp.) falling within the Major *Carex* Alliance. The remaining 30 species are restricted to East and Southeast Asia and comprise the Minor *Carex* Alliance. Our studies have also revealed the presence of three other previously unrecognised, early diverging, East and Southeast Asian lineages: a "Hypolytroides Clade" (sect. Hypolytroides, 2 spp.), which along with the Siderostictae Clade comprise the Minor *Carex* Alliance; a "Dissitiflora Lineage" (sect. Mundae, 3 spp.); and a morphologically diverse "Small Core *Carex* Clade" (sects. Graciles, Decorae, Mapaniifoliae, Euprepes, Indicae, ca. 70 spp.), which is sister to the temperate and species rich Large Core *Carex* Clade (> 1,300 spp.). Despite the relatively small number of species in these newly recognized lineages, its members exhibit an extraordinary range of morphologies that are rare or unique in *Carex* (e.g., densely capitate or cymose inflorescences, white stigmas and anthers, grass-like vegetative structures, wide pseudopetiolate leaves). Overall, our findings suggest that morphological diversification may have occurred in clades dominated by East and Southeast Asian species followed by canalization of a narrower range of phenotypes in species-rich, cosmopolitan lineages. Our results also suggest a Southeast Asian origin for the genus. Vietnamese *Carex* have not only afforded unprecedented insights into deep patterns of relationship within the genus, they have also upon closer examination revealed species groups that are hidden under a single name. One example of this unrecognized diversity is seen in *Carex kucyniakii* s.l. (Siderostictae Clade, Minor Carex Alliance), a taxon that represents a complex of three distinct species. These taxa are endemic to the southern slopes of the Hoang Lien Mountains and exhibit some of the most extraordinary morphological features found in the Cyperaceae including pseudopetiolate leaves up to 12 cm wide, and in some instances inflorescences suffused with pink or red. Each taxon is characterized by a suite of morphological and molecular traits that allows them to be consistently distinguished from one another and justifies their recognition as separate species. At the same time, these taxa share a number of attributes, including molecular synapomorphies, geographic range, and distinctive morphological features that show that they are closely related and constitute a monophyletic group. Despite being one of the largest genera in the Vietnamese flora, remarkably few *Carex* species have been described in recent years from this country, with the most recent discoveries dating back to the late 1970s. This contrasts with the unprecedented rate of taxonomic discovery that has taken place in China over the past 30 years. We are aware of other undescribed *Carex* species and propose that the sedge flora of Vietnam could reveal a comparable number of taxonomic novelties given a more focused attention by cariciologists.

T2-21-04

Relationships within the CDS Clade (Cariceae-Dulichieae-Scirpeae): New tribes, new genera and new species

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With approximately 40% of all sedge species (>2,050), the Cariceae-Dulichieae-Scirpeae (CDS) clade is a cosmopolitan lineage that is found in habitats ranging from deserts to tundra and from temperate wetlands to tropical rain forests. Although comprised of three tribes, the CDS clade is dominated by the Cariceae, a monotypic tribe for *Carex*. Long recognised as one of the world's largest plant genera, this taxon's diverse biogeography (e.g. amphiatlantic, bipolar, Gondwanan), intriguing cytology (holocentric chromosomes: n=6 to 56), and morphological singularity have made it an ideal model for studying speciation, ecology and biogeography. Despite many significant advances in our knowledge of relationships within *Carex*, our understanding of its relationships within the CDS clade remain unclear, a fact that affects the conclusions drawn in all *Carex* studies since outgroup relationships have a significant impact on homology assessment, character polarisation and biogeographical analyses. Over the past decade, we have focused our research on the relationships of Cariceae to the approximately 86 species and infraspecific taxa in tribes Scirpeae and Dulichieae that make up the remaining members of the CDS clade. Using representatives for all 18 genera currently recognised in CDS and ca. two thirds of the species that comprise tribes Scirpeae and Dulichieae, our five marker DNA sequence dataset (*matK*, *ndhF*, *rps16*, ETS 1f, ITS), in conjunction with morphological, anatomical and embryological data, suggest there are seven well supported major lineages within the CDS clade with Dulichieae and *Khaosokia* successively sister to a clade containing the previously recognised Cariceae, *Trichophorum*, *Calliscirpus*, *Zameioscirpus* and *Scirpus*+*Eriophorum* Clades. Although the backbone of our previous phylogenetic analyses has often been weak or unresolved due to a rapid radiation among CDS lineages (10 million years), we have since employed universal flowering plant enrichment probes for a subset of key taxa to generate a matrix of 461 nuclear genes using next generation sequencing technology. The results of these phylogenomic analyses are fully congruent with our Sanger-sequence datasets and morphology, but we now have unprecedented support for backbone nodes (100% BS) that were previously unsupported (<60% BS). Given these results, this talk will propose a new tribal and generic classification for the CDS clade, including the naming of four new tribes, a new subtribe, and the discovery of several new genera and species.

T2-21-05

Multiple approaches to unveil the phylogeny, biogeography and biodiversity of Himalayan sedges (Cyperaceae) of Pakistan

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Cyperaceae, the botanical family of sedges, consists of 5,500 species and 109 genera globally. In Himalayan Pakistan it is represented by 22 genera and 200 Species. Little information is available about the diversity, phylogeny and biogeography of Himalayan sedges. In this study, we are reporting: new field collection to herbarium specimens; morphology to character evolution; GIS mapping to biogeography; DNA bar coding to molecular phylogeny; Phytochemicals to chemotaxonomy. Further, by using the above data, we try to answer the big questions about the western Himalayan sedges diversity. Such as, "Is the western Himalayan sedges assemblage consist of immigrants from various other regions of the world?" Are they subjected to convergent evolution? This study also tested the hypothesis, whether diversity in western Himalayan Cyperaceae is primarily due to speciation during the uplift of the Himalaya (in case of endemic species). These basic questions are of prime interest—understanding the shape of plant biodiversity in this floristically rich region of the world—as well as applied interest: our basic understanding of species origins and distribution as a crucial first step to conserving sedge biodiversity in the region. This project will be a valuable addition to taxonomy of Cyperaceae worldwide and more broadly interpret Himalayan plant biodiversity globally.

T2-21-06

Taxonomic revision of genus *Eleocharis* R. Br. (Cyperaceae) in India

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Genus *Eleocharis* R. Br. (*Heleos* = a marsh, wet and *charis* = grace, beauty) is generally called as a beauty of marsh. It is characterized by the leaves reduced to the bladeless sheaths, inflorescence reduced to a single terminal spike, spikelets with few to many bisexual flowers, achenes usually with hypogynous bristles, style swollen at base, achene with a beak of persistent style base. The genus with about 300 species is widely distributed from tropical to temperate regions of both hemispheres. In India, it is represented by about 31 taxa. The genus was neglected in general floristic studies being an aquatic plants growing in muddy marshy areas difficult to collect and also due to their minute flowers and microscopic taxonomic characters and unavailability of standard literatures. Thus, number of specimens of *Eleocharis* were found to be wrongly identified in various herbaria of the country. Therefore an attempt has been made to revise the genus for India. The revision resulted into discovery of four novelties in the genus viz. *Eleocharis wadoodii* S.R. Yadav, Lekhak & Chandore, *E. khandwaensis* Mujaffar, Chandore & S.R. Yadav, *E. konkanensis*

Chandore, Borude, Kambale & S.R. Yadav and *Eleocharis* sp. nov. (in press). Similarly, *Eleocharis setifolia* (A. Rich) Raynal*, E. wichurae* Boeckeler and *E. yunnanensis* Svenson have been added to the flora of India. Some eight species of *Eleocharis* viz. *E. mitracarpa* Steud., *E. parishii* Britton, *E. philippinensis* Svenson, *E. quinqueflora* (Hartmann) O. Schwarz, *E. tuberculosa* (Michx.) Roem. & Schult., *E. uniglumis* (Link) Schult., *E. variegata* (Poir.) C. Presl, *E. yokoscensis* (Franch. & Sav.) Tang & F.T. Wang are reported from India on authority but their distribution in India is doubtful as there are no specimens of the species available in Indian herbaria (AHMA, ARUN, ASSAM, BSA, BSD, BSHC, BSI, BSJO, CAL, CALI, DRC, LWG, MH, NBU, PBL, PUN, RHT, SUK) of India. Therefore these species are excluded from the study. Presently, the genus is reported to have 31 taxa (25 species, 3 subspecies and 3 varieties) in India. The revision of the genus resulted into an addition of 10 taxa to the flora of India. Six taxa viz. *E. andamanensis* Govind., *E. atropurpurea* var. *vengurlaensis* Shaikh R.I & R.D. Tour, *E. khandwaensis* Mujaffar, Chandore & S.R. Yadav, *E. konkanensis* Chandore, Borude, Kambale & S.R. Yadav, *E. lankana* subsp. *mohamadii* Wadood Khan and *E. wadoodii* S.R. Yadav, Lekhak & Chandore are so far known to be endemic to India. This revisionary work on genus *Eleocharis* in India provides presentation of important morphological characters through photographs, line drawing and details of SEM characters of achenes useful in identification of Indian species. Botanical keys based on important taxonomic characters are also provided for easy identification.

T2-22: Mechanical forces in flowers: An evolutionary perspective

T2-22-01

Forces in flowers and their evolutionary significance

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Though many flowers demand physical force from their pollinators little is known about the functional and evolutionary significance of barriers in flowers. Among many open questions, two are of particular importance: Are weak insects excluded from pollen transfer? And do low forces have a selective significance? To get an insight into force dimensions, we measured floral forces with a highly sensitive force measurement device. We selected flowers with a force demanding pollination mechanism from six angiosperm families: Lamiaceae: *Salvia* (> 30 ssp.), Zingiberaceae: *Roscoea* x *beesiana*, Fabaceae: 11 genera (13 ssp.), Polygalaceae: *Polygala myrtifolia*, Calceolariaceae: *Calceolaria* (4 ssp.) and Asclepiadaceae: *Asclepias* (3 ssp.). Results fall into two groups: In Fabaceae, forces range from very low values up to more than 200mN. Compared to published data on bee forces, it is evident, that weak insects are excluded by some keel flowers. Surprisingly, in all other flowers, forces are rather weak not exceeding an average of 15 mN. They do not exclude bees from pollen transfer completely, but may have a functional significance during food plant selection. To test the functional significance of low forces, we constructed artificial '*Salvia'* flowers, i.e. a flower model with a lever-like barrier and a control model without any barrier. We trained honeybees (*Apis mellifera*) and bumblebees (*Bombus terrestris*) to the models and found, that both bee species were able to discriminate low forces. They preferred the model with no barrier as long as nectar rewards were equal. However, choice experiments indicated that the bees preferred the model with barrier when it offered a higher concentrated sucrose solution. Thereby, bumblebees were more successful on models with barriers than honeybees. We preliminarily conclude that flowers demanding low forces may play a selective role relative to the environmental conditions of the plant species. In the presence of competing bee species, they may contribute to niche formation by offering stronger bees a nectar source with reduced competition.

T2-22-02

The impact of floral developmental constraints on the evolution of flowers.

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Flower development and shape is regulated by the interaction of a genetic program and environmental factors, such as influence of pollination systems and biophysical forces acting on developing flowers. The initiation of floral meristems and floral organs is mainly regulated by spatial constraints. Three major factors are responsible for creating these spatial constraints and influence the morphology of flowers: time, size, and pressure. Changes in morphology are mainly induced by shifts in time of initiation of organs, pressure of previously initiated organs, and alterations of the size of the floral meristem, and these operate independently of genetic factors. A number of examples demonstrate this interaction and its importance in the establishment of different floral forms.

Delays in the timing of initiation of organs lead to the establishment of novel morphologies by absorption of tissue, such as in stamen-petal primordia, or by inducing shifts of organ positions, such as the phenomenon of obdiplostemony.

Floral meristem expansion will provide more space and will lead to an increase of stamen and carpel number, the development of hypanthia and accessory structures, and changes in merism. Merism is mainly established by a balance between the size of organs versus the size of the floral apical meristem.

Pressures can act centripetally by the action of external organs, or centrifugally by the inner meristem size directing the number and arrangement of organs.

It is demonstrated that changes in the physical environment of apical meristems has an important influence on ontogenetic processes leading to changes in floral configurations and major shifts in floral form, which is reflected in the phylogeny.

T2-22-03

Spatial constraints influence the shape of spikelets and flowers in Cyperoideae (Cyperaceae)

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Cyperaceae is a large grass-like family in Poales with two subfamilies, the small Mapanioideae and the species-rich Cyperoideae, each subfamily corresponding to a well-supported sister clade. Based on scanning electron microscopic observations we studied the development of the flowers and inflorescences of representatives in a wide range of genera of Cyperoideae.

Cyperoideae are characterized by spikelets composed of of wind-pollinated, typically trimerous, monocotyledonous flowers. The spikelets are the units of compound inflorescences, namely panicles of spikelets. The panicles are often compacted and contracted into dense head-like inflorescences. In such inflorescences, spatial constraints are obviously influencing the development of the spikelets as well as the individual flowers.

Reduction of the number of flowers in the spikelet, loss of the spikelet prophyll, metatopic displacements of the bracts subtending a flower, and reduction of the floral parts of the flowers are the main consequences of spatial constraints.

Within the flowers, spatial constraints may result in the reduction of the number of stamens to three instead of six, delaying or totally reducing the development of the abaxial floral parts, the loss of the perianth and modifications of the gynoecium. In the most derived subtaxa of Cyperoideae, the compacted inflorescence tends to take over the floral function, forming dense heads consisting of single-flowered spikelets. The question remains whether these evolutionary trends were driven by mechanical forces or by pollination strategies, or a combination of both.

T2-22-04

Development of actively morphing structures in plants

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Throughout the phylogeny of seed plants, flowers and fruits contain actively moving structures to facilitate pollination or seed dispersal. Examples include pollinator-induced movement of stamens and/or carpels, diurnal flower opening, and moisture-controlled dispersal of pinecones and wheat awns. These functional structures require unique cellular and tissue-level features that are precisely patterned and generated during development. In this talk I will present an overview of the biomechanics behind the moving parts in flowers and fruits and highlight the commonly found morphological and anatomical strategies. By understanding better about the hydrodynamic and tissue material features underlying the actively controled organ movement, we can identify the candidate key developmental events that are also likely to have been targetted by the evolutionary innovation of such morphing structures. I will then report our effort to establish an experimental platform to untangle the physico-chemical feedback loops regulating plant organ development, which can be used to test hypotheses generated above.

T2-22-05

Forces in flower ontogeny

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From an evolutive perspective, the flower can be understood as the historical outcome that serves reproduction and maintenance of the plant's lineage. At the same time, but from an organismic point of view, the flower can be seen as the outcome of the ontogenetic process in each individuum. Questions regarding the ontogenetic process of the flower involve multiple aspects including cell proliferation and growth, hormone flux, gene expression patterns, as many others. One of these elements that has received undeservedly little attention are mechanical forces itself. In this work we present a revision of a number of examples where mechanical forces have been shown to play a fundamental role in shaping the flower at different moments throughout ontogeny. The palette of action of these forces go from the early ontogeny at the microscopic level, - as the organ initiation patterns on the flower meristem in relation to the pressure exerted by inflorescence axes or neighboring primordia -, to the the macroscopic level in the late ontogeny, as the twisting of a pedicel of a resupinating flower. The premise here conceived is that without these forces ocurring during ontogeny, the recalled flower phenotype wouldn't be expressed as it is actually known, implying that the mentioned force plays a causal-generative role on the flower's phenotype, unaware of further genetic or molecular response coocurrence.

T2-22-06

Pollinator-driven floral evolution with and without pollinator shifts in *Impatiens* (Balsaminaceae)

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Floral diversity may evolve as a result of plant-pollinator interactions by two distinct processes: through the use of different functional pollinator groups, or divergent use of the same pollinator. However, the relative importance of these processes is largely unknown. Here we use the recently radiated, large plant genus *Impatiens*, characterized by variable, zygomorphic or asymmetric flowers, to understand the association between floral diversity and pollination ecology, and to study its evolution. A comparative field study of co-flowering *Impatiens* species in northern Thailand revealed that out of seven species, six are dependent on animal visits for pollination. Several different types of animals visited the flowers, but not all were effective pollinators. Four *Impatiens* species are pollinated by bees, whereas two species are pollinated by bees and butterflies. The main features that distinguish pollination systems included the shape and dimensions of the floral chamber and length of the (nectar) spur. Co-flowering species that shared bee pollinators deposited their pollen on different parts of the bee body, including through an unusual form of the asymmetric perianth. Based on our field-observations we predicted pollinators at the level of functional groups for a large number of *Impatiens* species. The majority of species was inferred to be bee-pollinated, and floral asymmetry was significantly more common among bee-pollinated species than those with other pollination systems. According to the phylogenetic analysis, shifts between functional pollinator groups occurred at least 17 times. Floral asymmetry evolved at least 11 times, never reversed, and was not associated with large clades. Shifts to floral asymmetry were uncoupled from shifts between functional pollinator groups, and always happened in lineages that were ancestrally bee-pollinated. These results are consistent with a scenario according to which floral diversity either evolves by means of pollinator shifts, or by divergent use of the same, frequently exploited pollinators. Once floral asymmetry evolves it does not appear to function as key-innovation that allows species to invade a new niche leading to clade proliferation. Further analyses, which take the co-occurrence of *Impatiens* species into account, could reveal whether competition between co-flowering species is an important driver of floral evolution.

T2-23: New data on early Cretaceous seed plants (two sessions)

T2-23-01

Diversity of corystosperms from the Early Cretaceous of Mongolia

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The corystosperms (=Umkomasiaceae) are a key extinct group for understanding the phylogeny of seed plants and the evolution of their reproductive structures, including the carpel and ovule of angiosperms. Corystosperms first occur in the Late Permian and appear to have attained their highest diversity and widest distribution in Gondwana during the Middle and Late Triassic. The group appears to have been extinct by the early Paleogene. Corystosperms have generally been thought to be distributed primarily in Gondwana, with only few scattered occurrences in the Northern Hemisphere. Recently however, study of a diverse, excellently preserved, conifer-dominated, plant assemblage from the Early Cretaceous Tevshiin Govi Formation at Tevshiin Govi, in central Mongolia, has revealed diverse seed-bearing organs of corystosperms, which can be recognized as three distinct species, all of which can be assigned to *Umkomasia*. All three *Umkomasia* species consist of a bract subtending an axis that branches distally, with each fork bearing a reflexed cupule and each cupule bearing a partially enclosed, single, triangular seed with the micropyle oriented towards the cupule stalk. In these respects all three species closely resemble the original material of *Umkomasia* from the Triassic of South Africa. However, the Mongolian *Umkomasia* species are associated with *Pseudotorellia*, a leaf with *Ginkgo*-like characteristics, rather than the pinnately compound *Dicroidium* leaves that are typical of Triassic Gondwanan Corystospermales. The three species of *Umkomasia* from Mongolia are distinguished by the number of branches of the main axis, the prominence of the extension of cupule stalk, as well as the number of cupule lobes. Together with the nearly contemporary North American permineralized seed cone *Doylea*, these species suggest that corystosperms were more diverse in the Early Cretaceous of Northern Hemisphere than previously supposed. They appear to exhibit an unusual combination of features seen in *Ginkgo* and conifers as well as other Mesozoic seed plants – such as *Caytonia* and *Petriella* – and as such raise important questions about our current understanding of seed plant phylogeny.

T2-23-02

Floristic composition of an Early Cretaceous swamp from Mongolia and the implications for seed plant evolution

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Fossils and molecular evidence suggest that all extant and crown conifer families were established by the Early Cretaceous. In addition ginkgophytes were an important element across Asia in the Early Mesozoic. However, recognition of the lineages that lead to extant conifers groups (e.g., Cupressaceae s. l., Pinaceae) and ginkgophytes remains incomplete, mostly due to an incomplete and inadequately understood fossil record. Exquisitely preserved lignite fossils (seed and pollen cones, foliage, and wood) from the Aptian-Albian (~100-125 Mya) of central Mongolia (Tevshiin Govi and Tugrug localities) provide taxonomic diversity and morphological and anatomical data that contribute to a more complete understanding of the complex pattern of conifer and ginkgophyte evolution. New Mongolian conifer fossils include at least seven taxa of seed cones, three types of pollen cones, at least five leaf morphospecies, and four wood types. *Krassilovia*, one of the seed cones, bears multilobed ovuliferous scales that suggest an affinity with extinct members of the voltzian clade of the Voltziales ("transitional conifers"); *Krassilovia* is associated with *Podozamites* foliage and also shows that voltzian seed cones with complex bract-scale arrangement and morphology persisted into the later Mesozoic. Three other seed cones show affinity with stem and crown Pinaceae, including the stem lineage taxon *Schizolepidopsis*; other Pinaceae cones include *Pityostrobus* and one the oldest records of *Picea* (spruce). Three additional taxa show affinities with the subfamilies Cunninghamioideae (*Elatides*, *Pentakonos*) and Taiwanioideae (*Stutzeliastrobus*) of the Cupressaceae s.l. The new Mongolian fossils also reveal that the enigmatic ginkgophyte *Umaltolepis* has a peltate, cupulate seed-bearing organ, unlike any other ginkgoalean fossil and extant *Ginkgo biloba*. *Umaltolepis* bore simple, strap-shaped *Pseudotorellia* leaves on well-developed short shoots. The new interpretation of *Umaltolepis* reveals that early ginkgophytes showed considerable morphological diversity as well as adaptations for wind pollination and ovule protection during the Early Mesozoic. Other major plant groups from Mongolia include corystosperms (*Umkomasia*), filmy ferns and bryophytes. However, conifers dominated the lignite floras. The deposition and taphonomic conditions of the lignite deposits suggest that these plants inhabited permanently flooded systems (e.g., forest-moor swamps), however, the presence of charcoal in the same sedimentary sequences suggest that fire events were also part of the Early Cretaceous landscape in Mongolia.

T2-23-03

Cretaceous/Paleogene angiosperm floras from Patagonia, Argentina

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The southernmost area of South America, Patagonia, is considered to be critical for understanding South American modern floras as it had a fundamental role in the origin and diversification of seed plants in the Southern Hemisphere. In this contribution, we will discuss the evolution of angiosperms in Patagonia from their first appearance during the Barremian (Early Cretaceous) to the Cretaceous/Paleogene boundary. Data was gathered from three major Patagonian basins: San Jorge Gulf, Deseado Massif, and Austral, and from the Chubut Extra Andean Region, representing 26 formations that were deposited during the earlier Cretaceous (Late Barremian, 130 MA) to the Cretaceous/Paleocene (Danian, 61.6 MA) which offer a wealth of fossil angiosperm pollen and leaves. We will report on the taxonomical components of the floras, dominating groups, the development of plant communities, and the environments in which they lived. As expected, during the Cretaceous the angiosperms went from being a minor component of the paleoecosystems that were dominated by gymnosperms and pteridophytes to be the dominating clade at the end of the Cretaceous (Campanian-Maastrichtian)/Paleocene (Danian). The fossil record confirms the presence of members of the ANITA grade, Chloranthales and several monocotyledons during the Aptian-Albian, while the eudicots and the rosids become more diverse and the ones dominating the paleoecosystems at the end of the Cretaceous. The first appearance of angiosperms in Patagonia dates from the Barremian Springhill Fm (Austral Basin), and they were represented by pollen grains assigned to the *Clavatipollenites* complex. Angiosperm leaves appeared later at the Early Aptian Anfiteatro de Ticó Fm (Deseado Massif Basin) as three morphotypes show clearly angiosperm characteristics. At end of the Cretaceous, the angiosperms from La Colonia (Campanian-Maastrichtian) and Lefipán (latest Maastrichtian) formations were undoubtedly the dominant components of the paleocommunities confirming the declining of the gymnosperms and pteridophytes and the rise of the angiosperms at the Cretaceous/Paleogene boundary in Patagonia.

T2-23-04

The early angiosperms of the Primorye region, Russian Far East

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Recently we discovered new localities of Aptian–Albian age with angiosperms in the Razdolnaya River, Partizansk, and Alchan River basins in Primorye region, Russia. The plant-bearing deposits have alluvial, sometimes volcanogenic origin. Hence it appears that the environments inhabited by these first angiosperms were high-stress and unstable. For the first time we have revealed in the Aptian coaliferous deposits and the Cenomanian red beds of the Razdolnaya River Basin the remains of the flowering plants. They are represented by extremely rare leaf fragments of unidentifiable monocots and dicots, as well as by dispersed cuticle of probable Platanaceae. The angiosperm pollen *Tricolpites* sp., *T. vulgaris*, *T. micromunus*, *Clavatipollenites hughesii*, *Quercites sparsus, Retitricolpites georgiensis* were obtained from these Aptian deposits. The Albian angiosperms are represented by *Sapindopsis variabilis*, *Laurophyllum* sp., *Cercidiphyllum sujfunense*, *Menispermites* sp., and unidentifiable dicots. The palynological assemblage has increasing numbers of angiosperm pollen including the new taxa *Asteropollis asteroides*, *Fraxiniopollenites variabilis*, *Rouseia delicipollis*, *Polyporites clarus*. The abundant leaves of platanoids are recognizable in the Cenomanian. The angiosperm pollen *Tricolpites micromunus*, *Fraxiniopollenites variabilis*, *Rouseia delicipollis*, *Polyporites clarus* were found. In the Partizansk Basin the early angiosperms were collected in the Albian deposits. These are rare isolated leaf fragments of *Laurophyllum* sp., *Sassafras ussuriensis*, *Sapindopsis* cf. *angusta*, *"Aralia" lucifera*, *Cissites* sp., *Sapindopsis* sp., *Artocarpidium* sp., and unidentifiable dicots. Recently in the south-western and central parts of the Partizansk Basin we have found imprints of early flowering plants, which are represented by entire plants, including rhizomatous stems with tubers and adventitious roots, branches terminating in fruits, and pinnatisect leaves, intact solitary flowers and heads of achenes or follicles. These angiosperms were described as *Achaenocarpites capitellatus* and *Ternaricarpites floribundus*. The Albian angiosperm pollen is represented by rare *Asteropollis asteroides, Cyclusphaera psilata*, *Clavatipollenites incisus*, *Fraxiniopollenites variabilis, Tricolpites micromunus*. In the Cenomanian of this basin the following flowering plants were found: *Araliaephyllum* spp., *Cercidiphyllum* sp., *Magnoliaephyllum* sp., *Platanophyllum* spp., *Menispermites* sp. In the Alchan River Basin the early angiosperms appear in the middle Albian. These are very rare fragments of small leaves of *Laurophyllum* sp., *Sapindopsis* sp., and unidentifiable dicots. During the late Albian the diversity and abundance of the flowering plants increase. They are represented by *Magnoliaephyllum* sp., *Sapindopsis variabilis, S. brevifolia, Cinnamomoides* ex gr. *ievlevii, Cissites* sp., *Sassafras* aff. *ussuriensis, Quercophyllum* sp., *Celastrophyllum* ex gr. *oppositifolius*, *C.* cf. *serrulatus*, *Lindera jarmoljukii*, *Menispermites* sp., *Kenella harrisiana, K. filatovii*. The extremely rare tricolpates *Tricolpites* sp. and monocolpates *Clavatipollenites incisus* occur in the Albian deposits. In the Cenomanian the platanoids mainly occur along with aquatic angiosperms. Among angiosperm pollen, including *Tricolpites* sp., *Clavatipollenites* sp., the new taxa *Tricolpites micromunus*, *Tricolporopollenites* sp., *Polyporites clarus*, and *Triporopollenites* sp. appear.

T2-23-05

Early Cretaceous vegetation and the emergence of angiosperms in Europe and North America

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The Early Cretaceous was marked by the initial rise of angiosperms, and also by the diversification of modern lineages of conifers (e.g. Pinaceae) and ferns (e.g.water ferns) that became important in later plant communities. The Early Cretaceous also saw the decline of ancient conifers, ferns, ginkgophytes and cycads, that were characteristic of earlier Mesozoic vegetation, as well Bennettitales, Erdtmanithecales and other seed plant lineages that are now extinct. This large-scale floristic transition is seen worldwide through the Early Cretaceous, although the details differ regionally as a consequence of palaeopalaeogeography and palaeoclimate. Most research on this transition has focused on the emergence of angiosperms, but changes in other elements of the vegetation through the Early Cretaceous are also important. Here we focus on the fossil flora collected at a large clay pit near Torres Vedras, northeast of Forte de Forca, Portugal, and illustrate the vegetational changes that took place in Europe and North America during the Early Cretaceous by comparisons with other mesofossil floras that range in age from Berriasian to Albian (ca. 145 to 100 myr BP). The Torres Vedras flora is likely the oldest of the Portuguese mesofossil flora so far described that contains angiosperms remains. There are many earlier mesofossil floras, all of which are devoid of angiosperms, and there are many later floras in which angiosperms are more common. Fossil assemblages from Torres Vedras are dominated by twigs and seeds of extinct conifers and fragments of fern foliage, some of which are fertile. Megaspores of Isoetaceae and Selaginellaceae are also common, as are chlamydospermous seeds (assigned to the extinct genera *Tomcatia, Quadrispermum* and *Ephedrispermum*). Angiosperms are not common in the Torres Vedras assemblages compared to remains of other groups of plants, suggesting that they did not dominate the local vegetation. However, the diversity of angiosperms is relatively high, with about 50 different species, represented mainly by forms with no clear modern relatives, or by forms related to early diverging lineages, such as ANA-grade angiosperms and Chloranthaceae. Other than pollen grains of probable Araceae there is no evidence of monocots at Torres Vedras and there are only few taxa of possible magnoliid affinity. Eudicots are extremely rare and are represented in the mesoflora only by two types of tricolpate pollen grain, one known from a single coprolite and the other (with irregular apertures) known from a single pollen sac. There is also one (perhaps two) kind (s) of pantoporate grains that could be of eudicot affinity. There is no evidence of any extant group of early diverging eudicot, or of later branching lineages of core eudicots, such as rosids or asterids. In mesofossil floras from later in the Early Cretaceous, from both Portugal and North America, angiosperms are more common, taxonomic diversity is higher, and there are several fossil species that can be assigned to various groups of extant magnoliids and early diverging eudicots. Unequivocal core eudicots are know only from about the latest Albian-earliest Cenomanian onwards (ca. 100 myr BP).

T2-23-06

The Early Cretaceous seed plants from Crato Flora, South America Brazil

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The Early Cretaceous (Aptian/Albian) Crato Formation from the Araripe Basin (Brazil) contains one of the best known paleofloras from low palaeolatitudes from this important time interval in the history of angiosperms. The flora is diverse characterized primarily by pteridophytes and non-angiospermous seed plants including many taxa related to the Gnetales. The gnetalean component includes stems and twigs with opposite branching, reduced or absent leaves and compact cone-like reproductive structures. Angiosperm macrofossils are also diverse in the Crato Formation, sometimes preserved as whole plants with roots, stems, leaves and reproductive organs organically connected. Many of the angiosperms were clearly herbaceous and several were interpreted as aquatics. However, the non-angiospermous component of the Crato flora shows structural features consistent with arid environments (fibrous leaves, sunken stomata, well-developed indumentum, possible deciduous branches). The Crato Formation (lacustrine-carbonatic) together with the upper part of the underlying Barbalha Formation (deltaic) constitutes the lacustrine Aptian/Albian sequence of the post rift phase of the Araripe Basin. The plant fossils occur in laminated limestones that were developed under tropical, arid and semi-arid climatic conditions, with long intervals of dry climate and periodic precipitation. Some of the fossils possess sufficient characters for a systematic assignment at the family or higher level, and many taxa have already been described including taxa assigned to Nymphaeales and Magnoliales as well as other eumagnoliid angiosperms and several types of monocots. Other plant fossils are less informative systematically, but provide useful information on habit and diversity of early angiosperms in the palaeoequatorial region. New data on angiosperms from Crato Formation will be presented together with a floristic analyses showing the diversity and taxonomic composition of the flora.

Among the new angiosperm fossils is a tiny floral structures borne in small spikelets, subtended at the base by a pair of bracts. The inflorescence has 16 small flowers, with stamens (the number is uncertain), preserved three dimensionally.

T2-23-07

New findings of the gymnosperms female reproductive structures and seeds (*Nagrenia, Ginkgo, Allicospermum*) from the Middle Jurassic of Angren, Uzbekistan

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The material studied comes from the Middle Jurassic Angren Formation in in the Angren coalfield (Uzbekistan). The first findings of ginkgoalean female reproductive structures in Angren were described as *Ginkgo gomolitzkyana* Nosova (2012). The fossil remains are represented by numerous detached seeds and some ovules with minor pedicel fragments. Those specimens lacked hairs and papillae on the ordinary cells of the outer integument cuticle. The investigation of the new specimens of seeds of *G. gomolitzkyana* suggests that this feature is not constant: hairs and papillae may occur irregularly on the surface of the outer integument epidermis varying locally from numerous to absent. Based on this I provided the emended diagnosis for *G. gomolitzkyana.* One more finding of a ginkgoalean female reproductive structure (collar complexes) from Angren was described as *Nagrenia samylinae* Nosova (2013), the only species of the genus *Nagrenia*. The specimens of *N. samylinae* are associated with the leaves of *Sphenobaiera angrenica* Nosova (2013) and share a similar epidermal pattern. The investigation of the morphological and epidermal features of the new findings of collar complexes from Angren permitted to describe two new species: *N. pilosa* and *N. leviana. N. pilosa* differs from *N. samylinae* in cuticle thickness and in having hairs and hollow papillae on the cuticle surface. Unlike these two species, remains of *N. leviana* are characterized by entirely preserved inner surface of the collars with a conical protrusion in the center that is similar to *Ginkgo biloba*. The co-occurrence of *N. pilosa* with leaves of *Ginkgo asiatica* Nosova and seeds of *G. gomolitzkyana* in one sample suggests that these remains may have belonged to the same plant. The sediments of the Angren Formation have yielded numerous remains of detached seeds. Besides *Allicospermum angrenicum* Nosova (2013) and *A. budantsevii* Gordenko (2015), I distinguish three other types of *Allicospermum* (unnamed species, preparing paper). Seeds of these species differ in sizes as well as in the structure of the outer epidermis of the seed coat and of the megaspore membrane. In shape and dimensions, the seeds of *Allicospermum* sp. 1 are comparable to *A. angrenicum* and *A. budantsevii*, but have a megaspore membrane that is 2.5–3 times thicker. Also they differ by the very thin outer integument cuticle lacking stomata. Seeds of *Allicospermum* sp. 2 are smaller than other seeds of *Allicospermum* from Angren. They have a thick outer integument cuticle, which also lacks stomata. The main difference of these seeds is unbranched sinuous bacula of the pattern layer of the megaspore membrane, arranged parallel to the foot layer. The seed of *Allicospermum* sp. 3 differs considerably from others by long resin bodies and the structure of the outer integument epidermis. There are numerous *Ginkgo*-type stomata, oriented along the axis of the seed. Flattened papillae occur in the most of the ordinary cells of the outer epidermis, and mycropilar cells have long papillae.

T2-23-08

The presumed ginkgophyte *Umaltolepis* has seed-bearing structures resembling those of Peltaspermales and Umkomasiales

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The origins and phylogenetic relationships of the five groups of living seed plants, including the single relictual species *Ginkgo biloba*, are poorly understood, in large part because of very imperfect knowledge of extinct seed plant diversity from the late Paleozoic and Mesozoic. Here we describe abundant and exceptionally well-preserved material from the Early Cretaceous of Mongolia of the previously enigmatic Mesozoic seed plant reproductive structure *Umaltolepis*, which has been presumed to be a ginkgophyte. The new material shows that *Umaltolepis* is a seed-bearing cupule, which was borne on a stalk at the tip of a well-developed short shoot. Each cupule is an umbrella-like organ with a central column that bears a thick, resinous, four-lobed outer covering, which opens from the base. Each cupule encloses four, pendulous, winged seeds that are attached to the upper part of the column. Evidence from morphology, anatomy and field association suggests that the short shoots bore simple, elongate *Pseudotorellia* leaves that have similar venation and resin bodies to leaves of living *Ginkgo*. *Umaltolepis* seed-bearing structures were previously poorly understood, but the Mongolian material shows that while very different from those of *Ginkgo* they are very similar to fossils described previously as *Vladimaria. Umaltolepis* and *Vladimaria* do not closely resemble the seed-bearing structures of any living or extinct plant, but are comparable in some respects to those of certain Peltaspermales (peltasperms) and Umkomasiales (corystosperms). Vegetative similarities of the *Umaltolepis* plant to *Ginkgo*, and the reproductive similarities to extinct peltasperms and corystosperms, support previous ideas that *Ginkgo* may be the last surviving representative of a once much more diverse clade, which included many kinds of extinct plants, several of which exhibited various degrees of ovule enclosure.

T2-23-09

Two oldest fossils of Altingiaceae from the Upper Cretaceous of New Jersey

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versity

We report two new fossil materials from the Turonian (Upper Cretaceous, ~90-94 million years B.P.) Raritan Formation of New Jersey. The fossils are charcoalifed and reserved detail with tiny pistillate inflorescences and fruits. The multi-features composition—solitary capitate infructescence, syncarpous bicarpellate, 2-locules, unique sterile phyllomes structure—indicate that the fossil taxa close affinities to modern Altingiaceae. Also, it was supported by the evidence of phylogenetic analyses of molecular sequences combined with morphological data. Compared with *Microaltingia* which found in the same formation, the significant difference is the morphology and position of phyllomes. *Microaltingia*'s gynoecium is surrounded by two-wheeled numerous short, sterile phyllomes, the new fossil species have enough open space between carpel and involucres for phyllomes. One with incompactly florets, granular phyllomes, haired at the base of style, the other is density florets, one circle wide triangular phyllomes longer than the style, and suspected the remaining perianth. The primitive types indicate the possibility of insect pollination and the latter supposed a bisexual flower though we did not find the *in situ* pollen grain in the stamen-like phyllomes. The tiny fruit possibility suggests wind-dispersed. Considering the importance of phyllomes to classification in Altingiaceae and the matrix tree structure, we erected a new genus *Paleoaltingia* and two new species.

T2-23-10

Radiography of Cretaceous ovuliferous cones – case study

Jiri Kvacek
National Museum Prague

X-ray and neutron radiography are becoming widely used non-invasive methods to investigate preserved fossil plants. Various modes of preservation require different approaches. X-ray and neutron radiography are both used to document 3D internal structures of preserved fossil plants. Charcoalified ovuliferous cones of *Pityostrobus andraei* (Coemans) Seward from the type locality Baume, from the Belgian Wealden were studied, using X-ray radiography. Bracts are short, but separate from the scale base. Distally, the ovuliferous scales broaden and thicken to form a rhomboidal apophysis, with a dorsal umbo. Their seeds are always two per cone scale. It represents one of the basal members of Pinaceae. Charcoalified ovuliferous cones attached to and associated with twigs of *Cyparisidium bohemicum* of the Bohemian Cenomanian were studied with X-ray radiography. The ovuliferous cone scales are flat, typically bearing two seeds per ovuliferous scale, characteristic of Pinaceae. However, the twigs are typically cupressoid. Flat ovuliferous scales with wingless seeds are similar to basal members of the Cupressaceae Taiwania and Cunninghamia. A silicified ovuliferous cone of unknown affinity from the Bohemian Cenomanian was documented using neutron radiography. The ovuliferous cone is characterized by a slender axis and densely, spirally arranged ovuliferous complexes, which are long and curved. They make a wide rhomboidal pattern on the cone surface, formed by the distal ends of the woody ovuliferous complexes. Seeds are large and wing-less. Botanical affinity of this plant is not known. It may be associated with dwarf stems of Dammarites albens Presl in Sternberg.

T2-23-11

Early cretaceous seed plant diversity in Burmese amber

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Several groups of seed plants had their rapid diversification in Early Cretaceous. In the ~100 mya Burmese amber, we found plenty evidence for these fossil floras, including conifers, yews, Gnetales, Bennettitales, and early angiosperms. Some well conserved flowers, fruits, and leaves indicated that specific groups of flowering plants, ranging from basal angiosperms to higher taxa of monocots and eudicots, may have reached great diversity at that time, and some extant clades may have originated earlier. Their emergence brought major changes into plant reproductive biology, and affected dinosaurs, insects, mammals, and other organisms in their evolvement and evolution. Traces of insect activities were also witnessed on the fossil floras, which provides new insights into the co-diversification of seed plants and their associated fauna. We'd like to share our findings in Burmese amber concretely in the following presentation, and collaborate on studying the ecological landscape of Early Cretaceous with paleontologist and botanists allover the world.

T2-24: The Origin of Plants: rocks, genomes and geochemistry

T2-24-01

Resolving the phylogeny of early land plants by integrating phylgenomics and fossil evidence using morphological evidence

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The relationships among the four main lineages of land plants, namely hornworts, liverworts, mosses, and vascular plants, are still controversial. The land plant grade hypothesis with the liverworts basal have been preferred for several years over alternative hypotheses such as monophyletic bryophytes sister to vascular plans or hornworts sister to all other land plants. However, studies using phylogenomic approaches challenged this hypothesis. In this study, we integrate phylogenomic evidence and morphological evidence using a total evidence approach to explore the relationships among the four lineages not only based on extant plant diversity, but also by integrating the early land plant fossil record. Using this combined approach, we test not only the support for alternative hypotheses in the context of consistency with transcriptome sequence data but also in the context of morphological evolution and divergence time estimates. To achieve this, we carry out several phylogenetic analyses to evaluate the phylogenetic signal in support to alternative hypotheses with focus on four hypotheses namely: "land plant grade with liverworts basal", "monophyly of bryophytes", " land plant grade with hornworts basal and mosses and liverworts sister", and "hornworts sister to vascular plants and mosses liverworts sister to mosses". Finally, we highlight the consequences of the alternative hypotheses on studies aiming to reconstruct the original of land plants and the ecological consequences of the diversification of land plants to the formation of terrestrial environments.

T2-24-02

Latest approaches in establishing early land plant phylogeny and a timescale for plant evolution

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The colonisation of plant life on land was one of the most significant evolutionary events to transform the planet. Major anatomical, biochemical and morphological innovations in the early evolution of land plants (e.g. complex body plans, leaves, rooting systems, symbioses with fungi) led not only to the evolutionary success of the lineage, but also to fundamental changes to global biogeochemical cycles, such as increased rates of continental weathering and sequestration of carbon over geological timescales. However, the nature and timing of the early evolutionary history of land plants and their interactions with the Earth is far from substantiated. There is currently no consensus on the fundamental phylogenetic relationships between living bryophytes (liverworts, mosses and hornworts) and tracheophytes, with conflicting results from numerous analyses using either molecular or morphological data. Support can be found for most hypotheses, including monophyletic bryophytes, each solution implying very different scenarios of the sequence and tempo of the origin of land plant characters. These conflicts are a consequence of a dramatic increase in the availability of molecular data and the development of more realistic models of molecular evolution. However, these models have yet to be applied to genome-scale data. Secondly, living bryophytes possess a mosaic of anatomical characters that are difficult to determine if derived from convergent or common descent. Our project aims to test the competing hypotheses of living plant phylogeny using a combination of phenotypic data with genome-scale sequence alignments with the latest computational models of molecular evolution. Genomic data is obtained from published sequences. Phenotypic data is in the form of a morphological character matrix of representative taxa across the plant kingdom, but with particular emphasis on bryophytes and 'lower' land plants. However, living taxa only represent a fraction of the diversity and disparity of early land plants that we known from the fossil record, thus fossil taxa are included in the phenotypic character matrix to understand their phylogenetic positions among their living relatives. One assemblage of particular importance in understanding early embryophyte evolution is a group of charcoalified fossils from rocks of Early Devonian age (~415 Ma), Shropshire, UK, that possess both bryophytic and tracheophytic characteristics. These rare fossils are preserved in three-dimensions to subcellular level by charcoalification. Traditionally they have been sectioned and characterised using Scanning Electron Microscopy. However, by using the latest techniques in synchrotron radiation X-ray Tomographic Microscopy, they can be characterised in a non-invasive and non-destructive method. Finally, after establishing the evolutionary relationships of living bryophytes and tracheophytes and the phylogenetic position of fossil taxa, we will determine the timescale of early land plant evolution. This will be achieved by applying the latest developments in molecular clock methodology, using molecular sequence data, models of evolution and fossil age constraints, with consideration of facies biases that can severely impact upon the fossil record. By determining the nature and timing of early land plant evolution it will then be possible to test hypotheses on the co-evolution of plants and the planet, particularly the far-reaching impacts on global biogeochemical cycles.

T2-24-03

The rise of land plants: Ecological context and geochemical implications

Paul Kenrick, *To Be Confirmed Others*
The Natural History Museum, London

The origin and early evolution of plants was part of the development of complex terrestrial ecosystems and, through their interactions with the physical environment, plants and associated microorganisms are thought to have had very broad impact on major geochemical cycles and on climate globally. Understanding how terrestrial ecosystems assembled and their broader impact requires the integration of evidence from several sources, including the fossil record, phylogenetics of living species and also the geochemical record. Furthermore, gauging the broader impact on Earth Systems depends to a large extent on identifying and measuring effects in modern analogues and analogue systems. A growing body of fossil evidence indicates that early terrestrial ecosystems most closely resembled modern biological soil crusts and cryptogamic ground covers (CGCs), which are communities that comprise bacteria, fungi, algae, lichens, bryophytes and arthropods. Critical comparisons between the biota of the earliest well-preserved terrestrial ecosystem (407Ma Rhynie Chert) and plausible modern CGC analogues demonstrates many points of similarity but also highlights some key differences in the nature of the growth forms of the plants, the absence of certain key animal groups (e.g., Oligochaeta, Formicidae, Isoptera) and the presence of important enigmatic organisms (e.g., *Prototaxites*). Symbioses between fungi and various autotrophic organisms including cyanobacteria, green algae and plants were present at the outset and were key elements. The evolution of lichens and mycorrhizal-like symbioses in plants is thought to have had a major effect on the geochemical carbon cycle principally through increasing the rate of calcium-magnesium silicate weathering. Likewise, the later evolution of roots amplified this process through physical disruption of regolith and by enhancing the development of soils, but the magnitude of these effects is poorly constrained. Furthermore, once vascular plants evolved and began to diversify recalcitrant complex organic polymers (e.g., lignin) became common in the environment, but in the absence of modern lignin decomposing fungi (e.g., Agaricomycetes) it is unclear how such matter was recycled and to what extent it contributed to soil organic carbon. Calibrated molecular phylogenetic trees provide a means of understanding when key organ systems (e.g., roots, wood) and relations (e.g., mycorrhizae) evolved enabling one to place constraints on when their effects would be expected to manifest in the rock record. Recent analyses however give highly divergent ranges and there is a need to develop better constrained calibrations. A second promising approach that we are developing is to characterise the micro-dissolution features in soil minerals. This could provide a general but indirect means of evaluating the nature of biotic weathering in sediments and palaeosols and thereby its broader effects on the environment. Finally, incorporating Ordovician-Devonian vegetation parameters inferred from fossils and calibrated phylogenetic trees into global vegetation models will provide insights into their likely effects on climate and carbon cycle.

T2-24-04

On the monophyly of bryophytes; Evidence based on organellar and nuclear protein-coding gene data

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Determining the pattern and timing of morphological changes that accompanied the origin and early diversification of plants on land is fundamental to our understanding the evolution of terrestrial ecosystems. The often cited consensus opinion is that the bryophytes diverged first, with either liverworts or hornworts identified as being the earliest-diverging lineage of land plants, and that therefore tracheophytes are derived from bryophyte ancestors. The primary molecular evidence for this conclusion comes from analyses that include chloroplast and nuclear protein-coding genes which can be shown to possess the greater-part of the supporting phylogenetic signal even when included in multi-genome data sets. Strikingly, when the protein translations of the same chloroplast genes are analysed, they predict a very different tree; one in which the liverworts and mosses are strongly supported as a monophyletic group, and together they are often grouped with the hornworts. Here we diagnose the main source of the conflict between analyses of chloroplast genes and their implied proteins as being due to the presence of synonymous substitutions in the gene sequences: non-synonymous substitutions and proteins, when modelled and analysed independently, give largely congruent results. Our analyses suggest that conventional substitution models are unable to adequately account for synonymous substitutions in protein-coding gene data and demonstrate that conflicting results can be attributed to the influence of a directional mutation pressure among synonymous substitutions that drives a composition bias. We find that currently the best supported hypothesis based on the evaluation of data from all three plant genomes is that bryophytes are monophyletic. If indeed the first divergence of land plants is a split between bryophytes and tracheophytes then the ancestral traits of the land plant ancestor could conceivably, and most likely would, have included some tracheophyte characters that were hitherto thought to have evolved subsequent to the diversification of bryophytes.

T2-24-05

Molecular dating of angiosperms in the genomic era

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The evolutionary timescale of angiosperms has long been a key question in biology. The oldest crown-group fossils date to the early Cretaceous (~140 Ma), yet fossils attributed to the angiosperm stem lineage date back to 247.2–242.0 Ma. Molecular estimates of the angiosperm evolutionary timescale have shown considerable variation, being influenced by differences in taxon sampling, gene sampling, fossil calibrations, evolutionary models, and choices of priors. However, a common theme in modern molecular dating studies is that the angiosperm crown age far predates the oldest fossils by a non-trivial amount of time, even by up to ~100 Ma. Did crown-group angiosperms really arise in the Triassic? Or are the substantially older molecular dating estimates a product of methodological biases? Here, we report on two of our studies in which we analyse plastid-level data sets to (1) evaluate the impact of genomic data and priors on Bayesian estimates of the angiosperm evolutionary timescale, and (2) compare different partitioning methods to account for rate heterogeneity across lineages, and investigate the impacts on the precision of molecular dating estimates. In our main analysis, based on a data set comprising 76 protein-coding genes from the chloroplast genomes of 195 taxa spanning 86 families, we find that crown angiosperms arose 221 (251–192) million years ago during the Triassic. Based on a range of additional sensitivity and subsampling analyses, we found that our date estimates were generally robust to large changes in the parameters of the birth-death tree prior and of the model of rate variation across branches. In our reduced analysis of 79 protein-coding genes from 54 taxa, we find that all genes are best considered to have been evolving under a single pattern of among-lineage rate variation. Despite this, increasing the number of relaxed clock models to two led to a dramatic increase in precision of posterior age estimates. For example, the uncertainty surrounding the age of crown angiosperms halfved. Additionally, further increases to as many as 20 relaxed clock models always led to increased precision of posterior time estimates, but the precision appeared to be close to the limit imposed by the uncertainty in fossil calibrations. Overall, our results suggest that increases in gene sampling are unlikely to produce substantial changes in estimates of the angiosperm evolutionary timescale. Instead, our understanding of this important question is likely to improve through increased taxon sampling, significant methodological changes, and/or new information from the fossil record. Additionally, increasing the number of molecular clock models may be an effective way to increase the precision of posterior age estimates if computationally feasible, but whether this results in spurious precision remains to be evaluated.

T2-25: Poaceae: Systematics and phylogeny of major Lineages

T2-25-01

A molecular phylogeny and classification of the Cynodonteae (Poaceae: Chloridoideae)

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Morphologically, the tribe Cynodonteae is a diverse group containing about 840 species in 100 genera and 21 subtribes, found primarily in Africa, Asia, Australia, and the Americas. However, there are still seven unplaced genera within the tribe. We have genetic evidence that rapid radiation has occurred within the grass tribe Cynodonteae and we want to identify the ecological and genetic factors that characterize the early evolutionary history of these grasses. The Cynodonteae are C4 grasses, i.e., with an extra system to fix carbon dioxide, that can outcompete other plants in warm, arid climates but we know very little about their early evolutionary history and how they became dominant in short grass and savannah grasslands. A modern classification of the Cynodonteae based on the study of molecular characters has resulted in major changes in the circumscription and alignment of genera. During the last 10 years we have conducted several phylogenetic analyses on nearly 600 species, subspecies and varieties (67% of the Cynodonteae species) using sequence data from eight plastid regions (*rps16*-*trnK* spacer, *rps16* intron, *rpoC2*, *rpl32*-*trnL* spacer, *rps3*, *ndhF*, *ndhA* intron, *ccsA*) and the nuclear ribosomal internal transcribed spacer regions (ITS 1 & 2) to infer evolutionary relationships and refine the current classification. *Allolepis, Jouvea, Kalinia*, *Lepturidium*, and *Sohnsia* do not align with other genera in our trees but occur as isolated branches in a New World clade along with other members of the Boutelouinae, Hilariinae, Monanthochloinae, Muhlenbergiinae, and Scleropogoninae. However, *Decaryella, Kampochloa,* and *Vietnamochloa* are still incertae sedis within the Cynodonteae and have not been included in any modern molecular study. In our new classification we recognize three new subtribes: Dactylocteninae that includes *Acrachne*, *Brachychloa, Dactyloctenium, and Neobouteloua*; Orininae with *Cleistogenes* and *Orinus*; and Zaqiqahinae with a single genus, *Zaqiqah*; resurrect Hubbardochloinae with seven genera; and describe six new genera: *Eleusinella* (Eleusininae) with two species, *Hyalopterus* (Eleusininae) with a single genus, *Orthacanthus* (Traginae) with a single species, *Triplasiella* (Gouiniinae) with a single species, *Tripogonella* (Tripogoninae) with three species, and *Zaqiqah.* Subgeneric classifications have been completed on *Bouteloua* (60 spp.), *Distichlis* (11 spp.), and *Muhlenbergia* (182 spp.). In the future we hope to clarify the branching pattern of the early diverging lineages (crown nodes) within the Cynodonteae and continue to refine our classification.

T2-25-02

Phylogeography and classification of *Stipa* (Poaceae: Pooideae: Stipeae)

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The genus *Stipa* includes approximately 144 species and is a dominant member of the Eurasian steppe grasslands. Earlier molecular phylogenies included a few species with up to 10 DNA markers or only two DNA markers and 52 species. Consequently, relationships among the species are not well resolved and the phylogeography has not been thoroughly explored. The subgeneric structure among species of *Stipa* is complex and the number of the natural groups is unknown. Our earlier molecular phylogeny using 10 DNA markers revealed two major clades within *Stipa,* Asian and European. The European clade includes more than 115 species (80%) and originated in the late Pliocene (2.5 – 3.6 mya) via rapid radiation. As a result, the lineage is genetically homogeneous and shows little polymorphism in nucleotide sequences when using conventional markers (cpDNA regions, nrDNA ITS). In our study we found low copy nDNA genes of the Conserved Ortholog Set (COS) - *AroB, Agt1*, and *At103* to be variable enough to resolve phylogenetic relationships among the major lineages and detect multiple origins. We clarify subgeneric relationships among the species of *Stipa* and detect the following distributional patterns: 1) broad diagonal distribution from W (SW) to NE in ser. *Pulcherrimae* (from West Atlas to the mountain ranges of the Central Asia) and ser. *Penicilliferae* (from the Alpine ranges to the Siberian grasslands); 2) eastern European longitudinal distribution of ser. *Rubentes* associated with the periglacial steppe zone; 3) another diagonal pattern SW?NE (Irano-Turanian to Mediterranean) is seen in sect. *Barbatae* which is most speciose in Central Asia and not present in Central and Eastern Europe; and 4) sect. *Smirnovia* that is restricted to Central Asia. We identify ancestral areas for all major lineages within *Stipa* and reconstruct a dispersal pattern for separate groups and for separate species. We exploit dating and location of rapid radiation to establish essential patterns of evolution within *Stipa* which was under the pressure of gradual aridification during the late Miocene-Pleistocene. We juxtapose the biogeographic patterns for the major clades with phylogeny, and investigate whether or not colonization and radiation events were triggered by climate shifts. Our study supports the premise that rapid radiation is a key factor in evolution and speciation within a lineage.

T2-25-03

Phylogenetic classification of the Poaceae (Gramineae): A comparison with the past

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Three modern worldwide classifications were produced just prior to or at the beginning of the molecular phylogenetic revolution [Clayton & Renvoize, Genera graminum, Kew Bull. Addit. Ser. 13: 1–389. 1986; Tzvelev, The system of grasses (Poaceae) and their evolution, Bot. Rev. 55: 141–203. 1989; Watson & Dallwitz, The grass genera of the world, CAB International, 1038 p. 1992]. Linnaeus published 40 grass genera in 1753 and this number has increased by an average of 8.5 per year up to 1986, when Clayton & Renvoize accepted 651. Since Clayton & Renvoize's publication (30 yrs) there have been 5.7 genera published per year (170 total). Currently there are ca.1500 legitimate generic names (typified by different species), roughly half of which are treated as heterotypic synonyms, the other half accepted. Based on molecular data two recent phylogenetic classifications were presented [Soreng, Peterson, Romaschenko, Davidse, Zuloaga, Judziewicz, Filgueiras, Davis, Morrone, A Worldwide phylogenetic classification of the Poaceae (Gramineae), J. Syst. Evol. 53: 117–137. 2015; Kellogg, XIII Flowering Plants, Monocots, Poaceae in Kubitzki, The Families and Genera of Vascular Plants, Springer International Publishing, 416 p. 2015] accepting 763 and 711 genera, respectively. These two classifications share 674 accepted genera, but diverge by 89 and 37 genera, respectively. Soreng *et al.,* listed 783 accepted and synonymous names as having been investigated using molecular methods while only 87 accepted genera were lacking DNA data. As of May 2016 only 50 genera were lacking DNA data entered in GenBank or in our own unpublished sequences. The number of accepted subfamilies, tribes, and subtribes reported in Soreng *et al.,* versus Kellogg are, respectively: 12 & 12,52 & 30, and 80 & 53. Obviously Kellogg used a broader concept and did not recognize tribes or subtribes when classifying monotypic lineages or those with few genera. In comparison with Soreng *et al.,* only *Alloeochaete*, *Danthonidium*, and *Phaenanthoecium* were placed in different subfamilies, and *Streptogyna* was left as incertae sedis by Kellogg, versus placement in Oryzoideae by us. At the rank of tribe Soreng *et al.,* and Kellogg placed 11 genera differently, mostly as incertae sedis in a closely related tribe or in a narrower tribal delimitation. Subtribal placements of genera differed more between the two classifications, primarily because of our narrower subtribal concept. Kellogg placed more genera in synonymy, i.e., lumped, especially in *Trisetaria* (7), *Elymus* (6), *Eragrostis* (4), but also in *Chinonachne*, *Ehrharta*, *Saccharum*, *Tristachya* (all 3 each), and in 30 other cases (all 1 each). However, the two classifications were remarkably similar, especially when compared to the discrepancies at all ranks among the three earlier classifications. The power of molecular phylogenetics to interpret evolutionary relationships to stabilize the classification is apparent. We are continually updating our current classification by expanding synonymy, adjusting tribe and subtribe membership, including new genera and new molecular results for existing genera that have been overlooked. Updated arrangements for tribes, subtribes, and generic alignments are noted across the family.

T2-25-04

A new phylogenetic classification of Poeae subtribes Poinae, Coleanthineae, and Miliinae (Poaceae)

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Subtribes Poinae, the largest subtribe in the grass family with over 700 species in 29 genera, Miliinae (1 genus, 5 spp.), and Coleanthineae (10 genera, 163 spp.) together form one of three clades in tribe Poeae, the largest tribe in Poaceae (118 genera, 2776 spp.). Here we present a revised classification of this clade based on molecular phylogenetic data (ITS, ETS, *trnT-trnL-trnF*, *MatK*, and *rpoB-trnC*). Our analysis includes 39 of the 40 genera (data lacking for *Agrostopoa*), with broad sampling within all of the large genera and all or most of the species in the small genera (>800 samples). We compare our classification with previous classifications based on morphology and/or molecular data, and discuss the evolution of several morphological characters. Subtribes Miliinae and Coleanthineae resolved as monophyletic with strong support [Soreng, Gillespie, Koba, Boudko, Bull. J. Syst. Evol. 53: 138-162. 2015]. However, Poinae sensu Soreng *et al.,* 2015 [Soreng, Peterson, Romaschenko, Davidse, Zuloaga, Judziewicz, Filgueiras, Davis, Morrone, A worldwide phylogenetic classification of the Poaceae (Gramineae), J. Syst. Evol. 53: 117–137. 2015], where Alopecurinae, Beckmanniinae, Cinninae, Phleinae were included in synonomy, is paraphyletic because Miliinae is embedded within it. The genus *Poa* is more closely related to *Milium*, and possibly *Phleum*, than it is to other genera traditionally placed within Poinae s.s. The latter genera, together with genera of Alopecurinae, Beckmanniinae and Cinninae, form a well supported clade. Rather than synonymyzing Miliinae in Poinae, thereby making that subtribe even larger and more heterogeneous, we prefer to recognize strongly supported clades within Poinae s.l. as subtribes. Here we redefine subtribe Poinae to include only the genus *Poa*, recognize both Miliinae and Phleinae as monogeneric subtribes, and subdivide the remaining 26 genera of Poinae s.l. into several well-defined subtribes, some of which are reticulate in origin. Morphological and habit characters traditionally used in the classification of the group, such as annual versus perennial, one-flowered versus multi-flowered spikelets, glume length relative to floret length, spike-like panicles, and awn development and insertion, were determined to be highly homoplasious among and within the subtribes.

T2-25-05

A molecular phylogenetic study of temperate grasses in Poeae chloroplast group 1 (Poaceae: Pooideae: Poeae) based on nuclear ribosomal and plastid DNA sequence data

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We conducted phylogenetic analyses of >2500 new DNA sequences from nuclear ribosomal (ITS and ETS) and five plastid regions to clarify relationships within and among genera and subtribes that are part of the grass tribe Poeae chloroplast group 1 and allies. A large clade comprises the subtribes Agrostidinae and Calothecinae, neither of which is monophyletic. The type species of *Deyeuxia* is allied with species of *Calamagrostis* s.s., thus *Deyeuxia* is a synonym of *Calamagrostis*, and relationships among species of *Calamagrostis* s.s. and related genera are mostly poorly resolved. *Agrostis* and *Polypogon* are distinct from *Calamagrostis* s.s. but are not reciprocally monophyletic. Species of *Agrostis* and *Polypogon* are intermixed in a strongly supported clade in plastid trees, including *Lachnagrostis*, and there is incongruence among data partitions in a subset of species. *Ammophila* and *Calamagrostis* s.s. are not distinguished as separate genera, and *Calamagrostis coarctata*, *Dichelachne*, *Echinopogon* and *Relchela* are closely related. Taxa of Sesleriinae are allied with Aveninae s.s. (*Arrhenatherum*, *Avena*, *Helictotrichon*, *Tricholemma*) in nuclear ribosomal trees, and with taxa of chloroplast group 2 in plastid trees. Koeleriinae is strongly supported and includes two major subclades: clade A comprises *Avellinia*, *Gaudinia*, *Graphephorum* and *Trisetum* sect. *Trisetum* p.p. (nuclear trees), *Koeleria*, *Rostraria*, *Trisetaria*, *Trisetum* sect. *Trisetum* p.p. and *Trisetum* sect. *Trisetaera*, and *Koeleriinae* clade B comprises *Calamagrostis*/*Deyeuxia* p.p., *Graphephorum* and *Trisetum* sect. *Trisetum* p.p. (plastid trees), *Peyritschia*, *Leptophyllochloa*, *Sphenopholis*, *Trisetopsis* and *Trisetum* sect. *Deschampsioides.* Within each clade most aspects of relationship are unresolved, and conflict between data partitions is identified. Affinities of *Lagurus ovatus* with respect to Aveninae s.s. and Koeleriinae are incongruent in nuclear ribosomal and plastid trees. All but one of the eight sampled species of *Deyeuxia* sect. *Stylagrostis* are part of the *Deschampsia* lineage, and these are transferred to *Deschampsia*. Substantial changes to the current classification will be needed to produce generic circumscriptions consistent with phylogenetic evidence.

T2-25-06

Reticulate evolution of Triticeae revisited: New insights based on multiple chloroplast and nuclear genes

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Triticeae is one of the economically important tribe in Poaceae. Due to complex reticulate evolution in the tribe, the genetic relationships among genera and among genomes are unclear, and the circumscription of genera remains questionable. In this study we analysed 26 of 27 genera in the tribe using two nuclear genes and 18 hypervariable chloroplast regions. Our well resolved phylogenies provide new insights into the evolution in Triticeae. (1) There are eight lineages in Triticeae, plus *Bromus*. (2) Genome Xe is a variant of genome P, genome Y is a variant of genome V, and genome Xm is closely related to genome Ns. (3) The origins of nine polyploid genera was proposed and *Elymus*, *Kengyilia*, and *Leymus* are of multiple origins involving several genomes. (4) The divergence time of Triticeae was dated to the Oligocene, but most genera originated in the Miocene and the speciation of polyploid species was in the Pliocene. (5) The cradle of Triticeae is deduced to be in Asia. (6) Taxonomic treatments of the genera are provided. This study clarifies generic relationships, genome relationships and the origins of hybrid genera in Triticeae, which is helpful for understanding the evolution in the tribe.

T2-26: Lamiales: Phylogenetic insight into the patterns and mechanisms of phenotypic diversity (two sessions)

T2-26-01

Phylogeny and systematics of Lamiales: Historical perspective and present concept

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We have come a long ways since the concept of Lamiales in its present form was first introduced in 1992. At the St. Louis IBC in 1999 a symposium on the phylogeny and systematics of Lamiales presented the first summary of what we know about this large clade. We now recognize over 1000 genera assigned to 26 families and including more than 22,000 species. With more than one-third of all species of Lamiales included in molecular phylogenetic studies, we now have a detailed understanding of the phylogeny of the clade. I will present the history of phylogenetic studies within Lamiales, an overview of the diversity at the family level, and conclude with a new dated phylogeny of the clade, which suggest that the age of crown-group Lamiales is approximately 92 million years (+/-12 MY).

T2-26-02

Probe into floral symmetry and A-class genes in a new model plant *Chirita pumila*

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The development and evolution of the flowers has always attracted much attention of botanists. In spite of extensive investigation in two classical model species *Antirrhinum majus* and *Arabidopsis thaliana*, there are still many unanswered questions in the fields of floral organ identity and floral symmetry. For example, the functions of A-class floral organ identity genes, so far limited to *Arabidopsis* and its close relatives, have not yet been testified in other lineages, and whether the BC model (lacking the A-function) is general in eudicots is still an open question. In the field of floral symmetry, it is still a challenge to decipher how related floral symmetry genes interplay to generate zygomorphic flowers in other Lamiales species, how they regulate their downstream targets, and how their own activities are controlled. *Chirita pumila* D. Don belongs to the Gesneriaceae family, one of the most basal groups in Lamiales, is an ideal model system to address these questions. Here we select *C. pumila* as a model to deeply address the function and regulatory networks of floral symmetry related genes, involving *CYCLOIDEA*-, *RADIALIS*- and *DIVARICATA*-like genes, and the role of A-class floral organ identity genes, mainly *APETALA1* and *APETALA2* genes. Real-time qPCR and *in situ* hybridization results hinted that *CpCYC1* and *CpCYC2* might have partially redundant function. However, knock-out of either one via a strong repressor SRDX motif was sufficient to generate the fully ventralized actinomorphic flowers due to concurrent down-regulation of another one, confirming the positive auto- and cross-regulation between them, as previously reported in *Primulina heterotricha*. Furthermore, knock-out of either *CpCYC1* or *CpCYC2* led to the up-regulation of *CpRAD* with *CpDIV*'s expression pattern unaffected. Further transgenic experiments involving *CpRAD* and *CpDIV* and transcriptome analyses are ongoing. In addition to function studies, we also focused on the regulatory mechanisms underlying the dorsal-specific expression patterns of *CpCYC1* and *CpCYC2* that play a pivotal role in establishing floral zygomorphy in *C. pumila*. We have identified a series of key elements in the proximal promoters of the two genes by sequential truncation transgenic experiments, and further function verification experiments are ongoing. As for the two A-class genes, we have performed real-time qPCR and *in situ* hybridization experiments. The results showed that the expression patterns of both *CpAP1* and *CpAP2* were different from the corresponding orthologs in *A. thaliana* and *A. majus*. Whether *CpAP1* and *CpAP2* have A-function and if they activate B- and C-function genes deserve further function studies.

T2-26-03

Phylogenetic pattern and underlying mechanisms of hummingbird-adapted flowers in the genus *Penstemon*

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Penstemon is ancestrally adapted to pollination by bees, and flowers adapted to hummingbird pollination have evolved through coordinated evolutionary transitions in a complex of physiologically and developmentally distinct floral traits. Using a phylogenomic RADseq approach on a subset of *Penstemon* species (sampled primarily from herbarium material), we identified an asymmetric pattern of pollination syndrome evolution: transitions from bee to hummingbird syndrome have repeatedly occurred with little evidence for evolutionary reversal. Results from QTL analyses and comparative morphological and gene expression studies suggest that loci of major effect and loci that pleiotropically influence multiple floral traits underlie adaptations to hummingbird pollination. This relatively efficient genetic architecture may help explain the frequency of evolutionary transitions to hummingbird pollination in this genus.

T2-26-04

Speciation patterns and processes in Gesneriaceae: New world *Columnea*, old world *Primulina*

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Phylogenetic analyses based on molecular data have revolutionized systematics and with well-supported phylogenetic hypotheses we are now able to examine processes that affect speciation as well as the patterns. These can resolve which morphological, biogeographic region, or environmental parameters are ancestral and how changes have occured across the evolutionary history of the group being examined. Likewise, methodologies also exist to infer which, if any of the above alter the rate of diversification. Here we examine two genera of Gesneriaceae from different parts of the world. *Columnea* is restricted to the New World tropics and is the largest genus in the nearly exclusively Neotropical subfamily, Gesnerioideae. Species are mostly epiphytic or epipetric and most are presumed to be hummingbird pollinated and the seeds dispersed by birds consuming the fleshy fruits. In contrast, *Primulina* is restricted to the Old World and is one of the larger genera in the Paleotropical subfamily Didymocarpoideae. Species in this genus are mostly epipetric and are found on calcareous outcroups or in caves where moisture levels are high. Pollination is presumably by bees and seeds are gravity or wind dispersed from dry dehiscent fruits. We examine biogeography and ancestral character state evolution in both of these lineages and investigate the parameters that are driving diversification.

T2-26-05

Say it with monkeyflowers *(Mimulus)*: Genetics, development, and evolution of phenotypic diversity and novelty

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The monkeyflower genus *Mimulus* (now *Erythranthe*) has long been recognized as a classic ecological and evolutionary model system that is particularly suitable for the studies of local adaptation, speciation, plant-pollinator interaction, mating system evolution, and species range limitation. Yet its potential as an excellent genetic and developmental model system has only been realized in the past few years. Instead of focusing on one specific story, in this talk I will present an overview of the sophisticated functional tools and genetic and genomic resources developed for this system, and highlight some recent progresses in using *Mimulus* to solve problems on the genetic control of ecologically important floral traits (that cannot be studied using *Arabidopsis*), developmental mechanism underlying the formation of periodic patterns in biological objects, molecular basis of inter-species phenotypic variation, and the origin and evolution of phenotypic novelty.

T2-26-06

Evolution of floral morphology in Bignonieae (Bignoniaceae): Implications for Community Assembly

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Flowering plants represent one of the most diverse groups of organisms in the Planet. A large portion of this diversity results from the multitude of floral forms encountered nowadays. An improved understanding of the patterns of floral variation and of the historical and ecological factors that led to the evolution of such diversity in floral forms is critical for a better understanding of the processes that led to the diversification of angiosperms. The tribe Bignonieae (Bignoniaceae) is the most diverse clade of Neotropical lianas and represents an excellent model for the study of floral evolution due to its high diversity of floral forms. In this study we characterize the: (i) pattern of evolution of discrete floral traits and floral morphologies in Bignonieae, and its potential associations with pollinators; (ii) phylogenetic signal and rates of evolution of continuous floral traits; (iii) pattern and magnitude of phenotypic integration among floral traits across the phylogenetic history of tribe Bignonieae; and, (iv) influence of phylogeny, floral morphology and abiotic factors for the patterns of species co-occurrence and the structure of communities of Bignonieae in the Neotropics. Our findings indicate high lability in the evolution of discrete floral traits and floral forms, contrasting the significant phylogenetic signal encountered in 16 continuous floral traits examined. However, the phylogenetic signal differs between traits of different floral whorls. Rates of evolution also varied among different characters, suggesting the action of different selective pressures or differential responses to selection in different floral parts. Overall, the patterns of phenotypic integration are constant during the history of Bignonieae, despite the homoplastic evolution of the magnitude of correlation among characters. This apparent evolutionary complexity, leading to different patterns in different traits, is not reflected in the ecological structure of communities given that neither floral morphology nor phylogeny influences species co-occurrence. On the other hand, we found species specialization to abiotic factors, suggesting that environmental filtering played a key role in the structure of communities of Bignonieae. These results reject the hypothesis that saturation caused by competition for pollinators is the main factor determining intra-community structure in Bignonieae (Funding: FAPESP, CAPES & CNPq, Brazil).

T2-26-07

Global Biogeographic patterns of diversification in Lamiales

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With over 20,000 species and a cosmopolitan distribution, Lamiales represent one of the major asterid radiations. Minimum estimates of the crown (~65-75 my) and stem ages (~89-113 my) suggest an origin and initial diversification at the time of the initial split of Gondwana (~100 my), thus suggesting that while vicariance may have played a role in their early diversification, the continental distributions within clades were likely the result of intercontinental dispersal. Using a dated Lamiales megaphylogeny and geographic data obtained from GBIF, we quantify the rates of intercontinental migrations, as well as transition rates between global biomes, to address how intercontinental migration events and rates of global biome evolution have led to present distributions in Lamiales, thereby obtaining a first pass at the dynamics of biogeographic history on a global scale for this large complex group.

T2-26-08

Evolution of floral symmetry and orientation as functional integration promotes speciation and diversification in Lamiales

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Flowers have provided an important model for studying the evolution of integration relevant to prezygotic mechanism in speciation and diversification. The association between floral orientation and symmetry was recognized as a floral complex trait very early the 19th century. However, the floral orientation to date has received little attention even though the floral symmetry has been of great interest to evolutionary biologists because the evolution of zygomorphy is considered a major trend in angiosperm radiation. In fact, the horizontal orientation, possibly the first step toward floral specialization, is widely linked to and co-varied with zygomorphy in angiosperms, especially in Lamiales. Recent study shows that the floral horizontal orientation and floral zygomorphy acting as a functional integration are controlled by a single CYC-like TCP gene via repressing the basal-dorsal floral tube, dorsal petals and stamens. This finding shed significant light on our understanding of the complex mechanisms behind floral adaptive evolution at molecular level in angiosperms. It has becoming increasingly apparent that floral horizontal orientation and zygomorphy play a key role in effecting specific-pollinator attraction, approach and behavior, on which scent and pigmentation traits develop further enhancing prepollination isolation. Given frequent variation in zygomorphic lineages, CYC-like genes could be hotspots of evolution in both floral orientation and symmetry, and dominate in the evolution of the complex prezygotic-isolation mechanism that underlies the rapid diversification of angiosperms.

T2-26-09

Inflorescence evolution in the Scrophulariaceae, with focus on the genus *Buddleja*

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Inflorescence architecture is important in the fitness and reproductive success of many angiosperm groups. Breeding system, pollinator attraction and behavior, ovary competition, and fruit dispersal and predation are all affected by inflorescence structure. As such, evolution in inflorescence architecture may be an important driver of plant adaptation and speciation. A diversity of inflorescence architectures has evolved in flowering plants, which may be correlated with different habitat types, pollinator communities, and life histories. In the Lamiales, including the family Scrophulariaceae, determinate cymose inflorescence units are common, but these cymes are arranged in compound inflorescences in various ways. *Buddleja* displays a diversity of inflorescence architectures across its 108 species, including racemose, thrysoid, verticillate, capitate, and paniculate forms. Using a recently inferred molecular phylogeny as a framework, inflorescence characters are mapped and analyzed to investigate evolutionary patterns in inflorescence architecture and correlations with environmental factors and other traits. We also survey inflorescence form across Scrophulariaceae to place inflorescence evolution in *Buddleja* within a broader context.

T2-26-10

Phylogeny and floral trait evolution in *Eremophila* (Scrophulariaceae, Lamiales)

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Tribe Myoporeae is a member of the large and cosmopolitan plant family Scrophulariaceae (Lamiales). Previously considered a family in its own right, Myoporeae was recently recircumscribed as a tribe within Scrophulariaceae sensu stricto based upon sequences of plastid genes. Myoporeae consists of seven genera, predominantly distributed throughout Australia. By far the largest of these genera is *Eremophila*, with ~220 species, ~60 subspecies and a large number of putative taxa still awaiting formal description. Commonly known as emu bushes, *Eremophila* species are significant, often dominant, components of the Australian arid and semi-arid zone flora, where they tolerate harsh, dry and saline conditions and provide food and shelter for a wide range of insect and bird species. Morphologically, *Eremophila* species are incredibly diverse particularly with regards to flower shape, colour and structure. This diversity is largely attributed to variation in modes of pollination. Eremophilas can be broadly divided into two groups, those that are bird pollinated (~20%) displaying brightly coloured, tubular flowers, and those that are insect pollinated (~80%) displaying purple, mauve or white flowers and patterns of spotting and banding. Species relationships within *Eremophila* have been unclear and the classification of this group to date has been based on morphology alone. With high throughput sequencing methods, we now have the opportunity to study the evolution of this group using substantial genomic datasets. The focus of our research has been to generate a comprehensive molecular phylogeny of *Eremophila* and related genera within tribe Myoporeae using plastid and nuclear data. This phylogenetic reconstruction using entire chloroplast genomes and high copy sections of nuclear DNA has provided greater understanding of evolutionary relationships both in *Eremophila* and Myoporeae, as well as biogeographic inferences for arid Australia. This molecular phylogeny provides a framework for inferring of patterns of floral evolution in *Eremophila*, with multiple floral trait transitions likely to have occurred during evolution of the group.

T2-26-11

Phylogeny, systematics and evolution of the largest hemiparasitic genus *Pedicularis* (Orobanchaceae)

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Pedicularis, with more than 500 species, is the largest genus in Orobanchaceae. Species of *Pedicularis* are biennial or perennial hemiparasitic herbs, and are primarily distributed in mountain ranges throughout the north temperate zone. Striking interspecific variation in floral traits in *Pedicularis* has given rise to controversy concerning species delimitation and infra-generic classifications. Evaluating DNA barcodes showed that nuclear ribosomal internal transcribed spacer (nrITS) or nrITS + *rbc*L can discriminate at least 78% of species in the genus. Furthermore, molecular methods are effective in helping to identify and discover cryptic species. Based on extensive sampling of *Pedicularis* (~ 257 species), with an emphasis on species from the Himalaya-Hengduan Mountains, phylogenetic analyses strongly recovered 13 major clades. There is little consensus between the phylogenetic tree and Tsoong's classification of *Pedicularis*. Only two of the 13 groups (15.4%), and 19 of the 56 series (33.9%) with more than one sampled species were found to be strictly monophyletic. Most opposite-/whorled-leaved species fell into a single clade, i.e. clade 1, while alternate leaved species were placed in the remaining 12 clades. Excluding the widespread *P. verticillata* in clade 1, species from Europe and North America fall into clades 6–8. Our results suggested that combinations of morphological and geographic characters associated with strongly supported clades are needed to elucidate a comprehensive global phylogeny of *Pedicularis*. Alternate leaves were inferred to be plesiomorphic in *Pedicularis*, with multiple transitions to opposite/whorled phyllotaxy. Anatomical observation on 39 *Pedicularis* species revealed two nectary forms [bulged (N = 27) or elongated (N = 5)] or the absence of nectaries (N = 7). Evolutionary inferences indicated that the bulged nectary should be the ancestral state, nectaries were independently lost in some beaked species, and elongated nectaries evolved independently in some clades of beakless species. Phylogenetic path analysis showed that nectary presence was indirectly correlated with beak length/pollinator behavior through an intermediate factor, nectar production. No significant correlation was found between nectary type and nectar production, beak length or pollinator behavior. The nectary in beaked species may be a vestigial structure retained during a recent rapid radiation of *Pedicularis*, especially in the Himalaya– Hengduan Mountains of south-western China.

T2-26-12

Phylogenetic relationships, character evolution, and biogeography of *Salvia* (Lamiaceae), with special focus on Iranian taxa

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Salvia L. is the largest genus in Lamiaceae (Mentheae-Salviinae), with approximately 1000 species that are mostly found in five major centers of diversity: Central and South America (500 spp.), Western Asia (200 spp.), Eastern Asia (100 spp.), Africa (60 spp.), and Europe (36 spp.). *Salvia* is characterized by a unique staminal structure of two theca separated by an elongated connective, which is attributed to a special pollination system referred to as a lever mechanism. This character evolved in parallel between the Old World and New World *Salvia* species. A high percentage of endemism and high morphological diversity are of special interest in understanding the evolutionary patterns within *Salvia*. Compared to New and Old World *Salvia*, taxa from Southwest Asia have not been well sampled in previous phylogenetic studies. Iran contains 62 species of *Salvia*, of which 19 are endemic, and the area is considered a center of diversity for *Salvia* in Southwest Asia. In this study, the phylogenetic relationships of Iranian species of *Salvi*a are reported for the first time. Two nuclear regions, ITS/5.8S and ETS, and the plastid locus *ycf1* were amplified and sequenced for 58 of the 62 species of Iranian *Salvia*. Maximum likelihood analyses for 274 species including Genbank data suggest that Iranian *Salvia* species are not monophyletic; instead, they are placed in three main clades. These clades are congruent with morphological characters. To assess the evolutionary patterns and diversification of *Salvia*, key morphological characters and habitat for 245 species were reconstructed across the phylogeny. In addition, biogeographic patterns of *Salvia* in the Old World were analyzed and will be discussed.

T2-27: Asian and Chinese bryology (two sessions)

T2-27-01

Systematic study of the genus *Yakushimabryum* and related genera in the Pylaisiadelphaceae (Musci)

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Phylogenetic relationships among the members of an epiphytic genus *Yakushimabryum* H. Akiyama, Y. Chang, T. Yamag. & B. C. Tan and related genera of the Pylaisiadelphaceae were studied based on phylogenetic analyses using cpDNA (*rbc*L, *rps*4, and *trn*L-F) and mtDNA (*nad*5) gene sequences as well as morphological features of type and authentic specimens. Our present phylogenetic analysis did not support the monophyly of the family. While, it suggested that *Yakushimabryum* to be monophyletic and should be treated as a distinctive genera in the family. The genus was revealed to be closely related to *Taxithelium* and *Pylaisiadelpha* in the family. It was revealed to include three species, all of which had been reported in East Asia. Systematic position of *Clastobryum*, which had been classified in the Pylaisiadelphaceae or Sematophyllaceae, was left ambiguous. One new genus *Orientobryum* H. Akiyama and a new species *Y. brevigemmium* H. Akiyama were recognized and described. Low genetic differentiation among geographically distantly separated populations were found in *Yakushimabryum spp.*, *O. oligonema*, *Pylaisiadelpha tenuirostris*, *Clastobryum scalare*, *Isocladiella surcularis*, *Gammiella ceylonensis*, all of which are epiphytes and reproduced by means of light and small propagules produced in leaf axils.

T2-27-02

Phytogeographic affinities of the moss flora of China relative to those of North America, Japan, and mainland Southeast Asia

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A phytogeographic affinity of one region with another refers to similarities of their current floras and is an outcome of their common evolutionary histories and past and contemporary connectivity. The existence of taxa (families, genera and species) is often expressed by their phytogeographic classifications. The level of similarities between two floras is commonly explained by their similarity coefficients. The moss flora of China contains a total of 2031 species and subspecific taxa belonging to 434 genera and 87 families. This study was a first attempt to analyze floristic composition and phytogeographical connections of the Chinese mosses based on distributional patterns of taxa. The distributional data corresponding to 2031 Chinese taxa were classified into northern, southern, southwestern, and widely spread elements according to their distribution records by provinces. The phytogeographic affinities of the Chinese mosses relative to the mosses of North America, Japan, and the mainland Southeast Asia for the first time were explored in this study, respectively. The Sørensen-Dice coefficients were used to measure the similarities between the two floras in the comparisons. The analysis shows that the moss flora of China not only holds Holarctic elements in the north of Yangtze River (northern flora), but also holds many tropical elements in the south of Yangtze River (southern flora). The northern flora shares more similarity to that of North America which has predominantly Holarctic temperate elements and a few tropical elements in the southern end. Their similarities are 100% at the familial level, 82.97% at the generic level, and 48.96% at the species level. The study also shows that the moss flora of northern China in contrast to the southern flora is more similar to that of Japan with 92.50% similarity at the family level, 81.23% at the generic level, and 61.48% at the species level. The southern flora and particularly the flora of southwestern (SW) China are more closely related to that of mainland Southeast Asia. The SW region alone represents nearly all the similarity between southern China and the mainland Southeast Asia with coefficients of 84.29% at the familial level, 68.42% at the generic level, and 39.57% at the species level. The data indicate that SW China is largely tropical in nature, although it also contains a significant number of temperate elements. Nearly all Chinese records of the following larger genera occur in SW China: *Bartramia*, *Brachymenium*, *Brachythecium*, *Bryoerythrophyllum*, *Bryum*, *Campylopus*, *Dicranodontium*, *Didymodon*, *Ditrichum*, *Entodon*, *Gollania*, *Hypnum*, *Leucobryum*, *Mnium*, *Neckeropsis*, *Orthomnionm*, *Philonotis*, *Pinnatella*, *Plagiomnium*, *Plagiothecium*, *Pogonatum*, *Pohlia*, *Rhizomnium*, and *Trichostomum*. This phenomenon suggests that the SW region occupies an important transitional position between the Paleotropical flora and the Holarctic flora, possibly serving as a significant corridor for the migration of moss species.

T2-27-03

All is about *Herbertus aduncus* (Dicks.) Gray, Species Delimitation in *Herbertus* (Marchantiophyta)

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No consensus has been settled on the taxonomy of the genus *Herbertus* since it was established in 1821. Much of the taxonomy of the genus, has in the past, been inaccurate and in a confused state in considerable measure, but has unfortunately been followed subsequently. The taxonomic uncertainties within the genus reside in the lack of global-scaled taxonomic studies and in the presence of a high degree of morphological variations. Because the genus is extremely variable from one local population to another as well as over wide geographic ranges, it has been suggested that the problem of delimiting species is unlikely to be solved without the study of large quantities of material. In the present study, an attempt on the species delimitation of *Herbertus* of the Northern Hemisphere was made based on morphology and multigene sequence data. Over six hundreds herbarium specimens were studied. Our results show that morphology alone offers limited useful taxonomical characters and that there are little genetic differentiations among currently recognized species *Herbertus aduncus*, *H. dicranus*, *H. hutchinsiae*, *H. longifissus, H. stramineus*, and *H. delavayi*, and among *H. sendtneri*, *H. armitanus* and *H. circinatus*. Our results do not support the current morphology-based species concept of the genus. Most of the species included in the present study are expected to be synonymized.

T2-27-04

Phytogeography of East Asian mosses with special emphasis on the East Asian element

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A survey is given of the distribution patterns of East Asian element of mosses. East Asia is one of the richest floristic regions in the world. This region roughly overlaps the Eastern Asiatic region, one of the phytogeographic regions which have been historically recognized on the basis of the distribution of flowering plants. This region was not significantly glaciated in the Pleistocene, which is one of the reason why this region has richer flora than that of Europe. For example East Asiatic *Nipponolejeunea* is only recorded by fossils in Europe. As compared with Europe, however, exploration of the moss flora of the East Asia has been ongoing, yet substantial gaps remain in our floristic understanding of this region. In the system of Takhtajan the Eastern Asiatic region belongs to the Holarctic Kingdom, is bordered by the Circumboreal region in the north, the Irano-Turanian region in the west and the Indian, Indochinese and Malesian regions of the Paleotropical Kingdom in the south. As the result the moss flora of the Eastern Asiatic region consists of several floristic elements such as circumboreal element, East Asian element, tropical element, cosmopolitan, *etc.*, but it is most characterized by East Asian element. The range of mosses is usually much wider than that of the flowering plants. Analysis of the range of the mosses of the East Asian element indicates that there are many taxa which show their limits of the distribution around the border of the Eastern Asiatic region recognized by the flowering plants. The causes leading to the accordance is discussed.

T2-27-05

Dr. Benito C. Tan 1946 to 2016 — The pillar and legacy of bryology in Asia

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The symposium on Asian and Chinese Bryology would be incomplete without a tribute to Dr. Benito C. Tan who has sadly passed away on 23rd December 2016. The research focus of Benito was in the taxonomy, systematics, and evolution of Asian mosses, as well as in the conservation and biogeography of the bryophyte Floras in Asia. He was undoubtedly the foremost expert on Asian mosses, specialising in one of the most difficult moss families, the Sematophyllaceae. His 40-year long career in Bryology has resulted in over 300 publications and significantly improve the knowledge of Asian bryophyte Floras. In 2004, he had been awarded the Richard Spruce Award by the International Association of Bryologists, for his outstanding research contribution in Bryology. A joyful and generous man, he has trained and guided numerous students into the study of bryophytes in Asia.

T2-27-06

Moss Flora of Russia project

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Recent studies considerably expanded knowledge of the moss flora of Russia, with especially great additions to the flora of its Asian part. A number of families, such as Pseudoditrichaceae and Andreaeobryaceae, previosly considered as endemic of North America were found in Siberia. More southern Erpodiaceae and Ortodontiaceae were discovered in Asian Russia, as well as Oedipodiaceae, a family with hyperoceanic distribution. In the last 25 years a number of moss species known in Russia raised from 1050 to 1280, and new findingds continue. This addition in fact is not 230, but over 300 species, as more that 70 species were excluded as erroneous or non-confirmed records in the course of revisions. In average 10 newly recorded species per year and two species new for science per year were being discovered in Russia during last twenty-five years. In 2017 the first out of six volumes of the Moss Flora of Russia was printed, while other volumes are planned to be accomplished in the next five to ten years. The flora comprises taxonomic revisions based on molecular and morphological data. Among others, it turned out that some species which were considered as circum-Holarctic, e.g., *Rhynchostegium riparioides, Schistidium apocarpus, Hedwigia ciliata, Polytrichum formosum, Brachythecium glareosum* are absent in Asian Russia, and all their previous records in Russia east of Urals belong to other species. At the same time, recently described from North America *Coscinodon yukonensis, Pylaisia steerei* and *Sphagnum alaskanum* appeared to be widespread in Asia; *Ditrichopsis clausii* described from Yunnan was found in Transbaicalia, and *Struckia enevis* described from Sichuan appeared to be widespread in Siberia, reaching eastern Yakitia, the coldest area in the Northern Hemisphere. Further taxonomic revisions, with the special attention to cross-comparison of East Asian, Siberian and North American specimens may reveal more similarity between these areas than it was previously thought.

T2-27-07

Taxonomy and phylogeny of *Leptolejeunea* (Spruce) Schiffn.

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Leptolejeunea is a pantropical, epiphyllous leafy liverwort genus of Lejeuneaceae, with approximately 50 extant species. Due to their small and easily degraded ocelli, it is hard to distinguish species of *Leptolejeunea* from one another. This genus has not been revised around the world until now. The definitions of species, subgenus and even the circumscription of genera are still unclear. Previous taxonomic schemes for the *Leptolejeunea* were re-evaluated in the light of more representative species derived from a Bayesian, maximum-likelihood and parsimony reconstructions with two plastid regions (*rbc*L, *trn*L-F) and nuclear ribosomal ITS region. The reconstruction revealed that *Leptolejeunea* as currently defined was not monophyletic, with *L. spinistipula* belonging to another genus. Some previous taxonomic proposals for subgenera of *Leptolejeunea* that do not conform to the strongly supported monophyletic groups here were recovered and four subgenera were proposed with two new subgenera *Neoleptolejeunea* and *Subasticta*. The consequence of molecular dating and ancestral area reconstructing indicate that *Leptolejeunea* may go through extinction events during the initial evolution period, which leads to two ancestral areas. Three molecular markers (chloroplast *trn*L-*trn*F and *trn*G, nuclear ITS region) were used to explore the circumscription of some ill-defined species. The accessions of the widespread species *L. elliptica* is discovered to be not a monophyletic group but nested with the species from the same origin regions respectively. Several new species discovered in Southeast Asia suggests more exploration and investigation needed in other tropics areas.

T2-27-08

Generic delimitation and biogeography of *Meteorium* and *Papillaria*, focusing on Asian species

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The genera *Meteorium* Dozy & Molk. and *Papillaria* Lorentz comprise two major clades within the family Meteoriaceae with a great number of species occurring mainly in the humid tropical and warm temperate forests. The identity of these two genera has gone through several changes during the last twenty years, especially when the traditional key characters to separate the two genera have been questioned. For example, Buck (1994) synonymized *Papillaria* with *Meteorium* with the argument that the number of papillae over the lumen of each laminal cell is an unstable character depending on the environment. Huttunen *et al.,* (2008) and Quandt *et al.,* (2004) favored recognizing the two genera using the structure of axillary hairs as a distinguishing morphological character. The generic delimitation by the latter authors is well-supported by molecular phylogenetic analysis but required some complex nomenclatural changes. However, the tangled taxonomical classification of *Meteorium* species still exists and species delimitation of these two genera remains unclear and needs further examinations, especially for South East Asian taxon. Within the genera taxonomy is hampered especially by plasticity of characters such as leaf shape and leaf laminal cell papillosity. This study aims to reconstruct a molecular phylogeny, to discuss the evolution of characters, to investigate morphological synapomorphies, and to refine the species delimitation of *Papillaria* and *Meteorium*, focusing on tropical Asian species. The biogeography of the two genera will also be reviewed.

T2-27-09

A taxonomic study of family bryaceae *s. l.* (Musci) in China

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The Bryaceae (*s. l.*) is one of the important moss groups with many species characterized by diverse sporophytic and gametophytic morphologies. However, the taxonomic system is confused, genera and species in it are considerable difficult to identifying with a global distribution. Recent researches based on molecular data have greatly changed understanding of relationships in the Bryaceae (*s. l.*). Based on observation in the field and specimen study at the laboratory, 85 morphological characters, including 55 gametophytic characters, 26 sporophytic characters and four habitat conditions, were used for cladistics analysis. Four chloroplast DNA sequences, *rps4*, *atpB-rbcL*, *trnL-trnF* and *trnG*, from 46 species, 65 specimen of Bryaceae (*s. l.*) and its related groups were studied. Combining morphological and molecular data in the analyses showed that the traditional Bryaceae should be divided into four families. Members of re-circumscribe Bryaceae include five genera, *Anomobryum*, *Brachymenium*, *Bryum*, *Plagiobryum* and *Rhodobryum* in China, total 77 species and two varieties. The genera *Orthodontium* and *Leptobryum* were respectively transferred from Bryaceae (*s. l.*) to family Orthodontiaceae and Meesiaceae. Although the molecular analysis showed that the group with the central genus *Pohlia* has more close relationships with genera of the Mniaceae than the Bryaceae, genera of Mniaceae differ from *Pohlia* in both gametophytic and sporophytic morphology. So we adopted views that the Mielichhoferiaceae was circumscribed as a single family. There are five genera, *Mielichhoferia*, *Pohli*a, *Pseudopohlia*, *Epipterygium* and *Synthetodontium*, in the Mielichhoferiaceae in China, total 36 species and two varieties. *Synthetodontium kunlunense* J. C. Zhao et Y. Y. Liu and *Pohlia mamtiminii* Y. Y. Liu & J. C. Zhao were named as new species. A new combination, *Synthetodontium gossypinum* (X. J. Li & M. Zang) J. C. Zhao & Y. L. Niu, was proposed. *Bryum blandum* ssp *handelii* (Broth.) Ochi was treated as *Bryum handelii* Broth. *Bryum petelotii* Thér. & Henr. and *Bryum yuennanense* Broth. were treated as independent species of *Bryum*. Six species of sensu lato Bryaceae were reported for the first time from China, e.g., *Bryum weigelii* Spreng. The genus *Synthetodontium* Card. was newly recorded in China. *Pohlia marchica* Osterw. and *Anomobryum concinnatum* (Spruce) Lindb. were reinstated at species level. *Pohlia minor* Schleicher ex Scheaegr. was reduced as *Pohlia elongata* var. *greenii* (Brid.) Shaw. Three propaguliferous Pohlious species, *Pohlia flexuosa* Harv., *Pohlia leucostoma* (Bosch & Sande Lac.) M. Fleisch. and *Pohlia proligera* (Lindb. ex Breidl.) Lindb. ex Arn. were correct identified in China. Determining how climate change may impact multiple ecosystem levels and lead to various species adaptations is necessary for both biodiversity conservation and sustainable biological resource utilization. We employed Maxent to project the potential distributions of species of Bryaceae (*s. l.*) under current and future climate change scenarios and determined the dominant environmental variables that affect changes in the distribution.

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T2-27-10

Molecular phylogeny and subfamilial classification of the family Pottiaceae (Bryophyta)

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The family *Pottiaceae* is the most generic and species rich family of mosses (*Bryophyta*), with around 1,400 species in 83 genera, comprising more than 10% of the extant moss species (Frey & Stech 2009). Widely distributed in the world, its species adapt to a wide range of habitat types including xeric, mesic and hydric, grow on various substrata including saxicolous, tericolous and corticolous, and possess a variety of life strategies including perennial, annual and ephemeral. The family exhibits a great variety of apparent morphological, physiological and genecological adaptations to their particular environments (Zander 1993). Among the various types of habitat where they are found, most species exhibit a great tolerance of hot and dry environments. Adaptation to such harsh selective pressures often leads to the presence of parallel or convergent characters which develop in response to the same environmental stimuli. In addition the difficulty in effecting fertilization or allowing for the maturation of sporophytes under xeric conditions leads to the lack of mature sporophytes which provide taxonomically important characters in mosses. These environmental features have complicated the phyletic assessment and classification of the family based on morphological criteria. The species concepts are often not well understood, and the family has been variously classified without an understanding of its phylogenetic relationships, leaving many ambiguous or poorly understood taxa unresolved (Satio 1975). A source of independent taxonomic criterion is therefore needed for the sound classification of this family, and during the past 20 years a number of molecular phylogenetic analyses have been conducted in an attempt to resolve relationships within the family. From the early 19th century, variously attempts at a subfamilial classification of the family have been proposed based on morphological criteria, while the limited number of morpho-molecular systematics have been proposed (Werner *et al.,* 2004, Zander 2006, Stech & Frey 2009). In this talk we review the current state of knowledge on phylogenetic relationships and classification at subfamilial level within the *Pottiaceae*, and reassess the subfamilial relationships within the family based on molecular phylogenetic inference using concatenated sequences of chloroplast *rbcL* and *rps4* genes, with codon substitution model which is a statistically higher precision model than nucleotide and amino acid substitution models for the evolutionary analysis of protein-coding sequences. The supposed ingroup species represent all the subfamilies of *Pottiaceae* recognized by Werner *et al.,* (2004): *Merceyoideae*, *Pottioideae* and *Trichostomoideae*. We also include the genus *Streblotrichum* whose phylogenetic position has remained ambiguous (Kucera *et al.,* 2013). The resultant tree confirms the monophyly of the *Pottiaceae* with four major clades within the family, corresponding to *Merceyoideae*, *Pottioideae*, *Trichostomoideae*, and the newly proposed *Streblotrichoideae*. Merceyoideae is resolved as the most basal clade within the family. *Pottioideae* comprise the sister-group to *Trichostomoideae* and this clade is sister to *Streblotrichoideae*. Based on the tree we recognize four subfamilies in *Pottiaceae*.

T2-27-11

Regeneration and vegetative proliferation of peat moss (*Sphagnum squarrosum*)

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Sphagnum (peat moss) are the major components of peat lands which are very important ecosystems for world water conservation and air carbon uptake. They has great financial potential for their utilization as pre-prepared 'growing media' that allow uniform high-quality plants to be grown at very high productivity levels. Sphagnum have unique morphological and developmental characteristics also. During their vegetative development process, there were 4 different tissues including filamental and unistratose thallose protonema, stem and mature leaf with the slender chlorophyllose cells and diamond shape hyaline cells. In Sphagnum, there are one-dimensional filament protonema, two-dimensional plate protonema, three-dimension leafy shoot and strong differentiated leaf cells. Besides the importance on earth ecologic system and potency on horticulture and other applications, Sphagnum are good material for plant cell develop and differentiation study. Sphagnum gametophore tissue culture system have been set up already while there is no report about stable protonema culture yet. The detailed developmental process of peat mosses vegetative growth were still unclear.Through continuously observation, we summed up the regeneration principle of Sphagnum gametophore fragments. The thallose protonema is the most interesting structure of Sphagnum. How does it look like? How does it developed from linear protonema? How does gametophore out growth? How phytohormones regulate Sphagnum development? They are the major questions that we endeavored to find answers. There are multidirectional cell development and regeneration relationship among the 4 vegetative tissues in Sphagnum vegetative tissues. A relative stable peat moss protonema culture method was set up based on that.

T2-27-12

Stress imprint of two moss species

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Plants are often exposed to stress environments, which generally induce morphological, physiological or genetic modifications in the plants. And, these modifications always improve the resistance of the plants to future stresses. This feature is described as 'stress memory' or 'stress imprint'. However, the evidences of moss's stress imprint are still lack. We studied the responses of two moss species widely distributed in South China, *Hypnum plumaeforme* Wils. and *Pogonatum cirratum* Bird. subsp. *fuscatum (*Mitt.) Hyv?nen, to high N, alone or combined with drought or low temperature stress, as well as their responses after a short term recovery or after a recurring stress. We found that after a 10-day recovery, oxidative stress induced by N application, drought or low temperature was significant decreased in both species, but it was still higher than non-stressed controls. At the same time, SOD activity and ABA content in the mosses after a short-term recovery were also higher than non-stressed controls, and we speculated that this might enable the mosses to better resist the future stress. To verify our speculation, we exposed mosses to recurring stress or cross stress of drought and low temperature with the time intervals of 7 days, 45 days and one year respectively. Our results showed that single drought or low temperature stress caused an increase in MDA content and inhibited the activity of antioxidant enzymes, however the level of MDA in *Hypnum plu-*

maeforme subjected to recurring stress or cross stress after a 7-day interval, was lower than corresponding single-stressed plants. The activity of SOD and CAT in *Hypnum plumaeforme*, exposed to recurring stress or cross stress were higher than corresponding single-stressed plants, showing the evidence of 'stress imprint'. However, those in *Hypnum plumaeforme* showed such rules only for recurring low temperature stress. If the second stress happened after a time interval of 45 days, MDA content in the recurring low temperature stressed or drought-low temperature stress treated samples was still lower than those of single low-temperature stressed samples, but for the samples subjected to recurring drought stress or low temperature-drought stress, no significant differences were found comparing with the single drought stress samples. The activity of SOD in *Hypnum plumaeforme* exposed to recurring or cross stress was still higher than corresponding single-stress plants, but this did not occur for CAT, suggesting that imprint of the first stress weakened gradually. Our results also indicated that after a time interval of one year, the memory of the first stress in the mosses disappeared. In summary, our results provide the first evidence for moss's stress imprint, confirming our speculation that mosses can 'remember' the stress and show higher resistance when subjected to recurring or cross stresses. However, this 'memory' is time limited.

T2-28: Evolutionary genomics and adaptation of *Arabidopsis* relatives

T2-28-01

What forces maintain genetic variation for large-effect trans eQTLs?

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The evolutionary forces that maintain genetic variation in quantitative traits within populations remain poorly understood. Specifically, how much of the variation present within populations is deleterious? Does balancing selection make an important contribution to within-population variation? We use gene expression as a model trait to address these questions by mapping the genetic basis of leaf gene expression ('eQTLs) within a single population of the outcrossing plant, Capsella grandiflora. First, we map local eQTLs and show that alleles at these loci are rarer than expected and exhibit a negative correlation between phenotypic effect size and frequency, consistent with the action of purifying selection. Second, we identify a number of eQTLs affecting large groups of coexpressed genes in trans and investigate the role of balancing selection in maintaining variation for these loci and the effect of these QTLs on traits. In total, our findings illuminate the role that both purifying and balancing selection play in shaping genetic variation for phenotype within populations.

T2-28-02

Population genomics of transposable elements in *Capsella*

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Transposable elements (TEs) comprise a significant proportion of eukaryotic genomes, but their importance as contributors to standing genetic variation in natural plant populations remains unclear. Given the action of host silencing mechanisms and the ability of TEs to move regulatory sequences around the genome, there is strong potential for TEs to have major contributions to gene expression variation. Here, we examined the population genomic variation for transposable elements and their contribution to gene expression variation in a large population of the outcrossing plant *Capsella grandiflora*. Most TE insertions were found at low population frequencies, particularly near highly expressed genes, consitent with the action of strong purifying selection. We observe a genome-wide pattern suggesting that rare TE insertions tend to drive gene expression towards extreme values, consistent with their widespread importance in maintaining natural variation in gene expression. We discuss the relative contribution of TEs and single nucleotide polymorphism in driving variation and evolution of gene expression.

T2-28-03

Evolutionary divergence between populations of *Arabidopsis lyrata* and its consequences for reproductive isolation

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Arabidopsis lyrata consists of disjunct populations belonging to two subspecies, ssp. *petraea* in Eurasia and ssp. *lyrata* in North America, which have diverged 180 KYA based on whole genome data analysis. The Central European populations have diverged about 135 KYA from those currently found in Scandinavia, after post-glacial colonization. A population in eastern Karelia (western Russia) likely is derived from an independent post-glacial colonization, as it is highly differentiated from other European populations. During the migration and colonization, populations have experienced bottlenecks, become locally adapted to their current climatic conditions, and diverged phenotypically in many traits. The divergence has also resulted in incipient reproductive isolation, found earlier as cytoplasmic male sterility in hybrids between populations of North America and Europe. More recent studies have also demonstrated that between Russian and North American populations there exists a low level of incompatibility at the seed stage, which is associated with high levels of transmission ratio distortion in F2 populations. The consequences of the various individual incipient barriers were also examined in an artificial hybrid zone comprising Russian and Swedish populations. We analyzed both the mating patterns and the fate of chromosomal segments in this experiment.

T2-28-04

Long-term balancing selection contributes to the adaptation of *Arabidopsis* relatives

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Balancing selection maintains genetic variations within a population, which plays important roles in the adaptation of organisms. What genes, genome-wide, are under balancing selection and to what extent these genes are involved in adaptation are largely unknown. To address these questions, we performed a genome-wide scan of shared polymorphisms, and then finally, trans-species polymorphisms - a robust signature of balancing selection, across two plant species, the model plant *Arabidopsis thaliana* and its close relative species *Capsella rubella*, which diverged about 8 million generations ago. Interestingly, we identified hundreds of genes with candidate sites, from which five of them, were confirmed to be under long-term balancing selection, involved in fundamental biochemical function or response to biotic or abiotic stress. Particularly, each of the five genes exhibited significant ecological diversification between its two haplotype groups, suggesting balancing selection must have been very important to the adaptation during a long time span. Our results highlight that in plants, beyond S-locus genes and resistance genes, other genes related with resistance to stress and fundamental functions are under balancing selection, which must have contributed to the adaption to diverse habitats.

T2-28-05

Gene expression and sequence variation in the genus *Boechera*: Implications for the evolution and maintenance of apomixis

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The genus *Boechera* arose through extensive and complex hybridization events that occurred multiple times among many diploid species in the genus, with current species being allopolyploid as well as diploid hybrids. Hybridization has been proposed to be the basis for gametophytic apomixis (diplospory), which is widespread in the genus. Apomictic *Boechera* species appear to be the only well-documented example of diploid apomixis in angiosperms, making the study of its genome evolution and transcriptional landscape of high relevance for the identification of genes relevant to the engineering of apomixis in crops, which would allow the genetic fixation of complex traits, including heterosis. Genomic differences between apomictic and sexual species have been reported with apomictic individuals being highly heterozygous, whereas sexual diploids having low levels of heterozygosity and high inbreeding. Furthermore, several genetic and cytological studies point to a particular genomic region of low recombination, and therefore high heterozygosity to be the diplospory-determining region in *Boechera*. We performed laser-capture microdissection of egg cells coupled with RNAseq and analyzed patterns of gene expression and sequence variation. We will present the results of our transcriptomic analysis of four sexual *Boechera* species and will compare them to four apomictic *Boechera* species. We will describe the enriched gene ontology terms and discuss their relevance in the establishment and maintenance of parthenogenesis. We have used the transcriptomic data to inquire the nucleotide variation between apomict and sexual species using SNPs from thousands of genes. This rich data set allow us to perform a comparative genome scan that describes the differences in nucleotide diversity between species of different reproductive modes, particularly validating the long standing observation that apomictic species have higher levels of heterozygozity and aiding in the delimitation the apomixis determining region. Moreover we have performed molecular evolution analyses and detected signatures of selection in the *Boechera* genome and will discuss the implications of this selective evolutionary force in shaping the maintenance of the different reproductive modes and hybrid networks in the genus.

T2-28-06

Population genomics of heavy metal adaptation of *Noccaea caerulescens*

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Noccaea caerulescens (Brassicaceae) is one of the rare metal hyperaccumulator plant species that evolved adaptation to multiple heavy metals, i.e. exposure to high levels of Zn, Ni, Cd or Pb. There are several accessions of this species, each with different metal accumulation and tolerance characteristics. All accessions hyperaccumulate Zn, preferentially in their leaves, and sometimes Ni, Cd or Pb. The species is widely distributed over North, West and Central Europe, though often found in separate local populations, especially the metallicolous ones. We use *N. caerulescens* as a model to study the evolution of plant adaptation to extreme environments. An inbred line of accession ‘Ganges’, originating from the south of France (Les Avinières, St.-Laurent-le-Minier), has been used for whole genome sequence analysis. This accession is extremely tolerant to high Zn and Cd exposure and hyperaccumulates these metals in its leaves. The whole genome sequence was assembled based on Illumina Hi-Seq and PacBio generated sequences. This assembly comprises around 30,000 predicted, annotated gene models, suggesting nearly all of the genic regions to be included in the assembly. When compared to *Eutrema salsugineum*, its closest relative of which a whole genome sequence is available, some 1000-1500 genes are found in more copies in N. caerulescens than in *E. salsugineum*. Many of these multiplied gene copies were previously found to be higher expressed in *N. caerulescens* than in related species, including several orthologues of genes known to be involved in mineral homeostasis of *Arabidopsis thaliana*. Quite a few of these genes show copy number variation (CNV) when comparing different metallicolous and non-metallicolous populations of *N. caerulescens*. Thus, CNV appears to have played an important role in the adaptation of *N. caerulescens* to heavy metals. Our progress in understanding the functional importance of this CNV will be discussed. Next to CNV, population genomics has revealed some signatures of selection associated with adaptation to metallicolous conditions. The function of the genes involved will be discussed.

T2-29: Apocynaceae: phylogeny, biogeography and diversity

T2-29-01

Phylogenomics of Apocynaceae: From tools to trees

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The advent of next generation sequencing and methods for targeted sequencing of nuclear genomes have created the opportunity for development of tools for phylogenomics in Apocynaceae. Sequencing of the genome and transcriptome of the common milkweed (*Asclepias syriaca*) have provided the basis for design of probes to specifically target putatively single-copy nuclear genes, which is combined with genome skimming for assembly of high copy targets, such as the plastome, in the Hyb-Seq approach. These tools were utilized to understand the evolutionary history of North American milkweeds (*Asclepias*), a recent rapid radiation of ca. 115 species that has been difficult to investigate using traditional molecular systematics approaches due to low levels of DNA sequence variation and problematic levels of incomplete lineage sorting. Phylogenetic relationships in *Asclepias* were estimated using DNA sequence data from ca. 768 nuclear genes and whole plastomes. The relationships of major clades in the genus are supported by both analyses, but produce conflicting results concerning species relationships within each clade. Nuclear loci exhibit low levels of phylogenetic signal and what signal exists is highly discordant among loci, providing challenges to accurately estimating the species tree with confidence. Building upon the *Asclepias* work, Hyb-seq tools were developed for wider use in the study of evolution of Apocynaceae. Genome skimming has produced the data to infer a plastome phylogeny of the family with sampling to the subtribal level and facilitated estimation of divergence times of major clades. Probes for targeted sequencing of ca. 800 single-copy nuclear genes that work across the family were also developed and have allowed a fresh look at both phylogeny and character evolution.

T2-29-02

The rapid radiation of Asclepiadeae (Apocynaceae) in Africa with special reference to the subtribe Asclepiadinae

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The family Apocynaceae consists of approximately ± 366 genera with at least 5 745 accepted names. Since the last XVIII IBC meeting in Melbourne increased resolved clade structure has lowered the number of genera slightly, a tendency that seems to be on-going. On the African continent there are some 1,679 taxa (including 13 naturalized taxa) and 174 genera (including 10 naturalized genera). With almost a third of the known species in the family found on the African continent, it is well-represented including members of all the subfamilies: Apocynoideae (± 29% of the genera); Rauvolfioideae (± 47% of the genera); Periplocoideae (± 69% of the genera); Secamonoideae (± 13% of the genera); and Asclepiadoideae (± 57% of the genera). Globally the Asclepiadoideae has three main centres of diversity: a Tropical Asian centre (± 38 genera); a tropical Central and South America centre (± 47 genera); and the Eastern and Southern Africa centre that is the most diverse (± 93 genera). Due to the recent radiation of the Asclepiadoideae, members are well-represented in Africa with ± 1,266 taxa mainly within the two large tribes: Asclepiadeae (± 36% of the taxa) and Ceropegieae (± 59% of the taxa). In an analysis of African members of the Asclepiadeae the largest six genera are: *Asclepias* (± 80 taxa); *Aspidoglossum* (± 39 taxa); *Cynanchum* (± 49 taxa); *Pachycarpus* (± 41 taxa); *Schizoglossum* (± 45 taxa) and *Xysmalobium* (± 44 taxa). These genera account for approximately 65% of the taxa and 71% of the genera in the tribe has less than 10 species. In this presentation an overview of the genera and species in the Asclepiadeae in Africa with specific focus to the centre of diversity in eastern and southern Africa, emphasis on the subtribe Asclepiadinae, and a synthesis of the genera and species of Africa will be presented. The presentation will also highlight some of the outcomes of the revision based on the morphological study and phylogenetic relationships of a selection of genera of southern African members of the subtribe Asclepiadinae. As a specific example, reference to members of the *Schizoglossum*-complex in the subtribe Asclepiadinae will be discussed.

T2-29-03

Pollination of *Ceropegia* pitfall flowers: Chemical mimicry and pollinator specificity

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Ceropegia L. (Apocynaceae, Asclepiadoideae) comprises more than 200 species that occur mainly in subtropical and tropical habitats of the Old World. Species in this genus are characterized by complex and non-rewarding deceptive pitfall flowers. Pollination is mediated by flies of different families, such as Ceratopogonidae, Chloropidae, Drosophilidae, Milichiidae, and Sciaridae. It has been suggested that floral scents mimic food sources, sex pheromones or oviposition sites of the flies and are key players leading to a highly functional specialization in terms of pollinator specificity. We identified flower visiting/pollinating flies of 14 *Ceropegia* species and analyzed their floral scent composition using dynamic headspace and gas chromatography coupled to mass spectrometry (GC/MS). We further tested for relationships between floral scent and visitor/pollinator patterns. Based on genetic similarities of the plants, we also checked for phylogenetic signals in scent chemistry and pollinator spectra. Gas chromatography coupled to electroantennographic detection (GC/EAD) and behavioral studies were applied to identify scent components responsible for pollinator attraction. We found that most *Ceropegia* species are associated with only one or two pollinating fly families or genera with strong differences in pollinator spectra among species. Floral scent chemistry was highly variable among species but did not correlate with flower visitor/pollinator patterns. We did not find phylogenetic signal in overall scent chemistry but in fly pollinator assemblages. Although scent patterns did not correlate with pollinator spectra, the highly specific pollination systems in *Ceropegia* still can be explained by floral scent chemistry and the resultant mimicry strategies. This is supported by more detailed chemical ecological studies on *C. sandersonii* for which we revealed the model mimicked by the flowers, i.e. western honey bees under attack.

T2-29-04

Distribution patterns in large Asclepiadoid genera

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Asclepiadeae, possessing pendent pollinia, constitute the largest tribe of subfamily Asclepiadoideae (Apocynaceae). From an African origin, an early split separates an exclusively New World lineage from a lineage centered in the Old World. The latter consists of three subtribes, Asclepiadinae, Cynanchinae and Tylophorinae, each of which comprising one large genus and few small relict genera. For all three subtribes, reconstruction suggests an East African origin. In Asclepiadinae, a New World and a southern African radiation contribute most to species diversity. In the New World, a niche shift to temperate climates, followed by a speciose radiation, has taken place. In Cynanchinae, the most speciose radiation is centered in Madagascar, and the New World has been reached only late in the history of the subtribe. Only single species extend beyond tropical and subtropical zones. Tylophorinae has centers of diversity in Africa and Asia, it is naturally absent from the New World. It comprises two lineages that have undergone a shift to temperate climates and subsequently radiated. One of these lineages comprises members that are neophytic to the New World and behave as noxious weeds in suitable niches. The phylogenetic history and possible adaptations enabling niche shifts will be discussed.

T2-29-05

The Apocynaceae, subfamilies Rauvolfioideae and Apocynoideae, in Cambodia, Laos and Vietnam

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In Cambodia, Laos and Vietnam there are 119 known species in 38 genera of Apocynaceae, subfamilies Rauvolfioideae and Apocynoideae (or the Rauvolfioid and Apocynoid informal groups). The diversity in Vietnam is much higher than in Cambodia and Laos which is a reflection of both the greater diversity of habitats in Vietnam but also the very much lower collection densities in Cambodia and Laos. The Rauvolfioids and Apocynoids show similar patterns of diversity in the Cambodia, Laos and Vietnam region compared to neighbouring Thailand. Based on their distributions in Thailand, and habitat availability across Southeast Asia, there are only very few species known from Thailand that would be expected to be found Cambodia, Laos and Vietnam that have not yet been found there, despite the lower collection densities. There are 17 known endemic species in Cambodia, Laos and Vietnam of which 16 are in Vietnam and one in Laos. There are, however, only two known endemic species of *Alstonia* and no known endemic species of *Wrightia* even though in neighbouring Thailand there are several narrowly endemic species of each genus in limestone habitats that are also present in Cambodia, Laos and Vietnam. New species of both of these genera, and possibly also of *Kopsia*, are expected with better exploration in the region. There is one endemic genus in the FCLV region, *Ixodonerium*. This genus has, unfortunately, never been investigated using molecular sequencing techniques. It shows affinities to *Anodendron* but does occur in a region of Vietnam known for its distinct plant diversity and high level of generic endemism in a range of families. Seven species of Rauvolfioid and Apocynoid Apocynaceae have been assessed under IUCN criteria as being threatened but many species are assessed as data deficient as no collections have been made for decades.

T2-29-06

Diversity of *Ceropegia* L. (Ceropegieae: Apocynaceae) in India

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Ceropegia L. (*Ceropegieae*: Apocynaceae) with about 244 species is widely distributed in the Old World ranging from the Spanish Canary Islands in the west, through central, southern, and northern Africa, Madagascar, Arabia, India, South-East Asia to northern Australia in the east. The genus is characterized by tuberous, fleshy or fibrous roots, solitary flower or flowers in cymose inflorescences, tubular corolla, uniseriate (*Ceropegia santapaui*) or biseriate corona, pair of follicles and comose seeds. Presently, in India *Ceropegia* is represented by 60 taxa (57 species, two varieties and one forma) of which 50 species are distributed in the peninsular India, nine in North-east India and one in Andaman Islands. Ceropegias are also known for endemism and 44 species are endemic to the country. Northern Western Ghats has 17 endemic species while Southern Western Ghats has only eight. Most of them are narrow endemics. Out of 60 taxa, nine are known only from type localities. Northern Western Ghats seem to be important region of diversification for the Ceropegias in India and probably most of the species are of recent origin. There are 24 non-tuberous species while 36 are with tuberous rootstock. Non-tuberous species are confined to tropical high rainfall areas with evergreen to semi-evergreen forests while species adapted to semi-dry or dry regions are always tuberous, a survival strategy during dry period of a year. 20 taxa are erect while 40 are twiners. *Ceropegia fimbriifera* Bedd., C. *mahabalei*, *C. nampyana*, *C. noorjahaniae* and *C. spiralis* become twiners under captivity. *Ceropegia* species show considerable variations in vegetative as well as floral characters especially with reference to colour, shape and size. *Ceropegia juncea* and *C. bulbosa* show Crassulacean Acid Meetabolism (CAM) pathway. Propagation and cultivation of Ceropegias is herculean task; however *Ceropegia candelabrum, C. elegans*, *C. gardneri*, *C. intermedia*, *C. juncea and C. maculata* can be propagated through stem cuttings. In the present study chromosome numbers for nine species are reported. All species show 2*n* = 22. New cytotype (2*n*=22) for *C. elegans* has been reported. *Ceropegia concanensis, C. ravikumariana* and *C. nampyana* are the new species described during the study. *Ceropegia gardneri* was earlier reported from Sri Lanka which has been now collected from Karnataka (India). In present study lectotypes for 17 binomials have been designated based on original material. Preliminary studies have revealed that the Indian species of *Brachystelma* nest in Indian clade of *Ceropegia* and African *Brachystelma* into clade of African Ceropegias. Even morphologically *Brachystelma* is very close to Ceropegias but differs only in its corolla divided almost upto the base. Taxonomically, inclusion of *Brachystelma* (ca. 120 species) in *Ceropegia* (240+ species) would solve part of the problem; however, the morphological concept of *Ceropegia* would be demolished, and the many name changes would be unpleasant. In future there is every possibility of merging *Brachystelma* into *Ceropegia*. Present revisionary study concludes that the further explorations in Indian subcontinent may result into novelties in Ceropegias.

T2-30: Evolution and development of the flower: from genes to development

T2-30-01

The underlying mechanisms of pollen wall development in *Larix decidua* and *Polemonium caeruleum*. experimental modeling of exine

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The sporopollenin-containing part of the pollen wall - the exine - is one of the most complex cell wall in plants. By tracing each stage of *Larix decidua* (Pinaceae) and *Polemonium caeruleum* (Polemoniaceae) microspore development at a high level of TEM resolution, we aim to understand the establishment of pollen wall in connection with organelle activities and to unravel the suggested self-assembly underlying mechanisms. In *Larix*, the sequence of events observed in the microspore periplasmic space, leading to exine emergence is as follows: the appearance in the periplasmic space of spherical units; their accumulation and arrangement to radial columns; initial sporopollenin accumulation on spherical units and their columns; the appearance of the primordial endexine lamella; multiplication of the white-lined endexine lamellae; the appearance of granulate ectexine after callose dissolution and final sporopollenin accumulation; the formation of intine. In *Polemonium*, in spite of different kind of the exine structure, the main steps of exine ontogeny are the same: spherical units, gradually transforming into columns, then to rod-like units (procolumellae); the appearance of the initial tectum; the appearance of the endexine lamellae; the appearance of the intine in aperture sites. The distinctions in *Polemonium* sequence is the emergence of a sponge-like layer and the foot layer on the base of it late in the microspore development. The sequences of the developmental events in both species fit well to the sequence of self-assembling micellar mesophases in colloidal solution with increasing concentration of surfactants: spherical micelles; columns of spherical micelles, in places rearranged to cylindrical micelles; laminate micelles, separated by stratum of water and seen with TEM as white-lined lamellae. The same fitness was observed in our ontogenetic previous studies on other species. A number of patterns were obtained in our modeling experiments in colloidal solutions in vitro conditions, when the influence of the genome is absent, in the course of condensation (a kind of self-assembly process), simulating structures at several stages of exine development in *Larix*. Therefore, pure physico-chemical processes as self-assembly, which are not under direct genetic control, play an important role in exine development and share control with the genome. It is highly probable that self-assembly is an intrinsic instrument of evolution.

T2-30-02

Perianth structure across Eudicotyledoneae and its relationship with diversification

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Flowering plants display an extraordinary diversity of floral structural configuration and morphological variation. Structural stabilization in the flower often allows architectural elaboration associated with specialized reproductive syndromes, including complex associations with pollinators. The eudicot clade (Eudicotyledoneae) contains over 70% of angiosperm species. It consists of an early-diverging grade of mostly depauperate lineages and a very large clade, Pentapetalae, which contains the vast majority of the species of the group. The early-diverging eudicot lineages encompass a wide variety of floral structural configurations, some of which lack a fixed floral pattern, and have little or no differentiation between perianth cycles. Within Pentapetalae the most common floral groundplan is characterized by (4- or) 5-parted flowers and well differentiated calyx and corolla. This basic Pentapetalae floral pattern has been greatly elaborated along many different lineages to constitute complex floral architectural configurations. However, it is not clear if this structurally stable Pentapetalae floral groundplan evolved one or more times within the clade; in which branch (es) did it originate; and if there is correlation between the calyx and corolla and the meristic pattern organization. In this study, we investigate the distribution of the bipartite perianth and meristic pattern across eudicots, and evaluate its relationship with increased rate of species diversification. We apply an exemplar approach on a 622 terminal dated phylogeny representing 295 eudicot families to document the distribution of three floral structural characters. Floral data was obtained from different electronic resources and combining direct observations of fresh and herbarium flowers. The evolutionary history and ancestral morphological characters were estimated with maximum likelihood using Mk model in BayesTraits. Subsequently, we conducted state-dependent diversification analyses to evaluate the potential link between these morphological attributes and the rate of diversification using the Hidden-State Speciation and Extinction (HiSSE) model. HiSSE also takes into account the possibility of unmeasured (hidden) states that also affect the dynamics of diversification. Finally, we analyze the correlation between the presence of a bipartite perianth and meristic pentamerous pattern. We found that the evolution of floral pentamery is decoupled from perianth differentiation in eudicots. Pentamerous merosity may have originated on the stem lineage of Pentapetalae, or after the diversification of this clade in subsequent branches. Our results provide an estimation of the number and phylogenetic placement of shifts towards a bipartite perianth, and reconstructions of the ancestral perianth structure in major eudicot lineages, including eudicots as a whole. These results provide a framework for a more detailed evaluation of the floral evolution in the Eudicotyledoneae, in particular, and the correlation between the different structural characters.

T2-30-03

Evolution, development and genetic basis of floral symmetry in *Anacyclus* L. (Anthemideae, Asteraceae)

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Most of the diversity in the pseudanthia of Asteraceae is based on the differential symmetry and sexuality of its flowers. Several studies have been In *Anacyclus* (tribe Anthemideae), where there are (1) homogamous capitula, with bisexual, mainly actinomorphic and pentamerous flowers; and (2) heterogamous capitula, with peripheral zygomorphic, trimerous and long-/short-rayed female flowers, we investigated the floral ontogeny and the evolution and expression of *CYC2* to infer their origin. The floral development in the capitulum of *Anacyclus* found separate sequence for disc and ray-flowers. The disc flowers, subtended by paleae, initiate acropetally. Their perianth and androecium initiation is unidirectional and simultaneous, and a late zygomorphy occurs by enlargement of the adaxial perianth lobes. In contrast, ray flowers, subtended by involucral bracts, initiate after the proximal disc buds, breaking the inflorescence acropetal pattern. Early zygomorphy is manifested through the fusion of the lateral and abaxial perianth lobes and the arrest of the adaxials. We found and interpreted atypical phenotypes with peripheral 'trumpet' flowers from natural populations. The peripheral 'trumpet' buds initiate after disc flowers, but maintain an actinomorphic perianth. Once established de developmental sequence, we investigated the genetic basis of the morphological diversity by using a candidate genes approach. We know that homologs of the *CYC/TB1* gene family have been independently recruited many times across the eudicots to control aspects of floral symmetry. The family Asteraceae exhibits the largest known diversification in this gene paralog family which is accompanied by a parallel morphological floral richness in its specialized capitulum inflorescence. In Asteraceae, whether or not *CYC/TB1* gene floral symmetry function is preserved along different tribes and gene lineages was unknown. In this part of the study, we used phylogenetic, structural and expression analyses with an emphasis in the highly derived genus *Anacyclus* to address this question. Phylogenetic reconstruction recovered eight main gene lineages present in Asteraceae: two from *CYC1*, four from *CYC2* and two from *CYC3*-like genes. The species phylogeny was recovered in most of the gene lineages, allowing the delimitation of orthologous sets of *CYC/TB1* genes in Asteraceae. The gene expression study included RNA qPCR and RNA in-situ hybridization. The quantitative real-time PCR results found that in *Anacyclus* three of the four isolated *CYC2* genes are more highly expressed in ray flowers. The expression of the four *AcCYC2* genes overlaps in several organs including the ligule of ray flowers, as well as in anthers and ovules throughout development. We concluded that despite the high diversity observed in the capitulum morphology of Anthemideae, the set of genes associated to floral symmetry detected in other tribes has conserved its function.

T2-30-04

Evolution of Darwin's peloric gloxinia (*Sinningia speciosa*) under domestication: Single gene speciation by pleiotropy

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Unlike most crop domestication that occurred through long periods of unconscious selection by ancient humans, horticultural plants were mostly domesticated with intentional selection over short time periods. The origin and spread of novel traits in the domestication process has remained unexplored in horticultural plants. The gloxinia (*Sinningia speciosa*) with attractive brilliant *peloric* flowers that influenced the thinking of Darwin has been cultivated since the early 19th century, but its origin and genetic basis has been unknown. By employing multiple experimental approaches including artificial hybridization, genotype-phenotype association, gene expression and functional repression, we determined that a single gene *SsCYC* encoding TCP protein controls the floral shape transition under domestication in gloxinia. In addition to the significant expression in the dorsal region of the second and third floral organs, a novel expression domain of *SsCYC* was observed in the dorsal basal floral tube that pre-pattern the development of the gibbous structure that force the floral tube orientate horizontally. We revealed that the loss-of-function of SsCYC protein caused by a *de novo* 10-bp deletion was targeted by intensive cross-based artificial selection, which underlies the evolution of the *peloric* flowers in gloxinia. Based on literature review and genetic reference combined with the findings herein, we further traced the origin of modern *peloric* gloxinias to a single individual with the mutant allele that originated in domestication 200 years ago. This study provides the first clear example for the evolutionary dynamics of horticultural plants. We also find that the floral orientation is causally associated with floral symmetry, acting as a key combinatorial complex floral structure relating to pre-pollination isolation in angiosperms, which is controlled by a single pleiotropic TCP gene. Our results suggest that pleiotropic changes with selective advantage could promote coordinated evolution of the complex integrated floral organs with subsequent speciation and diversification.

T2-30-05

Floral evolution and new hypotheses on perianth function in Magnoliidae

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With about 10,000 species in four orders (Cannellales, Laurales, Magnoliales, Piperales), the Magnoliidae are the largest clade of angiosperms outside Monocotyledonae and Eucotyledonae. Their floral biology and diversity of floral architectures greatly differ from those of other lineages. The magnoliid perianth is highly variable and includes a broad range of patterns present in other angiosperm lineages. Furthermore, traits such as perianth phyllotaxis and whorl number are more labile than in other flowering plants lineages. In this study, we investigate the diversification of the magnoliid perianth using a newly generated data set of seven floral characters scored for 198 exemplar species. This data represent more than 75% of the existing genera in the group and match the most recently published dated phylogeny of Magnoliidae. Ancestral states were reconstructed using maximum parsimony, maximum likelihood, and Bayesian reversible-jump approaches exploring multiple models of morphological evolution. The perianth morphology of the most recent common ancestor of all extant Magnoliidae is reconstructed as having at least two trimerous whorls of tepals. This ancestral morphology subsequently evolved further via gains (e.g. Winteraceae, Annonaceae, Magnoliaceae) or losses (e.g. Myristicaceae) of whorls, increases in the number of organs per whorl (e.g. Atherospermataceae, Hernandiaceae), or transitions from whorled to spiral phyllotaxis of the perianth (e.g. Calycanthaceae, Gomortegaceae, Degeneriaceae). Based on our current knowledge of magnoliid flower biology and these new results, we discuss functional roles of the perianth in floral evolution. For instance, we hypothesize that spiral perianth phyllotaxis has evolved independently in several magnoliid lineages as a response to floral bud predation.

T2-30-06

Variation on a theme: evolutionary-developmental insights into the Asteraceae flower head

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Plant reproduction relies on flowers and their arrangement into an inflorescence –two interrelated traits that exhibit impressive variation between plant lineages and constrain crop yields and horticultural forms. A key aim in evolutionary biology is to unravel how the modification of genetic networks has led to the diversification of reproductive plant architecture and floral form. We address this aim by seeking to understand how two fundamental biological processes, floral architecture and symmetry, have evolved in the daisy family Asteraceae (Compositae), with unique combinations of these traits. Much of the research on Asteraceae has concentrated on model plant systems with simple heterogamous capitula (e.g. *gerbera*, *sunflower*). We propose an original and novel evolutionary-developmental (evo-devo) approach to floral evolution, by bringing to this field the species of Asteraceae with secondary heads (=syncephalia). This unique inflorescence architecture has arisen from two rounds of capitulescence (i.e. inflorescence condensation followed by simplification) during their evolution. Syncephalous species which comprise ca. 70 genera from 11 tribes provide an invaluable source of material for deciphering the genetic basis of capitulum evolution since their genomes bear the signature of two inflorescence shifts toward capitulescence. Our approach, integrating evo-devo and genomics, provides a unique opportunity to ask fundamental questions relating to coordinated trait evolution, and to increase our understanding of how genetic pathways have been altered or co-opted during the evolutionary diversification of flowers.

T2-31: Key questions on angiosperm macroevolution: where do we stand, and where are we heading now? (two sessions)

T2-31-01

Measuring how the probability of long-distance dispersal depends on geographic distance, environmental distance, and co-evolving seed dispersal traits: BioGeoBEARS on a mega-phylogeny of angiosperms

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Organism traits must be important in historical biogeography. In particular, rates of dispersal (both range-expansion dispersal, and jump dispersal leading to founder-event cladogenesis) must depend to some degree on traits such as seed traits and the vectors that transport seeds (e.g. animals, wind, water). However, to date no full-likelihood historical biogeographical models have been available that allow geographic range and traits to co-evolve on the phylogeny, with traits influencing dispersal ability. I present an addition to the R package BioGeoBEARS that enables an evolving discrete trait to influence both anagenetic and cladogenetic dispersal. This model can be freely combined with models adding parameters for jump dispersal (e.g., DEC+J), distance as a predictor of dispersal (+x models, with dispersal rate multiplied by distance^x), and other variants (e.g. +n, where the multiplier is environmental distance^n). I use Maximum Likelihood to apply the model to a phylogeny of over 14,000 angiosperms with areas coded for over 50 biome/continent combinations, and show that each predictor causes massive improvements in model fit, as measured by change in log-likelihood (delta lnL). Parameter x adds 15231 lnL, n adds another 618, and 3 parameters for seed traits add 2344. The model infers that plants with seeds classified as wind-dispersed have the lowest average rate of dispersal at this global scale (muliplier 0.001), animal-dispersed intermediate (multiplier 1) and water-dispersed the highest (8.18). Seed traits are classified very crudely in this analysis, so there must be care in interpretation (winged maple seeds and dandelion seeds are both "wind dispersed", and sampling of species and traits is incomplete), but overall the approach shows great promise. The study of long-distance dispersal need not rely on “dumb luck” and near-miraculous events to explain dispersal events, but rather, in the main, the causes of long-distance dispersal can be inferred statistically and understood mechanistically. BioGeoBEARS updates are at http://phylo.wikidot.com/biogeobears.

T2-31-02

How (much) do flowers vary? –Unbalanced disparity among flower functional modules and a mosaic pattern of morphospace occupation.

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Angiosperm evolution has given rise to an overwhelming diversity of floral morphology. Applying morphospace analyses to a large dataset including extant and fossil species from the order Ericales, we quantify and explore phylogenetic and temporal patterns of floral morphological diversity (disparity). In addition, we compare disparity levels among the three functional modules of a flower, i.e., perianth, androecium, and gynoecium. We find that the floral morphospace of Ericales is organized as a continuous cloud, in which most clades occupy distinct regions in a mosaic pattern, indicating that different phylogenetic lineages have explored separate evolutionary trajectories of morphological diversification. Further, we show that disparity increases with clade size rather than age, likely because speciation is narrowly linked with floral diversity in angiosperms. The fossil taxa, although not closely related to each other, all fall in a narrow portion of the morphospace, suggesting that they share many plesiomorphic characters. Finally, our analyses clearly show that the androecium contributes more to total floral disparity than either the perianth or the gynoecium. Likely, this is due to particularly flexible ontogenetic patterns that allow for high levels of structural and functional diversity in the androecium of Ericales. The lower level of disparity in the gynoecium likely reflects architectural constraints exerted by the ubiquitous syncarpy in Ericales. The perianth with its simple organs displays the lowest disparity. Taken together, our analyses demonstrate that a morphospace approach is a powerful tool to disentangle phylogenetic and functional components of overall floral disparity in a major group of angiosperms.

T2-31-03

Combining macroevolutionary approaches and ecophylogenetics to uncover the role of pollinators in plant diversification

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The functional relationships between plants and their pollinators are thought to be important contributors to plant biodiversity. Phylogenetic approaches can elucidate the rate at which transitions to specialization occur during lineage diversification and how trait evolution affects plant-pollinator interactions within communities. On a more ecological timescale, studies on phylogenetic betadiversity in plants and pollinators have brought important developments to our understanding of the role pollinators play in angiosperm diversification. Here, we summarize the diversity and complementarity of approaches, focusing on i) how pollinator composition heterogeneity alters selection pressures on floral traits; ii) whether switches between pollinator functional groups affect diversification rates in angiosperm clades with diverse pollination modes, iii) the roles of functional traits versus phylogeny in assembling plant communities, and iv) the limitations and potential solutions to further improve the phylogenetic methods to elucidate the relationship between plants and pollinators. We put forth several lines of inquiry that we feel are especially promising for providing a more holistic view of plant-pollinator interactions that incorporates space and time. Using analyses on Antirrhineae (Plantaginaceae) as a case study, we show that shifts in pollinator preference can be observed upon dispersing into new regions, but these transitions do not necessarily confer increases in diversification rates in a clade. We also find a lack of correlation between floral colour and pollinator preference in the New World Antirrhineae, suggesting pollinator inferences based on pollination syndromes should be approached cautiously. Our multi-year studies of floral colour and phylogenetic structures of alpine communities in Canada and China show that the colour overdispersion with communities is not due to phylogenetic history, but due to the avoidance of interspecific pollinator moves and heterospecific pollen deposition, which suggests competition for pollinators plays an important role in plant diversification. The key to understanding the macroevolutionary history of plant-pollinator interactions is generating reliable phylogenetic reconstructions. Phylogenetic analyses heavily depend on the explanatory power of the phylogeny used as backbone. Therefore, the use of informative priors and assumptions in these analyses greatly affect the downstream analyses. Potential limitations to any phylogenetic analysis also include, but are not limited to, model selection, taxon sampling, missing data, and time calibration, and these should be addressed carefully to make reliable inferences from the phylogenetic data. Obtaining estimates of turnover in plants and pollinators over space and time are critical steps to understanding the contribution of pollinator shifts and pollinator breadth changes in plant macroevolution. Phylogenetic studies provide invaluable data to better explain plant-pollinator interactions in detail, and to elucidate the factors affecting plant biodiversity.

T2-31-04

Are floral nectar spurs a key evolutionary innovation? Phylogenetic, ecological and developmental insights from the Antirrhineae (Plantaginaceae)

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Floral nectar spurs are tubular outgrowths containing nectar that have evolved in multiple angiosperm families. Evolutionary variation in spur length may promote pollinator specialization by restricting the access of different pollinators to the nectar reward. Nectar spurs can thus potentially mediate reproductive isolation and eventually speciation. For this reason, they have been hypothesized as a key evolutionary innovation promoting diversification. Snapdragons and relatives (tribe Antirrhineae, Plantaginaceae) constitute an ideal study system to test this hypothesis at multiple evolutionary scales. The Antirrhineae include c. 30 genera of varying species diversity, including spurless and spurred ones. A comprehensive, time-calibrated phylogeny confirmed the recurrent acquisition of nectar spurs in the course of Antirrhineae diversification, and allowed us to test the key innovation hypothesis using state-of-the-art comparative methods. To understand the mechanisms underlying spur evolution at shallower evolutionary scales, we are investigating the role of spur length variation in speciation within a recently-diversified Western Mediterranean clade of the genus *Linaria* using an eco-evo-devo approach. Phylogenetic relationships were resolved using coalescent analyses of genome-wide sequence data generated by genotyping-by-sequencing. The sister relationship between the two species with the longest (*L. salzmannii*) and shortest (*L. clementei*) spurs suggested a role of spur length change in a recent speciation event. Nectar quantifications and pollinator observations indicated that this change was driven by a loss of nectar production in *L. clementei*, linked to a shift in the main type of reward offered to pollinators. A comparison of spur development in *L. salzmannii* and *L. clementei* indicated that contrasting spur lengths in *Linaria* can be achieved by changes in the initial phase of cell division generating the spur primordium.

T2-31-05

Macroevolutionary analyses questioned the role of angiosperms on insect diversification

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Phytophagous insects represent more than 25% of the global biodiversity. A remarkable diversity of lifestyles parallels this great diversity including mouthparts, gut morphological adaptations to the host-plant preference, and behavioural adaptations for use of particular plants. Interactions between insects and plants are considered as an important driver of diversification and evolutionary changes, yet we still know little regarding the macroevolutionary role and effect of angiosperms on insects. Insects appeared about 500 million years ago (Ma), while angiosperms are estimated to appear about 200-140 Ma. During the Cretaceous terrestrial revolution (between 125–80 Ma), the fossil record and phylogenetic trees revealed that angiosperms experienced an explosive radiation, which culminated in about 80% of the floral composition at the end of the Cretaceous. This angiosperm revolution likely provided new ecological and evolutionary opportunities for insects. However, there are contrasting supports for the role of angiosperms on insect diversification. Although angiosperms may have been a driver of insect diversification, other factors may have been at play such as climate change, or tectonic evolution. In this study, the aim is to investigate whether angiosperms had a role in the insect diversification, and to compare this impact to other time-variable determinants (temperature changes, gymnosperm diversity, and number of continents through time). Both family-level fossil and phylogenetic data were used to assess the role of each factor using macroevolutionary statistical frameworks. Very interestingly, both fossil-based and phylogeny-based analyses inferred the same results for each of the determinants of diversification. The rise of angiosperms correlated negatively with both origination and extinction, suggesting that origination and extinction decreased when the angiosperm diversity increased in the Cretaceous. The opposite role is inferred with the diversity changes of gymnosperms, which had a positive correlation with origination and extinction, thus indicating that insect origination and extinction increased with gymnosperm diversity. These results indicate that temporal changes in plant composition led to important Cretaceous turnover in insect diversification. Regarding abiotic variables, the temperature variations correlated positively with origination and extinction rates, signifying that insect diversification dynamics tracked global climate change. Strangely, the number of continents through time correlated negatively with origination and positively with extinction such that origination decreased and extinction increased when the break-up of Pangea and Gondwana occurred (vicariance events did not foster higher diversity). More importantly, the model with angiosperm diversity significantly outperformed the other models for both fossil-based and phylogeny-based analyses. The results question the positive role of angiosperms on the insect diversification. The rise of angiosperms was not a trigger of insect diversity, but instead a turnover, and may be explained because insects first diversified on gymnosperms before shifting to generalist-angiosperm associations. As the gymnosperms were replaced by angiosperms during the Late Cretaceous and Paleogene, the adaptations to gymnosperm were likely not advantageous as compared to adaptations on angiosperms. The concomitant decline of gymnosperms and rise of angiosperms led to the respective loss and evolution of feeding strategies and host associations (e.g. spore feeders were replaced by pollen feeders, sap suckers were replaced by nectar suckers).

T2-31-06

Testing the morphological clock using an angiosperm-wide floral dataset

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Probabilistic models of evolution are now widely used in all aspects of macroevolution, including molecular sequence evolution, branching processes (diversification), biogeography, and morphological evolution. Some of these models have been tested and improved considerably over the last few decades. However, models for morphological evolution, especially those for discrete traits, have remained little explored and are considered too simplistic by many biologists, despite their attractive statistical properties. In particular, most of these models make the assumption that morphological traits evolve at a constant rate through time and across lineages. It remains uncertain whether this assumption is reasonable for morphological rates, while many studies have now demonstrated that molecular and diversification rates of evolution vary in many groups across the Tree of Life. Further, most model-based inferences of discrete morphological evolution are still being conducted using the equal-rates (ER, Mk1) model, which assumes equal transition rates among all states of a character, despite the availability of more realistic, but also parameter-rich models such as the all-rates-different (ARD) model. Thus, accounting for heterogeneity and asymmetry in rates of trait evolution is considered to be a key challenge for macroevolution. The angiosperm-wide dataset of 27 floral traits scored in 792 species (367 families) compiled for the eFLOWER project provides an unprecedented opportunity to explore these questions at a very large taxonomic scale. Specifically, we ask: (1) Is floral evolution time-reversible (i.e., symmetrical rates)? (2) Do rates of floral evolution vary through time and/or across lineages? In other words, is there a floral morphological clock? (3) Last, what is the impact of model selection on ancestral state reconstruction? We address these questions by fitting different models of morphological evolution to the floral dataset using a maximum likelihood and a Bayesian approach. We find strong evidence for among-state rate asymmetry. For instance, out of 27 floral characters, 20 were best fit by an ARD model and 2 by unidirectional models, both supporting asymmetry, while 4 were best fit by a symmetrical (SYM) model and only one by an ER model. We also find strong signal for among-lineage rate heterogeneity, suggesting the absence of a strict floral morphological clock, in other words implying that floral character evolution has not been a homogeneous process throughout angiosperm diversification. However, importantly, and perhaps surprisingly, we find ancestral state reconstruction of these floral traits in key nodes of the angiosperm phylogeny to be remarkably robust to model choice and different assumptions on rate heterogeneity, with a few notable exceptions. Although further work on these and other traits and clades is necessary, and new models are required, we anticipate that relaxing the morphological clocks will represent an increasingly important development in the study of angiosperm macroevolution in the years to come.

T2-31-07

Drivers of origination, extinction, and dispersal of flowering plants in deep time

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Angiosperms originated at least 145 millions of years ago in a world dominated by non-flowering plants and survived through drastic geological and climatic changes and at least one mass extinction event. Since their origination they have undergone an unprecedented taxonomic, phenotypic, and functional diversification, which allowed angiosperms to become the most dominant taxon in several terrestrial ecosystems. However, which factors have influenced their diversification and rise to dominance remain debatable. Here we compile the largest fossil data set of flowering plants to date and analyze it to understand how origination, extinction and dispersal processes have contributed to shaping their diversity through time and across space. We use birth-death-dispersal models to assess the relative importance of different factors on the macroevolutionary history of flowering plants. In particular, we estimate whether changes in the angiosperm origination–extinction balance through time is affected by biotic interactions with other organisms (non-flowering plants and insects) on a macroevolutionary scale. We compare these effects with the impact of abiotic factors (e.g. changes in the configuration of land masses, climate change, and atmospheric composition) using a novel Bayesian analytical framework. Our results indicate that origination, dispersal, and extinction processes in angiosperms are heterogeneous in time and space and respond to multiple biotic and abiotic factors.

T2-31-08

Detecting hidden rate shifts, Type I errors, and revising our concept of angiosperm innovations using trait-dependent models of diversification

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The distribution of diversity within plants can vary considerably from clade to clade. Attempts to understand these patterns often employ state-dependent speciation and extinction models to determine whether the evolution of a particular novel trait has increased speciation rates and/or decreased extinction rates. It is still unclear, however, whether these models are uncovering important drivers of diversification in plants, or whether they are simply pointing to more complex patterns involving many unmeasured and co-distributed factors. Here we describe a new way forward by extending the popular state-dependent speciation and extinction models to account for the presence of unmeasured factors that could impact diversification rates estimated for the states of any observed trait, addressing at least one major criticism of BiSSE methods. Specifically, our model, which we refer to as HiSSE (Hidden-State Speciation and Extinction), assumes that related to each observed state in the model are "hidden" states that exhibit potentially distinct diversification dynamics and transition rates than the observed states in isolation. We also demonstrate how our model can be used as character-independent diversification models that allow for a complex diversification process that is independent of the evolution of a character, and how it can be used to potentially solve the rather serious issues related to Type I errors and "phylogenetic pseudoreplication". We will focus on the evolution of fruit type in campanulid angiosperms, and discuss the important ways in which HiSSE provides a more nuanced understanding of trait-dependent diversification.

T2-31-09

Radiation of the grassland biome during a falling CO_2 world

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Grasslands occur extensively around the globe and their productivity provides important services to humanity. Global scenarios for the year 2100 predicted that grassland ecosystems will experience a major biodiversity change. A better understanding of how a grasslands responded to past environmental variations will help us to comprehend present scenarios, inform conservation decisions, and predict the effects of future environmental change. Here, we explore the relationship between past atmospheric CO_2 fluctuations and the shifts in diversification rates of grasses (Poaceae) and daisies (Asteraceae), two exceptionally species-rich families (~11,000 species and ~23,000 species, respectively) dominant in grasslands. We introduce a novel species-diversification Bayesian model that simultaneously allows, for the first time, 1) the estimation of diversification-rates through time using phylogenetic trees with non-uniform incomplete sampling, and 2) the evaluation of correlations between speciation/extinction rates and environmental variables. The newly proposed model runs in the open-source package 'RevBayes' and provides a novel pathway to explore speciation and extinction rate shifts in hyper-diverse lineages, taking into account putative environmental drivers of biotic change. We found strong evidence that support a simultaneous increase in diversification rates both for daisies and grasses after the Cenozoic most significant reduction of atmospheric CO_2 (~34 Mya). Our estimations support earlier predictions that link periods of CO_2 starvation with the expansion of grasslands.

T2-31-10

The phloem of lianas: a comparative study across the tracheophytes

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The lianescent habit has independently evolved many times in the history of tracheophytes, converging into a number of morphoanatomical features related to the ability to climb and sustain a large canopy with a generally slender stem.The anatomy of lianas is very unique, combining structures related to an increased flexibility and a more efficient hydraulic system, such as cambial variants, very wide and long vessels associated with narrow ones, tall and heterocellular rays, fewer fibres and more abundant parenchyma. These convergences are so strong that even distantly related lineages look extremely alike in their wood anatomies, making them discernible mainly by the different cambial variants present. It is very likely that convergences of this type are also present in the phloem; however, that has never been explored. Therefore, the present work aimed to conduct a thorough analysis to determine any correlation between phloem characters and the lianescent habit within a phylogenetic framework. We used phylogenetically controlled pairs of lianas and self-supporting species across the entire tracheophyte phylogeny, comparing phloem 1) qualitatively by delimiting character and character states and checking whether they evolved in correlation with habit, or other aspects, using Pagel's 94 correlation test, and 2) quantitatively by means of paired *t*-tests.Our results showed that lianas, as a whole, have wider sieve elements, with wider sieve pores, and taller rays than their self-supporting relatives; aspects related, respectively, with a presumably more efficient conduction in the phloem and an increased flexibility for the stem. In self-supporting species derived from lianas, the sieve tubes lost the large width, but the rays and cambial variants tended to retain lianescent features, a phenomenon interpreted as phylogenetic inertia. Overall, however, modifications in the phloem of lianas are solely related to the width of sieve elements and ray height. All other anatomical aspects have remained equal in lianas and their self-supporting relatives, in contrast to what occur in the xylem anatomy.This study demonstrates that the phloem of lianas has undergone convergent evolution, generating, in the process, an efficient conductive system across all the tracheophytes, while retaining a strong phylogenetic signal for its other cell types.

T2-31-11

The time tree of order Malvales with the fossilized birth-death process

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The fossil record is an important source of information about the evolution of organisms through time. One way to analyze the evolutionary history of groups or organisms is by combining the information of the fossil record with molecular-based phylogenetic trees. The Fossilized Birth-Death Process is a method that estimates lineage divergence times along with speciation and extinction rates by taking into account the age and phylogenetic position of fossils. The order Malvales comprises 10 families and approximately six thousand species, most of which are important components of tropical forests around the world. There is a substantial number of fossils described for most of the families of the order, which makes this group an excellent system to study macroevolutionary processes by integratively combining the fossil record and molecular-based phylogenies. This study aimed to illustrate the evolutionary history of the order Malvales by applying the Fossilized Birth-Death Process (FBD). We generated a phylogeny derived from eight molecular markers for all the available malvalean genera in GenBank, extracted with PHLAWD. We reviewed the fossil record of each of the families and built a dataset containing the age and phylogenetic position of 126 fossils. We applied the FBD model to the phylogeny and the fossil dataset and compared the results against those with the "node dating" method (calibration points with a prior distribution). We obtained divergence time for all major clades within order Malvales as well as speciation and extinction rates of the order. The FBD model allowed us to use all the described fossils even if they are not circumscribed into a genus.

T2-31-12

Temporal patterns of extant species diversification of the families of flowering plants

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Flowering plants represent the structural and energetic basis of nearly all terrestrial ecosystems and their phylogenetic and ecological radiation has promoted the diversification of several biological lineages, including other plants, animals and fungi. Since their first appearance in the fossil record, angiosperms have gone through an exceptional species proliferation and an astounding morphological, functional and ecological diversification. More than 290,000 extant species of angiosperms have been described and the total number is estimated at ca. 350,000 species. Despite the many advances in the fields of comparative phylogenetics and paleontology, the question still remains whether the acquisition of extant species richness is dissociated from the proliferation of major phylogenetic branches. More specifically, present-day species richness in a particular family may have accumulated only recently (i.e., within the crown group), long after the time of origin of the family (i.e., stem group). The number of extant flowering plant species does not allow for a complete (or even nearly complete) sampling of species in phylogenetic studies. Thus, the most recent phylogenies have aimed at sampling representaive taxa of every flowering plant family (ca. 90% of the 434 families currently recognized in the Angiosperm Phylogeny Website). However, these phylogenetic trees include crown node age estimates for less than 30% of families. In addition, crown-group age estimates for several families are probably underestimated due to poor taxonomic representation in the phylogenies. Thus, the temporal pattern of acquisition of extant species diversity across families remains largely unresolved. In this study we address this question by estimating crown-group ages for over 90% of the families of flowering plants, which together represent 99.3% of the total present-day species diversity. Based on the most current version of the Angiosperm Phylogeny Website, we compiled a list of families and reviewed available phylogenetic studies for each family in order to select specific taxa representing the family's crown node. We then reconstructed a time-calibrated phylogeny using four cpDNA genes (i.e., rbcL, atpB, matK, ndhF) and two nuclear markers (i.e., 18S, 26S), with over 120 fossil-based calibration points. The resulting phylogeny represents a significant contribution to flowering plant systematics, allowing for a better description of the mode and tempo of evolution of extant species. This phylogeny will serve as a starting point for the more precise investigation of morphological evolution and lineage diversification in this key clade.

T2-32: The Juan Fernandez Archipelago: A model system for studying plant evolution and biogeography in oceanic islands

T2-32-01

Speciation in the endemic flora of the Juan Fernández Archipelago

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The process of speciation may be studied in two general ways. One approach is to study divergence before speciation is complete while the other infers process from comparing traits in distinct species. The latter approach will be employed in this presentation. Some 40% of the genera (lineages) have single species endemic to Robinson Crusoe Island (RC), which is nearer to continental Chile and older than the younger and more distant Alejandro Selkirk Island (AS), which has 16% endemics. Thus, geographical (peripatric) speciation accounts for a large percentage of the endemics in the archipelago. In some cases, the closest continental relatives are reasonably certain and changes associated with speciation are known. In other instances, the relatives are obscure. The largest lineage is the endemic genus *Dendroseris* (Asteraceae: Cichorieae: Sonchinae) with 11 species, eight on RC and three on AS. Sister species on RC are ecologically or altitudinally divergent; the three species on AS are the result of independent colonizations, with divergence subsequent to disperal. The species on RC do not appear to have postzygotic isolating factors, as hybrids between various species are known when they occur together in cultivation. The genus *Robinsonia* (Asteraceae: Senecioneae: Senecioninae) is dioecious, wind-pollinated, and consists of six extant species, five on RC, one on AS, and two extinct species. Two or more species occurring in close proximity sometimes grow in different microhabitats or have different flowering phenologies. In other cases, the factors isolating the species are not obscure. Molecular markers indicate that all species of *Robinsonia* are distinct genetically. The genus *Erigeron* (Asteraceae: Astereae: Conyzinae) is represented by six endemic species (apparently from a single colonization), five restricted to AS and one species on both islands. Species are rare and in some cases not easily distinguished morphologically. Molecular markers do not invariably group all individuals/populations assigned to the same species based on morphology. The species/populations of *Erigeron* on the younger AS are ostensibly in earlier stages of divergence than are species of *Dendroseris* and *Robinsonia* on RC. Several species of *Erigeron* may be isolated by habitat and altitude. Overall in Juan Fernández, differences in flowering time, autonomous self-pollination and pollinator specificity have not been significant in divergence and speciation. Other than hummingbirds, there are essentially no known animal pollinators in the archipelago. Wind, either directly or indirectly, is the most important mechanism for pollen distribution. Very few examples of interspecific hybrids have been documented, and there are insufficient data to judge whether any represent stabilized lines distinct from their parents, i.e., are homoploid hybrid species. The potential limitations of inferring modes of speciation for the flora of the Juan Fernández from comparative studies of extant species will be discussed in the context of the ontogeny of the islands, especially the loss of area and habitat diversity.

T2-32-02

Chromosome diversity and evolution in the flora of the Juan Fernández Islands

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Evolutionary changes in chromosome number, both polyploidy and dysploidy, have long been recognized as a common phenomenon among angiosperms. Biologists have been interested in explaining such cytological variations, and correlations have been sought between polyploidy and distribution. It is estimated that more than 60% of angiosperms exist at the polyploid level, in part due to reticulate evolution via allopolyploid hybridization. Chromosome number surveys of endemic plants in oceanic islands, including the Juan Fernández Islands, have revealed very little variation in chromosome number within genera despite high levels of endemism within these floras. Attempts to understand this chromosomal stasis have invoked suggestions of low levels of hybridization, short periods of geological time, and selection against chromosomal change in divergent habitats during evolution in the islands. Smaller karyological changes between sibling species at the same chromosomal level, however, can lead to reproductive isolation and subsequent genetic and morphological differentiation. These data, therefore, can be helpful for understanding taxonomic affinities within the endemic flora. As an example, the genus *Taraxacum* Wigg. (dandelion) is quite common in the temperate zones of the Northern and Southern Hemispheres. The basic chromosome number in *Taraxacum* is eight, and the genus reveals broad variation of chromosome numbers from $2n= 2x = 16$ to $2n = 12x = 96$. During an expedition to the Juan Fernández Archipelago, Chile, in February 2011, seeds of the endemic *Taraxacum fernandezianum* Dahlst. from Robinson Crusoe and Alejandro Selkirk Islands were collected. A karyological analysis of the two populations revealed both as triploid with $2n = 3x = 24$, with a formula 18m + 3sm + 3sm-sat (i.e., 18 metacentric, 3 submetacentric, and 3 submetacentric with satellite) chromosomes. These populations are karyologically identical to those reported in Japan and India from *Taraxacum officinale*. On this basis, we suggest that the previously designated endemic species, *Taraxacum fernandezianum*, is most probably *Taraxacum officinale*, a weed widely distributed throughout the world.

T2-32-03

Reproductive biology in the endemic plants of the Juan Fernández Archipelago (Robinson Crusoe Islands)

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We surveyed the reproductive features of the angiosperms of the archipelago including the pollination, breeding systems and sexual systems. The collection of original data was based on our team's hundreds of hours of field and lab experiments and observations combined with data from the limited existing literature. This is the first and most comprehensive analysis of an entire island flora. The Juan Fernández flora is not large. It is notable in terms of the proportion of endemics per unit area (the greatest for any island archipelago), and for unusual species (e.g., *Lactoris* and many Asteraceae, e.g., *Dendroseris* and *Robinsonia*). The reproductive biology is notable for several reasons. Although, the level of dioecy is not particularly high (9%), it is higher than that on the Canaries, and significantly less than in Hawaii. As studies move beyond the necessary alpha-ecological first steps to careful in-depth analyses of various taxa, our understanding of breeding systems, and of the incidence of dioecy, will change (i.e., more reports of cryptic dioecy as we discovered with *Pernettya rigida*). The pollination is notable for two reasons. First, because bird pollination, hummingbirds in this case, is prominent, serving some 10% of the flora; one of the hummingbirds is the only hummingbird endemic to an oceanic island. Secondly, because other than the hummingbirds, there are virtually no animal pollinators. The impressive pioneering work by Skottsberg cited a number of likely zoophilous flowers in the flora, but, given that there are no virtually no bees (virtually, though the just discovered new species might become more important), or flower-visiting Lepidoptera or Diptera, animal pollination is restricted to birds. Thus, we have concluded that the island-ubiquitous autogamy or geitonogamy, and anemophily, are the key breeding systems and pollination syndrome. That lack of potential animal pollinators, coupled with a relatively young flora, has led to another particularly notable feature: many more of the island endemics and natives have retained features that are similar to their presumed ancestors than expected. To be sure, a significant portion of the flora fits the usual island model that Carlquist (1974) recognized, where many of the flowers are small, green and, in terms of pollination, are not showy. However, a number of the features would seem to manifest retention of features of the colonizing forms (e.g., larger brightly colored corollas, the expected pollen to ovule ratios for biotically pollinated taxa, and the presence of nectar – all characteristic of zoophilous pollination) – presumably as a result of the lack of selection among species that are autogamous or geitonogamous and/or are wind pollinated. The flora of these little islands is indeed interesting in many ways. And, it seems, at least for the reproductive biology, to manifest an ability to "make do", to improvise success and sustainability, characteristics shared with its alternative namesake – the Robinson Crusoe Islands – from the literature.

T2-32-04

Modeling species diversity in the Juan Fernandez Archipelago

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Oceanic islands have long been investigated for insights on patterns and processes of evolution. One principal interest has been on understanding factors that regulate species diversity. The Equilibrium Theory of Island Biogeography, presented by MacArthur and Wilson in the 1960s, stressed the importance of size of island and distance from source area as the major factors controlling diversity. These dimensions constrain rates of immigration and extinction, which lead to an equilibrium number of species within a particular island. Despite stimulating aspects of this theory, it did not deal with many other aspects that impact species diversity, such as ecological diversity, inter-island dispersal, modes of speciation, and island subsidence and erosion. In the past decade, new dynamic biogeographic models have appeared that attempt to explain species diversity during the ontogeny of oceanic islands. After volcanic origin, islands subside on submarine tectonic plates and they are slowly eroded, such that after several million years the area and ecological diversity is much less than early in their formation. Eventually the islands disappear under the surface of the sea. The Juan Fernández Archipelago is a simple island system that contains only two main islands, each approximately 50 sq. kms.: Robinson Crusoe Island, 667 kms west of continental Chile, and 4 million years old; and Alejandro Selkirk Island, 181 kms further west in the Pacific Ocean, and 1-2 million years old. The main source area of the vascular flora is western South America. Because of their similar size, different ages, and direct East-West orientation from the major source area, the archipelago serves as an excellent model to test factors that might be contributing to species diversity. Since the flora of the archipelago is well known, we can begin with the species number on Robinson Crusoe Island

and attempt to identify factors that may have contributed to reaching this number. Using these same factors, we can then test their efficacy by applying them to the younger island and checking how accurately they predict the actual number of species. Important in these calculations are the distances from the major source area, the positions of the islands at their formation, their changing surface areas (size) over their ontogenies, intra-island cladogenetic speciation, and inter-island anagenetic speciation. By application of the earlier MacArthur and Wilson theory, the species diversity on the younger island is predicted at 18% higher than currently exists. By taking more dynamic factors into consideration, particularly island ontogeny, the actual number of species of Alejandro Selkirk Island can be closely approximated.

T2-32-05

Invasive plants and threats to the native flora of the Juan Fernández Islands and other archipelagos

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Biological invasion is a hot topic in conservation. Alien biota have had and continue to have a severe impact on biological diversity on oceanic islands whereby alien invasive plants such as the Strawberry Guava (*Psidium cattleianum*) on the Hawai´i Islands often benefit from habitat destruction by alien animals such as feral pigs. Lightheaded introduction of ornamental plants and poor understanding of their biology often leads to massive invasion. This is e.g. the case with the ornamental tree *Miconia calvescens* on Tahiti, where it replaces high proportions of the former native forest and drives the endemic forest understorey plants towards extinction. Alien plant invasion is also a hot topic on Robinson Crusoe Islands (Juan Fernández archipelago). The flora of the archipelago sums up to 475 taxa (excluding those behind garden fences) of which 207 (44%) are native and 268 (56%) are alien (introduced) taxa. Among the native plants 133 (64%) are endemics and 74 (36%) are also occurring on the continent. The high proportion (>55%) of alien plants on the Robinson Crusoe Islands is only surpassed by Easter Island (75%) among the Chilean islands and is fairly high compared to other oceanic archipelagos such as the Hawaiian Islands (44%) and the Galapagos Islands (29%) based on numbers published in the new millennium. Alien plants on the Robinson Crusoe Islands have increased continuously in number since the first records. A few of them such as the shrubs *Aristotelia chilensis, Rubus ulmifolius* and *Ugni moline* as well as the herbs and grasses *Acaena argentea*, *Rumex acetosella*, *Hypochaeris radicata*, and *Anthoxanthum odoratum* have become invasive. On Robinson Crusoe Island the invasive shrubs replace now a high proportion of native forest which is accompanied by a notable decline of endemic taxa. Additionally introduced animals cause several problems: Livestock (cattle) on Robinson Crusoe Island enters the native forests and the forest-grassland ecotone foraging the tree seedlings. Rabbits (*Oryctolagus cuniculus*) have increased in their number to an immense quantity on this island. Goats (*Capra hircus*) were introduced on both islands and are estimated at present to a population size of more than 2000 individuals on Alejandro Selkirk. On this island we found also a significant decline of endemic plants compared to data collected 100 years ago, while alien plants appeared with significantly higher frequencies in 2011. The most common aliens (*Anthoxanthum odoratum* and *Rumex acetosella*) were found at lower frequencies in 1917 and one (*Hypochaeris radicata*) was entirely missing in all old records. This may be due to a more recent arrival of *H. radicata* on Alejandro Selkirk and subsequent invasion of nearly all plant assemblages in less than 100 years. Goats certainly played a substantial role in this change. Other introduced animlas such as rats (*Rattus spp.*) and coatis (*Nasua nasua*) are dangerous for the native bird fauna. Besides the direct impact on endemic plants of which some show very small and declining populations herbivores such as goats and rabbits are effective dispersal agents for the seeds of alien plants. The native Austral Thrush (*Turdus falcklandii*) produces a dilemma for conservation decisions because the fleshy fruits of the invasive shrubs *Aristotelia chilensis*, *Rubus ulmifolius*, and *Ugni molinae* are mainly dispersed by this bird. Conservationists argue that control or even eradication of the bird would limit the spread of the invasive shrubs significantly.

T2-32-06

Genetic diversity and conservation in endemic species of the Juan Fernández Archipelago

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Located at 667 km from continental Chile, the Juan Fernández Archipelago is composed of two major islands: Robinson Crusoe (= Masatierra) and Alejandro Selkirk (= Masafuera), separated by 181 km, plus a much smaller island, Santa Clara. A fundamental difference between the major islands is their estimated geological age, approximately 4 my for Robinson Crusoe and 1-2 my for Alejandro Selkirk. The high level of endemic plant species (66%) makes the Archipelago a hot spot of biodiversity and a unique location for understanding the evolutionary processes that have occurred there. The effects of invasive plant species (*Aristotelia chilensis, Rubus ulmifolius* and *Ugni molinae*) and animals (*Capra hircus*, *Oryctolagus cuniculus* and *Turdus falcklandii*) on the endemic and native vegetation, as well as the human impact and natural processes on the islands such as erosion, have resulted in the delimitation of areas of protection based mainly on the diversity of endemic species in the different zones of Robinson Crusoe Island. The use of modern molecular tools such as AFLPs and SSRs, as well as traditional data based on isoenzymes, allows generation of information on genetic variation within many endemic species of the Juan Fernández Archipelago. This contribution identifies areas with the greatest genetic richness in each of the islands, using selected species and different molecular markers. Genetic diversity datasets from the following 13 species of endemic vascular plants of the Juan Fernández Archipelago were analyzed: *Drimys confertifolia* Phil. (Winteraceae); *Dysopsis hirsuta* (Müll. Arg.) Skottsb. (Euphorbiaceae); *Erigeron fernandezia* (Colla) Solbrig, *E. rupicola* Phil. (Asteraceae); *Gunnera bracteata* Phil., *G. masafuerae* Skottsb. and *G. peltata* Phil. (Gunneraceae); *Myrceugenia schulzei* Johow (Myrtaceae); *Rhaphithamnus venustus* (Phil.) B.L. Rob. (Verbenaceae); *Robinsonia evenia* Phil., *R. gayana* Decne., *R. gracilis* Decne. and *R. masafuerae* Skottsb. (Asteraceae). Neutral and outlier matrices were obtained, which allowed comparing levels of genetic diversity between each of the populations for each of the species of Robinson Crusoe and Alejandro Selkirk Islands, as well as geographic partitioning. Based on these values, the genetically most diverse regions are the southeast areas of Robinson Crusoe Island and southwest regions of Alejandro Selkirk Island. This study was financed by projects FONDECYT N° 1160794 and FWF N° P21723-B16.

T2-33: Evolution of mangroves: Phylogeny, conservation genetics and genomics

T2-33-01

Genetic and functional trait variation along three expanding black mangrove *(Avicennia germinans)* distributional ranges in the United States

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Climate-driven shifts in species' distributional patterns can have profound ecological consequences, particularly when these shifts involve ecosystem foundation species. Mangroves are (sub)tropical intertidal foundation species that influence both ecosystem function and associated community structure. With a warming climate, mangroves have experienced poleward range expansion worldwide and greater encroachment into areas historically dominated by temperate salt marsh. In the United States, mangrove expansion has been documented along three coastlines: Texas, West Florida, and East Florida. Northernmost range limits along these coastlines are dominated by black mangrove (*Avicennia germinans*), the most cold-tolerant mangrove species in the United States. We present findings from our assessment of both genetic and morphological variation among 41 *A. germinans* populations (a total of 1,103 individuals) that encompass this species' entire distributional range in the United States. We use both microsatellite molecular markers and functional leaf traits to outline population differences with a combination of classic population genetics, Bayesian, and multivariate approaches. The results of this study provide baseline information on *A. germinans* population structure in the United States and contribute to our understanding of the evolutionary and environmental forces shaping these populations. Continued research will focus on empirical approaches to test for patterns of local adaptation at mangrove range limits in the United States and the potential ecological consequences a shift from salt marsh to mangrove dominance may have on these coastal ecosystems.

T2-33-02

Global phylogeography of the mangrove genus Rhizophora: Separation and reunion across the ocean

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The global distribution of mangroves is attributed to interactions between geographical barriers and long-distance seed dispersal via ocean currents, which are manifest in species distribution, genetic structuring and evolutionary history. Extreme long-distance dispersal, especially, is an important process in mangrove biogeography, because such events can lead to changes in distribution ranges of mangrove species, to rapid diversification due to founder effects, and to secondary contact with sibling species. A mangrove genus *Rhizophora* (Rhizophoraceae) has been considered as a key taxon to reveal the global and disjunctive distribution patterns of mangroves because the plants dominate in most of the mangrove forests in both Indo-West Pacific (IWP) and Atlantic-East Pacific (AEP). Furthermore, the fossil records of the genus and family are relatively abundant due to the distinct shape of the propagules, which is useful features to trace historical distribution. Due to its wide distribution and presence of fossil records, however, comprehensive phylogeography on a global scale that includes all species have not been clarified. To investigate the phylogeography of the genus, we conducted chloroplast and nuclear DNA sequences, and microsatellites analyses in *Rhizophora* species collected worldwide. All molecular markers strongly supported the genetic disjunction between IWP and AEP, except for *R. samoensis* in the South Pacific Islands. The most recent common ancestor between IWP and AEP *Rhizophora* species were at 11 million years ago estimated by calibration on fossil records, indicating that the continental drift involving the closure of the Tethys seaways and the reduction of mangrove distribution followed by Mid-Miocene cooling could be key factors to promote their disjunctive distribution. DNA sequences and microsatellite supported that both lineages met in the South Pacific Island through the immigration of *R. samoensis* by an extreme long-distance dispersal and experienced gene exchange after long-term isolation. Global genetic disjunctions between IWP and AEP and its collapse by long-distance dispersal are key factors to explain current patterns of diversification of *Rhizophora*.

T2-33-03

Mangrove floristics and biogeography revisited: Corresponding biodiversity hot spots and ancestral discontinuities formed by common evolutionary processes

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This treatment provides a novel re-assessment of common biodiversity patterns and the evolution of mangrove plants based on the ancestral biogeography, extant floristics and current distribution records of all species entities. It is generally acknowledged that mangrove plants occur where they do in the world because past and current factors have influenced their dispersal, diversification and establishment. The premise here is that, while all 80 extant mangrove taxa from 18 family lineages with mangrove representatives responded individually, they did so under the overwhelming common influences of large-scale geophysical factors associated with continental drift and periods of glacial maxima. Accordingly, the combined distribution and phylogeny of all mangrove genotypes are likely to have shared common pathways from their genesis and dispersal to their current day distributions. In this treatment, I present the hypothesis where evolutionary processes of diversification, divergence and speciation have been driven largely by similar geophysical circumstances and events of isolation and reunion. As such, it is proposed that all species and their phylogenies may have been influenced by the same dominant factors and events, including: land barriers, water separation and climate conditions; albeit reflected differently in each genetic lineage depending on the dates and location of the genesis of each, and regards individual capacities and tolerances affecting mutation rates, dispersal capacities, propagule buoyancy, longevity and other species-specific factors.

T2-33-04

Mangrove genomes are extensively modified and point to novel mechanisms of adaptation

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Fewer than 100 species of woody plants, collectively known as mangroves, inhabit the interface between terrestrial and marine environments. Since mangroves have been suggested to be critically endangered at present (Duke *et al.,* 2007), the future could be uncertain when the sea level rises. Understanding how mangroves adapted to these habitats, which have experienced frequent sea level changes, may inform about their future. Here, we report the sequencing of the whole genomes of species in three mangrove genera (*Avicennia*, *Rhizophora* and *Sonneratia*) and the transcriptomes of 14 others, using the PacBio single-molecule real-time (SMRT) sequencing and Illumina paired-end/mate-paired short reads sequencing. High quality sequences of scaffolds are assembled (N50 = 2.3,5.4 and 5.5 Mb, respectively). The data show that the three species invaded new habitats independently within a relatively small window of time (43-54 million years before present), suggesting that climates favorable for invasion have occurred infrequently. In adapting to new habitats, mangroves, instead of expanding their genomes as might have been expected, have undergone extensive convergent reductions in both repetitive sequences and gene families. They have also convergently reconstituted their amino acid compositions across the genome. Amino acid changes between dissimilar residues, rarely found in other species, are much more common in mangroves. Their genetic diversity is extremely low as a consequence of small populations occupying shifting habitats under continual sea level changes. The genomes of mangroves suggest a precarious existence throughout the history. Hence, their ability to withstand the double assaults of rising sea levels and increasing human perturbations is hardly assured. Taken together, the genomic features suggest extensive modifications of mangrove biology for the adaptation to the intertidal habitats and open up new research avenues in adaptation, speciation and conservation.

T2-33-05

The integration and application of genetics in mangrove conservation

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Mangroves are a highly valuable ecosystem under severe anthropogenic threats. With the current rate of degradation, it is possible that the ecosystem services provided by mangroves will be lost or impaired within the next century. Here, we present an article prepared as a joint effort by mangrove conservation geneticists worldwide, advocating for a renewed focus to integrate and apply findings from mangrove phylogeny, biogeography and adaptive evolution to conservation planning. We highlighted three major areas of importance: (1) the determination of species identity and conservation priorities, (2) the identification of evolutionary significant units, and (3) the understanding of adaptation in the face of climate change. For each of these areas, we put forth suggestions on how state-of-the-art molecular data on mangrove evolution and ecology can inform and facilitate its conservation. Specifically, we present our work on the cold tolerance of mangroves as an example of how genetic data is essential for the effective conservation of mangroves. In this study, we conduct transcriptome analysis on individuals of *Kandelia obovata* and *Bruguiera gymnorhiza* under different temperature treatments to understand the molecular and physiological regulatory mechanism behind cold tolerance. Our findings have important implications on the distribution of mangroves, especially their response to climate change. Ultimately, we call for conservation efforts to aim toward "managing evolution", whereby the adaptive and evolutionary potential of mangroves is understood and preserved. This article serves as a mission statement for the field of conservation genetics in mangroves and provides a common ground for establishing international collaboration.

T2-33-06

A multiple disciplinary approach to unveil the evolutionary history of New World *Rhizophora*

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Humans' impact on Earth is undeniable such that people have become a geological force. Expectedly, living beings also are influenced by human activities. One of the phenomena that have severe impact on organisms are the current global climate changes (GCC), human-driven alterations on the climate properties, whose biological effects are unquestionable. Despite its global influence, different species may differently respond to these changes. Mangrove forests, for instance, are projected to be specially impacted such that distributional shifts are currently being reported in different regions of the world. Because retrospective information is a valuable input to make better predictions of the responses to a current changing world, our objective is to describe the evolutionary history of Western hemisphere *Rhizophora* species. Due to the complex nature of the ecological and evolutionary drivers of organisms' distribution, we are using a multiple disciplinary approach based on climatic niche, genetic diversity and transcriptome analyses of *Rhizophora mangle* and *R. racemosa* besides their putative hybrid *R. harrisonii* from the Atlantic-Caribbean-West Pacific biogeographic region. As a whole, the climatic niche pairwise comparison indicated that *R. racemosa* and *R. mangle* presented a similar (but not equivalent) climatic niche. Climatic niche models projected in three past moments showed a substatial regional heterogeneity in distributional response such that, for instance, whereas in North America there was a poleward expansion, in the South American Atlantic coast, we did not observe this latitudinal shift. Moreover, for *R. racemosa,* the Last Glaciation has reduced its climatic niche to few areas. It suggests that this species has been under cycles of population expansion-retraction since the Last Interglacial Period. These changes in species distribution may also provide cues to insterspecific hybridization, a relevant biological process for mangrove species, specially for Western World *Rhizophora.* Using genome-wide genetic markers, we observed that individuals identified as *R. racemosa* from the Pacific Coast of Central America are more closely related to *R. mangle* individuals from the same coastline than *R. racemosa* individuals from the Central American Atlantic littoral. Within species, we observed previously described genetic structure patterns indicating, for example, that the American continent is a major barrier to *R. mangle* gene flow whereas the Atlantic and Pacific oceans do not impede this species' migration. Moreover, in the South American Atlantic coast, for *R. mangle,* we found a clear subdivision of two populations (from North and South coasts of Brazil). We are currently analyzing the transcriptomes of *R. mangle* individuals from both North and South populations. These preliminary results provide cues from different perspectives that may shed light on the mangrove trees' response historical changes that may support better predictions of how they may respond to the current GCC.

T2-34: Evolution and ecology of bamboos I: Phylogenetics and evolution of woody and herbaceous bamboos, II: Ecology and biogeography of temperate and tropical woody bamboos (two sessions)

T2-34-01

Negative correlation between rates of molecular evolution and flowering cycles in temperate woody bamboos revealed by plastid phylogenomics

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Heterogeneous rates of molecular evolution are universal across the tree of life, posing challenges for phylogenetic inference. Here we present 15 newly sequenced plastid genomes in the temperate bamboo tribe Arundinarieae, including the first genomes ever sequenced from Madagascar representatives. The woody bamboos are noted for its extremely slow evolutionary rates within the rapidly evolving family Poaceae, supposedly caused by their mysterious monocarpic reproduction. However, the correlation between evolutionary rates and flowering cycles has not been formally tested. A data matrix of 46 plastid genomes representing all 12 lineages of the tribe was assembled for phylogenetic and molecular evolutionary analyses. We conducted phylogenetic analyses using different sequences (e.g., coding and noncoding) combined with different data partitioning schemes as well as using site-heterogeneous models, revealing conflicting relationships involving internodes among several lineages. A great difference in branch lengths were observed among the major lineages, and topological inconsistency could be attributed to the long-branch attraction (LBA). Using clock model-fitting by maximum likelihood and Bayesian approaches, we furthermore demonstrated extensive rate variation among these major lineages. Rate accelerations mainly occurred for the isolated lineages with limited species diversification, totaling 11 rate shifts during the tribe's evolution. As an example of recently radiated tribe, we further illuminate the universality of rate heterogeneity in plants. More importantly, we find a strong negative correlation between rates of molecular evolution and flowering cycles for Arundinarieae, in accordance with a putative generation time effect. The correlation identified will be helpful for studying the unpredictable flowering in these bamboos.

T2-34-02

Progresses in Bamboo (Poaceae – Bambusoideae) macroecology and diversification studies

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Bamboos are a seemingly well-known flagship taxon whatever the region, be it tropical or temperate. Its classification and phylogeny have been the subject of numerous studies, but much work is still needed, especially in notoriously difficult groups such as the *Dendrocalamus/Bambusa/Gigantochloa* complex or for addressing still debated questions such as obtaining supported phylogenetic relationships for the *Olyrea* (the herbaceous bamboos). The aim of this communication is to show the progresses made by our team in understanding bamboos evolution and ecology at a global scale, without focusing on a too specific group, as so much knowledge is still lacking to get enough resolution outside of the macroscale. Our study is thus focusing on macroscale patterns of selected morphological traits within a macro-ecological framework. Our results, exposed during this presentation, are heavily relying on distributional data and especially phylogenetic backbones produced through projects held in collaboration with Kunming Institute of Botany, RPC China, and Paris Muséum national d'histoire naturelle, in France.

T2-34-03

The phylogeny of temperate woody bamboos (Arundinarieae) based on ddRAD data

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The tribe Arundinarieae (the temperate woody bamboos) has long been recognized as a monophyletic group. However, the phylogenetic relationship within the tribe are still intricate due to the difficulty of detecting sufficient markers and their reticulate evolution history. Here, 422 species (417 temperate woody bamboos, and five tropical woody bamboos as outgroups) were sampled and candidate SNP markers were identified using the double digest restriction-site associated DNA sequencing (ddRAD-seq) for our phylogeny analysis. To improve data quality and obtain more robust phylogenetic topologies, the grouping method of Stacks and Bowtie2-Stacks mapping strategy were finally adopted in generic and higher level, respectively. Our results revealed 14 highly supported lineages within Arundinarieae using maximum likelihood inference. In this scheme, *Ampelocalamus calcareus* was the most basal branch to diverge from Arundinarieae, with the ADH clade as the second branch, thereafter followed by *Gaoligongshania megalothyrsa* and the core Arundinarieae which can be further divided into the monopodium clade and the sympodium clade according to their rhizome features. The monophyly of Arundinarieae and core Arundinarieae were well supported. Overall, compared with previous limited data sets consisting of either a single or a few genes, ddRAD data sets have been significantly advanced our ability to resolve historically difficult phylogenies. This well-resolved phylogenetic framework of Arundinarieae reflects a major step forward in our understanding of bamboo biodiversity and evolutionary history.

T2-34-04

Phylogeny of *Fargesia* (Poaceae: Bambusoideae) inferred from multiple DNA sequence data and chloroplast genome

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The majority of the woody bamboo species have been descried frequently without knowledge of the flowers so that the infrageneric taxonomy was mainly based on the subtle and variable vegetative features. But it has not been proven that either the vegetative-feature-based infrageneric taxonomy or the relationships between genera are cladistically sound. The infrageneric classification of *Fargesia* based on vegetative characteristics has not yet been tested by molecular phylogeny. To prove the infrageneric classification, we explored phylogenetic relationships within *Fagesia* based on three cpDNA (*mat*K, *psb*A-*trn*H and *trn*L-*trn*F), nrITS and *rpb*2 sequences. A representative sample of 50 bamboo species, including 37 species of *Fargesia* and nine species of *Yushania* was used to estimate the phylogeny of *Fargesia*. To estimate deeper-level phylogenetic relationships for *Fargesia*, we assembled a data set of whole genomes from 26 bambooos including 17 *Fargesia* species and 4 *Yushania* species, two newly added *Drepanostachyum* species (namely *D. melicoideum* and *D.saxatile*,) one *Bashania* species, one *Ampelocalamus* species and one *Pleioblastus* species by using next-generation sequencing. The phylogenetic trees of 5 DNA fragements showed that *Fargesia* (*sensu* Yi, 1988) is polyphyletic, with *F. crassinoda* and *F. damuniu* in *Thamnocalamus*, *F. ampullaris*, *F. semiorbiculata*, *F. gyirongensis* and *F. collaris* in *Drepanostachyum,* and the rest of the species of *Fargesia* and all species of *Yushania* in a *Fargesia* + *Yushania* clade. We suggest that *Yushania* should be merged into *Fargesia* and that the *Fargesia* + *Yushania* clade identified in this study should form the new *Fargesia* (s. l.). The phylogeny of genus *Fargesia* (s. l.) diverges into Clade A and Clade B, consistent with the changes in the morphology of flowers and inflorescences. Species of Clade A have compressed and unilateral inflorescences subtended by a series of spathe-like leaf sheaths; the flowers have three stigmas, blooming in spring, and Clade A tends to occupy the northern part of the distribution of *Fargesia* (s. l.), where it is colder in winter and spring. Species of Clade B have open inflorescences subtended by variably expanded but never spathe-like leaf sheaths; most have two stigmas, bloom mainly from June to August, and occupy the southern part of the distribution where it is warm. Reconstruction of ancestral states indicated that the derived "spathe-like leaf sheaths" type likely evolved as a result of an adaptive advantage of compressed inflorescences in colder temperature. There were several differences based on plastid phylogenomics compared with the previous results of 5 DNA fragments: 1) Clade A, two newly added species of *Drepanostachyum*, genus *Bashania* and *Phyllostachys* formed a strong supported clade. 2) Clade B was polyphyletic clade. Genus *Yushania* and some species of *Fargesia* in clade B constructed a strong support clade, which is sister to the clade of "*Drepanostachyum* + *Bashania* + *Phyllostachys* + Clade A". And the remaining species of clade B plus *F. crassinoda* and *Fargesia* sp. formed another strong support clade.

T2-34-05

Speciation of BDG Complex (*Bambusa, Dendrocalamus & Gigantochloa*): the molecular evidence of hybridization

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BDG complex (*Bambusa*-*Dendrocalamus*-*Gigantochloa*) have numerous species and are widely distributed in tropical and subtropical aeras. Most species with high economic value and extensive utilization belong to these three genera. The generic and infrageneric boundaries between/within these three genera are problematic, and the taxanomic status of many taxa are arguable. This study was conducted to uncover the potential hybrid origins in the woody BDG complex (*Bambusa*-*Dendrocalamus*-*Gigantochloa*) and the phylogenetic relationship and the taxonomic positions of Bambusoideae (Poaceae) by molecular analyses, both separated and combined, of nuclear (GBSS I partial regions) and chloroplast (*psdA-trnH*, *rp116* intron, and *rps16-trnQ*) sequences from the BDG complex, including 113 species, varieties, forms and hybrids from the three genera. All the samples were classified into eight basic taxa: hard thorn group of *Bambusa* subg. *Bambusa* (BBh), soft thorn group of *Bambusa* subg. *Bambusa* (BBS), *Bambusa* subg. *Leleba* (BLe), *Bambusa* subg.*Dendrocalamopsis* (BD), *Bambusa* subg. *Lingnania* (BLi), *Dendrocalamus* subg. *Dendrocalamus* (DD), *Dendrocalamus* subg. *Sinocalamus* (DS) and *Gigantochloa* (Gi). The taxa of hybrid origins in the BDG complex are verified and exclusively studied. The main results are as follows. 1. The taxa with soft thorns in *Bambusa* subg. *Bambusa* (BBs) are initially confirmed to be natural hybrid taxa. Its parental taxa are from the hard thorn taxa in *Bambusa* subg. *Bambusa* (BBh) and *Bambusa* subg. *Leleba* (BLe). Most BBs species' maternal origins are from BLe, except *B. lapidea*, *B.subaequalis* and a few species have maternal taxa from BBh. 2. Some species in *Bambusa* subg. *Lingnania* (BLi) are confirmed to be natural hybrids of *Bambusa cerosissima* or its closely related, as the maternal origin, and BLe, *Dendrocalamus* or *Gigantochloa*, as the paternal origins. 3. The hypothesis of *Bambusa* subg. *Dendrocalamopsis* (BD) as a natural hybrid taxa is further proved. Moreover, BD species have multiple parental origins. The result shows that natural hybridization are common in BDG Complex; it has significant reference for demarcation of the generic scope and taxonomic studies on BDG complex.

T2-34-06

Endemism, ecology and generic circumscription in woody bamboos in India

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Bamboos form a major component of the secondary forests and as pure strands in India mainly in North eastern states, Western Ghats and Andaman and Nicobar Islands. According to the present estimate more than 140 species are recorded in India of which 46 species are endemic to the country. Most of these are neoendemics. Two genera are described from and are endemic to India. The endemic genus *Stapletonia* with 2 species is reported from Arunachal Pradesh and Munrochloa, is reported from Penninsular India. Generic delimitation in the bamboos has stabilized in the subcontinent. Among the major dominant genera with maximum number of endemic species, *Bambusa* is represented with 10 species in NE region and *Ochlandra* with 9 species in Western Ghats. 2 species of *Dendrocalamus* are from NE region and 1 each from North and Penninsular India. 3 species of *Cephalostachyum* is endemic to North eastern states Arunachal Pradesh, Meghalaya, Nagaland and Sikkim. *Melocalamus* and *Sinarundinaria* are having 2 endemic species to India found in NE region. Species of *Schizostachyum* are found in Andaman and Nicobar islands and out of three species 2 are endemic to the country. Also genus *Dinochloa* is restricted to the Andaman and Nicobar islands of which 1 species *Dinochloa nicobariana* is an endemic species to the Nicobar Islands. Genera like *Chimonobambusa, Chimonocalamus, Drepanostachyum, Melocanna* and *Thamnocalamus* are having 1 species each endemic to NE India whereas *Pseudoxytenanthera* and *Teinostachyum* are having 1 endemic species from Kerala. 1 endemic species under *Gigantochloa* has been recently reported from Bastar district of Chhattisgarh state. This presentation is meant for comprehensive information on the endemic bamboo species found only in Indian territories. With a brief description of identifying characters of the species, accompanied with their illustrations or the photographs, local names, habitat and occurrence, information of flowering and new shoots, conservation status as per IUCN guidelines, distribution with map and specific notes.

T2-34-07

Madagascar's grasses and bamboos

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The dominant narrative regarding human arrival in Madagascar has long stated that people arrived 2,500 years ago to a largely forested island, with anthropogenic destruction responsible for the 65% of Madagascar's land area now covered in grasslands and savannas. Scarcity of lake bed deposits have made paleoenvironmental reconstruction difficult. One piece of the puzzle regarding Madagascar's environmental history remains missing: the Poaceae flora has never been fully documented or considered in an ecological perspective. Herbarium specimens of Malagasy grasses (family Poaceae) were studied, taxonomic revisions produced, and new species described during the six years of this project. Regular field work has targeted locations of poorly known endemic grasses and species knowledge has been built up throughout the island. Phylogenetic trees were reconstructed and dating was carried out in order to document all instances of Poaceae diversifications into Madagascar. Plot data recording species composition, phylogenetic diversity, and disturbance levels has been gathered at 80 sites. 217 of Madagascar's 541 Poaceae species are endemic, a level of endemicity in line with the grass floras of other subtropical islands. We have identified 97 endemic lineages (including 4 lineages of bamboos), which have colonised Madagascar primarily from Africa, with a mean age of 3.5 million years. Half of the grass dispersals into Madagascar were C4 lineages pre-adapted to open habitats. The earliest endemic lineage *Lecomtella mada-*

gascariensis arrived ca 22 million years ago. The largest endemic clade recovered corresponds to the bamboo subtribe Hickeliinae. Madagascar's High Plateau is home to a unique and endemic grass flora. A checklist if the Itremo Protected Area has revealed 99 species of Poaceae, with 20 restricted to the High Plateau. Phylogenetic diversity within grassy ecosystems of Madagascar decreases in areas with strong physical disturbance, indicating ecosystems dynamics typical of natural assemblages. We demonstrate that Madagascar is home to an ancient and diverse grass flora, with local species assemblages functioning similarly to natural ecosystems. This indicates that pre-human Madagascar was home to at least some tropical grassy biomes. Work continues to determine where these were located, and how can they be distinguished from anthropogenic landscapes resulting from forest destruction.

T2-35: Insights into the evolution and biogeography of the Compositae: Beyond the book (two sessions)

T2-35-01

The complicated phylogeny of the Gnaphalieae: Perspectives, outstanding issues and the path forward

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The publication of *Systematics, Evolution, and Biogeography of Compositae* coincided with the consolidation of new phylogenetic hypotheses that explained much of the diversity of the Gnaphalieae. A basal grade suggests a southern African origin of the tribe with multiple lineages spreading out around the globe. However, at the time significant discrepancies were emerging between nuclear ribosomal and plastid DNA trees. The Australasian Gnaphalieae and *Helichrysum* and its allied genera were proving recalcitrant to phylogenetic analysis. Few of the diverse South American Gnaphalieae had been included in molecular phylogenetic studies. Several large genera remained polyphyletic and the subtribal classification problematic. Since then progress has been patchy, leaving many opportunities. Important progress has been made with South American Gnaphalieae. Several African and Australian genera have been examined and refined. A major study of *Helichrysum* has been published as well as a separate detailed study of its satellite genus *Anaphalis*. Three informally named non-African clades; the FLAG, HAP and Australasian clade have proved useful hypotheses accounting for c. 140 genera (of c. 180) and c. 1,700 (of c. 2,100) species of the tribe. Some important genera are still probably polyphyletic (e.g. *Syncarpha*). Relationships among major clades, including those in southern Africa and the HAP, FLAG and Australasian clades are not robustly resolved. Hypotheses of allopolyploid origins for the Australasian and FLAG clade have been advanced, but lack independent tests. Within the HAP clade, clades with allopolyploid origin are also hypothesised, although these also require testing. Although these hypotheses of reticulate evolution have explanatory power, the devil in the detail is clear and conflict among DNA sequence phylogenies of different loci suggest those so far advanced may be just the tip of the iceberg. While many systematists consider monophyly to be a necessary characteristic of taxa this can be problematic when relationships are reticulate. While a number of species have been confidently excluded from some genera, accommodating them without erecting small or monotypic genera, or wholesale lumping, remains an issue. A corollary of an allopolyploid origin for the Australian and FLAG clades is that reduction in chromosome number and genome size has progressed in several genera of Australian Gnaphalieae and in the FLAG clade at least in *Leontopodium*. The Australian Gnaphalieae provide ample opportunities to study genome evolution after polyploidy, since many genera retain the putative ancestral complement of chromosomes while others have undergone varying degrees of reduction. Developments in sequencing technology provide the means to advance our understanding of Gnaphalieae phylogeny in two immediate areas. Firstly, whole plastid genome sequencing will increase the detection of robust incongruence between plastid and nuclear phylogenies and force more nuanced interpretation of both. More excitingly, the capacity to unpick the phylogeny of many nuclear gene families at once should enable tests of existing hypotheses of reticulate evolution and others that will emerge. Finally we will argue for a conservative new subtribal classification consistent with all the available phylogenetic evidence as an interim step until progress is made on the outstanding issues of phylogeny within the tribe.

T2-35-02

Multiple polyploidization events across Asteraceae with two nested events in the early history revealed by nuclear phylogenomics

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Biodiversity results from multiple evolutionary mechanisms, including genetic variation and natural selection. Whole-genome duplications (WGDs), or polyploidizations, provide opportunities for large-scale genetic modifications. Many evolutionarily successful lineages, including angiosperms and vertebrates, are ancient polyploids, suggesting that WGDs are a driving force in evolution. However, this hypothesis is challenged by the observed lower speciation and higher extinction rates of recently formed polyploids than diploids. Asteraceae includes about 10% of angiosperm species, is thus undoubtedly one of the most successful lineages and paleopolyploidization was suggested early in this family using a small number of datasets. Here, we used genes from 64 new transcriptome datasets and others to reconstruct a robust Asteraceae phylogeny, covering 73 species from 18 tribes in six subfamilies. We estimated their divergence times and further identified multiple potential ancient WGDs within several tribes and shared by the Heliantheae alliance, and also observed nested duplications in the early history of this family at core Asteraceae (Asteroideae–Mutisioideae) and shared by the sister family Calyceraceae. Our analysis of the history of Asteraceae reveals two rounds of WGDs that each coincide with an extinction event in the Earth history and are followed by two kinds of great increase of biodiversity in the family; the older one proceeded the divergence of at least 10 subfamilies within 10 My, with great variation in morphology and physiology, whereas the other was followed by extremely high species richness in the clade named Heliantheae alliance. Our results provide different evidence for several WGDs in Asteraceae and reveal potential association among WGD events, dramatic changes in environment and species radiations, providing a possible scenario for polyploids to overcome the disadvantages of WGDs and to evolve into lineages with high biodiversity.

T2-35-03

Big data for a big family: Phylogenomics of the compositae (Asteraceae)

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Phylogenomics, made possible by advances in Next Generation Sequencing, holds great promise for elucidating complex relationships in plant lineages. Such approaches make use of large-scale taxon and gene sampling to reconstruct phylogeny to address questions of evolutionary relationships. Despite enormous effort put forward to resolve the Compositae's evolutionary relationships through traditional molecular systematics, many areas of the phylogeny remain poorly understood, particularly along the backbone of the tree. Comprehensive and well-supported phylogenetic reconstructions at the family-wide level have been difficult to obtain due to the paucity of phylogenetically-informative universal markers for a family of this magnitude. Moreover numerous gene and genome duplication events coupled with large-scale and rapid radiations have further complicated phylogeny reconstruction. To date, molecular phylogenies for the Compositae have largely been based on molecular markers derived from Sanger sequencing focused at the tribal level and below with few exceptions. In addition, family-wide level phylogenies have largely been reconstructed using a metatree approach due to the difficulties associated with alignment issues for a common set of phylogenetically-informative markers across this large and diverse family. To overcome these issues, we have developed and implemented a targeted capture approach for low copy number nuclear markers for the Compositae using Next Generation Sequencing on the Illumina platform. Through the HybSeq method, we are able to sequence ~1000 nuclear loci from the COS II marker set along with chloroplast genomes as off-target capture reads. These data enable the comparison of nuclear and chloroplast phylogenies. We have employed both gene and species tree estimation methods, as well as maximum likelihood and Bayesian analyses. Much work remains going forward, but some key findings from our work thus far are that some subfamilies of Compositae are not recovered as monophyletic, and the backbone relationships surrounding these subfamilies in question remain tenuous. We hypothesize that a major cause of this discordance and low resolution in key areas is related to gene and genome duplication due to the polyploid nature of the family. We have begun exploring the use of transcriptomes to more precisely place genome duplication events on the tree, and on resolving issues of orthology and paralogy for the nuclear markers, all of which affects phylogenetic reconstruction for many major groups of plants that possess a rich history of polyploidy.

T2-35-04

Advances in our understanding of the evolution and biogeography of the compositae

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The Compositae (Asteraceae) are the largest and most diverse flowering plant family (ca. 24,000 spp/ 1,700 genera). Members occur worldwide in all but the most extreme habitats, reaching their greatest numbers in arid and temperate regions and on tropical and subtropical mountains. While many species have restricted ranges in areas that are threatened with high extinction rates (i.e., Pacific Islands, Cape Floristic Region, Tibetan Plateau), the family also includes some of the world's most noxious weeds (i.e., dandelion, ragweed, thistle) some of which cost the billions (in USD) annually. Numerous species produce novel secondary compounds that have many industrial and biomedical uses and others have been domesticated for food. In 2009 a comprehensive book was published that resolved some issues but left many questions unanswered, however, it triggered substantial new research based on new fossil and morpholgoical evidence including phylogenies and character evolution studies based on next generation methods such as gene target enrichment. It has been hypothesized that Compositae originated in South America and experienced a subsequent explosion in Africa afterward moving across the globe. But the placement of enigmatic taxa has cast some doubt on parts of the story. New data from traditional and next generation sequencing have provided additional insights on the radiation of the basal grade and the Cichorioideae bringing into question the monophyly of the both the Carduoideae and the Cichorioideae. In both cases one clade moves in and out of the respective subfamily and may disrupt the current hypotheses. Potential causes of this may be the type of data used in each analysis but it may also reflect a past history of hybridization. If two valid hypotheses exist then it brings up the fundamental question of which one should be used for studies in evolution and classification.

T2-35-05

Global biogeographic divergences and chromosome evolution of the paper daisy tribe Gnaphalieae

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The paper daisy tribe Gnaphalieae (Asteraceae) has complex biogeographic distributions characterized by disjunctions as well as radiations worldwide. Variation of chromosome numbers in the tribe is also extremely complicated with basic chromosome numbers ranging from 3-14. We herein explored the possible link between the global radiation and the complex chromosomal variation in this species-rich herbaceous tribe within a phylogenetic framework based on the hitherto largest taxon sampling, a total of 835 terminal accessions representing 80% of the genera, with nuclear internal transcribed spacer (ITS) and external transcribed spacer (ETS) sequences. Our broadly sampled study produces a robustly supported phylogenetic hypothesis for the backbone relationships of the tribe. The tribe experienced several dynamic early diversifications in southern Africa, resulting in at least three near-endemic groups (the *Relhania* clade, the *Metalasia* clade, and the *Lasiopogon* clade). Analyses using ChromEvol suggests that the ancestral base chromosome number for the tribe is most likely seven, and that the three basal lineages have experienced several chromosome gain or loss events. Four well-supported lineages are recognized in the crown group (i.e., HAP, *Gnaphalium* s.s., FLAG, and the Australasian group); and each of the four lineages has a world-wide distribution or independent large radiations. The HAP clade has a South African and diploid origin, followed by a radiation in southern Africa and several migrations towards the north of the African continent, the Mediterranean basin and Asia. These expansions and diversifications have been accompanied by a large number of genome duplications, as shown by several independent events of tetraploidy, hexaploidy and octoploidy. The FLAG clade is inferred to have a European diversification with a southern African and diploid origin, supporting a single dispersal/migration event in the early Miocene associated with both numerous duplications and dysploidizations. The Australasian group was likely founded by a single dispersal event from southern Africa that resulted in a radiation of more than 550 species, with numerous genome duplications as well as gain or loss events, as indicated by all chromosome base numbers presented in this group. Our data strongly suggested that Gnaphalieae originated in southern Africa during the Oligocene, followed by repeated migrations into the rest of Africa and the Mediterranean region, with subsequent entries into other continents during various periods starting in the Miocene. Expansions in the late Miocene to Pliocene appear to have been the driving force that shaped the global distribution of the tribe as forests were progressively broken up by the midcontinent aridification and savannas and grasslands expanded into the interior of the major continents. Both polyploidy and dysploidy are indicated as the most significant evolutionary trend in chromosome number within the tribe and may have partly or even mainly contributed to the recent colonizations and rapid radiations of the Gnaphalieae worldwide.

T2-35-06

Island-like mountain radiations in Asia: The case study of the genera *Saussurea* and *Jurinea*

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Evolutionary radiations represent events in which many species or lineages evolved from a common ancestor in a short period of time. Many plant radiations have been triggered by island-like ecological opportunities following mountain uplift; the mountain ranges with the steepest and widest environmental gradients, such as the Andes, are home of exceptional regional species pools (and also exceptional endemism rates), mainly derived from evolutionary radiations. The Himalayan-Qinghai-Tibetan Plateau (HQTP) and the adjacent Hengduan Mountains (HM) are considered one of the main biodiversity hotspots of the world thanks to its richness in species and endemics. Both regions show extreme altitudinal ranges compressed in short distances as a consequence of the collision of the Indian and Eurasian plates, and thus constitute ideal evolutionary scenarios to study diversification processes in mountain regions. We have identified two possible cases of alpine radiations in the *Saussurea*-*Jurinea* complex (Compositae-Cardueae), involving some 550 species in total. *Saussurea* shows an amazing number of species (more than 300) in the HQTP and Hengduan mountains, although a considerable number of species are also found on the west side of the mountains of Middle Asia (Tian Shan and Pamir-Alay). *Jurinea*, in contrast, has the highest number of species (150 sp.) in the Tian Shan and Pamir-Alay. Our general objectives are the following: a) To carry out an extensive sampling of *Saussurea* and *Jurinea*, especially centred in the two main radiation areas in the HQTP and Tian Shan mountains; b) To generate well-resolved phylogenies of both genera using a multi-loci approach through next-generation-sequencing (NGS) analyzed by Bayesian inference and parsimony, and explore coalescent-based species tree estimation with our NGS data set; c) On the basis of the new phylogenies, conduct phylogenetic comparative analyses and multi-model biogeographical inference to address the following questions: Do the alpine species of both genera in Asian mountains constitute clades with clearly higher rates of diversification than their lowland relatives? How many independent radiations took place in the complex? If several, did they occur at the same period, and are they comparable in terms of speciation rates? Which factors (intrinsic or extrinsic) shape species radiations, i.e., do the radiations follow a geographical model, an adaptive model, or a mixed model? Our results will be a major contribution to the study of alpine radiations especially in the HQTP, and will open a methodological pathway for the analysis of very large radiations in other genera.

T2-35-07

Phylogenetics of *Baccharis* (Astereae), a mega-diverse genus of compositae

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Baccharis L. is the fourth largest genus in the Compositae and the most speciose within tribe Astereae. With species number estimates ranging from 350 up to 500, *Baccharis* is among the largest plant genera in the Neotropics, and has long been considered a taxonomic nightmare. A comprehensive phylogenetic hypothesis for this American genus is necessary to test its monophyly, clarify the infrageneric relationships and shed light on its evolutionary and biogeographical history. Given its great morphological diversity and wide geographic distribution, phylogenetic studies based on molecular data to test the monophyly of the genus and investigate the relationships of its infrageneric groups are required, allowing the recognition of clades with evolutionary and biogeographical significance. Therefore, we provide the hitherto most comprehensive phylogenetic hypothesis for *Baccharis*. We performed Bayesian phylogenetic analyses based on four molecular regions from the nuclear and plastid genomes (ETS, ITS, *trn*H-*psb*A, *trn*L-F; in total 5041 aligned nucleotides) and including 248 species of *Baccharis*, from all previously segregated genera and all subgenera previously recognized, and 44 outgroups belonging to tribe Astereae, totalizing 1080 new sequences. Our results confirm subtribe Baccharidinae to be a monogeneric group, and support a wide circumscription of *Baccharis* as monophyletic, including the genera previously segregated from it (*Baccharidastrum*, *Baccharidiopsis*, *Heterothalamulopsis, Heterothalamus* and *Lanugothamnus*). Seven main lineages are recognized and treated as subgenera, all of which re-circumscribed as monophyletic lineages. *Baccharis* subgen. *Baccharis, B.* subgen. *Molina* and *B.* subgen. *Tarchonanthoides* roughly match previous subgeneric concepts, while *B.* subgen. *Pteronioides* and *B.* subgen. *Stephananthus* are synonyms. Two previously segregated genera (*Heterothalamulopsis* and *Heterothalamus*) and two formerly existing sections (*Coridifoliae* and *Oblongifoliae*) are placed at subgeneric rank with new circumscriptions. Four new sections are recognized, and three new statuses at the rank of section are proposed to accommodate taxa not corresponding to any previously described section, while 22 sections are reduced to synonymy. In total, we propose that *Baccharis* should comprise 433 species classified into 7 subgenera and 47 sections. This study represents a major advance in elucidating the relationships among species in such a large predominantly Neotropical plant genus, and thus constitutes the basis for further exploration of the evolutionary processes that have allowed the group to attain its wide geographic distribution, morphological variation, varied sexual reproductive strategies, and complex chemical contents.

T2-35-08

Analysis of genetic diversity and marker-trait associations in the medicinal non-model plant Chamomile (*Matricaria recutita l.*) using genome-wide snp-markers

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Matricaria recutita L. (syn. *Chamomilla recutita* (L.) Rauschert, commonly known as German chamomile, is a traditional medicinal plant already mentioned by Hippocrates, whose works can be considered as the basis of modern medicine, in the 5th century BC. In the last decades, rather limited research has been done on chamomile, often focusing on biochemical pathways analyses for medicinally-active compounds like alpha-bisabolol. NGS approaches are frequently used to exploit genetic resources and to improve breeding in major crops. Here, we applied genotyping-by-sequencing (GBS) to unravel the genetic structure of cultivated medicinal chamomile and create a first genomic resource for future applications to improve and facilitate breeding. A high number of SNP-markers were mined to characterize the genetic diversity in the panel of 91 plants from 33 predominantly cultivated varieties, populations and accessions, including diploids and artificially generated tetraploids, and from different geographic regions. 18 compounds (terpenoids) were measured by GC-MS from flower heads. Analyses of flowering time demonstrated that diploids generally flowered earlier than tetraploids. As revealed by STRUCTURE, PCoA and neighbor-joining analysis using 6495 high-quality SNPs, the outgroup *M. discoidea* was clearly distinct from *M. recutita*. The STRUCTURE analysis further showed that tetraploids tend to display lower genetic diversity than diploids, potentially reflecting their origin by artificial polyploidisation from only a part of the chamomile genetic background. No connection between geographic origin and genetic structure was found, but varieties with genotypes possessing high alpha-bisabolol content (e.g. ‘Manzana’) could be genetically discriminated. GWAS enabled us to resolve a number of SNPs significantly associated with flowering time and high alpha-bisabolol content. Four sequences characterized by these alpha-bisabolol-associated SNPs are involved in plant biotic and abiotic stress response in different plants species, whereas six of these sequences are described to play a role in universal metabolic pathways. One sequence associated with flowering time is described to be involved with self-pollination in *Arabidopsis thaliana*. Further research could lead to the development of reliable and cost-effective markers applicable for marker assisted selection in chamomile breeding.

T2-35-09

High altitude conservation biogeography: richness, turnover, endemism and regional hotspots of Andean Compositae

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The Andes is the largest, widest and highest mountain range in the world. It covers all possible climatic ranges, from the driest to the humid, and from the warmest to the coldest. From the biological point of view, it includes the Tropical Andes Biodiversity hotspot, considered the world’s most biodiverse hotspot. It has been estimated that along the Andes there are about 50,000 plant species (~ 50% endemic). However, the patterns that shape Andean biodiversity show differences between plant groups and are still not well understood. Being the most diverse South American angiosperm family, and showing high levels of endemism, the Compositae is an excellent model for the study of Andean biogeography. There are some 3,000-3,500 Andean species of the family, with both ancestral and recent origins. This work aims to understand the patterns of richness, species turnover and endemism of the Compositae family throughout the Andes, to help understand the mechanisms that have facilitated its radiation in these high-altitude environments, and to identify regional areas of importance for conservation (regional Compositae hotspots). As a first step in this long-term study program, all georeferenced Asteraceae records are being compiled and analyzed in the different databases of biological collections, and analyses are being carried out on the basis of richness estimators, diversity indexes, Beta-diversity models and sampling efforts. The results show three to four richness centres mainly in the northern and Central Andes of Colombia, Ecuador and Bolivia. The southern Andes show comparatively reduced collection efforts, but levels of endemism appear remarkable. These results disaggregated at the tribe level, allow to test hypothesis regarding the radiation of the different lineages within the family, identifying areas with sampling gaps, and regional priority areas for conservation efforts, eventually related to the ancestral uses of the species.

T2-35-10

Systematics of Chinese compositae

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The sunflower family (Compositae) is the largest and the most diverse flowering plant family, comprising
24,000–30,000 species and 1,600–1,700 genera. In China, Compositae are also the largest family, with approximately 2,336 indigenous species in 248 genera. In the past two decades, molecular phylogenetic analyses has contributed greatly to our understanding of the systematics of Compositae. Nevertheless, the large-scale analyses and knowledge about the relationships of Chinese Compositae at the generic level as a whole are far from complete due to difficulties in sampling. In this study, we presented a three-marker (*rbcL*, *ndhF*, and *matK*) phylogeny of Compositae, including 506 genera (i.e., approximately one-third of Compositae genera). The study sampled 200 Chinese genera (i.e., approximately 80% of Chinese Compositae genera). The backbones of the new phylogeny were largely congruent with earlier studies, with 13 subfamilies and 45 tribes recognized. Chinese Compositae were distributed in 7 subfamilies (Mutisioideae, Wunderlichioideae, Carduoideae, Pertyoideae, Gymnarrhenoideae, Cichorioideae, and Asteroideae) and 22 tribes (Mutiseae, Hyalideae, Cardueae, Pertyeae, Gymnarrheneae, Vernonieae, Cichorieae, Doroniceae, Senecioneae, Astereae, Anthemideae, Gnaphalieae, Calenduleae, Inuleae, Athroismeae, Helenieae, Coreopsideae, Neurolaeneae, Tageteae, Millieae, Eupatorieae, and Heliantheae). Chinese Compositae lacked 6 basal subfamilies and 23 tribes. Several previously ambiguous relationships were clarified. Our analyses also resolved some unplaced genera within Chinese Compositae. Finally, our phylogenetic tree was used to revise the classification for all genera of Chinese Compositae. In total, 255 genera, 22 tribes, and 7 subfamilies in China are recognized.

T2-35-11

An updated phylogeny of *Moquiniastrum* (Gochnatieae, Compositae) revealed by a novel molecular marker set using low cost phylogenomic methods

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The genus *Moquiniastrum* (Compostiae) includes 21 species and belongs to the Gochnatieae, a tribe located near the base of the Compositae phylogeny. The genus is unique in Gochnatieae based on the combination of dioecious flowers, 2–5 armed pubescence, and paniculiform capitulescences. Moreover, species of *Moquiniastrum* have a widespread distribution throughout South America in varied and sometimes disjunct habitats. We have designed and validated a 576-locus marker dataset for microfluidic PCR-based target enrichment in *Moquiniastrum*. Using this dataset we anticipate improved phylogenetic resolution not only in this genus, but more broadly within the tribe Gochnatieae. Ongoing research is focused on improving species-level resolution in each of the seven clades in Gochnatieae as well as resolving infrageneric relationships in the tribe, which have proven historically intractable. Our results will help to understand the biogeography and evolution of species of *Moquiniastrum* as well as traits that may be associated with transition from mesic and dry habitats throughout South America. The tribe Gochnatieae is the last branch of the South American base of the Compositae and followed by significant radiations into North America and Africa; as such, the findings in this study are critical to understanding broad scale biogeography and evolution in the family. Samples from more distantly related taxa in the Gochnatieae and Compositae suggest this phylogenomic marker set can be effective for phylogeny reconstruction at various scales of evolutionary distance in the family.

T2-35-12

Is the vernonieae (compositae) truly the “Evil Tribe?” Clues to the answer from phylogeny, biogeography and niche conservatism

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The Vernonieae (aka the Evil Tribe) includes 1500+ species distributed throughout the subtropics in both the New and Old World. It is unusual in that it has a nearly unbroken distribution in both hemispheres, running from Argentina through to northernmost Mexico with a similarly continuous distribution from South Africa through Ethiopia. Species are also found in eastern North America and the Caribbean in the NW, and in the OW in the Arabian Peninsula, SE Asia, Australia, and the Hawaiian Islands. The relationships among taxa within and between hemispheres and the biogeographical history of the tribe have been difficult to unravel, however. Twenty-four subtribes have been described based on DNA sequence data and morphology providing a framework to test putative relationships and clarify the tribe’s biogeography. Chloroplast and nuclear DNA sequences were obtained from 400+ taxa from across the tribe’s geographical range and including all subtribes. A dated phylogeny was produced using a BI tree in BEAST. This confirmed the origin of the Vernonieae in SE Africa/Madagascar, however, its origin was dated at ~45my, twice as old as previous estimates. Additionally, within 10 my of its origin the Vernonieae were established in all major geographic regions in which they are found today. Long-distance dispersal occurred directly from Africa to South America, Australia, Hawaii, and SE Asia. As previously suggested, the NW and OW lineages evolved independently once separated. There were, however, several cases of back dispersal from the NW to the OW (but not in the reverse direction). The nearly continuous distribution of the tribe in both hemispheres is associated with the nearly continuous creation of volcanic mountain chains from south to north in both Africa and South America (40-5 my). Coincident with the new habitat created by volcanic orogeny the world’s climate gradually became drier and more seasonal, shrinking the area occupied by tropical wet forests and opening up large expanses of sunny shrub/grassland habitats. The latter favor the family Compositae; most taxa prefer open sunny seasonal habitats. While retaining these family preferences, the Vernonieae are distinct in specializing on metal rich (especially iron containing volcanic) soils, and many taxa are equally well suited to limestone, bauxite, serpentine, and dolomite exposed by weathering and upthrust. The Vernonieae also grow from sea level to over 4,200 m. Additionally, the high mineral content of soils underlying savannahs and open areas of Africa and South America greatly expanded the possibilities for colonization in non-montane areas. And one other piece of family phylogenetic conservatism favoring the Vernonieae's ability to reach and colonize new areas is the production of vast numbers of wind-dispersed seeds, year after year. Rather than being called the "Evil Tribe" perhaps the Vernonieae should be renamed "The Lucky Devil tribe." Its extensive distribution and species radiations are due in large part to a fortuitous combination of phylogenetic/niche conservatism at the family level, edaphic specialization within the tribe, and the continuous formation of new areas open to colonization due to geo/tectonic events and climate change over the past 40 my.

T2-36: Systematics Agenda 2050: call for strategies for the future of systematic biology

T2-36-01

Systematics in latin America: current status and future challenges

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Latin America is a region united by culture, history, geography and diversity. Mainly tropical and subtropical, it contains the most diverse terrestrial ecosystems, the wettest and the driest areas on Earth, seven of the 25 Global Biodiversity Hotspots, six megadiverse countries, twelve major biomes, three of the world´s largest wetlands, and some of the largest and most pristine forests. Its immense plant diversity contains 90,000-110,000 plant species (30-35% of global plant diversity), in a land area of 9.6% of total land area worldwide. However, Latin American biodiversity is being lost or seriously threatened by human activities at all levels, being scenario for vast environmental problems, including the highest rates of deforestation (overall 4.6 million ha/year). The region includes seven of the 20 countries whose plant species are the most threatened. Although 21% of the land area is protected, the present configuration of protected areas is not always optimal, with strong imbalances when comparing ecosystem types. Numerous areas are still unexplored, there is no good knowledge of species distributions, or no checklists at all. Georeferenced databases are not always made available, and overall there is still poor knowledge on the majority of the species. In addition, there is a striking inequality in the knowledge of plant systematics within Latin American countries, and compared to developed countries, with no positive correlation between plant diversity and national researchers working on it. In some countries the community of taxonomists is down to a minimum, with no taxonomists at all for several groups of organisms. A bibliometric analysis of conferences at regional and worldwide level shows a relatively low collaboration index in Latin America, and a notable difference in the main research topics. Some of the 50 most common words in abstracts at the IBC or BSA meetings, e.g. phylogeny, phylogenetics, phylogenomics, genome and gene, are replaced at the CLB by words such as species, diversity, flora, and distribution. In the light of increased threats for plant species, the systematic agenda 2050 for Latin America must consider to: 1) carry out explorations and inventories guided by gap analyses, to allow a better understanding of local/regional plant diversity, and the implementation of important areas of conservation; 2) develop georeferenced databases, available to all the scientific community; 3) complete and monograph Latin American flora; 4) investigate and compile knowledge associated to plants; 5) promote research on micro- and macroevolution evolution and biogeography of Latin American plants; and 6) support capacity building, to incorporate new technologies, improve local research facilities and train Latin American taxonomists and systematists.

T2-36-02

Collections in 21st century science: More important than ever

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The 19th Century ushered in a new age as naturalists undertook large-scale collecting expeditions leading to field observations and preserved specimens in the short term, and to major scientific advances in the long term. Notable among these were the founding of Physical Geography, Meteorology, Ecology (Humboldt), Biogeography (Hooker), and the theory of Evolution (Darwin, Wallace). In the 20th Century collections were central to paradigm shifts, including theories of Continental Drift (Eigenmann) and Phylogenetic Systematics (Hennig, Brundin). Past expeditions provided tissues for all the cladograms as the era of Phylogenetics took over biological thought. Will this tide of collections-based scientific advancement continue? In the first 15 years of the 21st Century we have seen tree-thinking pervade the life sciences, leading to the emergence of Evolutionary Ecology, Evolutionary Medicine, and new Food Safety methods, and collections data increasingly are used for climate change studies. Collections are a gold mine of information and are now leading the way to advances in three main areas: collections contain vast quantities of genomic data accessible through Next-Generation Sequencing techniques and phylogenomics, allowing us to address big evolutionary questions such as the frequency of genome duplication and its role in species diversification; Open access to specimen data, allowing us to model changes in diversity through time; and Estimating extinction risk and conservation priorities, by linking collections and climate data with phylogenies. Our 'grand challenge' is to determine where we want to be with collections-based research in 2050 and plan a strategy to get there.

T2-36-03

The coming age of systematic biology: Global collaboration from big data to big impact

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Systematic biology is a discipline that aims to discover and classify all species on the earth, to reconstruct the species phylogeny and answer how species evolved, and to educate the public in biodiversity knowledge and biological conservation. Recent years have witnessed great progress in systematic biology, from big data collection to our understanding of phylogeny and genome evolution of living organisms. To further develop systematic biology and promote its impact on social services, I propose to strengthen global collaborations in two areas. One is to build shared platforms (DNA banks and photo banks) that aim to collect DNA and photos of all plant species. The shared DNA banks can greatly accelerate the reconstruction of tree of life and are very helpful to develop DNA barcodes for monitoring invasive species, while the shared photo banks can provide massive information for developing programs that identify species automatically using mobiles. The other is to sequence transcriptomes of all families and genera of land plants, which will provide unprecedented opportunities to interpret how plants adapt to different environments and which genes are important for plant adaptation.

T2-36-04

Strong, connected, demanded and alive: Four crucial characteristics for biological systematics in 2050

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Designing strategies for developing biological systematics (BS) in the next three decades requires integrating retrospect and prospect perspectives. Two elements will be crucial for BS and how to respond to people's demands towards this field: the concerns about the global-change-driven biodiversity threat and the changes in the human life, determined on the one hand by our aims, interests and priorities, and on the other, by the technologies incorporated into the common daily life. Imagining the interaction between these two aspects will be the theme of my talk. In the best possible scenario, the demands of society for BS in three decades time will not only be directly related to the so-called services, which plants have provided since the dawn of civilization, but also to a more basic and intellectual request for knowledge. In a world in which technology will facilitate better analyzing and interpreting complex systems, it is conceivable that citizens will aim at understanding biological systems, which unlike other natural sciences subjects, involve multiple organization levels, are spatio-temporally constrained and non-ideal for reductionist interpretations. In all, I envisage—or would like to imagine—a 2050 scenario in which BS is strong, efficiently integrating all relevant data from different sources, using the ultimate technologies to better achieve its goals; connected through truly distributed taxonomic information systems allowing full accessibility of information including collections; demanded by society, so that knowledge will be provided not only by the research community but also by the society in collaboration with the scientific community through the expansion of citizen science initiatives; and certainly alive, so that BS will continue to have the funding, the intellectual appeal and the human resources necessary for projecting its live well through the second half of the 21s Century.

T2-36-05

Global-scale spatial patterns of diversity in the Compositae family

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With an estimated 20-30,000 species, the Compositae account for one in ten flowering plants. They occur on all continents except Antarctica and in a wide range of habitats. Semi-arid to arid environments and mountain ranges are generally considered to be their centres of diversity, but no formal, quantitative spatial study has been conducted to date. We examined patterns of spatial diversity of the Compositae at the global scale, combining occurrence data from the expertly curated Global Compositae Checklist (GCC) and a genus level phylogeny derived from a supermatrix of Genbank and BOLD sequences. Spatial diversity metrics were weighted by land area of the regions used by the GCC. Species richness was highest in the South African Cape Province, Peru, Turkey and Iran. Oceanic Islands, the Cape Province, Madagascar, Western Australia, and the New Zealand South Island showed the highest Corrected Weighted Endemism. Phylogenetic diversity was highest in the Andes and Mexico. We also conducted a regression analysis of diversity scores against environmental variables to examine the correlates of high diversity in the family. Finally, we used the phylogeny for the estimation of ancestral areas and biogeographic events under different models, to compare the results against an earlier study by Funk *et al.,* that was based on a supertree and ancestral character reconstruction.

T2-37: Evolution of floral traits

T2-37-01

Spatio-temporal stability of an island endemic plant-pollinator interaction involving floral colour change

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Endemic oceanic island plant-pollinator relationships have generated particular interest because there is a theoretical mismatch in the expectations of the outcomes of dispersal and establishment of biotically pollinated plants to islands. The ancestors of endemic plants should, in theory, be more likely to establish on an island if they are generalists capable being pollinated by a wide range of taxa. At the same time, island pollinator faunas tend to be of rather low diversity and abundance, and plant reproduction is often pollinator limited, making pollination niche segregation more likely via specialisation on a single pollinator or functional group of pollinators. The relative importance of specialist versus generalist strategies for biotic pollination are therefore unclear for island plants. At the moment, however, too few detailed studies of oceanic island plant-pollinator interactions have been conducted to properly assess the generality of any patterns and further case studies are required, particularly over a longer time scales and across multiple populations to assess spatiotemporal variation in the interactions between plants and their pollinators. Since 2003 I have been studying the pollination ecology of a Canary Island endemic plant species, the Canary Wallflower *Erysimum scoparium* (Brassicaceae), on Tenerife. *E. scoparium* employs a flower colour change strategy to manipulate its principal pollinators, which comprise a guild of medium sized bees, dominated by an endemic solitary bee *Anthophora alluaudi* (Apidae). The interaction between *E. scoparium* and *A. alluaudi* is stable over that time period and in multiple populations over an altitudinal range of almost 1,000 m (range = 1,300 to 2,250 m.a.s.l). The period of study includes one of the driest winters recorded on Tenerife and I discuss the effect of changes in community composition on the ecology of this endemic plant-pollinator interaction, and the reproductive success of the plant.

T2-37-02

Flower beak in bumblebee-pollinated *Pedicularis*: Combined functions in controlling pollen removal

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Pollen release could be governed by floral structures that regulate pollen removal in the form of packaging or dispensing pollen grains, limiting pollen removal in a single visit of pollinators. As the pollen presentation theory (PPT) assumed, staggered pollen presentation corresponds to frequent visitation which reduces available pollen for each visit, increasing total pollen dispersal, while simultaneous pollen presentation could be more advantageous in a scarcity of pollinators. Flower shape is greatly diverse in a species-rich genus *Pedicularis* which species are exclusively pollinated by bumblebees. The upper lip forms a narrow-tunnel shaped galea enclosing the anthers in many species, and is extended into a long, slender beak. It has been hypothesized that the featured various beaks may govern pollen removal, however an empirical study remains unknown. We conducted series of experiments to test whether *Pedicularis* species match PPT predictions, and whether the beak structure could affect pollen removal by pollinators. We investigated anther dehiscence pattern in 10 *Pedicularis* species in a field station in Shangri-La, southwest China. Pollinator visitation, pollen removal and deposition by the pollinator in a single visit to 4 beaked species and 1 beakless species, as well as the time spent per visit were quantified. The differences of pollen removal/deposition between species with different beak lengths were also compared. We observed that five of the 10 *Pedicularis* species dehisced two of the four anthers in the first day and dehisced the remaining two anthers in the next day. Gradually dehiscing species could be either beaked or beakless, and received more than 2.5 times of pollinator visits than those simultaneously dehiscing species on average. Bumblebee pollinators removed 35-61% available pollen and deposited 17-102 pollen grains in a single visit, and the bees removed less proportion of available pollen in *Pedicularis* species with longer beaks. Although the beaked nectarless species provided only pollen as reward, their pollen production or P/O were even much lower than beakless nectariferous species, in conflict to the prediction of compensative sex allocation, but in accordance with a competition driven specialization model which based on the cost to male fitness. Our results confirmed the functions of *Pedicularis* beak structures as constraining pollen release to pollinators. Our results also suggested that, due to different pollination environments, the control of pollen removal by *Pedicularis* flowers could be fulfilled by non-conflict multiple mechanisms, and further supported the assumption that higher visitation of pollinators could drive plants allocating more to male function.

T2-37-03

Non-equilibrium dynamics and floral trait interactions shape extant angiosperm diversity

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9. University of Michigan
10. Universidad Nacional Autonoma de Mexico
11. University of Missouri
12. South Dakota State University

Why are some traits and trait combinations exceptionally common across the tree of life, whereas others are vanishingly rare? The distribution of trait diversity across a clade at any time depends on the ancestral state of the clade, the rate at which new phenotypes evolve, the differences in speciation and extinction rates across lineages, and whether an equilibrium has been reached. Here we examine the role of transition rates, differential diversification (speciation minus extinction), and non-equilibrium dynamics on the evolutionary history of angiosperms, a clade well known for the abundance of some trait combinations and the rarity of others. Our analysis reveals that three character states (corolla present, bilateral symmetry, reduced stamen number) act synergistically as a key innovation, doubling diversification rates for lineages in which this combination occurs. However, this combination is currently less common than predicted at equilibrium because the individual characters evolve infrequently. Simulations suggest that angiosperms will remain far from the equilibrium frequencies of character states well into the future. Such non-equilibrium dynamics may be common when major innovations evolve rarely, allowing lineages with ancestral forms to persist, and even outnumber those with diversification enhancing states, for tens of millions of years.

T2-37-04

Testing the Grant-Stebbins Hypothesis: Experimental sympatry between pollination ecotypes

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Under the classic Grant-Stebbins model, variation in pollinator assemblages across a geographic range leads to divergent specialization on the most effective local pollinator, resulting in reproductive isolation among populations. Alternatively, adaptation to a one pollinator may come at little cost to the effectiveness of another and therefore be unlikely to reduce gene flow among populations. I test this model in two species in the genus *Clarkia* with abutting ranges in California that are pollinated by a variety of diurnal pollinators, including bees, flies and hummingbirds versus by nocturnal hawkmoths. I ask to what extent geographical divergence in floral adaptation contributes to reproductive isolation upon experimental secondary sympatry. Using multiple populations of each species, I quantify ethological and mechanical floral isolation by exposing flower-naive hawkmoths to both homo- and heterospecific floral arrays. Although hawkmoths are more motivated to visit the hawkmoth-adapted species in single species arrays, they show no visitation preference in mixed arrays, and thus contribute no ethological isolation. For mechanical floral isolation, the likelihood of heterospecific pollen transfer depends on the ecotypic origin of the diurnally-pollinated species used in the trial. Populations with small flowers and narrow floral tubes are strongly isolated from the hawkmoth-adapted species, whereas larger-flowered populations experience no isolation. Counter to the Grant-Stebbins model, adaptation to a novel pollinator in one part of the geographic range has not automatically resulted in reproductive isolation; rather, the outcome of sympatric contact depends on the specific pairings of ecotypes. Whereas most floral isolation studies involve young plant species that are currently sympatric, my work shows weak ethological and floral mechanical isolation prior to widespread secondary contact, but also that genetic variation for floral isolation is abundant and could itself respond to selection.

T2-37-05

Functional differentiation and trait convergence in accordance with pollinator shifts in Merianieae (Melastomataceae)

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Pollinator shifts, i.e. shifts from one functional group of pollinators to another (e.g., bee to bird), are known to affect floral traits relevant for pollinator attraction, pollen deposition on the pollinator, and pollen pick-up by the stigma. The heavily debated concept of pollination syndromes summarizes these trait combinations under the assumption of convergent evolution of floral traits in response to similar selective pressures exerted by a given functional group of pollinators. Our study focuses on the Neotropical tribe Merianieae (Melastomataceae) as a model system for the study of pollinator mediated selection on floral traits. The Merianieae are characterized by repeated independent shifts from bee to hummingbird/bat, and passerine pollination, respectively. We combine qualitative trait mapping of 40 floral traits relevant for pollination with a modern micro CT 3D-geometric morphometric assessment of flowers across 60 Merianieae species. Our goal is (i) to pinpoint functional trait differentiation in pollen transfer mechanisms between the different pollination systems and (ii) to challenge the concept of pollination syndromes and evaluate its applicability in Merianieae. Our analyses indicate a clear functional differentiation in pollen expulsion and deposition mechanisms including elaborate vibratile buzz-pollination in bee pollinated systems, saltshaker-like pollen release upon stamen contact in hummingbird/bat pollinated species, and pollen release by a specialized "bellows"-mechanism in passerine pollinated species. Particularly strongly affected floral traits associated with the different functional groups of pollinators include reward type, stamen appendage shape, thecal wall structure and attachment as well as size of the staminal pore. Geometric morphometric analyses of 3D landmark data further support this pattern and reveal convergence in floral shape and function in relation to the functional group of pollinators. Thus, our preliminary results provide tentative support for the concept of pollination syndromes in the Merianieae.

T2-37-06

The macroevolution of floral hydraulic traits and strategies for maintaining turgor

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During the Cretaceous, early angiosperm leaf evolution was dominated by decreases in cell size that facilitated increases in vein and stomatal densities. Together these traits enabled leaves to more efficiently transport water and thereby increase photosynthetic assimilation rates. At the same time, flowers were rapidly diversifying morphologically in association with their animal pollinators. Due to their novel developmental pathways and unique functions, floral physiological traits are predicted to have evolved independently from those in leaves. Because flowers are heterotrophic, selection may have favored reduced water transport capacity in flowers and reliance on other physiological strategies to maintain turgor. Yet, how flowers remain turgid despite often being placed in the hottest, driest parts of the plant canopy is essentially unknown. Furthermore, with increasing aridity threatening plants globally, determining the physiological function of flowers is critically important to our understanding and predicting biotic responses to climate change. Here we present data from a series of studies employing a water balance framework to characterize the macroevolutionary patterns in floral hydraulic traits and physiological function. Variation in hydraulic traits among extant species suggests that monocot and eudicot flowers have few, if any, stomata, less leaky epidermises, lower vein densities, and lower hydraulic conductances compared to basal angiosperm and magnoliid flowers. The lack of stomata on the corollas of many monocot and eudicot species may minimize transpiration rates under cool, humid conditions but comes with the tradeoff of not being able to regulate transpiration rates under hot, dry conditions. As a result, selection in dry habitats favors species with low epidermal conductances. Reducing stomatal density is predicted to have relaxed constraints on the floral vascular system of supplying high fluxes of water. Indeed, the hydraulic structure-function relationships differ among major angiosperm clades, with basal angiosperm flowers having relationships more typical of leaves and monocot and eudicot flowers having lower vein densities than would be needed to optimally meet transpirational demands. While the upper limit of leaf vein density increased dramatically during the Cretaceous, the upper limit of floral vein density did not increase, but its lower limit declined. These results suggest that the hydraulic strategies used by flowers to maintain turgor underwent substantial transitions during early floral evolution. While magnoliid flowers rely predominantly on high investments in water (high hydraulic conductances and hydraulic capacitances), eudicot flowers rely more on osmotic gradients to prevent turgor loss and compensate for underbuilt vascular systems, leading to lower carbon costs in monocot and eudicot flowers. Untethered by high physiological costs, other morphological traits of flowers may more rapidly and more directly track pollinator preference.

T2-38: Plant DNA barcoding in evolution, ecology, and conservation (two sessions)

T2-38-01

Analyses of Andean and Amazonian Tree Communities in Ecuador using DNA barcoding

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4. The Atlanta Botanical Garden

The Amazonian and Andean rain forests of Ecuador are richly populated with tropical tree species that have rarely been studied in a community phylogenetic context. Studies that incorporate DNA barcoding can be used to test a broad set of environmental and evolutionary factors that may be affecting tree community structure. For example, to test abiotic and biotic factors that potentialy determine forest community structure along elevational gradients in montane forest communities, traditional diversity metrics (Shannon's and Simpson's indices) can be coupled with DNA barcode phylogenies. In lowland habitats, traits such as phytochemical content can be combined with DNA barcodes to better understand the distribution of medicinal plants. The goal of this presentation is to highlight two applications of DNA barcoding that (1) evaluate community phylogenetic structure and correlate phylogenetic analyses with diversity metrics among Andean tree species along an elevational gradient at the Siempre Verde Reserve, Ecuador and (2) construct a tropical tree community phylogeny using DNA barcodes to test for phylogenetic signal in the occurrence of phytochemicals among tree species within Yasuní National Park, Ecuador. In the montane forest at Siempre Verde, 595 individuals were tagged, collected and identified, comprising 36 families, 53 genera, and 88 species. Of these individuals, 152 were sequenced for the *rbc*L and *mat*K gene regions. Preliminary results indicate that species richness decreases with elevation but the number of stems of common species increases. Furthermore, significant clumping was found at the highest two plots within the transect for all three metrics (PD, MPD, and MNTD) tested. This result correlates well with findings based on Shannon's and Simpson's diversity indices; there are more closely related species at higher elevations. Habitat filtering may be the cause of diversity peaks at mid-elevations where clouds inundate the forest causing extreme differences in habitat above and below this elevation. Given that only four species span the transect, abiotic stressors could be limiting species distribution and central drivers of community structure. In the upland Amazonian forest of Yasuní, 337 common tree species were barcoded and trait distribution of phytochemical presence was determined for 181 genera distributed across 56 families. Of these individuals, 247 species were successfully sequenced for the *rbc*L and/or *mat*K gene regions with 67 of these classified as medicinal. Preliminary findings show that the mean phylogenetic distance (MPD), mean nearest taxa distance (MNTD), and Fritz and Purvis' D statistic support a random distribution of the medicinal trait within the phylogeny. Based on these findings it would appear that evolutionary processes controlling phytochemical distribution among tree species are weak. Overall our preliminary findings indicate that our use of DNA barcoding in a community phylogenetic context shows that plant communities are constructed in non-random ways along elevational gradients in Andean tree communities and traits such as phytochemicals are randomly distributed in lowland Amazonian tree communities. Future studies that include taxonomic, functional, and phylogenetic diversity along with more complete floristic sampling will improve statistical power and the ability to detect fine scale community structure patterns in both tree communities.

T2-38-02

Plant DNA barcoding: Advances in forest community studies in China

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DNA barcoding, a bio-technology characterized by standardization, universality and efficiency, is widely used in multiple disciplines including biology, environmental science, forensics and inspection, *etc.* Generally, these applications can be divided into the clade and community levels. Forest dynamic plots (FDPs) provide a unique opportunity to study large-scale research and multidisciplinary researches on forests including DNA barcoding. Presently, China had set up four, nine and six FDPs in tropics, subtropics and temperate zone, respectively. Amongst, at least seven FDPs (i.e., Dinghushan, Gutianshan, Changbaishan, Xishuangbanna and three FDPs in Taiwan) are available with plant DNA barcoding sequences (mostly *rbc*L, *mat*K, *trn*H-*psb*A and occasionally ITS2). The first community-level research article on plant DNA barcoding in China was published in 2011, utilizing a well-resolved DNA barcode phylogeny to explore tree-habitat associations in Dinghushan FDP. Subsequently, approximately a dozen of international and domestic literatures closely-related or comparative to this topic were published. The work is important and novel for since it demonstrates four previous progresses (e.g., species discrimination, community phylogenetic reconstruction, phylogenetic community structure exploration and biodiversity index evaluation), three major challenges (e.g., absent of impetus to sequence all woody species at forest community levels, lack of novel scientific hypotheses, and scarce of big-data analysis ability) and several possible directions. These attempts might be valuable to explore large-scale biodiversity patterns, preservation and utilization of biological resources, origin and evolution of life.

T2-38-03

Assessment of phylogenetic community structure for a mixed dipterocarp forest in Brunei Darussalam using DNA barcoding

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DNA barcoding is a fast and reliable tool to assess and monitor biodiversity and has a role to play in investigating processes that are responsible for species interactions and thus the community structure of forests. Here, we use DNA barcoding to assess phylogenetic community structure of a 25 ha forest dynamics plot in a mixed dipterocarp forest in Brunei Darussalam which is part of the Center for Tropical Forest Science - Forest Global Earth Observatory (CTFS-ForestGEO) network of plots. Leaves and bark of shrubs and trees were sampled from 70 subplots (10x10 m) that varied in relative elevation, convexity and slope. We generated DNA sequence data for the two widely used plastid coding regions *rbcL* (3,118 sequences) and *matK* (2,598 sequences) for 599 morphotaxa identified in the 70 subplots. These represented 24 orders, 69 families and 189 genera with Dipterocarpaceae (16%) and Euphorbiaceae (9%) as the dominant families. Based on the barcode sequences, we reconstructed phylogenetic relationships among the taxa. To compare phylogenetic resolution and to test if it has an influence on phylogenetic diversity (PD) metrics, we generated a second phylogeny using the program Phylomatic which is widely used to infer phylogenetic community structure. It estimates the phylogenetic hypothesis for the taxa in the study plot on the basis of the Angiosperm Phylogeny Group IV. The phylogenetic trees were largely congruent, but our community scale phylogeny obtained from barcode sequences showed higher resolution than the Phylomatic tree, which resolved only at family or generic level. Both phylogenetic trees will be used to calculate two widely used metrics of PD, Mean Phylogenetic Distance (MPD) and Mean Nearest Taxon Distance (MNTD). Further, Webb's Net Relatedness index (NRI) and Nearest Taxon Index (NTI) will be calculated. The metrics will be compared to a null model of community assembly to determine whether species are assembled into local communities at random or if they are more closely (phylogenetic clustering) or more distantly (overdispersion) related than expected by chance. Further, besides biotic interactions habitat filtering often plays a role in species interactions. We will investigate the influence of environmental factors on community structure.

T2-38-04

Plant DNA barcoding and conservation

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4. Queensland Herbarium

Currently conservation planning primarily rely's on identification of different community types, species richness or species endemism as measures of diversity and or uniqueness. None of these measures captures the genetic diversity among taxa and all communities and all species are treated as essentially of equal importance and of making equal contributions to diversity. Tallying the numbers of species restricted to particular geographic locations as a measure of uniqueness again does not incorporate the genetic distinctiveness of the taxa. DNA barcodes provide a standardised measure of genetic distance among unrelated taxa and with the development of comprehensive reference libraries for geographic regions and or vegetation community types are enabling assessments to be made for conservation planning that incorporate standardised measures of phylogenetic distance among taxa and hence better assessments for biodiversity conservation. We illustrate with examples of this application to the South East Queensland rainforest estate of Australia. While phylogenetic distance and species richness are correlated in rainforest species richness tends to underestimate the phylogenetic diversity. Here we have compared the diversity of different regional rainforest ecosystem types (RE) and identified limitations in the representativeness of the protected area network. We assessed how well rainforest plant biodiversity is conserved within biogeographic subregions and where to strategically target new protected areas to maximise biodiversity conservation. We looked at fine scale patterns and utilised this approach to identify the significance of the rainforests in the Fraser Island World Heritage area despite their relatively low species richness and to identify high diversity refugial areas on the Sunshine Coast that are under the greatest threats due to urbanisation and agriculture.

T2-38-05

Ten years of barcoding at the African Centre for DNA barcoding

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The African Centre for DNA Barcoding (ACDB) was established in 2005 as part of a global initiative to accurately and rapidly document biodiversity using short DNA sequences. Over the past decade, the ACDB has generated a DNA barcode library for African flora, contributing more than 21,000 DNA barcodes to the Barcode of Life Database. Although the ACDB was initially established to generate molecular data for systematics studies, its characteristics suggest that it has major implications for understanding the fundamental processes by which species diversity is recognized, distributed and maintained. Thus, research at the ACDB in the past decade has been a major step in that direction. Here, we present several case studies utilizing DNA barcode data generated at the ACDB, to explore questions relating to the evolutionary processes underlying the formation, distribution and maintenance of biological diversity in sub-Saharan Africa based on five major focus areas: (1) plant community dynamics in response to large mammal disturbances, (2) phylogenetically informed biogeographical regionalization, (3) species conservation, (4) species adaptive responses to environmental change e.g. responses of African trees to fire regimes, and (5) biological invasion. Together, our research at the ACDB demonstrates that DNA barcode data, integrated with systematics, ecology, evolutionary biology and conservation, can help to address questions about the mechanisms governing regional diversity patterns, especially in the face of climate change.

T2-38-06

DNA barcoding, genomics and plant identification

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Royal Botanic Garden Edinburgh

DNA barcoding is based on sequencing a few standard DNA markers to tell species apart. The approach was developed using Sanger sequencing technologies and workflows. DNA barcoding is now at something of a junction, between (1) Sanger sequencing of standard markers, (2) the use of new sequencing platforms to improve efficiency and recovery of standard barcode markers, and (3) extending the concept of DNA barcoding to give greater depth of coverage of more DNA regions per individual. In this talk I will provide an overview of the strengths, weakness and insights from standard DNA barcoding approaches, and explore the opportunities for improving the power of DNA barcoding with new sequencing technologies.

T2-38-07

DNA barcoding performs better in understory herbs than trees for species identification in a 25 ha subalpine forest dynamics plot of Southwestern China

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Abstract: Understory herbs contribute substantially to the species diversity of both temperate and tropical forests. DNA barcoding has been widely used for the identification of forest plants, but most of the applications were focusing on trees. In this study, a total of 880 specimens of both woody species and herbaceous species were collected in a 25 ha subalpine temperate forest dynamics plot, which represented 216 species (73 woody species and 143 herbaceous species) belonging to 141 genera and 64 families of seed plants. The performance of four DNA barcodes (*rbc*L, *mat*K, *trn*H-*psb*A, and ITS) were evaluated for the 216 species, which were represented by a minimum of two individuals for each species where possible. We quantified the rates of species discrimination (RSD) by the barcodes singly and in combinations using the tree-based method. Amplification and sequencing success were highest for *rbc*L (with 99% amplification success rate and 97% sequencing success rate, 99%/97%), and *trn*H-*psb*A (96%/92%) ranked the second, performed better than ITS (95%/91%) and *mat*K (93%/90%). The highest RSD of single barcode was shown for *trn*H-*psb*A (81%) and ITS (81%), followed by *mat*K (77%) and *rbc*L (75%). In combinations, the RSD of *rbc*L+*mat*K, *rbc*L+*mat*K+*trn*H-*psb*A, *rbc*L+*mat*K+ITS, and rbcL+*mat*K+*trn*H-*psb*A+ITS were 78%, 81%, 82%, and 86%, respectively. The four DNA barcodes performed pretty well in most of the taxa, but the species level resolution of some taxonomical complex taxa, such as *Acer, Litsea, Rhododendron, Salix, Clinopodium*, and *Poa* were unresolved. The RSD of the barcodes, either singly or in combinations, were much higher for herbaceous plants than that of woody plants. For example, the RSD of *matK* for herbaceous species was 83%, but only 68% for woody species.The RSD of the four barcodes in combination for herbaceous species and woody species were 88% and 82%, respectively. Our DNA barcode database would be a valuable resources for further community phylogeny studies of both trees and herbs in the forest dynamics plot.

T2-38-08

DNA barcoding reveals cryptic lineages of *Schistidium* at the interface between the natural and the anthropogenic environment

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Previous studies have shown that in the moss *Schistidium*, a narrow species concept better fits the observed diversity; this is in concordance with molecular genetic results. In our recent work (SYNTHESYS grant GB-TAF-3881) genetic barcoding assisted in identification of morphologically poorly-developed pioneer forms occurring on anthropogenic structures like masonry, that otherwise are only assignable to genus. We found unexpectedly high diversity, and were able to identify certain taxa; however, we could not identify all the recovered lineages. Similar taxa occur on man-made structures in geographically distinct regions throughout Europe. In order to increase their identification, sampling of high-quality determined material is needed for reference. Our new project (SYNTHESYS grant GB-TAF-5965) extends the previous study. Further samples and specimens from the extensive RBGE herbarium, which holds >600 *Schistidium* specimens from around the world, will be examined according to the current taxonomy: a subset shall be characterized morphologically with light and scanning electron microscopy (leaf tip structure, leaf and nerve surface, spore and capsule peristome structure), and DNA-barcoded for up to 4 loci (comprising *rbc*L, *mat*K, *psb*A-*trn*H and ITS2) to elucidate the complex taxonomy in *Schistidium*. Using this reference DNA barcode library for Western European species, we will not only increase our understanding of pioneer *Schistidium* species on artificial surfaces, but also target selected lineages for deliberate cultivation. Our secondary aim is to establish a sound technique for extensive moss cultivation on building structures. An effective way to enhance homogenous moss growth on urban surfaces offers several benefits: Moss cover on buildings can help retain water, reduce noise, and in building acclimatisation. Further benefits include reduction of air pollutants, and carbon sequestration. On the other hand, detailed knowledge of pioneer moss species on man-made structures can also allow specially tailored countermeasures where the occurrence of moss growth is deemed undesirable.

T2-38-09

Revisiting Darwin's naturalization conundrum: Explaining invasion success of non-native trees and shrubs in southern Africa

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Invasive species are detrimental ecologically and economically. Their negative impacts in Africa are extensive and call for a renewed commitment to better understand the correlates of invasion success. Here, we explored several putative drivers of species invasion among woody non-native trees and shrubs in southern Africa, a region of high floristic diversity. We tested for differences in functional traits between plant categories using a combination of phylogenetic independent contrasts and a simulation-based phylogenetic ANOVA. We found that non-native species generally have longer flowering duration compared with native species and are generally hermaphroditic, and their dispersal is mostly abiotically mediated. We also revealed that non-native trees and shrubs that have become invasive are less closely related to native trees and shrubs than their non-invasive non-native counterparts. Non-natives that are more closely related to the native species pool may be more likely to possess traits suited to the new environment in which they find themselves and thus have greater chance of establishment. However, successful invaders are less closely related to the native pool, indicating evidence for competitive release or support for the vacant niche theory. Non-native trees and shrubs in southern Africa are characterized by a suite of traits, including long flowering times, a hermaphroditic sexual system and abiotic dispersal, which may represent important adaptations promoting establishment. We suggest that differences in the evolutionary distances separating the native species pool from invasive and non-invasive species might help resolve Darwin's naturalization conundrum.

T2-38-10

Can DNA barcoding provide any help for conservation of the Turkish geophytes?

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Turkey is one of the most important plant biodiversity centre comprising more than 700 geophytes within approximately 10,000 vascular plant species. Many species of tulip, lily, snowdrop, hyacinth, orchid, cyclamen, crocus, and of the other bulbous plants naturally grow in Turkey. It is well known that DNA-based methods can be applied quickly and efficiently. Recently, studies have showed that the use of DNA barcodes might be a promising tool for identification and conservation of biodiversity. We aimed to investigate utility of eleven loci for barcoding in 36 geophytes, using both single region and multi region approaches. The study has been carried out on the taxa belong to more than a hundred samples from 36 geophyte species. The sequence data of each species belonging to the *atp*B, *atp*F-*atp*H, *rbc*L, *rpo*B, *rpo*C1, *trn*L-*trn*F and ITS (ITS1+ITS2) markers have been obtained. After basic studies on the row alignments, p-distance values of the sequences have been evaluated for their reliability. Consequently, the data have been analyzed with the Neighbor Joining and Maximum Composite Likelihood analyses applied for discrimination value. Based on the results, in consideration of sequence quality and PCR success as a basic requirement of being a barcode region, *rpo*B is one of the most distinguishing genes among them. Intraspecific variation of the species of the genera *Cyclamen* L., *Adonis* L., *Tulipa* L., *Fritillaria* L., *Narcissus* L., *Galanthus* L., *Muscari* Mill. *Arum* L. and *Orchis* L. are very low (<0.03) or absent according to *rpo*B sequences. Hence, this gene region can be used for barcode gene for the mentioned genera. However, the other markers have low discrimination value for the studied geophyte genera. Moreover, these markers have additional problems for being barcode as low PCR success, multiple bands and primer universality. Geophytes comprise many endemic and economically important plants in Turkey. Moreover, new geophytes are still discovered from the nature by botanical explorations. Hence, as a new tool for discovering biodiversity, barcoding of plant is not sufficient for identify and characterize of these plant species alone.

T2-38-11

Evaluation of DNA barcoding markers for Thai Epiphyllous Bryophytes

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Leaf-colonizing (epiphyllous) bryophytes are ubiquitous in tropical ecosystems and can be found on a number of vascular plant leaves, many of which are economically important plants. However, the studies on diversity of these bryophytes have been limited, because of minute and incomplete specimens, as well as lack of taxonomical expertise. The recent development of the DNA barcoding approach has allowed the taxon identification and species discovery of many obscure groups of organisms. We compared the efficiencies of six DNA markers (*rbc*L, *mat*K, *trn*L-F, *trn*H-*psb*A, nrITS1 and nrITS2) in their ability to amplify and discriminate among Thai epiphyllous species. The amplification success was relatively high (70-90%) in all of the markers, except for *matK* which yielded no success. The barcoding gap, as calculated from the difference between inter- and intraspecific genetic distances, was the highest in nrITS2, while the highest number of monophyletic groups were found in nrITS2 and *rbc*L. Consistent with the recommendation by the Consortium of Barcoding of Life Working Group, *rbcL* should be used a a main barcoding marker for epiphyllous species. The development of DNA barcoding as a tool for discovering currently poorly known species diversity will provide a rapid and reliable identification tool for epiphyllous bryophytes in Thailand.

T2-38-12

A mini barcode signature for the identification of Rhodiolae Crenulatae Radix et Rhizoma and its products

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The roots and rhizomes of *Rhodiola crenulata* (J. D. Hooker &

Thomson) H. Ohba have been included in the Pharmacopoeia of China as the authentic Rhodiolae Crenulatae Radix et Rhizoma, to enhance inner spiritual power, concentration and physical endurance. The accelerated and uncontrolled use of *R. crenulata* in China has led to deforestation, and rapid increasing commercial utility of the raw material has made other species of *Rhodiola* been sold as Rhodiolae Crenulatae Radix et Rhizoma in the market. DNA barcoding methods provide us an effective and quick way to identify *R. crenulata* and its product. However, the successful identification relies on a suitable barcode marker and a comprehensive survey of plants all over its distribution area. As the proposed internal transcribed spacer 2 (ITS2) is not sufficient to resolve heavily processed materials, we surveyed 308 individuals of *R. crenulata* from 16 populations from Yunnan, Sichuan, and Xizang provinces covering all its natural distribution area, to search a short barcode for the identification of processed materials. All ITS2 sequences were obtained for the 308 individuals, ranging from 209 to 223 bp in length. We detected six haplotypes in all individuals. Through comparing sequences from 185 other *Rhodiola* species, we found a 32 bp (5'-TACGAGGCCTTGCG-CCTTTGAGCTTT GCG CG T-3') nucleotide signature, which is unique in *R. crenulata*. Then, we designed primers to amplify the nucleotide signature region from processed materials. Out of the 23 samples we purchased online, 12 were from other *Rhodiola* species, and 3 were not *Rhodiola*. Thus, using nucleotide signature provides us an effective alternative for identify heavily processed traditional Chinese plant materials. We also evaluated the use of secondary structure of ITS2 for identification of *R. crenulata*. The results revealed that all of the ITS2 sequences had a common four-helix folding, but the structure of *R. crenulata* is different from that of its closest relatives. We thus propose that ITS2 sequence structure information could be used as an alternative mini-barcode when a full-length ITS is not present. Our results demonstrated that both a mini barcode from ITS2 and the structure of ITS2 are effective markers for identification of *R. crenulata*, after surveying representatives from all its distribution area. This study greatly broaden the application of DNA barcoding in identifying Chinese patent medicines and other products with degraded DNA.

T2-39: Ecology and evolution of parasitic plants

T2-39-01

Driving forces for rapid spreading of *Pedicularis kansuensis* in Western China

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Pedicularis L. (Orobanchaceae) is a large linkage of root hemiparasitic species widely distributed in the temperate zone of Northern Hemisphere, with about 800 species worldwide (2/3 of the species distributed in China). In most cases, they show rather patchy distribution patterns and do not cause significant reduction in plant productivity at a large scale. However, in the past two decades, dense occurrence in large scales and rapid expansion of *P. kansuensis* in the west of China have been severely reducing forage crop productivity in grasslands, hence threatening the local livestock industry. To better understand the spreading of *P. kansuensis* for a more effective and sustainable control against it, we investigated the driving forces for the spreading. Both lab-based studies and field trials have been conducted to understand the internal and external driving forces. Our results showed that high genetic diversity, high morphological as well as physiological plasticity conferred *P. kansuensis* with strong adaptation to various habitats. Climate changes with increased rainfall and temperature facilitated seed germination and dispersal of this root hemiparasite. Monoculture of forage grasses that function as preferred but vulnerable hosts of *P. kansuensis*, lack of quality control for forage and restoration plant seeds, and lack of quarantine control of seed transportation contributed the most to rapid spreading of this species over a large scale in a short period of time.

T2-39-02

Evolution of the organellar genomes in the Piperales with a focus on the holoparasitic Hydnoraceae

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Our knowledge of plastome evolution has greatly increased in recent years while comparatively little is known about plant mitochondrial genomes. In contrast to plant mitochondrial genomes, plastid genomes of photosynthetic flowering plants are usually highly conserved in size, structure and gene content. However, the plastomes of parasitic and mycoheterotrophic plants may be released from selective constraint due to the reduction or loss of photosynthetic ability resulting in size and gene reduction. The purge of plastome genes is well understood and appears to follow a very similar pattern. Piperales have both photosynthetic and nonphotosynthetic lineages and the holoparasitic genera *Hydnora* and *Prosopanche* have split from their photosynthetic ancestor presumably 100 MY ago. That makes the Piperales a particularly interesting system for studying organellar evolution. Genomic sequence data of the three species *Hydnora visseri*, *Prosopanche americana* and *Aristolochia fimbriata* allowed us to identify, characterize, and compare their plastomes as well as to obtain first insights into their mitochondrial genomes. Following the publication of the *H. visseri* plastome, it was comparatively easy to identify and characterize the plastome of *P. americana*. Despite the extreme reduction and sequence divergence of the plastomes to photosynthetic plants, they likely remain functional. Surprisingly, the assembly and characterization of the plastome of the photosynthesic *A. fimbriata* has proven to be more challenging. In contrast to the plastomes, the mitochondrial genomes of the two parasites are among the larger ones across the angiosperms and the gene set is surprisingly conservative, representing a fairly complete and functional set of common plant mitochondrial genes. While horizontal gene transfer (HGT) is fairly common in parasitic plants due to the physical connection to the host plant, there is no evidence for HGT based on phylogenetic efforts so far. Ongoing research in Piperales, including several more lineages has the potential to greatly complete our understanding of organellar genome evolution in nonphotosynthetic plants that diverged long time ago from their photosynthetic ancestor.

T2-39-03

Integrating nuclear gene data sheds further light on plastid genome degradation in parasitic plants

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Parasitism represents the most extreme interaction between plants, where the parasitic plant steals water and nutrients from a host plant by connecting directly to its vascular tissue. The secondary transition to a nonphotosynthetic way of life in plants has brought about massive reconfigurations at the molecular level. Particularly plastid genomes (plastomes) of nonphotosynthetic plants have experienced rampant gene losses and an acceleration of molecular evolutionary rates. Although general trends of reductive plastome evolution are known, we still have no understanding of the genomic reconfigurations pertaining to the parasites' nuclear genomes or of the coevolutionary forces acting on nuclear and plastid genes under relaxed selective constraints in parasitic plants. Here, we investigate the fate of nuclear-encoded photosynthesis-associated genes along the transition from an autotrophic to a nonphotosynthetic lifestyle. Our comparative genomic and molecular evolutionary analyses of 15 nonparasitic and parasitic species from the Broomrape family (Orobanchaceae), some of which suffering from extensive plastid gene losses, reveal a surprisingly long (physical) retention of genes directly involved in photo-autotrophic pathways in holoparasites. However, transcriptome data indicate that many of those genes play no more role in molecular and cellular pathways. Analysis of selectional regimes indicate that purifying selection is relaxed in most - but not all - nuclear photosynthesis elements in the various holoparasitic lineages. Our analyses also show that shifts in selection intensity have occurred already in the Orobanchaceae ancestor or set in as parasitism evolved within the family, that is, long before photosynthesis was lost. These results imply that already the transition to parasitism relaxes selectional constraints on photosynthesis genes. We also observe that the degradation of photosynthesis-associated genome fragments appears to proceed much faster in the plastome than in the nuclear genome. Our findings highlight the importance of integrating data from various genomic compartments for understanding mechanisms of molecular evolution in subgenomes and reconfigurations of biological pathways along lifestyle transitions.

T2-39-04

Plastome reduction in parasitic plants: Independent evolutionary events?

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Many parasitic plants are characterized specialized organs (i.e. haustoria) by which they obtain water and/or nutrients from host plants. There are around 4,000 parasitic flowering species placed in 12 orders and 19 families. Of them, about 90% of parasitic species (in four orders) retain photosynthetic capability (hemiparasites), while 10% of parasitic species (in 10 orders) have lost photosynthetic capability (holoparasites). In free-living plants, the plastome is very conserved in size (120 – 170 kb), gene contents (110 –130 genes) and a quadripartite structure (LSC, IRA, SSC and IRB). Loss of photosynthetic capability in holoparasites is often correlated with degradation or loss of photosynthesis-related and other non-housekeeping genes. To date, plastomes of 11 parasitic orders have been published or examined (except Ehretiaceae, Boraginales). In hemiparasitic taxa such as *Cassytha* (Lauraceae, Laurales), Krameriaceae (Zygophyllales), Orobanchaceae (Lamiales) and Santalaceae s.l. (Santalales), plastome size and/or gene contents showed high similarity to its non-parasitic relatives. Some NADH-polymerase (*ndh*) genes were found to be degraded or lost in some species, *Cassytha* ssp. had no inverted repeat region, and gene rearrangements were found in hemiparastic Orobanchachece. Genome reductions were found in all holoparastic plants. Genome size in holoparasitic plants varied from 11,348bp (*Pilostyles aethiopica*, Apodanthaceae, Cucurbitales) to 150,504bp (*Lathraea squamaria*, Orobanchaceae). Photosynthesis-related genes were (partitially) retained in *Cuscuta* spp. (Convolvulaceae, Solanales) and some holoparastic Orbanchachaceae (e.g. *Harveya* spp., *Lathraea* spp., *Orobanche californica*). Nevertheless, the photosynthesis-related genes, as well as some ribosomal protein genes, were lost in other holoparasitic lineages, i.e., *Cynomorium coccineum* (Cynomoriaceae, Saxifragales), *Cytinus hypocistis* (Cytinaceae, Malvales), *Hydnora visseri* (Hydnoraceae, Piperales), *Mitrastemon yamamotoi* (Mitrastemonaceae, Ericales) and *Pilostyles* spp., as well as in some Orobancheceae, e.g. *Christisonia hookeri*, *Cistanche phelypaea*, *Conopholis americana*, *Epifagus virginiana*, and *Orobanche* spp. Moreover, a plastome was not detected in Rafflesiaceae (Malpighiales) and Balanophoraceae (Santalales). Phylogenetic analyses have demonstrated that parasitism has evolved independently at least 12 times. When considering the evolutionary ages of parasitic lineages, plastome degradation follows a pattern: degradation of *ndh* genes in hemiparasites, followed by photosynthesis-related genes in newly derived holoparasites, loss of protein synthesis and other functional genes, and finally retention of ribosomal RNAs in ancient holoparasites, or complete elimination or with possible incorporation of plastome remnants into mitochondria and nuclear genomes. Seven ancient holoparastic lineages, i.e. Ericales, Cucurbitales, Malvales, Malpighiales, Piperales, Santalales, Saxifragales, have extremely reduced (or no) plastomes. By contrary, young holoparasitic lineages in *Cuscuta* and Orobanchaceae showed a gradual reduction pattern of plastomes.

T2-39-05

Parasitic plants: Genome scale analyses reveal core parasite genes and extensive horizontal gene transfer

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Parasitic plants, which obtain water and nutrients directly from a photosynthetic host plant, comprise 1.5% of all angiosperms. Phylogenetic analyses have resolved 12 independent origins of direct stem and root parasites, with a similar number of lineages having evolved the ability to parasitize mycorrhizal relationships (mycoheterotrophs). Parasitic plants have long challenged and enlightened our understanding of organelle genome evolution, the function and evolution of plant-plant interactions, and the dynamics of RNA and DNA transfer between species. Here, I will describe recent efforts by the Parasitic Plant Genome Project (PPGP) to leverage comparative transcriptomics and genomic-scale analyses to identify and study a core set of genes involved in parasitism in Orobanchaceae, the family of parasitic plants with the widest range of parasitic abilities. Current efforts to understand the function of parasite genes promises to lead to control measures for parasitic weeds, especially in parasites where RNA movement from host to parasite allows host-induced gene silencing (HIGS). The discovery of bidirectional movement of RNA and DNA between parasites and hosts (resulting in horizontal gene transfer, HGT) has opened up new dimensions in our understanding of parasite-host interactions and their long-term impact. Genome-scale survey of expressed genes has identified more than 50 high confidence functional HGTs in Orobanchaceae and more than twice that number in *Cuscuta*, a stem parasite with strong parasitic dependence. Results support the thesis that parasitic plants have stolen genes from their host plants via HGT and are using them in parasitic processes.

T2-39-06

Genomic basis of taxonomic complexity in hemiparasitic *Euphrasia*

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Hemiparasitism is a remarkably flexible life-history strategy whereby a given parasite can survive to flower without a host, but performs much better after host attachment. This strategy occurs in thousands of plant species, and is of special importance as it may represent an intermediate stage in the transition from free-living autotrophism to obligate holoparasitism. Here, I present recent work on the facultative hemiparasitic plant genus *Euphrasia* (Orobanchaceae). The genus includes incredible taxonomic complexity, which may in part be due to plasticity in response to host quality. Using large-scale population genomic analyses, in conjunction with a common garden study, I show surprising species relationships that have been obscured by plant performance in the field. Genotyping by sequencing data reveal deep divergence corresponding to ploidy, but limited support for species within a ploidy level. Plants grown on clover flower many months earlier than those without a host, while other morphological characters used in species delimitation show little plasticity. These results reveal the potential for common garden studies of non-specialised parasitic plants like Euphrasia for clarifying taxonomic boundaries and host preference, while also emphasizing the need to perform genomic studies of complex recent species radiations.

T2-40: Systematics and evolution of palms - a model group for the tropics

T2-40-01

Time-calibrated phylogeny of the American palms (Arecaceae) based on NGS sequence-capture methods

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During the last decade, resolution of the phylogenetic relationships within the palm family has been largely improved. Thanks to these efforts palms became a model group to investigate the origin of tropical rain forest and the evolution of plant diversity at a global scale. However, the genus-level phylogeny available today and the difficulty to resolve interspecific relationships within genera using Sanger DNA sequencing, strongly limit our abilities to reveal evolutionary processes occurring at a finer geographical scale. To overcome this difficulty, palm scientists, now organized in the Palm Phylogeny Working Group, recognize the need of generating new data towards a complete species-level phylogeny of palms. Next Generation Sequencing (NGS) and sequence-capture in particular, have been considered as promising techniques to achieve this goal. However, so far, no studies have evaluated the feasibility and advantages of NGS to estimate large species-level phylogenies on palms. To address this issue, we aimed to reconstruct the species-level phylogeny of the American palms, which represent about a third of all palm species. Sequence data were obtained using the sequence-capture procedure, with available probes designed for cross-taxonomic sequencing within Arecaceae. Nearly 30% of all American palms, including all subclades and 59% of the genera were sampled from private collections (Fairchild Tropical Botanical Garden, *Instituto Plantarum*, *Jardín Botánico del Quindío*, Montgomery Botanical Center) and during intensive fieldwork in Colombia and Panama. Emphasis was given to the richest clades: Bactridinae (55 species sampled/232 accepted species), Chamaedoreeae (80/123) and Geonomateae (60/84). Sequences data was processed using the bioinformatics seqcap_processor pipeline (https://github.com/AntonelliLab/seqcap_processor). Alignments were analyzed using Maximum Likelihood (RAxML) and Bayesian Inference (BEAST) methods. Divergence times were estimated using fossil data available for palms. Illumina sequencing of our gene libraries produced a mean of ca. 650,000 reads per sample that allowed the alignment of 326 targeted exons in Arecoideae. The phylogenetic analysis of the 50 alignments (totaling 21,380 bp) with the highest taxonomic coverage confirms the current palm classification and the monophyly of most of the subclades and genera. We will discuss the advantages and limitations of the sequence-capture method and notably the performance of the palm-probes, with the challenges imposed by differential loci capture among clades and loci variation. Our study is a promising first step towards the reconstruction of a complete species-level phylogeny of the American palms. This phylogeny will soon be complemented with the inclusion of all the remaining genera and 259 additional species that we have already sampled. Our result will contribute to better understand the causes and processes of plant diversification in the Neotropics. Preliminary results concerning the origin of palm diversity in Central America will be presented.

T2-40-02

Using a NGS approach to explore the macroevolutionary history of a Neotropical palm genera

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Understanding why the Neotropics host the greatest plant diversity on Earth is a question that has puzzled evolutionary biologists for a long time and is still a vibrant field of research. Recent phylogenetic comparative studies have brought new insight not only into the general patterns of diversity but also into the mechanisms that promoted the diversification of plant lineages. Our study focuses on *Geonoma*, a genus of small understory palms distributed from Southern Mexico to Southern Brazil. With 68 species it is one of the most diverse palm genus in the Neotropics and it occurs from sea level to above 3,000 m in Central and South American rain forests. Species delimitation has proved challenging because of high intra-specific morphological variation and 20% of *Geonoma* species are in fact considered as species complexes. *Geonoma* originated and diversified in the Neogene and this recent origin, combined with a slow rate of molecular evolution has led to poor resolution in current phylogenetic trees based on few genetic markers. We selected a target sequencing approach to obtain thousands of single copy nuclear DNA regions, for about 300 individuals and a taxonomic sampling covering 95% of the described species, in order to reconstruct a robust phylogenetic hypothesis and better understand the evolutionary history of this group. Using the phylogeny we investigated the biogeographical history as well as the drivers of diversification of *Geonoma* by exploring several intrinsic and extrinsic factors that could have played a role in the evolution of the group. Our results contribute to broadening the general knowledge about the emergence of the current outstanding neotropical plant diversity.

T2-40-03

Towards a species-level phylogeny of Indo-Pacific arecoid palms (Arecaceae): First insights from New Caledonian endemics

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Palms are a prominent plant family of rainforests, and humans daily benefit from hundreds of palm species as food, medicine or timber. Many palm species are endemics on small islands in the Pacific and Indian Oceans, which makes them a good model for comparative studies of the mechanisms of speciation in archipelagos. Such analyses were so far impaired by the lack of a well-resolved species-level phylogeny of the family, leading palm specialists to initiate an international cooperation to fill this gap. Our contribution to this effort will be: (i) to generate a genus-level phylogeny of all palms (180 genera) using a combined genome-skimming and targeted sequence capture approach (Hyb-Seq, Weitemier *et al.,* 2014, Applications in Plant Sciences 2: 1400042), (ii) to generate a species-level phylogeny of tribe Areceae (ca. 600 species) using the same approach adapted to palms (Heyduk *et al.,* 2016, Biological Journal of the Linnean Society 117: 106-120), and (iii) to combine those new phylogenies with ecological and morphological data in order to infer the biogeography of Areceae and the role of seed traits in their adaptation to changing climates and sea levels. The potential of this approach will be illustrated by the results of a pilot study bringing new insights to the evolution of endemic New Caledonian Areceae.

T2-40-04

Genome evolution and diversification in Palms

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Estimates of genome size variation and sequenced genomes have revealed no clear correlation between genome size and organismal complexity; i.e., the “C-value paradox.” Processes contributing to genome size variation include polyploidy (genome doubling), tandem duplication, expansion/contraction of repetitive regions such as transposable elements, illegitimate/intramolecular recombination, fractionation/diploidization, and selection for smaller genomes. The palms (family Arecaceae) are diverse, widespread, and ecologically successful components of tropical ecosystems, with >2,500 species. Based on available data across the family, genome size and chromosome number vary immensely. Modal ploidy levels are 16 and 18 within the species-rich subfamilies Arecoideae and Coryphoideae, while patterns of genome sizes based on C-values are less clear. Recent studies demonstrate strong correlations between genome size and species diversity, suggesting polyploidy potentially confers selective advantages due to the introduction of genetic novelty. Here we aim to test that hypothesis in palms and their close relatives, the commelinid monocots (grasses, gingers, dasypogonoids, *etc.*). We address potential correlations between ploidy level and C-value across the family, accounting for phylogeny, and reconstruct ancestral chromosome numbers and genome sizes. We use available genomic and transcriptomic data to investigate evidence for polyploidy among the commelinids and within the palms (at the subfamily level) by comparing the age distribution of paralogous sequences. Lastly, we investigate the mechanisms of genome size variation by exploring repeat type and content based on 'genome skim' data. Disentangling the causes of genome size variation in palms will help us understand the genetic conditions facilitating adaptive radiation and ecological success in other important clades.

T2-40-05

A molecular systematic study of the palm tribe Euterpeae

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The palm tribe Euterpeae, one of the 13 tribes in the subfamily Arecoideae, is distributed in the Neotropical region of Central and South America including the Caribbean. The Euterpeae is of economic importance for local people in the tropics, as it contains palms useful for harvesting palm hearts, acai berries, and oil for exportation. The ecological importance of the tribe is reflected in the fact that three Euterpeae species are among the 20 most abundant tree species in the Amazon. In this study, a molecular phylogenetic analysis was conducted using one plastid (*trnD-trnT*) and four low-copy nuclear DNA regions (*CISP4*, *PHYB*, *RPB2*, and *WRKY6*). We sequenced 34 individuals within 26 of the 31 species in the tribe, and 42 outgroup species to calibrate the tree using the fossil record of palms. Phylogenetic trees were reconstructed using maximum parsimony, maximum likelihood, and Bayesian inference methods. The Euterpeae and all its five genera were monophyletic. *Hyospathe* was basal to the rest of the genera, *Prestoea* and *Neonicholsonia* were sister, *Euterpe* was sister to the *Prestoea/Neonicholsonia* clade, and *Oenocarpus* was sister to the *Prestoea/Neonicholsonia/Euterpe* clade. This tree topology rejected Balick's hypothesis that *Oenocarpus*' hippuriform (horsetail shape) inflorescence was derived from *Euterpe*'s inflorescence by loss of the adaxial rachillae because *Euterpe* is a more recent genus. Furthermore, the hypothesis that *Euterpe catinga* is an ancestral species because it inhabits a unique and geologically old substrate type in Amazonia (white sand) was also rejected because it was found to be one of the most recent taxa within the genus and the tribe. Resolution at the species level was particularly challenging for *Prestoea* and *Euterpe* for which we should sequence many more DNA regions, and complete the taxon sampling by including the narrow endemic species unavailable for this study. Our phylogenetic analysis recovered quite different intergeneric relationships than those recovered in a previous maximum parsimony analysis based on 54 morphological and anatomical characters. The biogeographic history of the group will be analyzed in the future using the dated molecular phylogenetic tree.

T2-40-06

The de-evolution of diversity in Amazonia

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The Neotropics is recognized as harboring biodiversity hotspots, such as Amazonia, but there are some areas where diversity may have decreased over time, particularly in oligarchic forests. Understanding of the evolutionary origins and maintenance of oligarchic forests in the context of geologic change and historical climate can improve our predictions of how organisms, habitats, and Neotropical biomes may respond to comparable changes in the face of climate change. The genus *Mauritia* (Arecaceae) presents an ideal system for investigating the evolution of oligarchic forests, or the de-evolution of Amazonian biodiversity. The genus has a rich pollen record and next-generation sequencing techniques, gene capture probes, have been developed specifically for palms that we show are appropriate for investigating demographic structure on both ecological and evolutionary time-scales. Furthermore, *Mauritia* is the most widely used and economically important native South American palm. We hypothesize that historical extinctions in *Mauritia* correlate with increased diversification rates in eudicotyledonous plants, the expansion of South American savannas, and/or the constriction of lowland rainforests during the Neogene. The inference of biogeography and diversification is an integral window into the past that enables the investigation of how geographic regions, biomes, and communities assembled through time and how they may evolve in the future. By integrating data across fields, for example from geology, genetics, fossils, species distribution and abundance, and morphology, we can reach a more comprehensive framework for the understanding of biome evolution and extinction.

T2-41: Practical importance of polythetic approaches to the taxonomy of plant-associated Ascomycota

T2-41-01

Systematics and biodiversity of Didymellaceae, one of the largest families in the fungal kingdom

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The Didymellaceae is one of the largest families in the fungal kingdom, and most species in this family are known as plant pathogens causing leaf and stem lesions on a very broad range of hosts. We have recently assessed the Didymellaceae employing a polyphasic approach using morphological examination and multi-locus (ITS, *28S*, *tub2*, *rpb2*) analyses, with the family delimited to 17 well-supported monophyletic genera. As a result nine new genera and 84 new combinations were introduced respectively. Subsequently several more genera were added to this family, which are discussed in this paper. During our most recent survey in China and six other countries, 100 Didymellaceae isolates were obtained, of which 64 isolates represent previously undescribed species. Twenty-six novel species were described from 40 different host plants or other substrates, such as air, soil, water and faeces from several karst caves in China. For the fungal-host association in Didymellaceae, only a few genera exhibit certain level of host-specificity, for example, *Ascochyta* to Fabaceae, *Neoascochyta* to Poaceae, and *Phomatodes* to Cruciferae. Fabeceae, Poaceae, Asteraceae, Ranunculaceae, Rosaceae and Solanaceae are the six most common host families related to Didymellaceae species.

T2-41-02

The evolution of fungal taxonomy over two centuries: A case

study in the Ophiostomatales

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The Ascomycete order *Ophiostomatales* accommodates more than 320 species of mostly arthropod-associated species. These include some serious tree pathogens such as the causal agents of Dutch Elm Disease, as well as the human pathogens causing sporotrichosis. The taxonomic history of this order largely reflects events that shaped Ascomycete systematics over the past two centuries. The type species of the order, *Ophiostoma piliferum*, was described in 1823 by Fries in a cryptic, 11-word Latin paragraph without illustration. This style persisted during the 19th century in all the major attempts to describe and classify fungi. By the turn of the century, new taxa were often described as part of long, detailed studies on the biology, ecology or pathogenicity of fungi. By that time, the connections between sexual and asexual states were also understood, and in 1932 Buisman was the first to successfully cross two asexual isolates of an Ascomycete, *Ophiostoma ulmi*, in the laboratory to produce its sexual state. During the 21st century detailed morphological studies also led to species concepts being refined based on microscopy, ultrastructure, biochemical and physiological traits. However, throughout the century several monographs on genera in the order were hampered by their focus on either sexual or asexual state morphology, with generic concepts often contradicting each other. In 1992 ribosomal DNA sequences showed that the asexual human pathogen *Sporothrix schenckii* grouped among sexual, wood-infesting species in *Ophiostoma*. Soon protein coding DNA sequences were used together with rDNA sequences to distinguish between cryptic species, reflecting phylogenetic relatedness, rather than phenotypic traits that were often the result of convergent evolution, leading to taxonomic confusion. The ability to link sexually and asexually reproducing isolates provided the impetus for the One Fungus One Name movement that culminated in the abolishment of the dual nomenclature system, which allowed for one fungal species to have two or more names, at IBC in Melbourne in 2011. During the past decade population genetics has also changed the way taxonomists look at species, and hybridization and horizontal gene transfer often explain incongruence within phylogenies. Full genome sequences have now been produced for almost 30 Ophiostomatalean species, and phylogenies based on more than 2700 gene regions extracted from these genomes, formed the basis for the selection of five genes that were amplified and sequenced for more than 200 species in the order. The resulting phylogenies enabled the delineation of genera in the *Ophiostomatales* that clarified confusing and overlapping morphological traits that plagued the group's taxonomy for many decades. Robust phylogenies such as these also reveal and explain evolutionary processes that went undetected in morphology-based taxonomy. The current challenge for fungal taxonomists and nomenclaturalists is the incorporation of environmental nucleic acid sequences (ENAS) into taxonomic studies. One species, *Hawksworthiomyces sequentia*, known only from ITS sequences has recently been described in the *Ophiostomatales*, although the Melbourne Code does not provide for the valid description of such taxa for which no specimen exists.

T2-41-03

New Canadian lineages of ergot fungi revealed by multi-gene phylogeny

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Claviceps purpurea, the type species of the genus, was first described by Tulasne 1853 based on a specimen from rye. Other cereal crops (i.e. *Triticum* and *Avena*) and grasses (*Alopercuri*, *Dactylis*, *Lolium*, and *Poa*) were noted as additional hosts. To date, the reported host range has increased to more than 400 species in at least 19 genera belonging to 3 subfamilies of grasses (Poaceae). Unlike other *Claviceps* spp. that infect only a few genera in the same tribe, *C. purpurea* is unique in having a wide host range, leading to suspicion that it might be a complex of multiple species. Studies have confirmed cryptic speciation correlated with ecological niches. The taxonomy of *Claviceps* species occurring in Canada has been little explored. Among 600 specimens of *Claviceps* in the National Mycology Herbarium (DAOM), 80% are labeled as *C. purpurea*. To better understand species composition and genetic diversity of ergot fungi in Canada, we conducted multi-gene phylogenetic studies using recently collected samples from different parts of the country, along with historical specimens housed in DAOM. The loci sequenced include rDNA ITS, house-keeping genes (*EF1-alpha*, *BT* and *RPB2*) and ergot alkaloid synthesis genes (*easE*, *cloA*). Based on individual locus and concatenated alignments, the analyses recognized six lineages within the *C. pupurea* complex, among which three appear to be new. Morphological and phenotypic studies were undertaken to characterize these lineages.

T2-41-04

Using molecular dating in all cases of fungal classification or only in higher taxa?

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The classification and taxa delimitation in the Fungi are often subjective, being based on morphological differences and therefore other studies (e.g. phylogenetics) are needed to clarify unresolved taxa. The ranking of fungal species especially at the higher levels needs sets of evidence for their delimitation. Morphology and molecular data have been used for fungal identification and classification for many years. However, there are many problematic groups that need further collections and evidence and new techniques for their clarification. Many species also need to be restudied since they have been introduced repeatedly from different parts of the world. Thus, giving wrong names can cause many problems in identification and classification. The molecular dating technique is a key tool for interpreting the history of life of any organisms and it presently has become a common tool for many biologists in research areas. Molecular clock analysis can use information from anatomy embryology, biogeography, fossils and biological molecules. Fungal evolutionary study has used fossil evidence and molecular data to estimate the evolutionary events. Many thousands of papers have used molecular dating for estimating species divergences times. The evolutionary events can explain the rate of mutation of the interested organisms and present their history of life. The major factor that can cause mutation in each evolutionary can be estimated. Presently, researchers have increasingly used larger datasets that can estimate a much greater diversity of species. However, do we need to use the molecular dating in all cases? and do we presently have enough evident for molecular clock analysis?

T2-41-05

Secondary metabolites as chemotaxonomc and phylogenetic markers in an important order of Ascomycota

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The Xylariaceae (Xylariales, Sordariomycetes) are a large, hyperdiverse family of the Ascomycota, comprising macrofungi that produce conspicuous stromata on wood or dung, as well as microfungi that belong to the predominant endophytic mycobiota of seed plants. On the other hand, xylariaceous fungi are well-known as producers of bioactive metabolites with selective activities against human, animal and plant pathogens, as well as industrial enzymes, or as biocontrol agents. However, little is currently known about their taxonomy, phylogeny, biogeography, and ecology. Our recent work involves taxonomic and chorological studies on the tropical members of the family, to provide a basis for concise identification of the genus *Hypoxylon* and allied taxa. In addition, we have studied the stromata and cultures of these fungi for the occurrence of biologically active secondary metabolites. While some of these compounds constitute valuable chemotaxonomic marker molecules that helped to resolve species complexes, others show strong and selective activities in biological systems and may serve as lead compounds for development of anticancer agents, antibiotics or agrochemical fungicides. The results of our taxonomic work, with particular emphasis of a study of *Annulohypoxylon* and *Hypoxylon* in Argentina will be presented. A new multi-gene genealogy that resulted in the rearrangement of the Xylariales and the genera of Xylariaceae will be illustrated. Furthermore, some of our recent work on the discovery of novel secondary metabolites will be highlighted.

T2-41-06

Fungal biodiversity and functions in the pine barrens ecosystem

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Pine barrens ecosystem has acidic, sandy and nutrient-poor soils. Pines and oaks are the dominant trees, and other common plants in pine barrens include switchgrass and other grasses (Poaceae), sedges (Cyperaceae), orchids (Orchidaceae), blueberries and other heath family members (Ericaceae). Scarce attention has been paid to studies of fungi in the pine barrens, and much remains unknown about fungal biodiversity and functions in this ecosystem. The largest and most uniform area of pine barrens in the United States is the 1.4 million acre (57,000 km^2) pine barrens of New Jersey (NJ) located in the southern region of the state. The NJ Pine Barrens represents one of a series of barrens ecosystems along the eastern seaboard of USA and one of a series of similar ecosystems around the world. The podzolic soil in the NJ Pine Barrens is highly acidic (pH ~4.0 with very low cation exchange capacity), sandy, dry (low moisture holding capacity), nutrient poor (e.g. low in P, K, N) and containing elevated levels of soluble aluminum. During the 1600's and 1700's when settlers first came to this area they discovered most of the region's soils would not support the growing of grain crops from traditional European agriculture. Therefore they named the region “Barrens”. But why and how do wild grasses thrive in the pine barrens acid and nutrient poor soil? Do fungi play a role in aiding plant establishment and survival in such stressed environments? In order to address these questions, we have been studying root-associated fungi in the pine barrens using both culture method and Illumina metabarcoding method. Our data showed that with regard to fungal diversity, the pine barrens is anything but barren. We found that grass (Poaceae) roots in the pine barrens are one of the major reservoirs of novel fungi with 47% being undescribed species. Importantly, we observed that *Acidomelania panicicola* (Leotiomycetes), a new genus and species we described from switchgrass in the NJ Pine Barrens increases root hair growth of switchgrass and rice in low nutrient conditions. We also described another new genus *Pseudophialophora* (Magnaporthales, Sordariomycetes) that contains several pathogenic species. Eleven new species from the pine barrens have been published. Naming and description of a number of other new fungal taxa are in progress. We compared the root fungal community between the wild switchgrass from the NJ Pine Barrens and cultivated switchgrass in managed farms using both culture and metabarcoding methods. Both methods suggest that Leotiomycetes are dominant fungi in the switchgrass roots from pine barrens soils, while Sordariomycetes are dominant in the roots growing in the rich farm soils. Metabarcoding method uncovered a more diverse microbiome from the plant roots. More experiments are being conducted to test our hypothesis that *Acidomelania* and other Leotiomycetes play a role in increasing plant tolerance to abiotic stresses (e.g., low pH, low nutrients, drought) and contribute to improved establishment in poor soils. Results from this work will facilitate ecological and evolutionary studies on root-associated fungi.

T2-42: Evolution of land plants and their interaction with the environment

T2-42-01

Evolution of MIKC-type MADS-domain proteins and the origin of novelties in land plants

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MIKC-type MADS-domain transcription factors (MIKC TFs) represent a remarkable synapomorphy of streptophytes (charophyte green algae + land plants). Based on the presence of a keratin-like K domain they have the capacity to constitute different dimeric and tetrameric complexes in a combinatorial way. By binding to specific *cis*-regulatory elements on DNA they constitute floral quartet-like complexes (FQCs) that control diverse sets of target genes and hence developmental processes in land plants. The expansion and diversification of MIKC TFs is strongly correlated and probably causally linked to the origin of major novelties, such as ovules/seeds, flowers and fruits, during the evolution of land plants. These novelties have significantly shaped the way in which land plants interact with their environment. Since the constituents of FQCs are all paralogous MIKC TFs, events such as gene duplication, sub- and neofunctionalisation, paralog interference and escape form adaptive conflict all may have played important roles during the evolution of MIKC TFs in land plants. To better understand the proximate causes that generated major novelties in land plants, we study the interaction of MIKC TFs in a phylogenetic framework throughout the streptophytes. We will report about recent insights concerning the origin of FQCs during evolution and the molecular basis of FQC formation and function. We hypothesize that the unique phylogenetic trajectories of paralogous MIKC TFs interacting in FQCs facilitated the diversification of efficient developmental switches and hence the origin of novelties in land plants.

T2-42-02

A temporal evolutionary framework of land plants from multiple experimental molecular clock analyses

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Time-related information is essential to understand the history of life on earth. In this study, four aspects of data and methods were explored in a series of Bayesian statistical analyses of divergence time implemented in BEAUTi and BEAST, to develop a temporal evolutionary framework of land plants. First, four different matrices were constructed: a 5-gene (cp-*atp*B, cp-*rbc*L, mt-*atp*1, mt-*nad5*, and nu-*18S*) and 616-species matrix, and three phylogenomic matrices: chloroplast, mitochondrial, and combined chloroplast-mitochondrial. The 5-gene matrix sampled >70% families and the phylogenomic matrices included most orders of land plants. Second, one full- and two sub-sets of fossil calibration points were assembled, which spanned across the entire history of land plants and green algae. Third, different topologies of relationships among angiosperm-gymnosperm lineages and bryophyte-tracheophyte lineages were experimented, in addition to a series of phylogenetic constraints used in the analyses. Fourth, various combinations of rate, substitution, and speciation models were used in the analyses. These schemes of data assembly and analytic experimentations allowed comprehensive evaluation of data sufficiency and model adequacy that had not been done in most molecular clock analyses, and they offered quantitative assessment of robustness of the molecular clock analysis results. When fossils are still being discovered for many important groups of land plants, e.g., bryophytes and basal angiosperms, and molecular clock analyses seem to produce unreasonably old and a wide range of estimates for ages of some lineages, e.g., crown group angiosperms, it is imperative that molecular analyses are conducted rigorously and internally consistent and precise results are obtained.

T2-42-03

The importance of physiology for understanding the ecology and evolution of Paleozoic plants

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Paleoecology is necessarily founded on modern ecology, but that presents a distinct challenge: the modern world is utterly dominated by one highly unique lineage, the flowering plants, that have only been around for a quarter of land plant history. Thus, even when differences in form and structure are expressly recognized in the fossil record, implicit assumptions based on modern angiosperm ecophysiology can lead to very misleading expectations about the Paleozoic origins of vascular plant vegetation. I will present arguments that productivity of non-flowering plants has always been consistently low relative to what is seen among modern flowering plants. The implications of low productivity will then put important constraints on the potential biology of Paleozoic fossil plants. With this context, the early evolution of plant architecture and ecology across the vascular plants will be broadly considered.

T2-42-04

Synchronized radiations of fungi and plants linked to symbiosis

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Ecological interactions between fungi and plants, ranging from parasitism to mutualism and including plant decomposition by saprotrophic fungi, have been invoked as a central mechanism underlying their respective macroevolutionary success. Accordingly, their putative synchronized radiations might be detectable phylogenetically. Here we compare the origins of plant-fungal symbioses and saprotrophy, detecting linked and drastic shifts in diversification rates of both kingdoms in a comprehensive time-calibrated phylogenetic framework. The largest radiation during the evolution of land plants (tracheophytes) and fungi (Leotiomyceta) occurred simultaneously. However, fungi colonized land before embryophytes, likely facilitating their terrestriality through endomycorrhizal symbioses and possibly through endophytic symbioses aboveground. In contrast, the diversification of saprotrophic mushrooms occurred in the Late Paleozoic as terrestrial forests of seed plants (spermatophytes) started to dominate the landscape. The subsequent diversification and explosive radiation of Agaricomycetes, and eventually of ectomycorrhizal mushrooms, were associated with the evolution of Pinaceae in the Mesozoic, and the establishment of angiosperm-dominated biomes in the Cretaceous, respectively.

T2-42-05

Before angiosperms: Insect pollination during the Mid Mesozoic

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Gymnosperm-dominated floras of the mid-Mesozoic world were significantly different from the angiosperm-dominated floras that succeeded them. Diverse assemblages of Late Jurassic to Early Cretaceous gymnosperm groups were morphologically disparate at the highest taxonomic levels and included cycads, ginkgophytes, corystosperms, caytonialeans, bennettitaleans and gnetophytes that largely were replaced by the angiosperms that originated during the Early Cretaceous and continued to expand throughout the mid Cretaceous. Similarly, insect faunas occurring on gymnosperms previous to the advent of angiosperms were taxonomically distinct from those later associated with angiosperms. Much of the ecologic structure of these two evolutionary biotas can be gleaned from the myriad of plant-insect interactions present as pollination, herbivory and mimicry found in gymnosperm-rich deposits that essentially lack angiosperms. Such gymnosperm-rich compression-impression deposits include the Jiulongshan Formation, of latest Middle Jurassic age (about 165 million years old) in Inner Mongolia, and the Yixian Formation, of mid Early Cretaceous age (about 125 million years old) in Liaoning, both from Northeastern China. Other examples of well-preserved deposits include three, spectacularly preserved amber deposits of earlier Cretaceous age from Lebanon, Myanmar and Spain that range in age from 125 to 99 million years, spanning much of the Early Cretaceous. These compression-impression and amber deposits harbor many important plant-insect associations of the pre-angiosperm Mesozoic and provide fundamental insights into a lost world of plant and insect evolutionary and ecological convergences that parallel the analogs of today. The only difference is that the plant and insect interactors are taxonomically distinct while the associations--involving plant and insect morphologies, nectar and pollen rewards, mouthpart structure and functional feeding groups--are virtually identical. Four major insect pollination modes have been documented on gymnosperms during the pre-angiosperm Mesozoic. These documented gymnosperm-insect pollination modes include the four pollination modes of: (i) the thrips pollination mode consisting of mouthcone mouthparts engaged in punch-and-sucking feeding, pollen rewards and occurrences on ginkgoalean hosts; (ii) the beetle pollination mode bearing mandibulate mouthparts, a chewing feeding style, pollen rewards and hosted by cycads; (iii) the long-proboscid pollination mode found in scorpionflies, lacewings, flies and moths that possess a siphonate feeding style of imbibing pollination-drop fluids from deep-throated or tubular ovulate fructifications; and (iv) the labellate pollination mode made possible by the expanded labellum of brachyceran flies that fed by sponging from specialized ovulate cones of cheirolepidiaceous conifers. These pollination modes also exhibited four evolutionary-ecological patterns during the Aptian-Albian Gap in which the gymnosperm-to-angiosperm transition occurred. These changes included: early pollinator lineages associated with gymnosperms that became extinct; other gymnosperm-associated lineages that remained on gymnosperm hosts but significantly decreased in diversity, some surviving to the present day; still other gymnosperm-associated lineages that transitioned onto angiosperms via plant-host shifts; and later evolving pollinator lineages that originated with angiosperms, lacking any gymnosperm affiliation in their histories. This combination of several major pollinator modes and patterns of evolutionary-ecological change essentially resulted in the modern insect pollinator fauna.

T2-42-06

Embryophyte evolution as the main driver of global climate change and mass extinctions during the mid-Paleozoic Era

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The evolution of embryophytes over an extended, ~100-Myr-long interval of the mid-Paleozoic Era may have been the most important factor in driving contemporaneous climate change and triggering global biotic crises. The role of early vascular plants in elevating rates of burial of organic carbon, drawing down atmospheric CO_2 levels, and triggering a major glaciation during the Devonian Period was recognized twenty years ago (*Algeo, T.J. and Scheckler, S.E., 1998. Terrestrial-marine teleconnections in the Devonian: links between the evolution of land plants, weathering processes, and marine anoxic events. Philosophical Transactions of the Royal Society of London B: Biological Sciences, 353:113-130*). More recently, attention has shifted toward the possible role of early pre-vascular (bryophyte-grade) land plants in drawing down atmospheric CO_2 levels and triggering global cooling during the mid- to late Ordovician. As the record of fossil discoveries of the earliest land plants improves, evidence for such connections is accumulating. Both the Ordovician and Devonian events had profound effects on contemporaneous marine faunas, although of differing nature. The Ordovician cooling trend initially promoted increased animal diversification, probably due to invigorated oceanic circulation, improved ventilation, and enhanced nutrient upwelling and productivity. As marine productivity and organic carbon burial peaked in the late Ordovician, however, 'climatic overshoot' resulted in a hypercooling event (the Hirnantian glaciation at ~445 Ma) that led to a global mass extinction (the first of the so-called 'Big Five' mass extinctions of the Phanerozoic). The Devonian cooling trend occurred in a stepwise manner over an interval of ~30 Myr that resulted in repeated second-order biotic crises, culminating in the end-Devonian Hangenberg mass extinction at ~360 Ma (*Algeo, T.J., Berner, R.A., Maynard, J.B. and Scheckler, S.E., 1995. Late Devonian oceanic anoxic events and biotic crises: "rooted" in the evolution of vascular land plants. GSA Today, 5 (3): 45,64-66*). In this presentation, I will review the record of embryophyte evolution from the Ordovician through Devonian periods as well as contemporaneous changes in global climate, biodiversity, and marine and terrestrial environmental conditions. I will propose that embryophyte evolution was the key driver of global changes during a ~100-Myr-long interval of the mid-Paleozoic, and that long-term climatic cooling driven by expansion of terrestrial floras was ultimately responsible for setting the stage for the Permo-Carboniferous Ice Age, the most severe and most protracted (~350-290 Ma) glacial event of the Phanerozoic. The influence of embryophytes on global events was exceptional during the mid-Paleozoic because it represented a transition from a largely unvegetated to a fully vegetated world. Since the Devonian, global plant biomass has probably varied little (despite major changes in its composition, such as the Cretaceous advent of angiosperms), and, thus, land plants have not continued to have an outsize influence on global events.

T2-43: Natural history of *Ginkgo biloba* and its interaction with human

T2-43-01

Incongruent range dynamics between co-occurring Asian temperate tree species facilitated by life history traits - *Ginkgo biloba* as an example with new insights from plastid and mitochondrial genomes

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Postglacial expansion to former range limits varies substantially among species of temperate deciduous forests in eastern Asia. Isolation hypotheses (with or without gene flow) have been proposed to explain this variance, but they ignore detailed population dynamics spanning geological time and neglect the role of life history traits. Using population genetics to uncover these dynamics across their Asian range, we infer processes that formed the disjunct distributions of Ginkgo biloba and the co-occurring Cercidiphyllum japonicum (published data). Phylogenetic, coalescent, and comparative data suggest that Ginkgo population structure is regional, dichotomous (to west–east refugia), and formed ~51 kya, resulting from random genetic drift during the last glaciation. This split is far younger than the north–south population structure of Cercidiphyllum (~1.89 Mya). Significant (recent) unidirectional gene flow has not homogenized the two Ginkgo refugia, despite 2 Nm > 1. Prior to this split, gene flow was potentially higher, resulting in conflicting support for a priori hypotheses that view isolation as an explanation for the variation in postglacial range limits. Isolation hypotheses (with or without gene flow) are thus not necessarily mutually exclusive due to temporal variation of gene flow and genetic drift. In comparison with Cercidiphyllum, the restricted range of Ginkgo has been facilitated by uncompetitive life history traits associated with seed ecology, highlighting the importance of both demography and lifetime reproductive success when interpreting range shifts. Additional evidence comes from plastid and mitochondrial genome sequence data highlighting diversification during the Late Pleistocene.

T2-43-02

Demographic history of *Ginkgo bioloba* revealed by population genomics

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Ginkgo biloba L. (Ginkgoaceae) is one of the best-known and most distinctive trees worldwide, representing one of the four lineages of extant gymnosperm without living relatives. It is a living fossil that has been essentially unchanged in gross morphology for more than 200 million years. It survived glaciations as a relic in China followed by a human-aided global redistribution, and thus provides an inspiring example of how human can help a species survive and renew. Ginkgo's resilience has enabled it to become widespread and popular across the world. This resilient tree demonstrates an outstanding resistance or tolerance to both herbivores and pathogens, accounting in part for the longevity of the individual tree and also, in turn, for the longevity of this species. Despite substantial advances in recent two decades, knowledge gaps remains for ginkgo's natural history followed by human-assisted introduction. As a follow-up project of *de novo* sequencing of ginkgo genome (10.61 Gb), we resequenced the genomes of 300 big ginkgo trees selected from the evidenced refugial populations and introduced populations to 5× raw data coverage using BGISEQ-550 platform. We will investigate genome-wide variation patterns in ginkgo populations and will obtain a tremendous volume of high-quality single nucleotide polymorphisms (SNPs) after excluding sites with missing data in any accession. Using these population SNP data, we will be able to reconstruct the comprehensive picture of demographic and introduction history of ginkgo populations in China and to unveil the relevant mechanisms. Genes with significantly differential diversity can be identified in diverse populations, which might represent candidate regions undergoing selection either naturally or during introduction/ domestication. These genes will greatly facilitate the identification of functional variations and agronomically important genes. Dense variation data will also be useful for marker-assisted resource evaluation, breeding and gene mapping of ginkgo. The present study will provide a milestone case study of population genomics for non-model organisms in particular with large genomes.

T2-43-03

Strategies and approaches for population genome resequencing of non-model plants: Implication of genome rese-

quencing of ginkgo populations

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The "living fossil"*Ginkgo biloba* L. (Ginkgoaceae) is the sole extant species representing one of the four lineages of gymnosperm with a large genome (~10.61 Gb) composing of ~70% repeat sequences. Resequencing of ginkgo populations will be another milestone in illustrating population structure, demographic history, adaptation, secondary metabolism and origin of important traits (seed, pollen tube, carpel, sexuality and *etc.*) of ginkgo. Here, we elaborately selected ginkgo accessions from all over the world to perform whole-genome re-sequencing using BGISEQ-500 sequencing platform. To our knowledge, this is the first case study of genome resequencing on non-model plants with such large and complicated genome, which challenging our ability of handling enormous sequencing data. By using BGISEQ-500 along with efficient alignment and variants calling algorithm, we dramatically reduced time consuming and resource dependency. High-quality population-level genetic markers (SNP, INDEL, SVs) were generated for identification of genetic structure, demographic history and local adaptation. Combining phenotype information and genome-wide association study (GWAS), we further identified candidate region controlling important traits, especially dioecious reproduction. This study highlights the effectiveness and efficiency of the strategies and approaches which were successfully applied in population re-sequencing of ginkgo genomes, shedding new light on re-sequencing large and complex genomes of non-model plants.

T2-43-04

Regional characteristics in genetic variation and introduction history of *Ginkgo biloba*, inferred using giant old trees in Japan

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Ginkgo biloba, which is originated in China, is one of common planted tree species in Japan. In Japan, the species is spread by people and mainly planted in temples and shrines, it means that the species was introduced by association of buddhists. It is considered that initial introduction of the species into Japan had occurred during Asuka (ca. 600-700 A.D.), Kamakura (ca. 1190-1330 A.D.) or Muromachi (ca. 1400-1570 A.D.) period in the history. In the present, 70 giant old trees (over 10m in girth at breast height, GBH) live in Japan. Because *Ginkgo biloba* is long-lived, these giant-old trees are considered to be residuals in initial introduction stage into Japan. Therefore, these trees are considered to be representatives of genetic variations in the initial introduction. Here, we collected 272 samples from giant old trees, which are over 8 m in GBH, from Kyushu to Tohoku regions in Japan and analyzed using eight nuclear microsatellites previously developed to understand characteristics and history of the introduction for *Ginkgo biloba* into Japan.

T2-43-05

Gingo biloba, its close relationship with people

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Environment degradation has grown in recent decades because increased human population density, which has influenced the biological evolution in a way. The famous relic plant, *Ginkgo biloba*, is a representative example for this case. However, in another way, *Ginkgo biloba* influences human life in many aspects, such as, culture, foods, and medicine. In this study, based on the evidences from population demography, biological geography, and genetic information, we explored how *Ginkgo biloba* dispersed into the world from its wild population in eastern China and how it was used by human being. The results from this study will be useful to preserve the green earth and ourselves. Also, the methods used in this study will be extended in other fields.

T2-43-06

Application of Gydle bioinformatics tools to *Ginkgo* organellar studies

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The bioinformatics methods and software developed by Gydle allow the analysis of complex genomes using NGS data. These tools enable the genome assembly of genetically diverse organisms, and the study of genomic elements with different evolution dynamics across populations and species. We present a Ginkgo plastid genomes study demonstrating high-throughput tools for the assembly of complete chloroplast genomes from total DNA sequence data in plants with large nuclear genomes. The finished-level quality of assemblies provides unprecedented resolution for phylogeography analyses. We show that Ginkgo plastid genomes exhibit extremely low levels of sequence diversity, and present methods to identify often-overlooked sequencing and assembly artifacts in order to increase phylogenetic signals. The assembly of high-quality and complete plant mitochondrial genomes from total DNA sequence data is a more challenging task, owing to large variations in size (typically 300 kbp to 10 Mbp), the potential for recombination, repeat expansion and exchange with the plastid genome, and the different evolution dynamics of core genes with regard to other mitochondrial genome regions. Our results on Ginkgo mitochondrial genomes will be shown as well as comparisons between mitochondrial and plastid phylogenetic analyses. For large genomes such as Ginkgo, we present novel methods enabling the study of the gene space by combined assembly of RNA-Seq and DNA-

Seq data, and the application of comparative genomics based on simultaneous analysis of allelic diversity and copy number.

T2-44: Building and exploring the green plant tree of life (two sessions)

T2-44-01

Consensus and conflicts in genome and gene histories across the green tree of life

Jim Leebens-Mack
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Since their origin approximately one billion years ago, green plants (Viridiplantae) have formed the foundation for energy acquisition across the vast majority of ecosystems on our planet including marine, fresh water, terrestrial and agricultural systems. Great progress is being made on understanding ecological, molecular, biochemical and taxonomic diversity in green plants. Here we present a comprehensive phylogenomic estimation of relationships and patterns of diversification across the Viridiplantae. Analyses of nearly 400 universally single copy nuclear genes sampled from transcriptomes and available genomes for nearly 1,200 species representing all extant green plant diversity has yielded a robust phylogenetic inference for the evolutionary history of the Viridiplantae including repeated bouts of rapid diversification associated with developmental and ecological innovations. These diversification events are marked by a high degree of discordance between gene tree and estimated species trees. The implications of our phylogenetic inferences and gene tree - species tree discordance will be discussed.

T2-44-02

Plastome phylogenomics and evolution of the monocots

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Phylogenetic analyses of just a handful of genes provided an initial division of ca. 83 monocot families into 12 orders, but failed to provide decisive evidence regarding the relationships of several of these orders relative to each other (e.g., commelinids, Asparagales, Liliales) and the relationships of many families to each other within most of the orders. Here we present a phylogenomic analysis of relationships among monocot families and orders based on aligned sequences of 75 genes from the plastid genome and of entire plastomes for more than 350 placeholders. These analyses resolve relationships among all monocot families and orders with strong support for the first time and provide a backbone phylogeny on which to base many future studies of character-state evolution, ecological radiation, historical biogeography, and determinants of net rates of species diversification. Remarkably, however, even entire plastomes lack sufficient information to resolve a few deep but short branches (e.g., within Zingiberales) with 100% bootstrap support. Plastome-scale data also provide strong support for the placement of many mycoheterotrophic taxa; these have proven highly problematic in the past, due to loss of several plastid genes and greatly accelerated evolution of many others. A supermatrix approach extends the plastome backbone to produce a monocot phylogenetic hypothesis including placeholders for most monocot genera. Supermatrix phylogenies provide a means for testing several hypotheses regarding the drivers of extraordinary diversification in orchids, and for reconstructing historical biogeography in orchids and the order Liliales. In several orders, phylogenomic analyses point to biogeographic origins in Gondwana, with inferred overland dispersal via Antarctica when that continent had a subtropical climate and was connected to the Australian Plate and South America.

T2-44-03

From cacti to carnivores: A genome-wide perspective on Caryophyllales phylogeny

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The Caryophyllales comprise approximately 6% of angiosperm species diversity (approximately 12,500 spp. in 40 families) and are found on all continents and in all terrestrial ecosystems, from tundra to tropical rainforests, but are particularly diverse and abundant in semiarid, saline, and temperate environments. In conjunction with this ecological variation, the clade exhibits extreme life history diversity, ranging from tropical trees, temperate annual herbs, and long-lived succulent cacti to a diverse array of carnivorous plants, including sundews and epiphytic pitcher plants. This remarkable diversity has fostered a long history of research, making Caryophyllales one of the best-characterized major angiosperm clades with respect to morphology, ecophysiology, and life history. For example, the Caryophyllales exhibit repeated origins of such fundamental morphological and ecophysiological traits as a differentiated perianth, betalain and anthocyanin pigmentation, C4 and CAM photosynthesis, and leaf and stem succulence. Hence, resolving the backbone phylogeny of Caryophyllales and its constituent families with confidence would provide the necessary framework for future comparative evolutionary analyses of these and other key traits. Recent multigene phylogenetic analyses have helped to clarify the backbone relationships among many Caryophyllales lineages, but the core Caryophyllales in particular contain several relatively ancient and apparently rapid radiations that have so far proved difficult to resolve (e.g. early core Caryophyllales, early Portulacineae, and early phytolaccoids). To resolve these relationships, we employed transcriptome sequencing to generate a large data set of over 150 transcriptomes for key Caryophyllales taxa. Phylogenetic analyses of these data have helped to resolve the backbone relationships of Caryophyllales while simultaneously revealing high levels of gene tree incongruence at certain key nodes.

T2-44-04

Deciphering the genetic and genomic basis of extreme plant adaptation in the Caryophyllales

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Modern transcriptomic and genomic techniques offer the ability to explore the patterns and drivers of plant diversification on a scale never before possible. The plant order Caryophyllales contains ~12,500 species in 40 families with an estimated crown age of approximately 67–121 Ma. Species of the Caryophyllales are found on every continent including Antarctica, and in all terrestrial ecosystems as well as aquatic systems. Familiar members of the group include cacti, diverse array of carnivorous plants (e.g., the sundews *Drosera* and pitcher plants Nepenthes), and a number of crop plants (e.g. beet, spinach, and quinoa). Using a data set of genomes and transcriptomes from over 300 species across the Caryophyllales we found evidence for at least 21 ancient genome duplication events, which is more than previously known in all of angiosperms. From the data set of >15,000 genes per species, we discovered that many gene duplication events were associated with key adaptive trait changes, such as evolution of pigmentation and plant carnivory. Our work integrates not only new molecular phylogenomic and computational methods, including several developed for this study, but also field- and collection-based research to connect rapid phenotypic shifts to their genetic and genomic bases.

T2-44-05

Complete plastid genome sequences and the green plant tree of life

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The plastid genome has a long history of use in plant molecular systematics. From the initial single gene *rcb*L studies in the early 1990s through today, the plastid genome has served as the workhorse of plant molecular systematics. With the advent of second generation sequencing technologies, sequencing of entire plastid genomes for large numbers of taxa has become feasible. We report on the phylogeny of 1,899 green plant taxa using complete plastid gene sequences. Overall support of the phylogeny, as assessed by bootstrap analysis, is high with many historically problematic nodes receiving high support, and the relationships supported by the tree consistent with previous hypotheses based on plastid data. Our analysis supports previous findings of the Zygnematales as sister to the land plants. We recover a monophyletic bryophte clade, with mosses, liverworts and hornworts sister to the vascular plants. Within the gymnosperms, we recover support for the Gne-Cup hypothesis. *Amborella* alone is recovered as sister to the remaining angiosperms, followed by Nymphaeales, Austrobaileyales, and Chloranthales and Magnoliales. In addition to reviewing the topology of the plastid phylogeny, we will make comparisons to a comparable nuclear gene-based phylogeny and discuss the challenges of filtering transcriptome data to generate reliable sequences.

T2-44-06

Rosaceae nuclear phylogeny and fruit type evolution in the context of geological times and genome duplication

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Fruits are the defining feature of angiosperms, likely have contributed to angiosperm successes by protecting and dispersing seeds, and provide foods to humans and other animals, with many morphological types and important ecological and agricultural implications. Rosaceae is a family with ~3,000 species and an extraordinary spectrum of distinct fruits, including fleshy peach, apple, and strawberry prized by their consumers, as well as dry achenetum and follicetum with features facilitating seed dispersal, excellent for studying fruit evolution. To address Rosaceae fruit evolution and other questions, we generated 125 new transcriptome and genomic datasets and identified hundreds of nuclear genes to reconstruct a well-resolved Rosaceae phylogeny with highly supported monophyly of all subfamilies and tribes. Molecular clock analysis revealed an estimated age of ~101.6 Mya for crown Rosaceae and divergence times of tribes and genera, providing a geological and climate context for fruit evolution. Phylogenomic analysis yielded strong evidence for numerous whole genome duplications (WGDs), supporting the hypothesis that the apple tribe had a WGD and revealing another one shared by fleshy fruit-bearing members of this tribe, with moderate support for WGDs in the peach tribe and other groups. Ancestral character reconstruction for fruit types supports independent origins of fleshy fruits from dry-fruit ancestors, including the evolution of drupes (e.g., peach) and pomes (e.g., apple) from follicetum, and drupetum (raspberry and blackberry) from achenetum. We propose that WGDs and environmental factors, including animals, contributed to the evolution of the many fruits in Rosaceae, which provide a foundation for understanding fruit evolution.

T2-44-07

Phylogenomics of angiosperms from the 1KP Consortium

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The 1KP initiative provides transcriptome resources and phylogenetic inference across the approximately half a billion species of *Viridiplantae*. Angiosperms represent ~3/4 of *Viridiplantae* and account for ~2/3 of the transcriptomes assembled by 1KP. Phylogenomic analyses using a supermatrix comprising ~800 species of angiosperms and over 400 nuclear genes provide a comprehensive view of evolutionary history from the perspective of the nuclear genome. Notable phylogenetic results include: *Amborella* sister to all other extant angiosperms, with subsequent divergence of Nymphaeales and Austrobaileyales prior to the radiation of *Mesangiospermae*, within which relationships remain poorly resolved and/or supported. Ceratophyllaceae are sister to all other mesangiosperms, with monocots sister to a clade of magnoliids + (Chloranthales + eudicots), but with weak support. Within magnoliids, there is strong support for Magnoliales + Laurales sister to Piperales + Canellales. Relationships among basal eudicots are not strongly supported, but Trochodendraceae are sister to Buxaceae + core eudicots. Within core eudicots, there is general support for *Superrosidae* and *Superasteridae*, although the placement of Santalales within *Superrosidae* requires further attention. As found previously with nuclear data, the COM clade of *Rosidae* is sister to malvids, whereas nearly complete plastomes place it with fabids. Other key points of angiosperm phylogeny will be discussed and comparisons made between nuclear and plastid trees. The transcriptome resources of 1kp can be used in a variety of ways, at both deep and shallow evolutionary scales, and provide for discovery of putatively single-copy nuclear genes for use in phylogeny reconstruction of other groups.

T2-44-08

Phylogenomic analysis of plastid genomes confirmed the position of Mitrastemonaceae and revealed rapid radiation of Ericales

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Ericales is a highly morphologically heterogeneous order sister to Euasterids in the largest Asterids clade in angiosperms. It comprises 22 families including a parasite family Mitrastemonaceae. Previous molecular studies have circumscribed some major clades of the order, but deeper nodes are largely unresolved, and Mitrastemonaceae has never been incorporated in phylogenetic analyses of Ericales. A total of 105 plastid genomes covering all families of Ericales were obtained here to explore the interfamilial relationships in the order and ensure the systematic place of Mitrastemonaceae. Our results showed that the plastid genome of Mitrastemonaceae was highly reduced at a size of ~26 kb and lack of inverted repeat region compared to other members of Ericales. Fourteen conserved protein coding genes and four rRNA genes found in plastome of Mitrastemonaceae were selected in all plastomes of Ericales and other 32 orders of Eudicots to verify the position of Mitrastemonaceae. Results revealed that the parasite family was placed in Ericales, but the interfamily relationship was uncertain. Phylogenetic relationships within Ericales were reconstructed using Maximum likelihood method base on 83 genes of 105 Ericales species with outgroups of 29 Cornales and three Caryophyllales. Some strongly supported clades were resolved: (1) Marcgraviaceae- (Tetrameristaceae-Balsaminaceae) was sister to the remaining families of Ericales; (2) Polemoniaceae-Fouquieriaceae subsequently formed a clade sister to the remainder of the order; (3) (Primulaceae-Ebenaceae)-Sapotaceae; (4) Pentaphylacaceae-Sladeniaceae; (5) ((Actinidiaceae-Roridulaceae)-Sarraceniaceae)- ((Ericaceae-Cyrillaceae)-Clethraceae); (6) (Styracaceae-Diapensiaceae)-Symplocaceae. Phylogenetic relationships/position of some families and clades were unresolved. The systematic position of Mitrastemonaceae and Lecythidaceae within Ericales was also not fully confirmed in this study. Very short internal branches of families in the phylogenetic tree may indicate a rapid radiation diversification of Ericales, which posing a particularly difficult challenge for phylogenetic inreference of Ericales.

T2-44-09

Revisiting the relationships among bryophyte lineages with increased taxon and gene sampling, and phylogenomic approaches

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Bryophytes comprise three monophyletic lineages: the mosses, liverworts, and hornworts. The extant members of one of these lineages, or some combination of the three, represent the sister group to the vascular plants and, as such, play a key role in understanding the evolution of terrestrial plants approximately 480 million years ago. Several molecular phylogenetic studies using chloroplast genomes and a limited set of nuclear genes in the 1990s and early 2000s led to the consensus view that bryophytes comprise a paraphyletic grade with liverworts and mosses successively sister to a clade composed of hornworts and vascular plants. However, alternative topologies have been proposed and supported by both molecular and morphological data sets. More recently, phylogenomic studies employing hundreds of nuclear markers have suggested that the consensus view of successive sister groups might not represent the true relationships, with growing support for the monophyly of mosses and liverworts, with the hornworts remaining somewhat unstable in their placement. Here, we discuss past, current, and ongoing efforts to reconstruct bryophyte relationships, with an emphasis on understanding conflicting phylogenetic signal among genes and datasets.

T2-44-10

Completing the plant tree of life

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Evolutionary trees are powerful tools for prediction, species discovery, monitoring and conservation. Through comparative analysis of DNA sequence data, the backbones of the plant and fungal trees of life are relatively well understood, and many subcomponents have been studied in great detail. However, DNA data are still lacking for numerous genera and the vast majority of species of plants and fungi, preventing their accurate placement within this evolutionary framework and hindering downstream science. To better understand how the world's plants and fungi are related to each other and how they have evolved, we have initiated a project at the Royal Botanic Gardens, Kew to complete the Plant and Fungal Trees of Life (PAFTOL). We will utilise our collections and work with our collaborative networks to produce extensive new DNA sequence data (whole plastid genomes, 100s of nuclear loci) for a representative species from each genus of plant and fungus using high-throughput sequencing technologies. This comprehensive investigation of phylogenetic relationships will be a rich resource enabling the discovery and study of evolutionary patterns in the plant and fungal kingdoms, and will provide a unifying framework for comparative research. The project is an essential step towards the compilation of genomic data for all known species. This talk will focus on the plant tree of life component of the project (focusing on angiosperms), highlighting current progress in sampling, phylogenomic methodologies, bioinformatic approaches and collaborative opportunities.

T2-44-11

Tree of Life: China Project

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China is ranked among the top six megadiverse countries of the world and has 31,362 species of vascular plants belonging to 3,328 genera and 312 families. We herein reconstructed the Tree of Life of the Chinese vascular plants at the generic level based on five molecular markers by sampling 6,098 species (representing 93% vascular genera from China). The comprehensive large phylogeny supports most relationships among and within families recognized by recent molecular phylogenetic studies for lycophytes, ferns (monilophytes), gymnosperms, and angiosperms. For angiosperms, most families in Angiosperm Phylogeny Group IV are supported as monophyletic, except for a paraphyletic Dipterocarpaceae and Santalaceae. The infrafamilial relationships of several large families and monophyly of some large genera are well supported by our dense taxonomic sampling. To facilitate further application of such large-scale phylogeny to other biology fields, the SoTree (http://www.darwintree.cn/flora/index.shtml) software was introduced to enable the efficient generation of the phylogenetic trees by providing sub-datasets with interested species lists for studies concerning the origin, ecology, and biogeography of the local flora in China. On the basis of a robust phylogenetic framework, we further investigate the temporal divergence patterns of the Chinese angiosperm flora using a dated mega-phylogeny. Our results showed that the majority of Chinese generic flora (61%) originated during the Neogene and Quaternary, and the remaining genera diverged in the Paleogene (32%) and the Cretaceous (7%). We found evidence for an older divergence history in East China with higher phylogenetic diversity (PD), while a younger divergence history in West China with lower PD. Our data suggest that West China represents an evolutionary cradle while East China is a museum for Chinese angiosperm genera.

T2-44-12

Evolution of genus *Ludwigia* L. (Onagraceae) and polyploid evolution of section Macrocarpon

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Ludwigia L. (Onagraceae) is a worldwide wetland genus of plants that includes 82 species in 23 sections. This genus has a wide distribution, with endemic groups of species in North and South America, Africa, and Asia. Several species are of medicinal or horticultural interest; some are invasive weeds in wetlands worldwide. With a comprehensive multilocus phylogenetic analysis representing 73% of species in *Ludwigia*, we have tested the current classification of the genus and its validity as a reflection of the phylogenetic relationships of the species. Our results suggest that the North Temperate Haplostemonous (NTH) group, the *Microcarpium* complex, and sections *Ludwigia*, *Jussiaea*, and *Macrocarpon* are monophyletic clades, while others, including the largest section, *Myrtocarpus*, are paraphyletic and/or polyphyletic. Our results indicate that hybridization and polyloidy have each played important roles in the evolutionary history of the genus. We discuss the origins of several polyploid taxa. Moreover, based on our multilocus phylogeny, the divergence time estimations suggest that sect. *Ludwigia* and the *Microcarpium* complex split at ca. 26.7 mya during the late Oligocene Period of the Paleogene Era, sect. *Jussiaea* diverged ca. 41.0 mya during the middle Eocene, and the crown age of sect. *Macrocarpon* is ca. 11.5 mya during late Miocene. Furthermore, ancestral range estimations show that the NTH clade likely originated in North America, while other taxa originated in Central or South America, followed by several independent dispersal events to the Old World. The ancient state reconstruction analyses support the following character state pairs as plesiomorphic/derived: diplostemony/haplostemony, seeds free/seeds embedded, pluriseriate seeds/other patterns, diploid/polyploid, and tetramery/pentamery or more. We explored polyploid evolution of section *Macrocarpon* as a model for the genus, using both genome skimming and target enrichment methods. This section contains two diploids (*L. bonariensis*, *L. lagunae*) and one tetraploid (*L. neograndiflora*), all with restricted distributions; a fourth polyploid, *L. octovalvis*, distributed throughout the warmer regions of the world. Phylogenies based on organelle genomes together with 32 enriched putative nuclear single copy genes suggest that *L. lagunae* is closely related to *L. bonariensis,* and *L. bonariensis* may have contributed a genome to the tetraploid *L. neograndiflora*. A series of Mexican diploid populations grouped within *L. octovalvis* deserve taxonomic recognition. Hexaploid *L. octovalvis* likely arose from tetraploid strains through the functioning of an unreduced gamete. The methodology, involving organelle genomes and nuclear single copy genes, that we have used in this study should be useful to further studies of *Ludwigia* and other polyploid taxa.

T2-45: Plant genome size diversity and evolution: From genomics to ecology

T2-45-01

Plant genome size diversity: Towards a comprehensive understanding across angiosperms

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In recent years, research into genome size evolution in land plants has increased significantly, uncovering a huge diversity across different taxonomic lineages. Despite such interest and the vast amount of data now available (> 9,500 species collated in the Plant DNA C-values database (http://data.kew.org/cvalues/), many plant families are still lacking a single estimate. Certainly, such taxonomic gaps are particularly notably in angiosperms, where nuclear DNA contents are only available for c. 2,095 genera out of the c. 13,000 genera currently recognised and > 9000 species (c. 2.5% of the c. 352,000 species recognised). At the family level, this translates into estimates for about 262 families out of 416 (*sensu* the Angiosperm Phylogeney Group - APG IV*), i.e. nearly 62% of the currently accepted diversity. With the currently available data, we have been able to unveil a staggering diversity of nuclear DNA contents in angiosperms that spans up to 2400-fold. Notwithstanding, the question still remains as to (i) what is the full extent of diversity of genome sizes in angiosperms? and (ii) what is yet to be uncovered? In order to bring further robustness to our current understanding of angiosperm genome size diversity, we have set up a project aimed at filling most –if not all- the taxonomic gaps, starting at the family level. In the age of high throughput sequencing technologies, C-values provide important baseline information not only for genomic sequencing projects, but also to shed light on why such genome size diversity exists and what impact does it have on plant evolution. Our goal is to provide at least one estimate per family recognised by APG IV, focusing our efforts on those lineages largely unstudied. So far we have obtained first C-values for 216 genera, which represent new additions to 82 families that previously lacked any genome size information. These data have already highlighted the existence of plant lineages with distinctive genome size profiles and contribute towards our knowledge of how ups and downs of genome size evolution in plants, may be linked to and impact on the diversity of intrinsic and extrinsic processes which are shaping plant genome diversity.

T2-45-02

Ecological consequences of angiosperm genome size under macronutrient limitation and herbivory stress.

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Genome size (GS) is a fundamental trait influencing cellular, developmental and ecological parameters in angiosperms. There is huge variation in GS among species, yet most genomes are small despite the prevalence of polyploidy in many angiosperm lineages. Nucleic acids are amongst the most nitrogen (N) and phosphate (P) demanding molecules, and GS may thus influence a plant's nutrient demands and its ability to grow in environments where N and P are limited. We first test the hypothesis that large genome sizes are costly to build and to maintain by analysing the impact of N and P treatment on the abundance of angiosperm species with a range of genome sizes, ploidy levels, and competition strategies in natural plant communities at the Park Grass Experiment (Rothamsted, UK), the world's longest running ecological experiment. We further test whether GS influences how species and plant communities respond to herbivore pressure in long-term exclusion experiments at Silwood Park (Imperial, UK). Support for macronutrient limitation of plants with larger GS was found in the Park Grass Experiment, where plants with large genomes (e.g. 1C-value > 5 pg) are under greater macronutrient limitation in comparison to plants with small genomes. Polyploidy further influences the abundances of plants with large GS. In terms of herbivory, the key finding is a highly significant negative association between GS and rabbit herbivory. Our results suggest that ploidy and genome size are functional traits which contribute to how plants respond to nutrient and predation stress, thus influencing plant abundances, community composition, and hence, potentially food web and nutrient cycling dynamics.

T2-45-03

Population perspectives on genome size evolution through polyploidy: Dynamics of unreduced gametes and phenotypic divergence within species

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Variation in genome size, achieved through multiplication of genome copy number (i.e. polyploidy), is widespread in plants and represents a unique source of phenotypic and genomic novelty with important implications for adaptation and speciation. Biologists have long sought to explain the prevalence of polyploidy by studying its genetic, genomic, and phenotypic consequences. Ultimately, however, the influence of these mechanisms on genome size evolution must be interpreted in terms of their impact on key population processes (formation, establishment, persistence). This perspective is lacking for all but a few species despite the abundance of intraspecific variation. Here, I present insights into two aspects of the population biology and evolutionary dynamics of polyploidy: 1) polyploid formation via unreduced gametes, and 2) polyploid establishment via rapid phenotypic and ecological divergence. Polyploid formation is governed by the frequency and distribution of unreduced gametes, and mating patterns within populations, neither of which are well understood in natural populations. Using new techniques in flow cytometry and high throughput screening of gametes across multiple species, I identify factors governing the persistence of 2n gametes and enhancing the likelihood of polyploid formation. In contrast to unreduced gametes, the role of phenotypic divergence in polyploid establishment is well recognized; however, the immediate effects of genome duplication on phenotype, and the factors determining the magnitude of divergence are limited to only a few cases. I will present recent evidence from two species (*Chamerion angustifolium, Arabidopsis thaliana*) that suggest the effects of genome doubling are highly variable within species but dependent on progenitor genotype and phenotype. These results suggest that the phenotypic impact of genome multiplication and therefore the potential for polyploid establishment may be non-uniform within species and across the geographic range. Collectively, this research highlights the importance of a population perspective when explaining the prevalence and uneven distribution of genome size via genome multiplication. Through further work, we hope to better understand why some taxa are predisposed to polyploid evolution and others not; and under what conditions new polyploids will arise, establish and evolve.

T2-45-04

Retrotransposons and whole genome duplications shape genome (size) variation

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The dynamics of retrotransposons (RT) and whole genome duplications (WGD) are major molecular drivers of genome size, but their causes and consequences among plant populations deserves further attention. As reported in both autopolyploid Buckler Mustards and allopolyploid wild wheats, hybridization coupled with WGD induces conflicting interactions among RT families and promotes their proliferation. The prevalence of gene flow as a proximal cause of RT dynamics and genome size variation among plant populations can now be addressed with adequate tools. Available evidence further indicates that RT proliferation supports genome fractionation and species diversification, but the relationship with genome size variation and to what extent this is non-neutral has to be elucidated. Here, I highlight recent insights on interactions between RTs and duplicated genes during the colonization of stressing environments, and discuss how pseudogenisation and innovation may impact on genome trajectories towards either downsizing or obesity.

T2-45-05

Repeat and genome size dynamics in *Nicotiana* polyploids of different ages

Steven Dodsworth

Royal Botanic Gardens, Kew

Genome size in most angiosperms is dictated by changes in repetitive element (repeat) abundance, that often can constitute a majority of nuclear genomic DNA (gDNA). The other main mechanism by which plants increase their total gDNA is through rounds of polyploidy, or whole genome duplication. Post-polyploidisation, however, changes also occur to the repeat content of genomes, and this can be dramatic. In the genus *Nicotiana* (Solanaceae) polyploidy is pervasive, with allotetraploids being formed recurrently over different timescales and with alternative parental lineages. This sets *Nicotiana* as an excellent model system in which to look at changes in genome size and structure post-polyploidisation. Here I present the results of high-throughput sequencing across the genus in order to compare repeat profiles and genome size dynamics. In young allopolyploids formed less than 1 Mya (e.g. *N. rustica* or *N. tabacum*) the polyploid genome is almost additive for its two putative parental subgenomes, and this is also reflected in repeat dynamics. However, for much older allopolyploids (c. 4-7 Mya) genome downsizing is the norm, and concurrent with this is much restructuring of the repetitive fraction of the genome. In all cases there also seems to be significant bias as to gDNA loss, in favour of retaining maternal DNA, i.e. as predicted by the cytonuclear discordance hypothesis. In summary this presents a timescale for the diploidisation process that occurs in most angiosperms at some point following rounds of polyploidy, where genomes are restructured and streamlined to resemble their diploid ancestors.

T2-45-06

Influence of genome size on stomatal size and photosynthetic efficiency in *Fritillaria* (Liliaceae)

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3. Petersham Lodge

The genus *Fritillaria* (Liliaceae), which comprises ca 140 species of bulbous perennial plants, includes species with the largest diploid genomes so far reported (1C-values ranging from 30.8pg to 100.4pg). This contrasts strikingly with the majority of angiosperms which are characterized by much smaller genomes (data for >10,000 species show the modal 1C-value is 0.6 pg /1C, and mean = 5.1 pg/1C). While there is ongoing research into the molecular dynamics underpinning the origin and evolution of such large genomes, our understanding of how this impacts various physiological processes is more limited. This talk will focus on how photosynthesis is affected by genome size via its impact on stomatal size. Previous studies across a diverse range of plants have shown that there is a broad correlation between genome size and stomatal guard-cell size, and that this may, in part be driven by changes in atmospheric CO_2 concentration over geological time (Franks *et al.,* 2012). Given that guard-cell size is likely to influence, for example, gas and water exchange dynamics needed for photosynthesis and hence potentially photosynthetic efficiency, we have probed this subject further by analyzing three different factors that contribute to photosynthetic efficiency in ten species of *Fritillaria* that differ in genome size (37.41 - 89.2 pg/1C). The approaches used were (i) infra-red gas analyser (IRGA) to investigate the rates of CO_2 uptake and Rubisco activity, (ii) pulse amplitude modulation (PAM) fluorometry to gain insights into the efficiency of light energy harvesting for photosynthesis, and (iii) stomatal measurements (size and density). The results shed light on how genome size interacts with other plant traits and abiotic factors to influence photosynthesis dynamics in these genome giants.

T2-46: Plant phylogeography in East Asia

T2-46-01

A review and meta-analysis of plant phylogeographical studies in the Ryukyus

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Phylogeographical studies on continental archipelagos generally seek explanations for geographical patterns of genetic differentiations in the history of island connections and separations. Old straits, thereby, have been considered to be the most likely barriers in the determination of genetic boundaries among island populations. However, caution should be taken when discussing phylogeographical patterns based on geological history. Biogeographic patterns have several possible explanations, of which historical and ecological biogeography are contrasting approaches. Historical biogeography seeks explanations in geological processes such as sea level change, crustal deformation and plate tectonics, which acted thousands to millions of years in the past. Ecological biogeography, in contrast, attempts to explain biogeographical patterns by the effects of more recent and current environments. The theories of historical and ecological biogeography have developed separately, but any biological phenomena are not the result of a single factor, either historical or current environmental. It is therefore necessary to analyze and explain biogeographical patterns from ecological as well as historical biogeographical viewpoints. The islands of the Ryukyus provide interesting settings for biogeographic studies that are rarely available elsewhere on the Earth: chain-like arrangement of the islands stretching over a thousand kilometers, landbridge formation/split during the past few million years, and latitudinal climatic gradient. Preceding phylogeographical studies in the Ryukyus chiefly considered the oldest straits that split the landbridge, the Tokara and Kerama gaps, as the most effective barriers that caused phylogeographic differentiation. Of the phylogeographical surveys so far conducted on terrestrial plants from the Ryukyus, some revealed large genetic differentiations across the Tokara and/or Kerama gaps, while others found a lack of differentiation across either gap. At this stage, there is no clear trend in the dispersal strategies/modes of studied plants on whether they showed genetic differentiation across the gaps. I therefore presume that ecological factors had (and still have) an additional role in the origin and maintenance of lineage divergence in the Ryukyus. It is feasible, for example, that the spatial configuration of the islands, including their isolated distribution, small size, and low elevation, imposes obstacles to the dispersal through limiting the availability of habitats for some plant species. In addition, adaptive processes should not be excluded from consideration. If an environmental selection gradient and related ecophysiological differentiation exist among populations, migration would be limited and, as a result, populations would be genetically differentiated even at neutral genetic loci. Examination of ecological factors (e.g. latitudinal low-temperature gradient) and related physiological differentiations among populations (e.g. cold-tolerance differentiation along the latitude) can offer explanations for the different phylogeographical patterns without assuming stochastic events. Integration of geohistorical and ecological explanations is essential to advance our understanding of plant phylogeography in the Ryukyus.

T2-46-02

Spatial and temporal evolution of the Arid Central Asia inferred from molecular phylogeography of desert plants

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The formation and evolution of the Arid Central Asia (ACA) was associated with the uplift of the Qinghai-Tibet Plateau (QTP) and the development of East Asian Monsoon system (EAMS). However, most of the evidences came from loess sediment records. To fully understand the spatial and temporal evolution of the ACA, here, we thoroughly investigated molecular phylogeography of one zonal constructive shrub *Reaumuria soongarica* and one pioneer annual plant species *Agriophyllum squarrosum*, both of which were widely distributed across all the desert ecosystems from the ACA. Based on cpDNA, nrITS and 13 nuclear loci, we found a complete divergence between the western and eastern populations could be traced to 2.96 ~ 0.8 Ma in *R. soongarica*. However, ecological niche modeling supported that the frequent historical global climate changes could comprehensively fragmented the habitat of this desert shrub. Thus, the present distribution of the desert ecosystems were shaped by both the long term habitat fragmentation driven by the uplift of northeast QTP and Pleistocene climate oscillations. Both cpDNA and nrDNA supported that *R. soongarica* could bidirectionally colonize into the Gurbantunggut Desert (the north desert) from the eastern and western since ~ 0.37 Ma, inferring to the asynchronous aridification of the regional ACA. Approximate Bayesian Computation (ABC) simulation strongly suggested scenario that the North group could originate with two hybridizations between the East and West groups, which could be facilitated by the multiple aridification events in the late of Pleistocene. As the desertification processes of the ACA were much more influenced by development of East Asian Winter Monsoon, which strongly supported by wide stratifigraphic records from the marginal lakes and loess around ACA. Our molecular data on cpDNA and nrITS indicated that *A. squarrosum* could originate from the north Gurbantunggut Desert or the neighbor area since ~1.6 Ma, and subsequently underwent three waves of colonisation into other deserts and sandy lands corresponding to several glaciations during late Pleistocene. The rapid population expansion and distribution range shifts of *A. squarrosum* from monsoonal climate zones suggested that the development of the monsoonal climate significantly enhanced the population growth, and unbalanced gene flow of *A. squarrosum* which could be an indicator for the historical movements of sand dunes in response to global climate change. Therefore, more efforts should be focused on profiling the gene flow of these desert plants with genomic data. These studies on psammophytes exemplified how the desert ecosystems of the ACA responded to the geological and paleo-climate changes, for the first time from the molecular phylogeographic view.

T2-46-03

Secondary contact, hybridization and polyploidization add to the biodiversity in the Hengduan Mountains, exemplified by the widespread *Corallodiscus lanuginosus* (Gesneriaceae)

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Corallodiscus lanuginosus is distributed 12,000 km across its northeast-southwest boundaries in Central Asia, showing its highest morphological diversity in the Hengduan Mountains, in Southwest China. We investigated the mechanisms behind the high morphological diversity exhibited by this species. We acquired molecular sequence data across the distribution range, reconstructed a dated phylogeny, projected the resulting clades onto geographic maps and linked the results with inferred ploidy levels of the populations. We found strong geographic patterns for the ITS clades but with extensively overlapping ranges, particularly in the Hengduan Mountains region. We used the presence of ITS polymorphisms to infer a high level of hybridization events mainly in areas of clade overlap, where we also inferred repeated tetraploidization events to have taken place. We deduced an origin of *C. lanuginosus* in the mid Oligocene. Historical pre- and post-Quaternary climate oscillation-driven expansions and contractions of distribution ranges have apparently allowed secondary contacts, hybridization and introgression to have occurred that shaped the current patchy distribution of morphological diversity in the species. Polyploidization is seen as a reinforcing genetic barrier added genetic diversity in space and time particularly in the Hengduan Mountains.

T2-46-04

Range expansions of plant species in subtropical China during the Quaternary

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Range expansions have occurred repeatedly in the history of most species and they are presently occurring at an increasing rate owing to rapid climatic changes. Because subtropical China was not glaciated during the Quaternary, most phylogeographic studies revealed that multiple refugia and limited range expansion from refugia seem to be a paradigm for plant species in this region. However, this conclusion could be incomplete because early phylogeographic studies in the region biased toward endangered species with narrow distributions. Recently, rapid advances have be achieved concerning range expansions in subtropical China during the Quaternary climatic oscillations. 1) Extensive range expansions may be the rule rather than exceptions for plant species, as the region underwent profound climate changes throughout the Quaternary; 2) Molecular dating suggested that the time for range expansion events varied among different plant species, most species occurring in the Holocene or the last interglacial period. Glacial expansions have also been implicated in several studies. 3) Due to the relatively mild Quaternary climate in subtropical China, the genetic signature of multiple range expansions across different glacial-interglacial cycles was likely to be preserved. Phylogeographic inference in subtropical China is prone to be complicated by multiple range expansions; 4) With increasing cases of range expansion, several recolonization routes, which largely coincide with major mountains in subtropical China, have been identified, especially through the integration of phylogeographic and geospatial data. These new findings provide important insights into evolutionary histories of plant species in subtropical China and have profound implications for biodiversity conservation in the face of recent climate change.

T2-46-05

Biogeographic history of alpine flora in the Japanese Archipelago

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High mountains in the Japanese Archipelago are one of the southernmost areas in the enormous ranges of arctic-alpine plants as well as alpine plants distributed around Beringia. One can hypothesize that alpine flora in this region may represent ephemeral occurrence of cold adapted species that dispersed southward during the last glacial maximum (LGM) and have survived the postglacial warm climate in relict populations on high mountains. On the other hand, the origin of alpine flora may have predated the LGM and populations of alpine plants may have persisted in Japan for prolonged periods in the Pleistocene. Recent phylogeographic studies on various alpine plants consistently revealed genetic divergence between populations in northern and central Japan, which implies prolonged persistence of alpine plants in central Japan. In this talk, I will show recent progresses on phylogeographic studies and provide insight into the biogeographic history of alpine flora in Japan. Analyses based on coalescent simulations demonstrated that genetic divergence of alpine plants between northern and central Japan predated the LGM. In contrast to the genetic uniqueness in central Japan, genetic similarity as well as recent divergent history was revealed between northern Japan and the Kamchatka Peninsula. Accordingly, these findings suggest that alpine flora at least in central Japan is unlikely the ephemeral occurrence of cold adapted species in the postglacial warm period but has persisted for several cool-warm climate cycles in the Pleistocene. On the contrary, alpine flora in northern Japan would originate from alpine and/or arctic-alpine plants that spread widely in northern East Asia during the LGM, while its persistence in the Pleistocene remains to be examined.

T2-46-06

Phylogeographic history of lithophytic plant genera disjunct in geologically active regions in East Asia

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East Asian warm-temperate zone includes peripheral regions characterized by rapid geological movements and earthquakes due to active faults. The geological movements have continued to produce landslides, forming complex landscapes full of rocky outcrops, steep slopes and waterfalls. These structures harbor the habitats for lithophytic plant groups, which prefer to survive on rocks or rock-associated environments. Among these groups, two genera of *Shortia* (Diapensiaceae) and *Tanakaea* (Saxifragaceae) show remarkable disjunct distributions between the Japanese Archipelago-Ryukyu Archipelago-Taiwan and Yunnan-Sichuan regions. Contrary to most lithophytic plants, they have shallow root systems with fine roots extending on the surfaces of rock-associated habitats, which requires year-round wet microenvironment to maintain populations. The specific ecological requirements and their conservatism would have led to similar disjunct distribution in *Shortia* and *Tanakaea*. Therefore, these genera can be suitable model systems to investigate the relative impacts of geological activity and historical fluctuations of rainfall on phylogeographic structures and population demographies in East Asia, where most plant phylogeographic studies focused on temperature changes in late Pleistocene. This study will present the results of comparative analyses of *Shortia* and *Tanakaea* using chloroplast genome sequencing and ddRAD-sequencing/nuclear microsatellite markers to discuss the roles of environmental drivers that led to present disjunct distribution patterns.

T2-47: Taxonomy and conservation of ferns and Lycopods (two sessions)

T2-47-01

Phylogenomics analysis resolve the backbone of tree of life in extant ferns

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Exant ferns are monophyletic group and the closest living relatives to seed plants and originated about 380 million years agao. However, the evolutionary relationships of extant ferns remain controversial based on different molecular evidences with different morophological interpretation. Here we aimed to resolved the early evolutionary relationships in ferns using high-quality RNA sequencing data obtained by high-throughput sequencing for 69 species representing all extant 11 orders and 30 families. We compared and analysed two supermatrices of homologous proteins encoded by 2,392 genes and 1,334 genes. From this analysis, we obtained a new topolgical structure with strong support by optimisation of the ML methods.These results allowed us for the resolution of key nodes that had been unclear in the past and revealed the evolutionary relationship of vertical annulate sporangia from exannulate sporangia. This well-resolved tree of ferns will constitute a framework for further studies of evolution and development in land plants.

T2-47-02

Diversity of ferns and lycophytes in Brazil

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This paper presents the diversity of ferns and lycophytes in Brazil, after the previous compilation published in 2015. The methodology to estimate the diversity consisted in collecting data from regional checklists, taxonomic revisions, selected databases, and literature in general. The results show 1,310 species: 1,143 of ferns and 167 of lycophytes. This number is 4.35% higher than the previous one (1,253 spp.), reported in 2015. Were recognized 39 families and 150 genera (vs. 36 families, 133 genera in 2015). The 10 most diverse families are Pteridaceae (196 spp.), Dryopteridaceae (184), Polypodiaceae (164), Thelypteridaceae (97), Hymenophyllaceae (89), Selaginellaceae (80), Aspleniaceae (78), Lycopodiaceae (64), Anemiaceae (61), and Cyatheaceae (45). The three most diverse genera are *Elaphoglossum* (87 spp.), *Selaginella* (80), and *Asplenium* (74). The richest phytogeographic domain continues to be in the Atlantic Rainforest with 895 species which also has the largest number of endemic and threatened species, followed by the Amazon Rainforest (516), Cerrado (263), Pantanal (34), Caatinga (29), and Pampa (10). Minas Gerais remains as the richest state (666 spp. vs. 657 in 2015).

T2-47-03

Community phylogenetics of Florida ferns: Insights from integrated analyses of trees, traits, and climate data

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Community phylogenetic methods enable us to examine the dynamics of plant community and ecosystem assembly in the context of species' evolutionary histories. Combined with ecological, functional, and climatic data, we can take an integrated approach to understanding the various biotic, abiotic, and evolutionary components driving patterns of diversity in communities at different temporal and spatial scales. The state of Florida in the United States has the richest fern flora of any state in the continental U.S. It is home to 149 species of ferns, including ~120 that are thought to be native. We are using community phylogenetic methods to investigate community structure and the ways in which trait associations and climatic factors shape the assembly of fern species at the county level. We have built a phylogeny for all species of Florida ferns using chloroplast markers, and constructed a matrix of functional trait data for all species using morphological descriptions in floras. We find that phylogenetic diversity and species richness are strongly correlated, as expected, across the state of Florida, and several traits show strong phylogenetic signal. We are also using species distribution models, which can be used to examine species' contemporary geographic ranges and identify the climatic factors that may have shaped these distribution patterns. These models can also be used to forecast anticipated shifts in distribution in response to changing climates. We have quantified the climatic niches of all fern species in Florida, and are examining the specific factors that drive each species' distribution, and how these relate to functional traits and species' evolutionary histories. Preliminary results suggest that species diverge in their tolerance for warmer temperatures and greater amounts of precipitation, with phylogenetic signal appearing at the family level and generic level. Species from the same genus and family tend to occupy similar temperature and/or precipitation regimes. Projected distributions for the year 2070 indicate that a few species may be able to expand their distributions in the future, if they are able to disperse to areas with newly appropriate climates, but the vast majority of Florida fern species are likely to experience severe reductions in their geographic distributions.

T2-47-04

Nuclear phylogenetics of ferns and its implications for taxonomy

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Much of our understanding of fern phylogeny, and thus the foundations of classifications that seek to recognize monophyletic groups, derives from inferences from plastid sequence data. This reliance on plastid data has potential weaknesses: plastid data come from a single linkage group so plastid-only studies can't corroborate their inferences with independent data; approaches that involve multiple gene trees (such as "species tree" inference) are unavailable; and being uniparentally inherited, plastid data are ill-suited for studies of hybridization or allopolyploidy. Fortunately, genomic resources for ferns have become much more available in the past five years, and there has been a corresponding increase in the use of nuclear markers for inferring fern phylogeny. Here, I'll discuss some of the taxonomic implications of these studies, ranging from those focused on the deep relationships among fern lineages to those involving more fine-scaled patterns, such as the prevalence of allopolyploid species. New opportunities bring with them new challenges, and nuclear data are no exception; I'll close with a discussion of some of the difficulties associated with using nuclear data for fern phylogenetics, and ways that the pteridological community may be able to move forward to an increasing robust classification.

T2-47-05

Phylogeny and biogeography of *Isoetes*

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The lycopods of the genus *Isoetes* have long held a fascination because of their fossil relatives within Isoetales, including some of the first trees. But the extant species also hold some intriguing mysteries. *Isoetes* are a mostly semi-aquatic genus with species all over the world. They are heterosporous and ligulate just like their closest living relative *Selaginella*. But the sister groups diverged long time ago, in the vicinity of 380 million years before present, and in their adaptation to aquatic environments the ancestors of present-day *Isoetes* likely lost many similarities to the ancestors of *Selaginella*. Because they are also substantially different on a molecular level it has been difficult to use outgroups in phylogenetic analyses of *Isoetes*. Their very uniqueness makes it difficult to place them within a framework. The phylogeny presents some intriguing insights into the history of the genus. Despite the high similarity between species there is well supported evidence for such old patterns as a Gondwana clade and a corresponding Laurasia clade. In my presentation I will disclose new details on the phylogeny and biogeography of *Isoetes*.

T2-47-06

Current condition of diversity information on Asian ferns and lycopods: An overview through the Asian Red List compilation

Atsushi Ebihara
National Museum of Nature and Science

Integrating diversity information on ferns and lycopods on an Asia-wide scale is an urgent and important task for taxonomists possessing massive knowledge about species of the region since it is not always possible to obtain sufficient information about Asian species from worldwide database and name index projects (e.g. The Plant List, GBIF, IUCN Red List *etc.*). The number of species (including subspecies and varieties, excluding hybrids) in Asia (excluding New Guinea) is estimated to be 4,650 according to our integrated checklist based on the latest flora and monographs. For screening globally threatened species, it is essential to extract candidate species on unified criteria throughout Asia, but it is quite difficult mainly due to difference in the degree of progress of local check-listings and red-listings. We therefore extracted candidate species by excluding certain LC (Least Concern) species from the total check list. As a result, 2,185 candidate species remained after exclusion of 2,465 LC species, and a 1-degree gridded preliminary distribution map showing the species density was produced based on the information from presently available sources (online specimen databases, floras, monographs, original descriptions *etc.*). It would be most important to refine the list by adding more information on taxon status, up-to-date local conservation status and distribution records in collaboration with as many specialists as possible.

T2-47-07

A worldwide molecular phylogeny of the morphologically cryptic genus *Botrychium* (Ophioglossaceae)

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Background — The description of biodiversity is an essential starting point for any study related to evolutionary biology and conservation. Among these investigations, molecular phylogenies have led systematists to characterize more taxa, sometimes having similar morphologies despite their deep phylogenetic divergence, and among them, moonworts (*Botrychium* s.s.) are an emblematic example of morphologically cryptic species. Previous molecular studies on this genus did not include a complete taxonomic sampling of the taxa, nor multiple specimens from throughout the known geographical range of each taxon, and therefore to investigate the evolutionary relationships of its major clades, we increased both the taxonomic representativeness as well as the phylogenetic resolution by including additional chloroplast markers. *Emphasizing main questions and goals* — Our study aims to better understand basic phylogenetic relationships within *Botrychium*, then retrace the maternal lineages of allopolyploid taxa, and ultimately identify species diversity centers in the northern hemisphere. *Materials and methods* — Out of 35 published and thirteen putatively new taxa, we sampled from 5 to 15 populations per taxon throughout their geographical range (Asia, Europe, North America, Oceania, and South America) for a total of 365 specimens, most of which were genotyped with 20 nuclear enzyme loci to confirm taxa identification and provide evidence from both maternal and paternal parentage of allopolyploids. We then analyzed four chloroplast regions (*matK* intron, *trnH*^{GUG}–*psbA* and *trnL*^{UAA}-*trnF*^{GAA} intergenic spacers, and *rpL16* intron region) on those specimens and we conducted a phylogenetic analysis based on Maximum Likelihood (ML) and Bayesian Inference (BI) methods to explore the genetic diversity and phylogenetic relationships between taxa. *Results* — We found strong support for the monophyly of the three major clades: Lanceolatum, Lunaria, and Simplex-Campestre, and resolved 17 subclades among those. Our results suggest multiple origins for at least four polyploid taxa. The Simplex-Campestre clade had the largest number of species despite having a similar total number of haplotypes to the Lunaria clade (62 and 59 respectively), which has the broadest worldwide distribution. In total, 47 taxa are considered in this new molecular phylogeny based on multiple accessions per taxon. *Conclusions and perspectives* — Our results support the taxonomic delineation of nearly all moonwort taxa previously described on the basis of morphology, chloroplast, and nuclear data. These results have also revealed several new morphologically cryptic taxa as well as substantial genetic diversity hidden within common species. Our study also strongly corroborates previously hypothesized maternal parentage of allopolyploid taxa, and provides support for multiple independent hybridization events generating the same allopolyploid taxon. Strong asymmetry in maternal parentage of allopolyploids by certain diploid species raises questions and provides clues about the understanding of how the process of polyploidy takes place within natural populations. Finally, our large sampling also provides evidence of long-distance migratory ability of *Botrychium* taxa on intra– and inter–continental scales, as well as probable directions of migration. Vectors of migration undoubtedly involve wind dispersal of spores and possibly bird migrations from the Northern to Southern Hemisphere, and further studies are needed to retrace the phylogeography history of the genus, especially in Asia where the taxonomic diversity remains poorly known.

T2-47-08

Toward a community-derived classification for extant lycophytes and ferns

Eric Schuettpelz
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Phylogeny has long informed pteridophyte classification and, as our ability to infer evolutionary trees has improved, classifications aimed at recognizing natural groups have become increasingly predictive and stable. The ordinal and familial scheme by Smith *et al.,* (2006, *Taxon* 55: 705–731) was the first higher-level pteridophyte classification published in the molecular era. Founded on the principle of monophyly, while also recognizing the importance of maintaining well-established names and circumscriptions, this classification established a new standard in fern taxonomy. Over the past decade, many important advances have been made in our understanding of relationships, but only some of these were reflected in subsequent higher-level schemes. The Pteridophyte Phylogeny Group (PPG) was established to provide a modern, comprehensive classification for lycophytes and ferns, down to the genus level, utilizing a community-based approach. A global assemblage of 94 pteridologists ultimately came together to produce a classification treating an estimated 11,916 species in 337 genera, 51 families, 14 orders, and two classes. This first installment of the PPG classification is not intended as the final word on lycophyte and fern taxonomy, but rather a summary statement of current hypotheses, derived from the best available data and shaped by those most familiar with the plants in question. The hope is that it will serve as a resource for those wanting references to the recent literature on pteridophyte phylogeny and classification, a framework for guiding future investigations, and a stimulus to further discourse.

T2-47-09

Plastid phylogenomics resolve the deep relationships of Polypodiales

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A series of molecular phylogenetic studies in recent years have reconstructed framework of ferns. However, the circumscriptions of some families remain controversial and their relationships are not thoroughly resolved. Polypodiales represents 80~90% of the species diversity of ferns, and the relationships among the basic groups of Polypodiales remain unresolved or poorly supported, and phylogenetic positions of some families in Eupolypods are not completely resolved. The primary objective of this study is to explore the application of plastid phylogenomics in resolving difficult backbone relationships of Polypodiales. We sequenced plastomes from 37 species representing 19 families of polypod ferns, and constructed the phylogeny of Polypodiales based on all sequenced plastomes of ferns. Plastid phylogenomics can provide large chloroplast gene data to resolve difficult deep nodes in Polypodiales. Our results supported the sister relationships between Dennstaedtiaceae and Pteridaceae (BP > 90), and the Eupolypod clade was sister to the Dennstaedtiaceae-Pteridaceae clade (BP = 100). The Didymochlaenaceae-Hypodematiaceae clade was the firstly diverged lineage in eupolypods I (BP = 100), and Didymochlaenaceae was sister to Hypodematiaceae (BP = 100). The plastid genomic features, such as gene order, intron gain/loss, may also offer independent tests of phylogenetic hypotheses. The pseudogene is universal in ferns, and the pseudogene of *rps16* was found in all nine Polypodiaceae species with sequenced plastomes, while it only was found in *Odontosoria* (Dennstaedtiaceae) except for Polypodiaceae. Furthermore, *rps16* of *Elaphoglossum* (Dryopteridaceae) and *Lomariopsis* (Lomariopsidaceae) contains 2-3 terminators.

T2-47-10

Asplenium sect. *Thamnopteris* (Aspleniaceae): The morphological delimitation of species and molecular phylogeny based on chloroplast DNA sequences

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The section *Thamnopteris* in *Asplenium* (Aspleniaceae), or called *A. nidus* complex, is unique in morphology (fronds simple, entire, with free veins connected at margin) and in cytology (only tetraploids and a hexaploid reported). Some 20 species are estimated within this group and the morphological limits between species are confusing. To determine the morphological delimitation of species we conducted comprehensive comparisons of morphology as well as phylogenetic analyses based on two datasets of chloroplast DNA sequences. Our results showed that most species in the complex can be clearly circumscribed by the combination of four morphological characters, i.e., type of stipe scales, shape of fronds' midrib, density of sori, and pattern of perispores. Phylogenetic analyses indicated that *A.* sect. *Thamnopteris* is not monophyletic. In *A.* sect. *Thamnopteris* the species sampled in our analyses were well resolved into two clades, *A. nidus* clade and *A. phyllitidis* clade. The clade *A. nidus*, morphologically supported by narrow scales, comprises *A. australasicum*, *A. goudeyi*, *A. harmanii*, *A. musifolium*, *A. nidiforme*, *A. nidus*, *A. oblanceolatum*, *A. setoi*, and *A. wagauensis* (ined.). A species outside *A.* sect. *Thamnopteris*, *A. amboinense*, was resolved to be sister to *A. nidus* clade. In the clade *A. phyllitidis*, which is supported by broad scales, clustered seven species: *A. antiquum*, *A. antrophyoides*, *A. cymbifolium*, *A. humbertii*, *A. palawanense* (ined.), *A. phyllitidis*, and *A. riswanii*. Some species with fronds finely dissected, such as *A. beccarianum*, *A. prolongatum*, *A. sampsonii*, and other species outside the complex, were suggested to be sister to *A. phyllitidis* clade. The re-circumscribed *A. nidus* is still a complex which contains at least three cryptic species and currently we fail to find stable morphological characters to distinguish them.

T2-47-11

Taxonomy and evolutionary history of the neotropical fern genus *Salpichlaena* (Blechnaceae)

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The neotropical fern genus *Salpichlaena* (Blechnaceae) is interesting both because it has an unusual climbing habit and because there has been much debate during the past three decades about how many species the genus contains. Some researchers recognize 2 or 3 species whereas others consider there to be just one. The habit of these plants has caused some difficulty for taxonomical work. They have twice pinnate twining leaves that can become more than 15 m long, and the pinnae vary considerably in both size and shape according to their distance from the rhizome. We have now systematically observed 62 morphological traits on 183 herbarium specimens of *Salpichlaena*. E We also sequenced three plastid genes (*rbcL*, *rpoC1* and *rps4*), two plastid intergenic spacers (*trnH-psbA* and *trnG-trnR*) and a nuclear gene (*pgiC*) from 46 *Salpichlaena* and nine out-group specimens to run phylogenetic analyses. We found that pinna texture, shape of pinna apex, type of pinna margin, shape of the scales on the costa and presence of buds on pinna axils are the most informative traits for distinguishing the species. Our preliminary results indicate the presence of at least three species in the genus *Salpichlaena*, and that their distribution is related to geographical and ecological factors.

T2-47-12

Lycophytes and ferns of Natmataung National Park in Myanmar

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The flora of Myanmar was extensively documented by British botanists and plant hunters in the early nineteenth century. Dickason (1946) reported 460 species of lycophytes and ferns as first records for Myanmar. More recently, Thet YuNwe (2009) studied the fern diversity of Kyaik-Hti-Yoe Wildlife Sanctuary, reporting 68 species. Because of lack of man powers and available funds, the flora, especially lycophytes and ferns are pooly known. As a part of compilation of lycophytes and ferns of Myanmar, field works were conducted in Natma Taung National park, Chin State, Myanmar from 2015 to 2017 to make a checklist of lycophytes and ferns. Natma Taung National Park in southern Chin State, western Myanmar, was established in 1994 and is located close to the border with India and Bangladesh. It covers around 723 km^2 of Chin Hills and contains the highest peak in southern and central Myanmar: Mt. Natma Taung (Mt. Victoria, elevation 3,051 m). As a result, we found some lycophytes and ferns which include first recorded taxa from the flora of Myanmar.

T2-48: Plant diversity and evolution in Africa: Challenges and opportunities in a changing world

T2-48-01

How threatened is the flora of continental tropical Africa? A response to reaching Targets 1 & 2 of the Global Strategy for Plant Conservation (GSPC)

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Two of the GSPC Targets for 2020 are: 1) an online world flora, and 2) an assessment of the conservation status of all known plant species. Progress toward achieving both targets is far from sufficient. Tropical African biodiversity face a high level of threat linked to explosive population growth and resulting expansion of industrial activities such as agriculture and mining. Knowing distribution of plants across the continent and estimate extinction risks is thus of major conservation importance. However, distributional data for plants remains insufficient and lack a general standardization. Estimates of conservation status are available for only a limited portion of species (around 11%). To speed up this process, we have developed 1) a mega database of tropical African plants called RAINBIO and 2) a new approach to undertake fast-track preliminary conservation assessments based on the IUCN categories under criteria A and/or B. RAINBIO represents the first-ever near-comprehensive database of vascular plant occurrences in sub-Saharan tropical Africa, currently comprising distribution information for over 22,000 native species. This allowed us to generate a check list of known species at regional and country levels contributing to Target 1. Our cost-effective and efficient conservation assessment approach enabled the preparation of a preliminary working set of potentially threatened species at multiple scales contributing to Target 2. We used this approach on RAINBIO and processed over 18,000 species in less than 24 hours. Our results suggested that around half of tropical African plants are potentially or likely threatened by extinction. We identified areas with high concentrations of threatened species. Synthesizing databases and applying new fast-track approaches to entire floras provides a powerful example of how we could achieve

GPCS Targets by 2020.

T2-48-02

The grasses and grasslands of Madagascar: A multidisciplinary investigation

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The dominant narrative regarding human arrival in Madagascar has long stated that people arrived 2,500 years ago to a largely forested island, with anthropogenic destruction responsible for the 65% of Madagascar's land area now covered in grasslands and savannas. Scarcity of lake bed deposits have made paleoenvironmental reconstruction difficult. One piece of the puzzle regarding Madagascar's grasslands remains missing: the grasses themselves have never been fully documented or considered in an ecological perspective. Herbarium specimens of Malagasy grasses (family Poaceae) were studied, taxonomic revisions produced, and new species described during the six years of this project. Regular field work has targeted locations of poorly known endemic grasses and species knowledge has been built up throughout the island. Phylogenetic trees were reconstructed and dating was carried out in order to document all instances of Poaceae diversifications into Madagascar. Plot data recording species composition, phylogenetic diversity, and disturbance levels has been gathered at 80 sites. 217 of Madagascar's 541 grass species are endemic, a level of endemicity in line with the grass floras of other subtropical islands. We have identified 97 endemic lineages of grasses, which have colonised Madagascar primarily from Africa, with a mean age of 3.5 million years. Half of the grass dispersals into Madagascar were C4 lineages pre-adapted to open habitats. The earliest endemic lineage *Lecomtella madagascariensis* arrived ca 22 million years ago. Madagascar's High Plateau is home to a unique and endemic grass flora. A checklist if the Itremo Protected Area has revealed 99 species of Poaceae, with 20 restricted to the High Plateau. Phylogenetic diversity within grassy ecosystems of Madagascar decreases in areas with strong physical disturbance, indicating ecosystems dynamics typical of natural assemblages. We demonstrate that Madagascar is home to an ancient and diverse grass flora, with local species assemblages functioning similarly to natural ecosystems. This indicates that pre-human Madagascar was home to at least some tropical grassy biomes. Work continues to determine where these were located, and how can they be distinguished from anthropogenic landscapes resulting from forest destruction.

T2-48-03

Contrasting dispersal histories of broad- and fine-leaved temperate Loliinae grasses: Range expansion, founder events, and the roles of distance and barrier

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Successful colonisation after long-distance dispersal (LDD) depends on the availability of suitable habitats and competitive ability. Here we address the hypothesis that two widely distributed sister grass lineages (broad- and fine-leaved Loliinae; hereafter BL and FL) that differ in their habitat requirements and competitive ability also differ in their biogeographic history, with particular reference to LDD. Sampling was designed to represent all genera, subgenera and sections, and the geographical distribution of the Loliinae, particularly in the less explored Southern Hemisphere (sub-Saharan African taxa were studied for the first time). We reconstructed a comprehensive phylogeny of the Loliinae, based on nuclear and plastid markers and estimated divergence times using fossil calibrations. Biogeographical events were estimated using analysis of range evolution, comparing different models. Numbers and rates of dispersals were estimated for BL and FL using stochastic mapping with best-performing and baseline biogeographical models, and examined for correlation with distance, disjunction type, and phenotypic syndrome. The most recent common ancestor of the Loliinae likely split at the Oligocene-Eocene transition (22.50 ± 3.95 Ma), pre-dating previous estimates, whereas the ancestors of the BL and FL Loliinae likely began to diversify in the Early Miocene (18.91 ± 4.15 and 17.50 ± 3.50 Ma, respectively). A model of range evolution integrating founder events and scaling of dispersal by shortest distance between areas performed best among a set of alternative models and recovered a mean of 83 dispersal events in Loliinae. Overall dispersal rates were significantly higher in BL than in FL. Per-route dispersal rates showed a significant negative exponential relationship to shortest distance but were not affected by phenotypic syndrome or disjunction type. Loliinae originated in the Northern Hemisphere and evolved through recurrent LDDs. Sub-Saharan Africa was colonised four times. A continental colonisation of Tropical Africa from the West Palaearctic was inferred to have occurred in the BL lineage in the Early-Mid Miocene, and from there to S. Africa and Madagascar in Late Miocene to Quaternary, resulting in a Tropical African-South African clade (including *Pseudobromus*). By contrast, a transoceanic colonisation of Tropical Africa from S. America was inferred to have occurred in Late Miocene (Messinian) – Early Pliocene leading to the origin of the FL Afroalpine clade; it was followed by two Late Pliocene – Quaternary dispersals from there to Madagascar and to the Mascarenes, thus expanding the distribution of the Tropical African 'sky islands' flora to the high peaks of the Indian Ocean islands. Higher competitive ability, potentially related to the broad-leaved syndrome (i.e., tall strong-rhizomatous habit, long-living individuals, occupancy of more stable habitats), may explain higher dispersal rates in BL compared to FL Loliinae. However, the dominant factor influencing dispersal in both BL and FL Loliinae is the distance between suitable areas.

T2-48-04

Multiple origins and a narrow genepool characterise African tea germplasm: Concordant patterns revealed by nuclear and plastid DNA markers

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Despite the highly economic value of tea in Africa, its genetic and geographic origins remained largely unknown. We collected 439 samples across 11 countries in Asia and Africa to investigate the origin and genepool composition of African tea based on 23 nuclear microsatellites loci (nSSRs) and three cpDNA intergenic spacer regions. Our results concluded that the African tea represents a potpourri originating from multiple introductions over time. The nSSR analysis revealed that the majority (79%) of tea accessions collected in Africa belong to Indian Assam tea which have likely originated from India and/or Sri Lanka. China tea was initially introduced from China directly to Africa. Patterns of nSSR variation also showed that Chinese Assam tea, genetically distinct from Indian Assam tea, has rarely been used in African tea breeding efforts, as only 4% of the African tea accessions possessed this genotype. We found a total of 22 cpDNA haplotypes, which grouped into three main geographic clades that were concordant with the distribution of microsatellite genotypes. Several private cpDNA haplotypes were identified in Chinese Assam tea in Southern Yunnan province of China. Therefore Chinese Assam tea may be important for the enrichment of African tea gene pools. Our results will be a useful guide in future breeding programmes and allow a strategic approach to the conservation of tea germplasm in Africa.

T2-48-05

Connectivity in the extremely fragmented African ‘Sky Island’ flora: Historical migration corridors or long-distance dispersal?

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The flora on the isolated high mountains of eastern Africa is renowned in biogeography. These mountains were mainly colonized by lineages pre-adapted to frost in remote areas of the world, some possibly before the formation of the current high mountains, some at the time of their final uplift phase, and some long after their formation. By combining ecological niche modeling and genetic analysis, we here address the relative importance of long-distance dispersal (LDD) and migration corridors potentially formed during past climates in shaping the isolated floras on different mountains. We are currently performing comparative ecological and genetic analyses of 29 plant species (AFLP genotypes of ~4000 plants from ~800 populations). A preliminary analysis revealed relatively high median genetic divergence between mountains (F_{ST} = 0.24-0.47), but we found large variation both among different species and among different mountains. In some species, there was virtually no differentiation among mountains, whereas populations of other species were extremely divergent. The phylogeographic histories thus seem to differ considerably among species. Our ecological niche modeling work suggests that most alpine populations on different mountains remained isolated from one another even during the Last Glacial Maximum, when the alpine habitat extended 1000 m further downslope and was eight times larger than today. Intermountain gene flow may therefore to a large degree depend on LDD, a highly stochastic process, at least for species that exclusively occur in the alpine zone of the mountains.

T2-48-06

Exploring the phylogenetic composition of pteridophyte flora of Eastern Afromontane Biodiversity Hotspot

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Studies on the fern and lycophytes established a reliable taxonomic and geographical framework to identify the factors that shaped the extant diversity of these plants in East African Biodiversity Hotspot (EABH). The formation of geological young mountain chains, such as the Andeans and the Himalaya, has been recognized as major factors contributing to the assembly of extant pteridophyte diversity hotspots in South America and Asia. In contrast, relatively little attention has been given so far to the impact the rise of the East African Mountains on the local and global diversity of ferns and lycophytes. Here, we present the first comprehensive analyses on the phylogenetic composition of the fern and lycophyte diversity of this region. This analysis enables us to explore the support for previously introduced hypotheses such as the existence of geographically differentiated pteridophyte floras in Africa such as the East African pteridophyte flora and the Central-West African lowland forest pteridophyte flora. The analyses include also the reconstruction the role of niche conservatisms, long distance dispersal, and local adaptive radiations on the accumulation of ferns and lycophyte diversity across Africa but specifically to the East African Biodiversity Hotspot. Given the distinct geographic and geological structure of this region, it is expected that the evolutionary processes shaping the extant plant diversity of this region are distinct from those shaping the accumulation of species diversity of the main mountain chains in Asia and South America. Instead, some evidence suggested that the accumulation of the plant diversity of the mountains is more comparable to those on Oceanic islands

T2-49: Origin and diversification of the East Asian flora

T2-49-01

The diverse origins of *Lobelia* in China

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Lobelia originated in southern Africa, but has since colonized all habitable continents and many oceanic islands. The 2011 revision

by Thomas Lammers recognized 18 sections of *Lobelia*, five of which are represented in the flora of China. Section *Delostemon* is centered in Africa, but one lineage colonized southern and eastern Asia, with three species present in China. Section *Stenotium* also has an African origin, and independently reached China, where it is represented by the widespread *L. heyneana* and the rare *L. hainanensis*. Section *Hypsela* originated in Australia, with *L. nummularia* and *L. chinensis* derived from separate dispersal events. Section *Speirema* is endemic to southeastern Asia, with three species that range north into China. Section *Rhynchopetalum* originated in eastern Asia (following dispersal from Madagascar) and subsequently dispersed to Africa and South America, but also gave rise to section *Speirema* and the Hawaiian endemic sections *Revolutella* and *Galeatella*. In total, the Chinese species of *Lobelia* are a diverse assemblage derived from five independent, intercontinental dispersal events.

T2-49-02

The East Asian origin of the giant Lobelias

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The cosmopolitan radiation of *Lobelia* that began about 20 million years ago (mya) has two lineages derived from African ancestry. Precise reconstruction of the early biogeographic details of the first lineage is not possible because it colonized the Mediterranean, North America, South America, and Australia in such rapid succession. The second lineage had a slower tempo, with initial colonization of Madagascar before an extended period of diversification in eastern Asia. The four distinct East Asian lineages provide compelling evidence of the evolutionary center of origin for the giant lobelias. However, the giant lobelias comprise two lineages that dispersed in different directions about 10 mya. One lineage colonized the ancient Hawaiian archipelago and also reached isolated, tall islands in the South Pacific. The other lineage dispersed to East Africa, where it colonized ancient crystalline block mountains and the tall volcanos as they arose, but also reached Ethiopia (across the desert of northern Kenya), West Africa (across the Congo basin), and Brazil (across the South Atlantic Ocean).

T2-49-03

Origin of East Asian Campanuloideae (Campanulaceae) diversity

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In Eastern Asia, the Campanuloideae (Campanulaceae) clade is represented by 14 genera and over 190 species. Almost half of these are endemic to this region. Results from phylogenetic and biogeographic analyses, using both plastid and nuclear loci, will be presented for this group, with special emphasis on East Asian taxa. Evolution of the Eastern Asian campanuloids appears to be the result of numerous, independent movements from Africa, Eurasia, and North America. Movement of the largest East Asian clade (platycodonoids) occurred approximately 37 mya from an African ancestor. More recently, we found evidence for 13 additional movements into this region, primarily from Eurasia and a single dispersal event from western North America. Results from diversification analysis will also be presented in the context of genome and character evolution.

T2-49-04

Evolution of Musaceae: Diversification of bananas was driven by the geological and climatic history of Southeast Asia

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Tropical and subtropical Southeast Asia, which harbors most of the Musaceae biodiversity, is one of the most species-rich regions in the world. Its high degree of endemism is shaped by the region's tectonic and climatic history, with large differences between northern Indo-Burma and the Malayan Archipelago. Here, we aim to demonstrate a link between the diversification and biogeography of Musaceae and the geological history of the Southeast Asian subcontinent emphasizing northern Indo-Burma. Age estimates of the Musaceae family are inferred using a phylogenetic framework encompassing 163 species from all Zingiberales families. Evolutionary patterns within Musaceae were investigated using ancestral area reconstruction and diversification rate analyses. All three Musaceae genera - *Ensete*, *Musa* and *Musella* - originated in northern Indo-Burma during the early Eocene. *Musa* species dispersed from 'northwest to southeast' into Southeast Asia with only few back-dispersals towards northern Indo-Burma. Musaceae colonization events of the Malayan Archipelago subcontinent are clearly linked to the geological and climatic history of the region. *Musa* species were only able to colonize the region east of the Wallace line from the late Miocene onwards after the availability of emergent land.

T2-49-05

Uplift-driven diversification in the Hengduan Mountains, a temperate hotspot of biodiversity

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A common hypothesis for the rich biodiversity found in mountains is uplift-driven diversification—that orogeny creates conditions favoring rapid in situ speciation of resident lineages. We tested this hypothesis in the context of the Qinghai-Tibetan Plateau (QTP) and adjoining mountain ranges, using the phylogenetic and geographic histories of 18 groups of plants to infer the tempo (rate) and mode (colonization vs. *in situ* diversification) of biotic assembly through time and across regions. We focused on the Hengduan Mountains region, which in comparison to the QTP and Himalayas was uplifted more recently (since the late Miocene), is smaller in area, and richer in species. The time-calibrated phylogenetic analyses, which made no prior assumptions about when any region was uplifted, showed that about 8 million years ago, the rate of *in situ* diversification increased in the Hengduan

Mountains, significantly exceeding that in the geologically older QTP and Himalayas, and marked the point at which cumulative speciation overtook colonization. By contrast, in the QTP and Himalayas during the same period, the rate of *in situ* diversification remained relatively flat, with colonization dominating lineage accumulation. This indicates that the Hengduan Mountains flora has been assembled disproportionately by recent *in situ* diversification that coincides temporally with independent estimates of orogeny, and is the first quantitative evidence to support the uplift-driven diversification hypothesis.

T2-49-06

Plant radiations in hotspots of biodiversity surrounding the Qinghai-Tibet Plateau

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Orogenesis is often considered the main driver for the radiation of plants in mountains. Yet, the role of climate oscillations and key opportunities as well as key innovations as contributors to the establishment of high levels of biodiversity around the Qinghai-Tibet Plateau (QTP) is often overlooked. In order to understand the contribution of orogenesis to plant radiations and the importance of the QTP to the diversification of temperate plants, we investigated the evolutionary history of two taxa with high species numbers in the QTP region, Gentianinae and *Saxifraga*. We performed molecular dating, historical biogeography, diversification rate and climatic niche evolutionary analyses. Contrary to the "out of Tibet" hypothesis, not all lineages that radiated in the QTP region originated there, and immigration contributed to the high species richness of this region. Similar to other studies, we found a significant delay between the start of orogenesis and radiations. In fact, our studies underline the complexity of processes and factors underpinning plant radiations in mountain systems. For example, rather than geomorphological processes, evolution of key innovations such as berry-like fruits and the cushion life form as well as habitat shifts and niche evolution were found to be important processes connected to these radiations. Interestingly, even in closely related lineages occupying the same life zone, radiations are not necessarily governed by the same factors. In conclusion, the underlying causes for plant radiations in mountains can be multi-faceted and do not necessarily derive from orogenesis, but rather result from an intricate interaction of lineage-specific traits and complex geological settings (a prerequisite) and/or climatic modifications providing key opportunities.

T2-50: Systematics of Orchidaceae

T2-50-01

Relationships of Liparis species in Russia based on nuclear and plastid sequences phylogenetic analysis

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Liparis is an orchid genus containing over 400 terrestrial and epiphytic species widely distributed in tropical and temperate regions of the world. Six species of *Liparis* are known in Russia: *L. loeselii* (L.) Rich. is found in European part of Russiaand Siberia; the other five species (*L. japonica* (Miq.) Maxim., *L. krameri* Franch. et Savat., *L. kumokiri* F. Maek., *L. makinoana* Schlechter and *L. sachalinensis* Nakai) are distributed inRussia's Far East. The last one probably is a narrow endemic to Sakhalin island. *Liparis* is one of the most complicated genera in Orchidaceae family, in spite the fact that numerous researches were dedicated to the systematics of this genus. Numerous morphological features – such as size of perianth parts, shape of lip and amount of its twisting, length of inflorescence, pedicle and petiole, shape of leaf blade – are usually used in *Liparis* species delimitation. However, intraspecific variability of these features makes *Liparis* species difficult for delimitation. Taking this fact into consideration, the number of *Liparis* species in flora ofRussiaturns out to be an open-ended question. We analyzed sequences of nuclear (*nrITS*) and chloroplast (*trnL-F* spacer; *psbA-trnH* spacer; partial *ycf1b* sequence) DNA in order to discover additional molecular features applicable for delimitation of *Liparis* species. 37 accessions of 6 species of *Liparis* distributed inRussiawere involved in analysis, including critical specimen from Kunashir (*Liparis sp. nov.*?). The final set contained 52 accessions. The results of *nrITS* analysis show that representatives of Far East populations form 3 well supported clades. Critical specimen from Kunashir occupies its own isolated position and have specific nucleotide substitutions. Furthermore the results of analysis indicate paraphyly of genus *Liparis*. Results of chloroplast *trnL-F* and *psbA-trnH* spacer analises are coherent with results of *nrITS* analysis. The results of *ycf1b* analysis did not revealed specific substitutions or indels for the studied species. This fact eliminate *ycf1b* marker from the group of potential molecular-diagnostic features.

T2-50-02

RADseq elucidates relationships within phylogenetically challenging groups of European Orchids

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Intensive molecular and morphological research has led to robust phylogenies of most orchid genera that occupy Europe and Asia Minor. However, at least three genera apparently underwent recent radiations that have generated extensive species complexes wherein morphological and especially molecular divergence are at best subtle. Consequently, the delimitation, rank and relationships of species remain highly controversial. The three clades – Bee Orchids (*Ophrys*), Tongue Orchids (*Serapias*) and Helleborines (*Epipactis* subgenus *Helleborine*) – are each characterised by unusual pollination mechanisms: pseudocopulation in *Ophrys*, shelter provision in *Serapias*, and near-obligate autogamy in many of the *Epipactis* taxa. We have exploited recent technical advances in applying next generation sequencing (NGS) approaches to non-model organisms in order to re-evaluate phylogenetic relationships among putative species within *Ophrys* and *Epipactis* subgenus *Helleborine*. Detailed, long-term field sampling across the full geographic range of both genera has allowed us to apply the RADseq NGS technique to comprehensive suites of taxa. The resulting matrices of single-nucleotide polymorphisms each contain several thousand phylogenetically informative characters, inevitably generating phylogenetic trees that incur stronger statistical support than any previous trees. The RADseq trees broadly corroborate the topologies the more rigorous of the previous candidate-gene trees but differ in many details, as well as challenging some previous assertions of both species status and species circumscription. In addition to elucidating relationships within these much-discussed clades of orchids, the presentation will outline the advantages and disadvantages of RADseq for reconstructing phylogenies and inferring evolutionary mechanisms, as viewed through the lenses of these two novel case-studies.

T2-50-03

Phylogenetic analysis and morphological revision of the orchid genus *Holothrix*

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The African orchid genus *Holothrix* includes between 46 and 55 species, 23 of which occur in South Africa. A treatment of the Southern Africa taxa based on morphology was published by Immelman in 1996, but a comprehensive revision is lacking. Furthermore, no phylogenetic analysis has ever been performed. There is also uncertainty at the generic level as the genus *Bartholina* shares a similar ecology, as well as some key morphological characters with *Holothrix*. Therefore, the separation of these two genera might be artificial and needs reassessment. The main aim of our study was to reconstruct the first phylogeny for *Holothrix* and its allied genus *Bartholina*. We included 32 species of the *Holothrix* alliance as well as 19 outgroup taxa and gathered 3461 molecular characters from three different DNA regions: ITS (nuclear), matk and *trn*L-F (plastid). Bayesian inferences, maximum parsimony and maximum likelihood were used to examine the phylogenetic relationships. The resultant phylogeny is generally very well-resolved and most of the clades are strongly supported. *Bartholina* is deeply nested within *Holothrix* but the two together form a monophyletic clade, strongly supported by molecular characters as well as by floral morphology. The three different sections defined by Schlechter (i.e. *Euholothrix*, *Tryphia* and *Scopularia*) appear polyphyletic. In the light of the molecular phylogeny, we identify the taxonomic significance of several morphological characters and lay the foundation for a new classification of the *Holothrix* lineage.

T2-50-04

Phylogenetic affinities and diversity of New Zealand potato orchids (*Gastrodia*)

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Gastrodia is one of the largest genera of non-photosynthetic myco-heterotrophic orchids. It comprises about 70 species and although the highest diversity of species and endemism occurs in Asia, *Gastrodia* is well represented across Africa, Oceania and islands of the Indian and Pacific Ocean. The southernmost limit of distribution of *Gastrodia* is in New Zealand (NZ) where it reaches ca. 47°S. Five species of *Gastrodia* occur in NZ. Two of them are self-pollinating and the other three out-crossers, depending fully on insect pollinators for fertilisation of their flowers. Only one of these species, *G. sesamoides*, does also occur in other nearby landmasses. The remaining species are endemic to NZ, and have a contrasting extent of occurrence and conservation status. In this talk we will present results from phylogenetic analyses of nuclear and mitochondrial DNA sequences that suggest long distance dispersal of *Gastrodia* to NZ, followed by local and recent species diversification. Diversification seemed to have been accompanied by changes in floral structures and, consequently, breeding systems. This study represent one of the first outcomes of collaboration between orchid researchers from multiple international institutions that aims to understand biogeographic patterns in *Gastrodia* and phylogenetic affinities among species.

T2-50-05

The power of movement in orchids, a kinematics study of *Porroglossum* (Pleurothallidinae) evaluated in the context of their phylogeny reconstructed using nextgen GBS data

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Charles Darwin was fascinated by the power of movement in plants. In particular, he had a strong interest in the various types of climbing plants, leaves exhibiting sleep movements, and insectivorous plants exhibiting fast-action snap-traps. Had Darwin known about the orchid genus *Porroglossum*, he surely would have considered these small plants of subtribe Pleurothallidinae the most remarkable of orchids because physical stimulation of the flower's labellum causes it to actively snap inward thrusting pollinators against the column. *Porroglossum* is composed of 53 described species, most of them endemic to Ecuador, that are distributed throughout the Andean cloud forests of South America. Recently, we reconstructed the evolutionary history of *Porroglossum* using a combination of nuclear (*XDH*), ribosomal (nr*ITS*), and plastid (*matK*, *ycf1*, *rpl32-trnL*) markers, but the backbone of the phylogeny and interspecific relationships remained unresolved. Additionally, a majority of the species were characterized by relatively short branch lengths indicating the possibility of a recent origin for the genus. To compensate, we turned to genotyping by sequencing (GBS) for phylogenetic inference at the species-level. By utilizing high-throughput computing, we implemented the Stacks pipeline to generate thousands of SNPs from the raw sequence data for phylogenetic inference. Support values for the clades recovered using traditional Sanger sequencing were bolstered (100% bs) and the tree was fully resolved. For our kinematics study we recorded high-speed videos (60fps) of the active, floral snap-trap of all species at the orchid nursery Ecuagenera in Gualaceo, Ecuador. For each video, we included a scale bar (e.g., a ruler), applied a single stimulus to the callus of the labellum, and did not cease recording until the labellum of the flower was fully closed. As a result, we obtained >900 videos spanning 30 species of *Porroglossum* (>20 samples per species). By utilizing the program Tracker, we were able to calculate the average time to snap-trap closure for each species as well as the average velocity and acceleration of the snap-trap for each species. Furthermore, we implemented statistical methods in R to map the aforementioned continuous traits for each species onto the GBS phylogeny to calculate phylogenetic signal. By doing so, we documented strong phylogenetic signal when using both Pagel's λ (λ = 1.0840, p = 0.0014) and Blomberg's *K* statistic (*K*= 1.7001, p = 0.0027) indicating that closely related species resemble each other more than expected by chance in relation to time, velocity, and acceleration.

T2-50-06

Biogeography and diversification of Zygopetalinae (Cymbidieae, Orchidaceae)

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Determining historical biogeography is fundamental to understanding diversification in Neotropical Orchidaceae and the mechanisms underlying spatial patterns. Widely distributed throughout tropical America, subtribe Zygopetalinae comprises 36 genera and more than 430 species adapted to live as epiphytes in temperate premontane and humid lowlands forests below 1500 m. This habitat's specificity is reflected in the high level of endemism exhibited in the group, with almost 70% of the species distributed in small areas within any one country. To assess the historical biogeography of these orchids, we inferred phylogenetic relationships from three plastid DNA sequences, *matK*, *ycf1*, and *trnL*-F intergenic spacer and the nuclear ribosomal ITS region. Bayesian, maximum likelihood, and parsimony analysis of combined datasets revealed phylogenetic results, which were congruent with previous studies. We also calculated divergence dates, an ultrametric analysis using BEAST. Based on a crown clade age of 11.8 My, Zygopetalinae orchids appeared by middle Miocene, and their diversification happened in the last 5 My, coinciding with major geologic events, such as the uplift of the Andes and the final emergence of the Panama Isthmus. Biogeographic reconstruction for some genera of these orchids indicated a clear origin in South America and recent dispersal events to Central America, like in *Benzingia, Chaubardiella, Chondroscaphe* and *Warczewiczella;* meanwhile for others, *Dichaea*, *Cryptarrhena*, *Huntleya* and *Kefersteinia,* the pattern is unclear. The northwest of South America was identified as the center of diversification and the richest area in endemic species; two other regions were also found, one in Central America and the other in the Southeast of Brazil. Given the high habitat specificity in the group and the increasing alteration of the natural forest in some of these rich areas, the protection of the Andean montane and humid lowland forest is a priority for the conservation of these Neotropical orchids. This study provides information that highlights the vulnerability of Neotropical Orchidaceae.

T2-51: Systematics and evolution of Liliales and Asparagales

T2-51-01

The Monocotyledonous underground: Excavating the evolutionary history of geophytes

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Geophytes, plants with resting buds located beneath the soil surface, are able to survive periods of unfavorable growing conditions by lying dormant below the soil surface. Examples of this alternative lifestyle include rhizomes, corms, tubers and bulbs. These adaptations are hypothesized to have evolved in response to an increase in seasonality, and are renowned landscape beautifiers in areas such as the Mediterranean Basin or the Cape Floristic Region. In addition to these geographic hotspots, geophytic lineages can be found across the globe and are distributed across the plant tree of life. However, geophytic taxa are most prevalent within the monocot clade and include members such as ginger, taro, arums and tulips. Interestingly, some monocot clades appear to favor a certain underground adaptation whereas other groups contain a concoction of different traits. This breadth of diversity has led many of these groups to become highly prized for both their horticultural and agricultural value, which has warranted researchers' attention for centuries, yet a majority of studies on the groups possessing these adaptations have primarily focused on select clades. Broad phylogenetic inferences of these traits have yet to be carried out. Here we attempt to answer several questions related to these fascinating adaptations in order to uproot their evolutionary complexity. We dig into questions such as: (1) Do certain climatic variables (e.g., precipitation vs temperature) favor certain geophytes? (2) Does climate or shared ancestry play a larger role in geophyte evolution? (3) How has biogeography affected the evolution of these traits? (4) Are there selective advantages for any of the traits? Using a transcriptomic dataset, in addition to a large, comprehensive phylogeny we investigate these questions in the monocot clade. By digging deeper into the complex evolution of geophytic lineages we can bring to the surface clades in need of more research in order to fully tease apart the complex evolution of geophytism, highlight geographic areas that favor geophytism and may need conservation, and predict the future response of these groups to climate change.

T2-51-02

Overall view on genus *Dipcadi Medik.* (Asparagaceae) in India

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Genus *Dipcadi* Medik. of about 55 species with main center of diversity in Africa is represented by about 9 species in India of which 5 species are endemic to the country. It largely remained under explored in India from the point of view of taxonomy, distribution, phenology, ecology, reproductive biology, cytology and understanding of diversification of the genus in the region.

Taxonomically the genus is difficult because of unvarying external morphology of species and there are no good qualitative characters to differentiate and delimits of the species. The species of the genus are primarily distinguished on the basis of taxonomically bad quantitative characters. Distribution, phenology, odor of flower, chromosome number, karyotype and overall morphology are collectively of considerable importance in the taxonomy of Indian species of the genus and their reproductive isolation and diversification in India. Therefore, concentrated efforts were made to collect populations of *Dipcadi* from various localities from all over India and to grow them in garden under similar ecological conditions. Continuous and critical observations were made on their morphology, phenology, floral characters including odor of flowers and blooming time and stigma receptivity during last decade to assess Indian species of *Dipcadi*. Different diagnostic features of taxonomic value have been identified to delimit the species and probable mechanisms operating in speciation and diversification of the genus have been identified. Cytological data combined with morphology is found to be useful in distinguishing and delimiting *Dipcadi* species in India. On the basis of chromosome number, the Indian species can be grouped into three groups viz. *Dipcadi* species with 2n = 12,20 and 22. Flowering period, somatic chromosome number (2n), length of flower, extent of union of perianth lobes, length of bract, width of leaf and odor of flower are of diagnostic value. Odor of flower has played significant role in reproductive isolation of populations and speciation of the genus in India. The paper gives systematic account of the genus in India, a botanical key for determination of the Indian species and discusses speciation in the genus with reference to India.

T2-51-03

Comparative genomics and phylogenomics of East Asian tulips (Amana, Liliaceae)

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The genus *Amana Honda* (Liliaceae), when it is treated as separate from Tulipa, comprises six perennial herbaceous species that are restricted to China, Japan and the Korean Peninsula. Although all six Amana species have important medicinal and horticultural uses, studies focused on species identification and molecular phylogenetics are few. Here we report the nucleotide sequences of six complete Amana chloroplast (cp) genomes. The cp genomes of Amana range from 150,613 bp to 151,136 bp in length, all including a pair of inverted repeats (25,629–25,859 bp) separated by the large single-copy (81,482–82,218 bp) and small single-copy (17,366–17,465 bp) regions. Each cp genome equivalently contains 112 unique genes consisting of 30 transfer RNA genes, four ribosomal RNA genes, and 78 protein coding genes. Gene content, gene order, AT content, and IR/SC boundary structure are nearly identical among all Amana cp genomes. However, the relative contraction and expansion of the IR/SC borders among the six Amana cp genomes results in length variation among them. Simple sequence repeat (SSR) analyses of these Amana cp genomes indicate that the richest SSRs are A/T mononucleotides. The number of repeats among the six Amana species varies from 54 (*A. anhuiensis*) to 69 (*A. kuocangshanica*) with palindromic (28–35) and forward repeats (23–30) as the most common types. Phylogenomic analyses using likelihood inference and Bayesian analysis based on these complete cp genomes and 74 common protein-coding genes strongly support the monophyly of the genus, and a sister relationship between Amana and Erythronium, rather than a shared common ancestor with Tulipa. Nine DNA markers (*rps15–ycf1*, *accD–psaI*, *petA–psbJ*, *rpl32–trnL*, *atpH–atpI*, *petD–rpoA*, *trnS–trnG*, *psbM–trnD* and *ycf4–cemA*) with number of variable sites greater than 0.9% were identified, and these may be useful for future population genetic or phylogeographic studies of Amana species.

T2-51-04

Accelerated rates of climatic-niche evolution in Old World after migrating from the New World: Evidence from spatiotermporal diversification history of the Greenbrier family (Liliales: Smilacaceae)

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Explain differences in species richness between regions and among clades is an important topic in biogeography. The diversification rate of clades is a key parameter for understanding both patterns. Many studies have showed examples in animal and plant groups with regional and cladistical difference in speciation rate. These disparities might correlate to climatic shifts and topographic heterogeneity. Smilacaceae is a widespread monocot group with both Old World and New World distribution. We combined phylogenetic, fossil records, and climatic data for 150 species of greenbrier family (Smilacaceae) to assess the spatiotermporal diversification focused on Old World and New World clades. Old World clade of Smilacaceae underwent a distinct increase in speciation rate around Late Eocene to Middle Miocene (34 Ma-15 Ma) compare to their New World allies. Accelerated diversification in the Old World Smilax is associated with climatic-niche evolution among species triggered by intensive topographic change and dramatic climatic fluctuation during the period of accretion of the Indian subcontinent to Eurasia.

T2-51-05

Phylogenomics and historical biogeography of Asparagales

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Phylogenomics refers to the use of phylogenetic trees to explain genome evolution and to use of genome-scale data to reconstruct phylogenetic trees. As more complete genomes are sequenced thanks to the now widespread availability of next generation sequencing technologies, phylogenomic has advanced rapidly in the past decade and recent studies have demonstrated the power of this approach. Asparagales is the largest order of monocots (14 families, 1122 genera, and 26,070 species; 50% of monocots), and it is economically important as ornamentals and crop plants. Although a number of studies have generally clarified the relationships among the big families as like those of Asparagaceae s.l., Alliaceae s.l., and Xanthoreaceae in Asparagales, the exact relationships of families, especially in lower asparagoids distributed in the Southern Hemisphere, are unclear. Here, we present the phylogenomic analyses of relationships among all 14 families of Asparagales based on 79 coding genes of chloroplast genome. Based on the phylogenetic framework, fossil-calibrated chronogram, we also provide the historical biogeography of Asparagales taxa with a focus on their main routes for the migration between the Northern and the Sourthern Hemispheres.

T2-51-06

A hidden evolutionary story in Liliales from organelle genome data

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The order Liliales include potentially medicinal and economically ornamental species which are either worldwide or natively distributed. Exploring organelle genome data of this order will improve the understanding of genomic evolution and contribute to further research on conservation and biodiversity. Therefore, we sequenced chloroplast genome of 45 representative species which cover all 10 families of Liliales and did a comprehensively comparative analysis. The results showed highly conserved chloroplast genomes in the photosynthetic group and a significantly structural reorganization in Corsiaceae which contain mycoheterotrophic taxa. Among Liliales families, Melanthiaceae possess a higher frequency of genomic events including gene duplication, deletion, and inversion. Further investigation revealed that deletion of gene and inversion in chloroplast genome was occurred in accordant with the diversification of species (ie. *Veratrum, Trillium,* and *Colchicum*), suggesting that genomic events reflected the phylogenetic relationship in Liliales. Furthermore, by comparing junction among large single copy (LSC), small single copy (SSC) and inverted repeat (IR) regions of chloroplast genomes from basal angiosperm to most of monocotyledon orders, we proposed that the border of LSC/IR could be categorized into 5 types which expanded from *rpl2* to *rps3* genes. In contrast, the SSC/IR junction was commonly located within ycf1 coding region which was supposed to be a potential region for developing marker among angiosperms. Additionally, the first data of mitochondrial genomes in Liliales demonstrated a contrasting trend between photosynthetic groups and mycoheterotrophic species in which a higher rate of gene transferred from chloroplast to mitochondrial genome was found in the former. Overall, organelle genomes of Liliales improve the understanding of plastid and mitochondrial genome evolution of angiosperms in general and of monocotyledons in particular.

T2-52: The evolution of apomixis in natural plant populations

T2-52-01

The role of ploidy and niche shifts, apomixis, residual sexuality and mutational load in the formation, dynamics and evolution of agamic complexes

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Polyploidy is one of the most relevant factors promoting plant diversity. Polyploid plants frequently associate with diploid parents to form interbreeding complexes in which each cytotypes gene pool retain genetic cohesivity whereas allowing a certain level of recurrent genetic exchange. In some cases, the genomic shock resulting from a change in ploidy is accompanied by a reproductive shift from sexual to asexual seed formation, or apomixis. Because apomixis skips meiosis and syngamy, it highly restricts gene flow and modifies the genetic structure of natural populations. Additionally, in absence of recombination deleterious mutations can′t be purged by selection, and stochastic loss of individuals with the least-loaded mutational class would lead to an irreversible increase of mutational load in asexuals (a phenomenon called *Muller's Ratchet*). Thus, deleterious mutation accumulation is expected to drive clonal lineages to a genomic decay and extinction. However, 1) the ratchet mechanism is efficient in small diploid and obligates asexual populations, while polyploids mask recessive mutations and buffer the effect of mutation accumulation, 2) apomictic plants in polyploid complexes usually are widespread over extended geographic areas (depicting a pattern of *geographical parthenogenesis*), and 3) apomixis in plants is hardly ever obligated and variable levels of residual sexuality are normally observed among genotypes. Using frequencies of meiotic recombination events observed in progenies of apomictic plants, a mathematical modeling for predicting the dynamics of mutant frequencies as a function of time show that low levels of meiotic recombination may counteract the ratchet mechanism. Under various selection coefficients and plant-specific mutation rates, low recombination rates suffice to prevent facultative apomictic plants from the accumulation of slightly deleterious mutations. Uniparental reproduction, cytotype niche differentiation, fixation of adapted genotypes and small proportions of new recombinants in facultative apomicts facilitate range expansion by coping and occupying new ecological niches, and help to explain geographical parthenogenesis in sexual-asexual plant complexes. High levels of intragenomic (allelic) diversity, epigenetic variability, and physiological plasticity further enhance fast ecological flexibility, adaptation, and dispersal. Finally, occasional re-stabilization of meiotic pathways, reversals to sexuality in apomictic lineages and establishment of new sexual populations will be constrained by *Allee* effects, but when possible, the occurrence of new sexuals in areas far from the parental species can promote divergent evolution and speciation. Phylogenetic patterns of apomixis and biodiversity parameters support this hypothesis and place a potential role of facultative apomixis as a promoter of plant biodiversity.

T2-52-02

Next-generation sequencing of apomictic fern (*Woodwardia*

orientalis) and non-vivipary fern (*Woodwardia japonica*) reveals candidate genes for vivipary character

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Vivipary is an asexual reproduction (apomixis) process which occurs in plants diversity due to environmental stress and lack of phyto-hormone function. Pseudo-vivipary describes that plants produce apomictic or asexual propagules like bulbils or plantlets in place of sexual reproductive structure. The objective of our present study is to compare Chinese pseudo-viviparous medicinal fern, *Woodwardia orientalis* (W.O) and non-viviparous fern, *W. japonica* (W.J) in order to reveal their functional viviparous mutant genes for viviparous character and spore dormancy. The transcriptomics data was acquired in our study using newly developed Illumina Hiseq^{TM} 2000 RNA sequencing. Among differentially expressed genes (DEGs), 6997 up-regulated genes and 39912 down-regulated genes were identified in mature leaf of *W.orientalis* and their vivipary buds, respectively. Of them, 75 up-regulations and 39 down-regulations of transcription factors were also exhibited. All of these DEGs were associated with 112 metabolic pathways. In addition, all of them are involved in starch and sucrose metabolism, phenylpropanoid, secondary metabolites, and hormones biosynthesis pathways. Moreover, all the genes in fatty acid, brassinosteroid biosynthesis and basal transcription factors were up-regulated. Totally, 12936 simple sequence repeats (SSRs) in *W. orientalis* and 9920 SSRs in *W. japonica* were known as possible molecular markers, which may helpful for genetic diversity studies. All the unigenes were annotated to numerous databases (Nr, Swiss-Prot, GO, COG, KEGG) to understand their functional classification. In pseudo-vivipary fern (W.O), we identified the vivipary mutant genes (*NOT2/NOT3/NOT5* family isoform 2), ABA content reducing gene (*Abscisic acid deficient 4*) and spore dormancy reducing gene (transcription factor viviparous 1). The pseudo-vivipary fern (W.O), exhibited a special type of protein, dehydrin which performs cellular protection against osmotic stress while the same gene was not present in non-vivipary fern (W.J). Moreover, DELLA protein DWARF8 reduces or represses gibberellic acid (GA) inducible genes and also present in viviparous fern (W.O). There are no genes which are responsible for reducing spore dormancy, ABA content, gibberellic acid (GA), and vivipary mutant genes in non-viviparous fern, *W.japonica*. In addition, greater expression level of ABA (Abscisic acid) and GA (Gibberellic acid) inducing genes in non-vivipary fern (W.J) and lower expression of these same genes found in vivipary fern (W.O) transcriptomic sequencing data. Furthermore, significant functional genes were identified to confirm their presence in mature leaves, vivipary buds of *W. orientalis* and mature leaves of *W. japonica* using RTPCR technique. However, our present study opens the entry to understand the molecular mechanism of pseudo-vivipary fern for the first time in pteridophyte's scientific research.

T2-52-03

Progress towards understanding the causes of geographical parthenogenesis in diploid sexual and autopolyploid apomictic easter daisies (*Townsendia hookeri*: Asteraceae).

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Ecological divergence between sexual polyploids and their diploid progenitors permits new polyploids to avoid minority cytotype interactions that would limit their establishment. These dynamics are potentially different when polyploids are apomictic, because apomixis can protect nascent polyploids from hybridization. Further, apomicts that retain male function can reduce recruitment of sexual diploids by siring interploidy hybrids with sexual diploid mothers, suggesting that sexual diploids are threatened by the presence of apomicts, even when apomictic polyploids are the minority cytotype. *Townsendia hookeri* includes autopolyploid (triploid and tetraploid) apomicts and sexual diploids. The ranges of the two cytotypes are distinct, but overlap in southern Wyoming and Northern Colorado (USA), with the sexuals extending to southern Colorado, and the apomicts extending north to southern Alberta and British Columbia (Canada). Species distribution models reveal evidence of differentiation in climatic niches of the cytotypes, but suggest that diploids may have the capacity to spread further into the polyploid range. Crossing experiments in the field indicate that pollen from apomicts can readily fertilize ovules of sexual diploids, producing viable offspring of varying DNA content, mostly above diploid levels. Along with the results of niche modeling, our crossing results suggest that sexual expansion could be limited by reproductive interference rather than ecological factors. In order to test the role of ecological divergence in limiting the distribution of apomicts and sexuals, we set up a large- scale experiment that includes common gardens that span allopatric and sympatric parts of the range of each cytotype. Eight transplant gardens were established in the summer of 2014, and are being monitored for survival, growth and reproductive traits, with a separate experiment to look at early establishment success. Our predictions, that apomictic polyploids would have low fitness in sexual diploid sites, is not supported to date, and apomicts show advantages in early life stages. The study of ecological and evolutionary interactions between cytotypes in an apomict such as Townsendia hookeri, emphasizes the need to consider the selective pressures that act on diploids, during and after the establishment of asexual polyploids.

T2-52-04

Effects of light and temperature stress on the expression of apomixis in *Ranunculus*

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Apomixis, the asexual reproduction from seed, occurs in natural plant populations in many angiosperms lineages. Most apomictic species retain the ability for facultative sexuality and thus reproduce with variable frequencies of sexual and apomictic seed formation. The factors determining the frequencies of sex and apomixis are still not completely understood. Abiotic factors, like temperature and length of the photoperiod affect growth and development of plants. Unfavorable and stressful environmental conditions are supposed to play an important role in the expression of apomixis and might influence the apomictic or sexual pathway directly. One of our model system is the hexaploid (allopolyploid) natural hybrid *R. carpaticola* x *cassubicifolius* (*R. auricomus* complex), a forest understory plant native to the western Carpathians, Slovakia. We hypothesized that light stress would enhance the production of reactive oxygen species and oxidative DNA damage in the flowering buds. We consider meiosis as a repair mechanism of DNA damage, and hence we expect under stress a higher frequency of sexuality. We tested if variation in facultative apomictic (aposporous) reproduction in this species was affected by light stress (16.5 h photoperiod, compared to 10 h in the control). We analyzed two developmental stages: the ovule directly after megasporogenesis (via microscopy) and the seed stage (via flow cytometric seed screening, FCSS). The prolonged photoperiod in *R. auricomus* resulted in higher frequencies of sexual megaspores versus aposporous initial cells, while the frequencies of sexual versus asexual seeds were not affected, suggesting that only meiosis and sporogenesis are sensitive to light stress. A concomitant change of secondary metabolite profiles at the early developmental stage supports the hypothesis that oxidative stress could have affected megasporogenesis. Our second model plant, *R. kuepferi*, occurs with diploid and polyploid cytotypes in the European Alps. A correlation between mode of reproduction and temperature was observed in natural populations. While the predominantly obligate sexual diploids grow in lower altitudes, the facultative tetraploid cytotype is more widespread at higher elevations with lower mean annual temperature. We tested experimentally if low temperature and repeated frost treatments triggered the production of apomictic seeds in diploid and tetraploid *R. kuepferi* plants (FCSS). We observed a slight but significant increase of apomictic seed production in cold treated diploid *R. kuepferi* plants, while the likewise slight increase in tetraploids was not significant. Light stress and low temperature can affect the mode of reproduction in *Ranunculus*. However, in natural populations and environments a combination of diverse abiotic and temporary stressful growth conditions is likely. Facultative apomixis might be an adaptive strategy for plants to reproduce under unfavorable environmental conditions. The actual impact of stress most likely depends not only on the severity or combination of parameters, but also on the reproductive stage and on ploidy level of the plant.

T2-52-05

Adaptive advantages of partial clonality

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Plants are notorious for the kaleidoscopic diversity of their reproductive systems. For example, many plants undergo both sexual and clonal (apomictic, asexual) reproduction simultaneously. Thus, in contrast to the either/or choice often evoked to discuss the evolution of sex, plant sexual/clonal reproductive strategy may be tuned on a much finer scale. Which factors act to calibrate it? Partial clonality might improve the potential for adaptation to new selective pressures. Using sexual reproduction to create new and clonal reproduction to maintain well-adapted genotypes, populations of partially clonal plants could be able to combine the "best of both worlds". We tested this hypothesis with a mathematical model, comparing different selection schemes across the whole range from completely sexual to completely clonal reproduction. For each scheme, we investigated which rates of clonality make adaptation at the population level most probable, fastest and most efficient. We found selection for a heterozygous genotype (heterosis) to be the only selection scheme with clear adaptive advantages for partial clonality. This ties in well with the frequent coincidence of apomixis and hybridization in plants, and adds another angle to our understanding of the evolutionary maintenance of apomictic systems. We discuss further questions raised by our result, as well as its implications for the frequencies of sexual reproduction expected to be found in natural plant populations.

T2-52-06

Genome evolution in *Taraxacum agamospecies* and comparisons with triploids

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Taraxacum officinale agg. (dandelion) includes triploid (2n=3x=24) agamospecies showing extensive and characteristic morphological variation. A key question asks how the apomictic species gain variation without sexual recombination? We looked at three apomictic microspecies using molecular markers and next generation sequencing to reconstruct the chloroplast genomes and other genome regions. In particular, we will report on the nature, organization and abundance of the repetitive DNA present in the genomes. We have identified these elements in unselected genomic raw-read DNA sequence data from three agamospecies. Most of the repetitive DNA can be identified as being retrotransposon-related, although a significant proportion of the sequence reads are repetitive but unrelated to known retrotransposon sequences. Few satellite repeats were found. Most retrotransposons show similar abundance in the three microspecies. A few classes show substantially different copy numbers in the accessions, which is also seen in the contrasting chromosomal distributions, indicating amplication, homogenization and loss of sequences during separation of the taxa. IRAP markers (Inter-retroelement amplified polymorphisms) can be used to group and classify the microspecies, showing that retroelements are active and related to evolution. The results from nuclear repetitive DNA will be compared with chloroplast sequences (http://dx.doi.org/10.1371/journal.pone.0168008) and the genomic organization of repetitive DNA in other triploid species associated with humans, saffron crocus and banana. The contribution of repetitive sequences to genome evolution and variation in *Taraxacum* will be considered in relation to phylogeny, selection and generation of variation.

T2-53: Role of plant-pollinator interactions in generating and maintaining biodiversity

T2-53-01

Plant-pollinator interactions, reproductive isolation, and the diversification of flowering plants

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Flowering plants are spectacularly diverse, and flowers themselves have been implicated in species diversification because of the effects they have on plant mating patterns. In comparative studies across the angiosperms, we typically see higher diversification rates in plant lineages with animal pollination and floral traits indicative of specialized plant-pollinator relationships. Although most coevolutionary relationships between plants and pollinators are diffuse and do not involve cospeciation, divergence in plant-pollinator interactions can contribute substantially to plant reproductive isolation, act synergistically with other forms of ecological divergence, and allow closely related species to coexist without reproductive interference. Floral divergence can involve adaptation to different pollinators or more subtle changes in the way a plant uses the same type of pollinator, and importantly, floral isolation can evolve as an indirect byproduct of floral adaptation or through natural selection to reinforce reproductive isolation *per se*. I explore both types of floral divergence and modes of selection primarily using the Neotropical spiral ginger genus *Costus*, which has rapidly diversified into dozens of species pollinated by either orchid bees or hummingbirds. I ask how strikingly novel flowers that fit a new type of pollinating animal evolve, when often the inferred transitional states appear maladaptive, and to what extent an evolutionary shift in pollinators contributes to reproductive isolation and coexistence. Traits that deter ancestral pollinator types are crucial to providing reproductive isolation, but may evolve late in the process of a pollinator shift. I further investigate how divergence in flower shape can allow two closely related spiral ginger species to use the same hummingbird pollinators while risking little cross contamination of pollen. In this case, I ask whether natural selection to reduce hybridization in sympatric areas has driven the divergence, and how selection interacts with the genetic architecture underlying the flower morphology. Overall, we see that floral divergence proceeds along pathways facilitated by existing traits and genetic correlations, takes advantage of pollinator assemblages that are already in place, and can be the target of direct natural selection upon secondary contact. This work helps elucidate the myriad ways plant-pollinator relationships can contribute to reproductive isolation and promote plant diversification.

T2-53-02

The diversity and evolution of pollination systems in the ANITA grade of earliest-diverging angiosperms: New insights

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Pollination in phylogenetically early-diverging flowering plants has been thought to mostly be generalized, but this hypothesis is based on field observations of less than 10% in the ANITA grade families. The most species-rich family Schisandraceae (*Illicium*, 40 species; *Kadsura*, 16 species; and *Schisandra*, 22 species) holds great potential for understanding the origin of traits that are characteristic of more derived evolutionary lineages. *Schisandra* and *Kadsura* species bear separate staminate and pistillate flowers. *Illicium* was historically segregated in the Illiciaceae, and bears bisexual flowers, but is now known to be unambiguously sister to the *Schisandra-Kadsura* clade. We try to use an integration of pollination biology, molecular phylogeny, and character-evolution inferring methods to analyze coevolutionary/pollinator shift relationships between Schisandraceae plants and their pollinators. Our field work finds that, in Asia, gall midges are effective pollinators of Schisandraceae. Trees of Subgen *Cymbostemon* bear red bisexual flowers which are pollinated by gall midges, but intriguingly exhibit post-anthetic floral thermogenesis as a pollinator reward; this suggests that floral heating-possibly an ancestral trait in the family-has been co-opted for post-anthetic nurturing of gall midge larvae. While in Subgen *Illicium* often bear white flowers and was pollinated beetles. Our field observation and previous studies also found that species in *Schisandra* and *Kadsura* pollinated by gall midges (Cecidomyiidae), which have a special visiting behavior. In this presentation, I will show a highly diversified species-specific pollination mutualism between the base angiosperm family, Schisandraceae, and a resin midge genus, *Resselillea*. In this mutualism association, the female resin midge visit the flowers and lay eggs in the flower (male and female or bisexual) at night, and the larvae feed on the post-andthetic secretion of flower for growth. These results also suggest that the diversification of insect-pollinator mutualism in early angiosperms may have been facilitated by the exploitation of preexisting sensory biases of herbivores.

T2-53-03

Quantifying integration of native and alien pollinators on the Galápagos Islands

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Natural ecosystems are increasingly threatened by biotic homogenization due to the expansion of some "winning species" and the concomitant extinction of "looser species" - a situation persistently fuelled by anthropogenic disturbances and species introductions. One key question arising under such a scenario is to what extent these new winning species will fully replace the functions lost with the extinction of the indigenous biota. The functional role of species is to a large extent rooted in the way that species engage in complex interaction networks with other co-occurring species. Indeed, ecological networks have proved a most valuable way of analysing an entire ecological community as a coherent unit. Although species and interaction turnover can be high between spatially or temporally separated networks, recent studies also suggest that species roles can be conserved and that species do not interact with a random assemblage of the potential partners. Phenological and morphological matches likely drive such non-random choice of partners. If species differ in their interaction behaviour it is therefore unlikely that 'winning' alien species will replace the roles of 'loosing' indigenous species. Here, we investigate this by focusing on alien and native insect pollinators on the Galápagos Islands. Using network analysis as a tool for understanding functional roles, we quantified whether alien and native pollinators are integrated differently into pollination networks on these islands. Surveys were undertaken at five major islands within the Galápagos archipelago *viz*. San Cristobal, Santa Cruz, Santiago, Pinta and Fernandina. In predefined areas on each island, plant species were surveyed for pollinators during the peak flowering season for two consecutive years, allowing the construction of quantitative pollination networks. If aliens and natives were functionally equivalent, the community response to sequential removal of either natives or aliens would be similar. Performing 200 iterations, we sequentially and randomly removed all alien pollinators from a given community and at each step we re-calculated structural network descriptors (e.g. weighted nestedness and specialization) to quantify how these removals affected the network structure. Subsequently, we repeated the process but instead randomly removed an equal number of native pollinators. This allowed us to compare how the two different removal scenarios affected the network topology, and essentially to quantify whether alien and native species are integrated similarly. For several islands, but especially the two most invaded ones (San Cristobal and Santiago), there were substantial differences between the native and alien removal simulations, suggesting that native and alien pollinators are indeed integrated differently. Tentatively, we infer that small individual differences in the integration of native and alien pollinators on the Galápagos can produce large accumulated differences at the community level. These findings provide a piece to the puzzle of understanding how the threatening biotic homogenization may influence natural communities, and cautiously suggest that an exact replacement of lost functions is highly dubious.

T2-53-04

Ecological speciation through floral scent/pollinator divergence in Asian Mitella

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Floral scents are among the key signals used by pollinators to navigate to specific flowers. Therefore, evolutionary changes in floral scents should have strong impacts on plant speciation through pollinator shifts. Nevertheless, few cases of ecological speciation initiated by floral scent changes are reported, and thus the presumed divergent selection acting on the floral scents has rarely been demonstrated. Here we present an ongoing research project that aims to extensively isolate and analyze genes involved in floral scent production in Asian *Mitella* (Saxifragaceae), where floral scent composition repeatedly switched between species adapted to pollination by long-tongued and short-tongued fungus gnats. With the combined use of RNA-seq, recombinant protein assay, and linkage analysis with interspecific cross, we successfully identified several genes involved in the production of the key floral scent compounds for pollinator specificity in Asian *Mitella*. Similar approach would be effective for any plant lineages that utilize scents as the major signals for communicating with their mutualists and/or antagonists.

T2-53-05

Patterns of flowering phenology and floral Trait diversification in two Neotropical Gesneriaceae Genera

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Pollinators have long been recognized as important selective agents in floral trait evolution. Evidence for pollinator-mediated selection on traits associated with pollinator attraction and pollen transfer effectiveness is compelling in many plant taxonomic groups. However, the role of biotic interactions on the diversification of flowering phenology patterns is not yet well understood. Here, we use a comparative framework to assess patterns of diversification of morphological and phenological traits in the subtribe Gesneriinae and the genus *Achimenes* in the sister subtribe Gloxiniinae. Pollinator shifts in both genera are associated with floral trait diversification, but not with phenological patterns. In the *Achimenes* in particular, phenology appears to be constrained by a geophytic growth form, a condition that may have triggered evolutionary changes in floral traits to reduce interspecific hybridization or competition for pollinators. This study highlights the importance of studying phenology along with other morphology and other floral traits for a more comprehensive understanding of the factors underlying floral diversification in different plant lineages.

T2-53-06

Pollinators determine assembly of flower colour patterns in communities of mass flowering annual daisies

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Communities are thought to be assembled either neutrally or non-randomly (through ecological sorting, or evolutionary interactions). In addition to abiotic processes structuring communities, biotic interactions can also play a key role in how communities are assembled. For example, the pattern of assembly of floral phenotypes (or pollination syndromes) within plant communities likely reflects ecological filtering or floral evolution in response to the community of available pollinators and the nature of interactions between plants for pollination services. Overdispersion of floral phenotypes within communities would be expected if competitive interactions between plants favour partitioning of the pollinator resource. Clustering of floral phenotypes would be expected when plant-plant interactions are facilitative, or when filtering or floral divergence occur in response to differences in the available community of effective pollinators across communties. Here we assess the landscape-level patterns of community assembly of flower colour patterns in annual mass daisy flowering displays, in the winter rainfall deserts of South Africa. While the open shape of daisy inflorescences does not preclude visits from any insects, annual daisy species in these communities exhibit a wide array of bullseye colour patterns which may represent a syndrome trait associated with particular pollinators. Using a null modelling approach we show that colour patterns are clustered within communities, with the dominant colour pattern varying across the landscape. This suggests different communities are dominated by pollinators with dissimilar preferences, and pollinators either facilitate the co-occurrence of plant species with similar floral phenotypes, or plant species converge in phenotype in response to effective pollinators. Analysis of community visitation networks demonstrates that the dominant flower colour pattern within communities is associated with particular fly pollinator species. If different fly species prefer particular floral phenotypes, we expect pollinators to exert divergent selection on floral phenotype. We find turnover of dominant colour patterns across the landscape corresponds to turnover in the fly pollinator community, and experimental evidence suggests that fly species exhibit divergent preferences for colour patterns. Together these lines of evidence suggest non-random assembly of flower colour patterns resulting from convergence of multiple species within communities on traits favoured by dominant fly pollinators.

T2-54: Ranunculaceae and the Evo-Devo of the flower

T2-54-01
Perianth evolution in Ranunculales

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Angiosperms are characterised by one of the most striking evolutionary novelties of land plants, the flower, which presents an extraordinary diversity in shape, size and colour in relation with pollination, a key step in the life cycle of flowering plants. This evolutionary novelty accounts for the fact that angiosperms are by far the most successful clade of land plants. In most angiosperm species flowers possess a differentiated perianth composed of sepals and petals, but a significant number of species displays an undifferentiated perianth composed of a single category of organs, called tepals. Ranunculales are one of the few orders that includes species with either a differentiated or an undifferentiated perianth. This order is the first diverging order within eudicots (the largest clade of angiosperms), it is therefore a key group to understand the evolution of the flower. Progress has been made towards the elucidation of phylogenetic relationships in the Ranunculales, providing now a relatively robust framework to address the key issue of the ancestral vs. derived nature of a differentiated perianth within the order as a whole. Here, we present ancestral state reconstructions for several perianth characters including perianth differentiation, symmetry, shape of petals and presence/absence of nectaries, with a focus on Ranunculaceae.Our results challange the traditional hypothesis of petals having evolved repeatedly in the family.

T2-54-02
The ABC model of flower development in non-core eudicots: A functional synthesis from studies in the ranunculid *Thalictrum thalictroides*

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The elucidation of the "ABC model" of flower development in the 1990's promised a mechanistic basis for J.W. Goethe's intuitive idea that all floral organs must have arisen from a leaf *bauplan*. Yet the first attempts to induce floral organs from leaves by overexpression of B- or C-class genes failed to produce full conversion. The discovery of a fourth gene class, the E-class, filled this gap by elegantly demonstrating the recapitulation of floral organs in seedling leaves via overexpression of all four gene classes. The "extended" ABC model was complete and the ensuing floral quartet model clarified how these transcription factors, belonging mostly to the *MADS*-box gene family, interacted as tetramers while binding and looping DNA to turn on flower development cascades. As conserved as the ABC model appeared, first among the distinct model systems *Arabidopsis thaliana* and *Antirrhinum majus*, later in other eudicots and in monocots, much remained to be elucidated in light of mounting evidence for the importance of gene duplication in the evolution of development. Multiple duplication events affecting the *MADS*-box gene family and flower morphologies that diverged drastically from those described originally required modifications to the canonical model. As part of this evolutionary journey into the tinkering of the ABC model, be it via gene duplication, changing protein-protein interactions or changes in binding affinities and downstream targets, functional studies in a variety of plant groups began to emerge. In the past few years, my research group has functionally characterized all B, C and E class genes in *Thalictrum thalictroides*, a representative of the ranunculids (the sister lineage to the rest of the eudicots). Our work contributes to a broader effort to fill the gap in functional knowledge outside of the core eudicots in order to better understand the evolution of this important lineage of floral *MADS*-box genes. To that end we used Virus Induced gene Silencing, gene expression, protein-protein interactions and mutant analyses. Here, I attempt to summarize these efforts, highlighting particular cases of sub- and neo-functionalization, as well as redundancy, and ending with a proposed ABC model for this plant and its modifications in floral homeotic mutants.

T2-54-03
Ranunculales as new model order to gain insight into the molecular base of floral trait evolution

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Ranunculales, the sister group to all other eudicots, is an amazingly diverse plant order. Many species with diverse floral architectures are now amenable to genetic manipulation with VIGS enabling the functional characterization of key gene regulating floral development. Comparison between Ranunculales flower architecture and the respective genes regulating this architecture allows to address questions concerning the evolution of floral traits. We summarize recent advances towards understanding the evolution of floral variation and present recent results on the *CYCLOIDEA* gene function in Ranunculales.

T2-54-04
Identifying the genetic basis of a key innovation: How Aquilegia got (& lost) their spurs

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As this symposium is testament to, the Ranunculaceae are well known for their diversity of floral morphology. Much of this variation is due to the striking diversity of petal shape, from their complete absence to elaborate modifications such as tubular nectar spurs. Such nectar spurs are especially interesting as they have independently evolved within the Ranunculaceae as well as across the Angiosperms and these origins are strongly associated with bursts of species diversification. While we now know that many aspects of petal development appear to be highly conserved, little is known about the genetic basis underlying the great diversity of petal shape. Ideally, we would like to know how cell division and expansion interact to sculpt petal shape and what genes control these processes. In addition, we would like to know how novel organ shapes initially evolve and the genetic basis of morphological variation between taxa. We are using the dramatic development of the Aquilegia nectar spur to address these questions. In particular, we are investigating the genetic basis of the very early initiation of petal nectar-spur development. Key advantages to using Aquilegia is the great diversity among species in spur morphology from completely lacking to 180 mm in length, the fact that fertile hybrids can be formed between most species and a high quality reference genome sequence. To identify candidate genes underlying spur development we are using a QTL approach to first identify genomic region (s) associated with spur development. We are then using comparative gene expression profiles of stages of petal development across multiple species to narrow the list of candidate genes. These studies, combined with functional analyses of candidate genes will be discussed.

T2-54-05

Morphological development of petals in Ranunculaceae

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The petals, or the honey-leaves, are of great divergence in morphology in Ranunculaceae, i. e., tubular, bilabial, cup-shaped, flat, concaved or scaled at the base, with or without spur or succate. The previous observations showed that although the petals differ in mature morphology, they showed great similarity in the early development stage. The petal primordia are all hemispherical, rounded and much smaller than the sepal primordia, a relatively long plastochron exists between the last sepal and the first petal and differentiate into a blade and a short stalk. Thus, we assumed that the different morphology of the mature petals might be due to the morphological repatterning of petals in the development. To prove the hypothesis, the morphological development of the petals from 22 species from 20 genera, recovering all ten petalous clades and the major morphological types, in Ranunculaceae was observed by scanning electron microscope (SEM). The young petal undergoes the following developmental stages to the mature petal after it differentiates into blade and stalk. In the first stage, a depression appears at the base of the blade and the nectary tissue will appear in the depression in the later development. In the second stage, two bulges appear at the base of the depression that makes the petal bilabial and the bulges will be the upper lip of the petal and thus the blade will be the lower lip. In the third stage, two bulges become larger and fuse with one another at first and then fuse with the margins of the blade in each side, or each of the bulges fuses with the margin of the blade at first and then fuses with one another, or the bulges stop further growth and the depression deepened to form the succate or the spur. In the fourth stage, the lips, the two fused sides and the stalk growth in different speed. The divergence of development of different petals happens mainly in the third and the fourth stages and less divergence in the second and then the first stages. For example, a tubular petal of *Helleborus thibetanus* undergoes the following developmental stages: a depression appear at the base of the blade, then two bulges appear at the base of the depression, and then the bulges fuse with one another to form the upper lip, the upper lip fuses with two margins of the blade that makes the petal oblique cup-shaped, the growth speed of the upper lip is faster than the two fused sides and that of the later is faster than the lower lip that makes the petal to be tubular; a spurred petal of *Aquilegia yabeana* undergoes the following developmental stages: a depression appears at the base of the blade, then two bulges appears at the base of the depression, the depression deepens to form the spur and the bulges stop further growth. According to the molecular systematic results, the genera in two basal most clades, *Glaudidium* and *Hydrastis*, are apetalous. *Coptis* and *Xanthorhiza* are in the next basal most clade, the petal in this clade only has, or sometimes not clear, the depression at the base of the blade and the nectary tissue appears in the depression and the petals in these genera are not bilabial. The rest of the genera form the core Ranunculaceae in which two bulges appear at the base of the depression. The appearance of the bulges can be considered as a key innovation and let the petals get the diverged potential in morphology and finally resulted in the divergence of the genera. The development processes of the petals in different petalous clades, or even in different petalous genera in the same clade, are different. The bulges might be lost in *Consolida* clade, in this case, the spur is well developed, or only one bulge appears in *Ranunculus* and *Nigella* clades, or both the bulges and the depression might be lost in *Adonis* which has the flat petals without nectary tissue. The growth of the bulges which becomes the upper lip of the petal or the depression which becomes the succate or the spur can result in the hidden of the honey from the nectary tissue and thus can increase the difficulty for the nectar foraging pollinators and avoiding the non nectar foraging insects and then increase the pollination efficiency and increase the adaptability to different nectar foraging insects.

T2-54-06

Ranunculaceae and the Evo-Devo of the flower

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Flowers show tremendous diversity in size, color, structure, symmetry, display, and function, *etc*. The mechanisms that underlie the origin and diversification of the flower, however, remain largely unclear. One of the obstacles that prevent us from knowing more about the underlying mechanisms of flower evolution is the lack of suitable model systems. To solve this problem, we have developed the basal eudicotyledonous family Ranunculaceae into a model system to address important evolutionary developmental questions that cannot be easily addressed by using all the existing models. In this talk, I will first introduce the advantages of using the Ranunculaceae as a model and then present our new results on three topics: 1) the mechanisms that underlie parallel petal losses within the Ranunculaceae; 2) the molecular basis and its flexibility of the floral organ identity determination program in *Nigella damascena*, a species with spiral flower; and 3) the tempo, mode, and mechanisms of character evolution during petal elaboration within the genus *Nigella*.

T2-55: Neuro-pollination Biology

T2-55-01

The specific neural circuit of pollinating flies responses to volatile compound of orchid flower.

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Pollinators display innate attractions to a range of volatile compounds of flowers, and this vital plant-insect interaction shapes the ecological and evolutionary fates of flowering plants. However, the neuronal and behavioral basis of these interactions remain poorly understood. In our study of the deceptive pollination system of *Cypripedium bardolphianum*, we first found out the precise *Drosophila* species of pollinators which mostly are female *D. immigrans*, and identified ethyl tiglate, one of the floral volatile compounds as the key “attractive cue” in this interaction. Behavioral experiments revealed that Ethyl tiglate intrigues female flies in the role of aggregation pheromone, feeding signal and ovipositing signal, which should promote the probability and latency of landing of these pollinators. Furthermore we found out that the specific neural circuit, V glomerulus in antennal lobe of flies, mediated the olfactory signal of ethyl tiglate, which referred as “pollination glomerulus”. And the volume of this “pollination glomerulus” of pollination flies was enlarged compared to that of the non-pollination flies. Taken together, our study demonstrated that vital floral volatile compound ethyl tiglate exhibits innate multifunctional attraction of specific *Drosophila* species to accomplish the pollination task and the specialized expanded neural circuit mediating this olfactory signal evolved to serve the dramatic preference of ethyl tiglate in pollinating *Drosophila* species.

T2-55-02

The sweet scent of pollination success

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In order to forage efficiently pollinators need to make informed choices about individual flowers. In a natural environment however, floral odors are quickly intermixed with other volatile compounds from the environment such as green leaves or neighboring flowers. Due to this the hawkmoth *Manduca sexta* might need to additionally evaluate individual flowers at a close range before investing energetically costly hovering time. When we tested hawkmoths in a semi-natural free flight tent assay we did find that the moths approached wild-type *Nicotiana attenuata* flowers just as often as neighboring flowers in which the emissions of the main flower compound had been genetically silenced. In spite of this, nectar uptake was drastically reduced in scentless flowers, indicating a low degree of foraging success by the moth. Further tests in a wind tunnel revealed that also pollen take-up and pollen delivery on scentless flowers was strongly reduced due to a lower time investment of the hawkmoth into odor-free flowers. However, as these tests indicated that the hawkmoth detected volatiles of individual flowers mainly in the close vicinity, we hypothesized an additional mechanism for close-range odor detection in the moth. Using morphological, molecular and electrophysiological approaches we demonstrated the presents of additional olfactory neurons on the moth proboscis, which responded strongly to floral volatiles. To further explore the behavior significance of these neurons, a costume build two-choice assay was developed, in which in a freely flying moth only the proboscis was exposed to the flower odors. Through this we could demonstrate that the proboscis neurons were indeed sufficient to mediate the increased time investment into scented flowers and could dues also help to explain the higher foraging and pollination success of the moth on these flowers. Taken together, our results highlight the importance of volatile signals for plant-pollinator interactions and thus also for the co-evolution of these two mutualistic partners.

T2-55-03

Linking brain activity to odour-guided behaviour in the pollination model *Manduca sexta*

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Most hawkmoths feed on floral nectar, and are therefore important pollinators of night-blooming plants. To locate these nectar sources, moths use mainly olfactory cues. The hawkmoth *Manduca sexta* is predominately nectaring at flowers of the desert plants *Datura wrightii*, and *Agave palmeri*, which emit very dissimilar floral bouquets. While *Datura* has a strong, sweet smell, *Agave* has a weaker scent, reminiscent of rotten fruits. Odours are coded as spatial activation patterns across the antennal lobe, the first olfactory neuropil of the insect´s brain. We investigated if the valence of an odour is represented already at this early processing level. Using *in vivo* calcium imaging, we first established a functional atlas of the antennal lobe by stimulating the moths with a panel of 80 monomolecular compounds belonging to different chemical classes (terpenes, aromatics, aldehydes, alcohols, esters, ketones, acids, and nitrogen-bearing molecules). A subset of these compounds was used as diagnostic odours that enabled us to identify 23 activity spots, so-called olfactory glomeruli. These functional units of the antennal lobe had distinct response spectra but were mainly broadly tuned, indicating a combinatorial olfactory coding strategy. In order to study the innate significance of the tested odours, i.e. which chemicals might act as feeding stimuli, we next tested the compounds in two-choice experiments (odour *versus* solvent) in a wind tunnel. We found that foraging moths were highly attracted by some of the odours, especially by aromatics and esters, which are characteristic components of *Datura* (aromatics), and *Agave* (esters) flowers. Linking our behavioural results with the obtained functional activity patterns in the brain revealed that innate olfactory valence is encoded in the moth's antennal lobe by a group of glomeruli that is preferentially activated by attractive odours.

T2-55-04

Floral odor signals guide optimal flower selection in a hawkmoth-pollination system

Markus Knaden, *Alexander Haverkamp, Brynn Cook, Julia Bing, Bill Hansson*
Max Planck Society

In order to forage efficiently, pollinators need to make informed choices about individual flowers. This is particularly important for pollinators such as hummingbirds or hawkmoths, which feed during hovering flight - making their decisions particularly costly. If possible these pollinators should therefore recognize rewarding flowers already at a distance or at least before engaging into energetically demanding hovering maneuvers. Darwin already predicted that the best flower choice for a hawkmoth pollinator should be those flowers, which match its proboscis in length and that this foraging advantage would drive the co-evolution between hawkmoths and the flowers they pollinate. However, it has been unclear whether hawkmoths innately recognize and prefer matching flowers. Here, by measuring both the energetic costs and gains of the hawkmoth foraging in a free-flight respiration chamber, we demonstrate first that hawkmoths do indeed obtain the highest net reward from matching flowers. Moreover, we reconstructed the moth flight behavior from 3D video recordings and show that the hawkmoth preferred matching flowers most and did respond to the best matching flower already at the first encounter with the floral odor plume.

T2-55-05

Experimental test of pollination syndromes using hawkmoths and tobacco flowers

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The pollination syndrome concept continues to be debated as an organizing principle in floral ecology and evolution. A primary assumption undergirding syndromes is that pollinators show pre-existing sensory bias for specific floral traits, such as colors and scents, which translates into innate preference. Another assumption is that certain floral morphologies or shapes are easier for certain pollinators to learn to manipulate as they seek floral rewards, making them more profitable. When such flowers physically match the dimensions of their pollinators, efficient pollen transfer should result. In combination, these two features provide the ethological (attraction and preference) and mechanical (handling and fit) aspects, respectively, of Verne Grant's concept of floral isolation. We used a lineage of South American tobacco plants (*Nicotiana*; Solanaceae) and a laboratory colony of flower-naïve, inexperienced hawkmoths (*Manduca sexta*; Sphingidae) to test aspects of innate preference and handling efficiency. Individual hawkmoths were flown in no-choice flight cage assays with blooming *Nicotiana* plants whose flowers represented bee-, hummingbird-, settling moth- and hawkmoth-pollinated phenotypes. To assess pre-existing bias, we measured the latency (time to response) and the overall proportion of test moths responding to each *Nicotiana* species. To assess handling, we measured discovery time and success rate for moths to probe the nectary of each *Nicotiana* species with their extended proboscis. All bioassays were filmed and data were scored blind by student volunteers on video. As expected, *M. sexta* moths showed the shortest median latency (25 sec) and highest proportion (100%) of responses to *N. alata*, a hawkmoth-syndrome species, at rates that were twice as responsive as the next most attractive species. Conversely, moths showed lowest responses and highest latencies in response to *N. forgetiana* and *N. rastroensis*, two red-colored, poorly scented hummingbird-syndrome species. Median nectary discovery time (2 sec) did not differ appreciably between species, with slightly lower values for the small, white, upright flowers of *N. bonariensis*, a settling-moth syndrome flower. However, median success rate (40%) was at least four-fold higher at flowers of *N. alata* than at any other tobacco species. These results are largely consistent with the expectations of the syndrome concept, and reinforce recent studies showing that mechanical fit between *M. sexta* tongue length and *Nicotiana* floral tube length predicts energetic profitability in this same system. We will discuss the relative contributions of scent, color and floral posture to these results and those of other studies using similar model systems.

T2-55-06

Perfume-collecting male oil bees? Evidence for a novel pollination system involving *Anthurium acutifolium* (Araceae) and *Paratetrapedia chocoensis* (Apidae, Tapinotaspidini)

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It has been known since Stefan Vogel's observations in 1969 that solitary female oil bees collect fatty floral oils and thereby pollinate the flowers of various specialized oil-secreting plant taxa. The bees collect the oil with the help of distinct hair tufts on either their legs or their abdomen. The female bees use this oil-reward as food for their larvae and/or to line their brood cells. Similar adaptations are also known from male oil bees, although the purpose of their oil-collecting behavior has not yet been clarified. Based on an investigation made in the tropical lowland of Costa Rica, we describe a novel pollination system involving male *Paratetrapedia* oil bees and the tropical herb *Anthurium acutifolium*. We present ultrastructural morphological details of bee and plant structures involved in this interaction and the composition of floral scents likely mediating pollinator attraction. Inflorescences of *A. acutifolium* are visited almost exclusively by male *P. chocoensis* oil bees. During their visits on anthetic *A. acutifolium* inflorescences, the bees press a patch of hairs located on their abdominal sterna 3 onto the inflorescence surface. Due to this behaviour, both on staminate and pistillate stage inflorescences, the bees' abdomens and legs become loaded with pollen and also get in contact with receptive stigmas. In contrast to what has been observed in other angiosperms visited for the collection of fatty floral oils, our anatomical investigation shows that the inflorescences/flowers of *A. acutifolium* do not have specialized structures for oil secretion, i.e., they do not have any elaiophores. However, these inflorescences are strongly scented during the time they are visited by the bees. Gas chromatography/mass spectrometry (GC/MS) analyses of dynamic headspace floral samples revealed that inflorescences of both anthetic phases emitted scent bouquets consisting mainly of aliphatic esters, indole and uncommmon terpenoids (megastigmanes). Interestingly enough, our data suggest that the unusual floral scent of *A. acutifolium* is a perfume reward collected by male *P. chocoensis*. This pollination system thus bears a remarkable resemblence with the interactions between perfume-collecting male euglossine bees and their preferred flowers, discovered by Stefan Vogel half a century ago.

T2-56: Polyploidy: early effects and their evolutionary implications

T2-56-01

Effects of abiotic stress on duplicated gene expression and alternative splicing in polyploid *Brassica napus*

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Alternative splicing is a fundamental aspect of gene expression because differential AS patterns can alter the protein products produced and/or level of transcripts, which can have phenotypic consequences. In this study we investigated the effects of abiotic stress treatments on expression levels and alternative splicing of homoeologous genes in *Brassica napus*, which is an allotetraploid derived from *Brassica rapa* and *Brassica oleracea.* We conducted a comprehensive transcriptome analysis by strand-specific transcriptome sequencing to examine expression levels and alternative splicing of homoeologous genes in *B. napus* under heat stress, cold stress, and drought stress compared with untreated plants. Cold stress resulted in the greatest changes in gene expression compared to the other stress conditions. More genes had decreased expression levels under the stress treatments than increased expression levels. There were more gene pairs with A_t subgenome biased gene expression than C_t genome biased expression among the homoeolog pairs (27,360) analyzed. Overall the C_t subgenome showed a bias in alternative splicing patterns (quantitatively and qualitatively) in all conditions. The plants under cold stress and drought stress shared more changes in alternative splicing patterns than either with heat stress. These results provide insights into the effects of abiotic stress treatments on homoeologous gene expression and alternative splicing patterns. Some of the changes in expression and alternative splicing may help the plants cope with abiotic stresses.

T2-56-02

The effect of polyploidy on transcriptome size and dosage response in natural and synthetic polyploids

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"Genome downsizing" is a commonly observed phenomenon in polyploid evolution, in which the size of the polyploid's genome is smaller than would be expected given the genome size of its diploid progenitor (s). Much less is known, however, about the response of the overall transcriptome to polyploidy. We have shown previously that *Glycine dolichocarpa*, an allopolyploid ($2n = 80$) relative of soybean (*G. max*, $2n = 40$) shows "transcriptome downsizing", having a leaf transcriptome that is around 1.4x the size of its two diploid progenitors (i.e., 0.7x per genome), both of which have similar transcriptome sizes. We have extended this observation to the leaf transcriptomes of other *Glycine* allopolyploid species, and also to the root transcriptome of *G. dolichocarpa*, all of which show an approximately 1.5x larger size in polyploids. Knowledge of transcriptome size permits the dosage responses of each individual gene to be estimated, and we have found that the distribution of responses is unimodal, with the mode at around 1.5x per cell, which equates to ca. 0.75x per gene copy. The biological relevance for this response remains unknown. Because these natural *Glycine* allopolyploid species are all several hundred thousands of years old, it is also unclear whether this downsizing is an immediate effect of allopolyploidy, or a function of parallel divergence in these independently formed polyploids. To explore the earliest stages of polyploidy, we have measured transcriptome size in a synthetic *Arabidopsis thaliana* polyploid. As in *Glycine*, the *Arabidopsis* C24 autotetraploid exhibits leaf transcriptome downsizing (0.8-0.9x expression per genome), as well as a unimodal distribution of gene dosage responses centered near 0.8x per gene copy. Estimates of transcriptome size per cell are complicated by the fact that, unlike in *Glycine*, *Arabidopsis* leaf tissue includes endopolyploid cells, and the degree of endopolyploidy varies between accessions. Allowing for endopolyploidy, average transcriptome size on a per cell basis is only about 1.1-1.2x larger in the tetraploids than in their diploid parents. Approximately 20% of genes are dosage compensated (no change in expression following a doubling of copy number), and these are enriched for primary metabolic functions, including translation and photosynthesis. Approximately 34% of genes show a 1:1 dosage effect (a doubling of transcription following a doubling of copy number), and these are enriched for GO terms relating to signal transduction and transport, similar to responses in *Glycine* allopolyploids. The fact that a doubling of gene content does not produce a doubling of transcription, even in the first generations after genome duplication, indicates that less than doubled gene expression is an immediate effect of genome duplication, and perhaps an emergent effect of polyploidy.

T2-56-03

Allotetraploidy generates extensive homeologous expression rewiring and phenotypic diversity at the population level in rice

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Allopolyploidization, i.e., concomitant merging and doubling of two or more divergent genomes in a common nucleus/cytoplasm, is known to instantly alter transcriptome dynamics, a phenomenon referred to as "transcriptomic shock". However, the immediate effects of transcriptomic alteration in generating phenotypic diversity at the population level remain under-investigated. Here, Mass ARRAY-based Sequenom platform was employed to assess and compare orthologous, allelic, and homeologous gene expression in leaf and root of randomly-chosen individuals from populations of parental subspecies (*indica* and *japonica*), *in vitro* "hybrids" (parental mixes), reciprocal F1 hybrids and tetraploids. The data revealed that hybridization and whole genome duplication (WGD) have opposing effects on allelic and homeologous expression in the F1 hybrids and tetraploids, respectively. Whereas hybridization exerts strong attenuating effects on allelic expression differences in hybrids, WGD augments these differences and generates extensive and variable homeologous content and expression patterning among tetraploid plants. Coupled with the extensive phenotypic diversity among tetraploid individuals, our results provide experimental evidence in support of the notion that allopolyploidy catalyzes rapid phenotypic diversification in higher plants. Our data further suggest that homeolog content reshuffling rather than alteration in total expression level may be an important feature of evolution in young allopolyploids.

T2-56-04

Genomic and gene expression divergence between two subgenomes in tetraploid *Oryza minuta*

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Polyploidization have long been recognized as a common phenomenon and played important roles in the evolution of plants. Recent studies show that polyploidization is usually accompanied by extensive genome modifications, and the fractionation process and gene expression divergence occurred preferentially toward one subgenome, such as in *Zea mays* and *Brassica rapa*, etc, although the precise mechanisms remain largely unknown. Of about 20 wild species in the rice genus, approximately one half of them are tetraploids that originated through interspecific hybridization and genome doubling, representing a unique system for polyploid research. In our previous studies, we determined the origins of BBCC tetraploids and found that all the BBCC species were formed through polyploidization within the last one million years. In this study, we used the tetraploid *O. minuta* and its diploid progenitors as a study system. We sequenced the genomic regions surrounding *DEP1* locus by isolating orthologous BACs from OMAP BAC libraries, in conjunction with published sequences from three other genomic region, and selected 36 low-copy genes that harbor diagnostic single-nucleotide polymorphisms (SNPs) between the two subgenomes for cDNA pyrosequencing analysis. We compared the homoeologous genome sequence of BBCC tetraploid with its diploid progenitors and investigated the differentiation of two subgenomes at genetic and expression level by DNA sequences analysis and cDNA pyrosequencing. The results indicated various divergent patterns of duplicated genes and intergenic regions in the two subgenomes of *Oryza* tetraploid, including the biased gene structure variation and different expression patterns among three tissues. These findings provide several novel insights into our understanding of the consequences of genome merger and doubling in plants.

T2-56-05

The genomic drivers of ecological divergence in sibling *Dactylorhiza* allopolyploids (Orchidaceae)

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Early-generation allopolyploids need to quickly accommodate divergent genomes into one nucleus by adjusting organization and function, thereby altering ecological properties and adaptive success. With powerful genomic approaches we are screening the genome-wide natural diversity among ecologically-divergent, sibling alllopolyploids in *Dactylorhiza* (Orchidaceae) in order to identify the mechanisms that, after iterative genome doubling, drive adaptation to different environments leading to isolation. The array of *Dactylorhiza* polyploids are of different ages, 15,000 years or younger. With restriction-site associated DNA sequencing (RADseq) we document a genome-wide absence of genetic differentiation between the allopolyploids. In addition, we bring evidence of frequent gene flow between the polyploids in sympatry, which points toward a strong divergent selection required in order to maintain the observed phenotypic divergence. With RNAseq and smRNAseq we document the progression through time of alterations in gene expression and regulation after allopolyploidization, and their importance to the ecological properties of the allopolyploids. Our experiment includes 31 transcriptomes isolated from wild individuals collected on a European-wide scale, after they have been grown in uniform conditions. We observe a general trend of increased overexpression of genes in the youngest polyploid lineages in comparison to the oldest *D. majalis*, whose transcriptome generally resembles more closely those of the diploid parents. We estimate the diploid parental species to have diverged 10 MYA; they show today highly divergent transcriptomes, indicating a significant genomic shock associated with the origin of the allopolyploids. The phenotypic divergence between the polyploids is mediated by a general parental dominance in opposite directions in the sister polyploids, a patterns retained partly also at the level of transgressively expressed transcripts. The differential expression between the polyploids affects several genes related to metabolic processes and responses to stimuli, with a putative ecological function. For example, several genes in the nitrate metabolic pathway appear to have been significantly down-regulated in *D. traunsteineri*, which became adapted to nitrogen poor environments. The differential posttranscriptional regulation via smRNAs is less significant, and affects a different population of transcripts, processes and functions. We conclude that the major transcriptomic divergence among the diploid parental species of these polyploids became reconciled in different ways in the sibling *Dactylorhiza* polyploids, most probably as a result of an interplay between stochastic genomic alterations and distinct selection pressures specific for divergent environments. We finally discuss the importance of qualitative versus quantitative expression alterations in allopolyploids, and their role for diversification in general.

T2-56-06

Insights into reticulate evolution and hybridization in the phylogeny of willows (*Salix* L.) using RAD sequencing

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Hybridization is a frequent phenomenon in flowering plants, nevertheless, the evolutionary impact is still controversial discussed and in detail poorly understood. The genus *Salix* is an interesting model system for studying ancient and recent hybridization. Willows have the highest frequencies of hybrid formation among all flowering plants and polyploidy occurs frequently, ranging from diploid to octoploid species. Crosses between distantly related species from different morphological sections appear regularly in natural populations. Ecological and geographical crossing barriers can break down easily after secondary contact hybridization. Information on morphological patterns and presence of polyploidy suggest that the evolutionary history of the genus has been shaped by reticulate evolution and ancient hybrid speciation. However, traditional DNA Sanger sequencing markers could not resolve interspecific relationships within Salix. Especially the big and species-rich clade of northern hemisphere shrub willows (subg. *Chamaetia* and subg. *Vetrix*) was so far unresolved. It remained unclear whether polytomies in phylogenetic trees were the result of conflicting phylogenetic signals or of low genetic divergence. In deeper time levels the evolutionary history might have been further influenced by incomplete lineage sorting. We used the next generation sequencing approach RAD sequencing (Restriction site Associated DNA sequencing) to analyse the phylogenetic relationships of representatives of the 16 European sections of the *Vetrix-Chamaetia* clade. By mining a large number of single-nucleotide polymorphisms (SNPs) we could reconstruct the phylogenetic history and the temporal framework for reticulate events within the genus. We will discuss methods of discriminating incomplete lineage sorting from hybridization. We will further present experience on the applicability of this genomic marker system on high polyploids to understand evolutionary origin, parentage and diversification of polyploid willow species.

T2-57: Evolutionary and biogeographic studies of gymnosperms in the genomic era

T2-57-01

Gnetales is sister to the pine family but experienced convergent evolution with angiosperms: evidence from a comparative transcriptomic analysis

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After decades of molecular phylogenetic studies, evolutionary relationships among major clades of seed plants are still controversial, especially the phylogenetic placement of Gnetales. What factors are responsible for the phylogenetic conflicts? To address this question, and to resolve the phylogeny of gymnosperms, we conducted a comparative transcriptomic analysis with a sampling of 22 genera representing all 13 families of gymnosperms and 13 species representing main lineages of angiosperms. Multiple data sets containing up to 1,135,848 sites across 1,114 loci were analyzed, using partitioned and unpartitioned concatenations as well as coalescence approaches. Our study generated a consistent and well-resolved phylogeny of seed plants, which strongly supports Gnetales as sister to Pinaceae (the Gnepine hypothesis), and cycads plus *Ginkgo* as sister to the remaining extant gymnosperms. It is interesting that the values of d_N, d_S, ω, R_N and R_S are very similar between angiosperms and Gnetales, implying that these two groups might have experienced similar selective pressures in their evolutionary histories. Convergent molecular evolution, rather than compositional heterogeneity and heterotachy, could be responsible for the topological discordance between gene trees of seed plants.

T2-57-02

Advances in using Hyb-Seq for molecular evolutionary studies of Pinus and Pinaceae

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Large multilocus datasets offer an outstanding opportunity for evolutionary studies of closely related species, but there remains a great need to evaluate the effect of alignment length, number of loci, evolutionary rate, and clocklike behavior on improving the accuracy of phylogenetic results. Early molecular studies of Pinaceae based on the nuclear, plastid, and mitochondrial genomic compartments mainly found agreement with morphology-based phylogenetic hypotheses among genera in the family and within *Pinus*, its largest genus. However, single locus studies of closely related species of *Pinus* have given results that are at odds with each other or with other sources of evidence such as morphology and anatomy. We adopted the Hyb-Seq method, target capture of nuclear loci combined with high copy number plastid genomes and nuclear ribosomal DNA, to characterize representative lineages of Pinaceae and *Pinus*. RNA probes for target enrichment of 1,000 low-copy number nuclear genes were based on published studies of *Pinus taeda*. Fewer than 25% of these genes have been mapped to chromosomes. High-copy number plastome and nuclear ribosomal sequences were also obtained. Using stratified taxonomic sampling (within *Pinus* subsections and among principal lineages of Pinaceae) we inferred phylogenetic relationships using multispecies coalescent methods that account for both incomplete lineage sorting and introgression. We tested the hypothesis that loci with clocklike substitution rates give more accurate phylogenetic inferences than those with unequal rates.

T2-57-04

The intergeneric plastid phylogenomics of Gymnosperms

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The phylogenetic relationships among major clades of gymnosperms have been addressed by multiple studies. However, relationships among some genera are still not fully resolved. We carried out plastid phylogenomic study including all 86 gymnosperm genera. Relationships among most genera in gymnosperms were fully resolved with strong support except those in one Podocarpaceae clade. Different lineages show different degree of substitution rate variation and plastomic rearrangements. Correlation between nucleotide substitution rates and plastomic rearrangements was revealed and should be explained by the improper mismatch repair system. This comprehensive plastid phylogenomics offers important insights into deep phylogenetic relationships and diversification of plastome structures of gymnosperms. The robust phylogenetic backbone we provide establish a framework for future comparative studies on gymnosperm evolution.

T2-57-05

Evolution and speciation of the Chinese white pine, *Pinus armandii*

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Speciation is a fundamental issue in evolutionary biology and it is responsible for the world's biodiversity. The process of species diversification is strongly linked with historical and ecological factors. The Hengduan Mountains, in the southeastern fringe of the Qinghai-Tibetan Plateau (QTP) in southwestern China, is one of the world's biodiversity hotspots. It is characterized with a series of parallel, roughly north-south running high ridges, separated by deep valleys. These massive mountains provide an exceptional range of climates, and thus would be an excellent system to investigate how genetic, geographical, ecological and evolutionary factors interact to create species. *Pinus armandii* Franch is an endemic and dominant species of coniferous forest in central and southwest China. Two distinct genetic groups or ecotypes, i.e., the northern and southern provenances, which roughly divided by Gongga Mountains, the highest mountain in the eastern QTP, have been recognized by previous provenance test and cytoplasmic DNA markers. In this study, transcriptome data was used to reconstruct the evolutionary history of *P. armandii* complex. Population genomics analysis further confirmed the significant genetic differentiation between the north and south populations of *P. armandii*. Many lines of evidence suggested that speciation has occurred in *P. armandii*. In the process of speciation, the massive Gongga Mountain played a key role as a physical barrier in the initial isolation of the north and south populations, and the contrasting environments resulted from the formation of Gongga Mts. subsequently accelerated their differentiation. This study sheds novel insights into the extremely high species diversity of conifers in the Hengduan mountains region.

T2-57-06

Biogeography and ecological niches of Asian *Gnetum* (Gnetales)

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Macroevolutionary patterns are the result of complicated processes, including speciation and extinction events as well as dispersal and vicariance, which may operate over long time periods. It can therefore be particularly difficult to infer the evolutionary history of a group if a fossil record is deficient. This is true for *Gnetum*, a gymnospermous genus that comprises 30-40 tropical species with the life forms trees, shrubs and lianas. Asia hosts the majority of the species and is the hotspot diversity region of the genus. Time-calibrated phylogenies have indicated the Gondwana continent as the ancestral region of *Gnetum*, and its subsequent breakup appears to have facilitated early divergence of the genus. However, no investigations that explicitly elucidate historical processes behind current biogeographic patterns have so far been performed, and the biogeography of Asian *Gnetum* is particularly poorly understood. To address the latter question, we will first address the delimitation of the sister-species of the remaining Asian clade, *G. gnemon*, an arborescent species with a broad distributional range across tropical regions of the Southeast Asia. Second, ancestral areas of Asian clades of *Gnetum* will be inferred using an extensive sampling of species. We will also investigate whether geological or climatic variables exert considerable influences on the diversification of Asian *Gnetum*. Modelling of environmental niches of Asian *Gnetum* species will be conducted in the context of a new phylogeny. The study enriches the knowledge of species delimitation, biogeography and ecology of *Gnetum*. Furthermore, it attempts to uncover mechanisms that drive diversification in extant gymnosperms.

T2-58: Orchid pollination

T2-58-01

Pollination of *Specklinia* (Orchidaceae: Pleurothallidinae) by nectar-feeding *Drosophila*

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Background: The orange flowered species of *Specklinia* (Orchidaceae: Pleurothallidinae) form a monophyletic but morphologically diverse group. Fruit flies, *Drosophila* spp., have been reported to be pollinators of *Specklinia endotrachys*, one of the most morphologically aberrant and variable species in the group. Based on morphological, geographical, ecological and molecular data, we have found that *S. endotrachys* represents a species complex. Objective: Our aim was to discover which strategy of pollinator attraction is employed by orange flowered *Specklinia*. Methods: Pollinators and visitors of species of the complex, that includes *S. endotrachys*, *S. dunstervillei*, *S. pfavii*, *S. remotiflora* and *S. spectabilis*, were observed and caught in the wild and in a green house in Costa Rica. Using DNA barcoding more than 10 closely related species of *Drosophila* were identified, including *D. hydei* and *D. repleta*. The flies are arrested by the aggregation pheromones ethyl tiglate, methyl tiglate and isopropyl tiglate, to which at least *D. hydei* was shown to be very sensitive in ElectroAntenography (EAG) tests. Using SEM of the sepals we were able to detect glands on the abaxial surface and stomata and nectar on the adaxial surface. Results: TEM photographs of the glands were identified as possible osmophores and the stomata as nectar secreting stuctures. Visible nectar drops on the adaxial surface of sepals are secreted by those nectar-secreting stomata, encouraging male and female *Drosophila* to linger on the flowers for several hours at a time. The flies frequently show courtship behavior, occasionally copulating. Several different *Drosophila* species can be found on a single *Specklinia* species. Conclusions: Species of the *S. endotrachys* group share a similar pollination syndrome based on aggregation pheromones and there seems to be no species-specific relationship between the orchids and the flies. However, it is not expected that *Specklinia* species will hybridize naturally as their populations do not overlap geographically.

T2-58-02

Exploring the evolutionary origin of floral organs of the emerging orchid model *Erycina pusilla*

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Background: thousands of flowering plant species attract pollinators without offering rewards, but the evolution of this deceit is poorly understood. Rewardless flowers of the orchid *Erycina pusilla* have an enlarged median sepal and incised median petal ('lip') to attract oil-collecting bees. These bees also forage on similar looking but rewarding Malpighiaceae flowers that have five unequally sized petals and gland-carrying sepals. The lip of *E. pusilla* has a 'callus' that, together with winged 'stelidia', mimics these glands. Different hypotheses exist about the evolutionary origin of the median sepal, callus and stelidia of orchid flowers. Results: the evolutionary origin of these organs was investigated using a combination of morphological, molecular and phylogenetic techniques to a developmental series of floral buds of *E. pusilla*. The vascular bundle of the median sepal indicates it is a first whorl organ but the morphology of the epidermal cells reflects convergence of petaloid features. Expression of some MADS-box gene lineages is low in the median sepal, possible correlating with its petaloid appearance. A vascular bundle indicating second whorl derivation leads to the lip. Some *MADS*-box gene lineages are highest expressed in lip and callus, consistent with current models for lip identity. Six vascular bundles, indicating a stamen-derived origin, lead to the callus, stelidia and stamen. Some *MADS*-box gene lineages are highest expressed in the stamen. Other *MADS*-box gene lineages are highest expressed in the stelidia, suggesting these may be required for fertile stamens. Conclusions: the median sepal, callus and stelidia of *E. pusilla* appear to be derived from sepals, petals, and stamens, respectively. Duplications, diversifying selection and changes in spatial expression of different *MADS*-box genes shaped these organs, enabling the rewardless flowers of *E. pusilla* to mimic an unrelated rewarding flower for pollinator attraction. These genetic changes are not incorporated in current models and urge for a rethinking of the evolution of deceptive flowers.

T2-58-03

Deceptive pollination of New Zealand *Corybas* (Orchidaceae)

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There are over 100 species of orchids in New Zealand (NZ). Although most genera are also found in neighbouring landmasses over 70% of NZ orchid species are endemic. *Corybas*, a genus of terrestrial orchids locally known as spider and helmet orchids, comprises 22 species and ca. five entities which taxonomic status requires further study. Pollination of *Corybas* has puzzled botanist and orchid enthusiasts for decades. Fungus gnats (*Mycetophila*, Diptera) have been reported as occasional floral visitors, and based on these observations, it has been suggested pollination by brood-site deception occurs in these orchids. How does this mechanism exactly operate and the degree of specificity of such interaction has remained largely unstudied. Similarly, whether this deceptive mechanism has promoted diversification of *Corybas* in NZ is unknown. To answer these questions we will present evidence from floral scent analyses, scanning electron microscopy and observations under UV light of the floral structures as well as DNA analyses of floral visitors, eggs and larvae collected from flowers and mushrooms. Also, preliminary data from *ex situ* bio-assays using virgin flowers and naïve pollinators reared in captivity will be presented.

T2-58-04

Pollination and reproductive isolation in *Cryptostylis* orchids

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Sexually deceptive orchids provide a unique opportunity to study plant speciation due to their extreme pollinator specificity, which can rapidly drive diversification via pollinator-mediated speciation. The genus *Cryptostylis* presents an exception to the norm of a different insect species pollinating each sexually deceptive orchid species, with all five Australian species of *Cryptostylis* being exclusively pollinated by the ichneumonid wasp *Lissopimpla excelsa*. Despite frequently occurring in sympatry, the species do not hybridise. We aimed to identify mechanisms that could be contributing to reproductive isolation, and to compare traits relevant to pollinator attraction across species. In a mark-recapture experiment we found that 20% of individual wasp pollinators visited multiple *Cryptostylis* species, and displayed the necessary behaviour for inter-species pollen transfer to occur. Different ploidy levels have previously been reported for two Australian species, leading us to investigate this further across all five species. Flow cytometry revealed inter-species differences in orchid genome size, which may suggest that ploidy differences are contributing to the maintenance of reproductive isolation between the species. Hand cross-pollinations have been conducted to compare the percentage of viable seed resulting from intra- and inter- species pollination events. To determine whether an inter-species difference in requisite fungal symbionts could be preventing the establishment of hybrids, fungal isolations and sequencing of peloton rich orchid sections have been undertaken. Floral orientation (which varies among species) was artificially manipulated, yet pollinator sexual attraction and positioning behaviour in relation to the floral parts remained constant. Floral shielding experiments, where orchids are placed inside a black shield to remove visual cues, indicated long-range pollinator attraction to be chemically mediated. One semiochemical that attracted the pollinator was isolated using bioassay-guided fractionation, with extracts being fractionated via column chromatography and high-pressure liquid chromatography. This semiochemical was found to be present across Australian *Cryptostylis* species. Investigation of one of 18 Asiatic *Cryptostylis* species, *Cryptostylis arachnites*, revealed that this species does not contain the semiochemical. To provide further insight into the evolution and diversification of this genus, it is aimed to determine the pollination strategy of Asiatic *Cryptostylis*. Further, a comprehensive phylogeny of the genus will be created, which will yield insights into the phylogenetic relationships between the Australian and Asian taxa.

T2-58-05

Floral modifications for pollination by Microdiptera (Chloropidae) in the genus *Corunastylis* (Orchidaceae)

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Flies 1.5 - 2.5 mms pollinate Corunastylis species in eastern Australia. The orchids' high conversion ratio of ovaries into fruit (75-95%) is explained based on the evolution of modifications that exploit Microdiptera including secretory staminodia, trembling labella and summer-autumn flowering.

T2-58-06

Wasp pollination in two sympatric orchids: *Coelogye fimbriata* and *Panisea cavaleriei* (Orchidaceae)

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Orchidaceae is one of the most species-rich and highly evolved plant families and is well-known for its special pollination mechanism.Pollination by a single wasp species is rare in orchids except in species with sexually deceptive flowers that are pollinated exclusively by male insects. Consequently, finding and interpreting examples and attraction mechanism of wasp-pollinated orchid species that are pollinated by deception, but lack sexually attractive characters, becomes a key point in understanding the evolutionary ecology of wasp-orchid interactions and even pollinator-orchid interactions. Moreover, it is also important in discussing the effect of deceptive strategies in speciation, diversification and evolution of orchids and even angiosperms. In this study, we focused on the wasp-pollination system in two sympatric and co-flowering orchids in Yachang Orchid National Nature Reserve: *Coelogye fimbriata* and *Panisea cavaleriei* with nectarless flowers which were pollinated by females of the same *Vespula* species (Vespidae, Hymenoptera). Quantitative study of floral scent and color were employed in order to learn the deceptive strategies to wasps. The floral odor was considered to be the important cue to attract the female wasps in both two orchids. Reproductive isolation is one of the key steps in speciation and the foundation of species independence, especially in sympatric and co-flowering species. Mechanical isolation mediated by *Vespula* sp. was the prezygotic isolation mechanism between *Coelogye fimbriata* and *Panisea cavaleriei*.

T2-59: Phylogenomics and Evolution of Legumes

T2-59-01

Plastid phylogenomics reveals deep relationships of Fabaceae

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The family Fabaceae (Leguminosae) is the third largest angiosperm family in terms of species richness with c. 770 genera and over 19,500 species. In ecological and economic terms the family is also one of the most important plant groups of the world and it has been recently the focus of numerous taxonomic, phylogenetic and evolutionary studies. However, the classical and long standing three subfamilies classification has been questioned, and categories of many tribes, subtribes and clades need to be clarified. Despite remarkable advances over the last 20 years, some deep phylogenetic relationships within the family remain unresolved or weakly supported. Plastid phylogenomic analyses were conducted on 350 species representing 300 genera of Fabaceae. Trees obtained from different data sets and/or tree reconstruction methods are largely consistent. We found better support for some short nodes when noisy sites or saturated genes are excluded from the data matrix. The major phylogenetic relationships of Fabaceae were fully resolved with high support, and most deep relationships are clarified.

T2-59-02

A phylogenomic approach to study the evolutionary origin of Detarioideae, a clade of ecologically dominant tropical African trees.

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Tropical rain forests are one of the most biologically diverse habitats on the planet. Yet their description and the understanding of their evolutionary history are far from complete. West Central Africa represents the area of greatest biodiversity richness in tropical Africa and has the highest percentage of untouched pristine forest in all of Africa and Madagascar. Our study is focused on the recently established subfamily Detarioideae (Leguminosae), which are the dominant tree components of West Central African forests. Detarioideae includes 81 genera (and approximately 750 species), 58% of which occur in Africa and Madagascar (with 329 species). Our aim is to generate the first fully sampled phylogeny, including genera never sequenced before, using a phylogenomics approach (sequence capture). Our bait design includes 283 nuclear genes (359,222 bp) and we will obtain plastid genome sequences as well. We use this new phylogeny to study the biogeographical history of the group and investigate biome shifts within the subfamily. Detarioideae originated in Africa-South America and are retained as a Primary Forest clade in its origin according to our analysis. Implications for the origin of the group and its role in shaping African diversity are discussed.

T2-59-03

Solving difficult phylogenetic problems in Leguminosae using multiple genome-scale sequence data sets

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Several parts of the legume phylogeny suffer from lack of resolution. Here we investigate whether genome-scale data can resolve these problems, with a focus on three specific parts of the legume phylogeny: (1) the root of the Leguminosae and relationships among the six subfamilies, (2) relationships among the major lineages in the recently recircumscribed Caesalpinioideae and (3) the large polytomy formed by the clade that includes the c. 37 genera in the non-monophyletic tribes Ingeae and Acacieae p.p. and c. 1,850 of the c. 3,300 species in the mimosoid clade (the former Mimosoideae). We harness the power of large datasets including fully sequenced genomes, transcriptomes, complete chloroplast exomes/proteomes and hybrid capture sequence data for 967 low copy nuclear genes for the recircumscribed Caesalpinioideae. We explore different phylogenetic reconstruction methods using heterogeneous models of molecular evolution on both DNA and protein sequence data and gene-tree species-tree reconciliation methods. Our results show that the root of the legumes remains problematic to resolve, even with large volumes of data. After dissecting the phylogenetic signal among hundreds of nuclear genes, we find that significant numbers of genes support each of the different possible rootings. We suggest this might be caused by incomplete lineage sorting, rapid diversification of the early legume lineages, extinction of early legume diversity, whole genome duplication, or most likely, a combination of these factors. Within the recircumscribed Caesalpinioideae, we can resolve the most probable branching order of the major lineages in the subfamily. Previously, lack of resolution across this part of the tree has hindered progress towards a phylogenetic classification of the legume family, even though it was clear that former subfamily Mimosoideae needed to be included in a recircumscribed Caesalpinioideae to avoid having to recognize a large number of additional, small subfamilies. Our results provide the basis for a new tribal classification, and recognition of additional clades with evolutionary significance (e.g. a petiolar nectary clade and an aggregated pollen clade). Within the large clade combining former tribes Ingeae and Acacieae p.p., large scale plastid and nuclear gene data resolve several major subclades for the first time, supporting some previously hypothesized informal generic alliances based on morphology while rejecting others. Furthermore, substantial geographic and ecological structure is apparent across this large clade, but this requires further investigation to understand the relative contributions of dispersal limitation and niche conservatism in shaping the mimosoid phylogeny. Future research will focus on mimosoids, and aims to infer a robust backbone phylogeny for the clade with complete sampling of genera and dense sampling of larger genera using the same set of genes via further hybrid capture work. Our results have implications for revising the tribal classification of the legume family and our study has wider significance in terms of methods for inferring phylogenies of large (plant) clades combining different types of (genome-scale) molecular data.

T2-59-04

Polyploidy, nodulation, and Legume genome evolution

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Polyploidy has played a major role in shaping the genomes of species in the legume family (Leguminosae, Fabaceae). It is now known that independent whole genome duplications took place in the ancestors of several of the major lineages of the family; only one of these, in the papilionoid subfamily (Papilionoideae), has been well characterized. Symbiotic nitrogen fixation involving soil bacteria (rhizobia) housed in plant-produced nodules occurs in most members of the Papilionoideae, and more sporadically in the Caesalpinioideae (within which the predominantly nodulating mimosoid clade is embedded), but is absent outside of these two sister lineages. The scattered distribution of nodulating lineages in Caesalpinioideae has raised the question of the number of origins of the phenomenon in legumes. Assessment of nodule homology using morphological, anatomical, biochemical, or molecular phylogenomic approaches has produced equivocal results. In an effort to determine whether non-nodulating legumes have lost nodulation or never had it, we have identified conserved noncoding sequences (CNS) in the genome sequences of papilionoid legumes, with over 100,000 CNS shared between soybean and *Medicago truncatula*; we are assessing the association of CNS with genes involved in nodulation. In addition to characterizing an important component of legume genomes, understanding the evolution of CNS could help elucidate how genes were recruited for nodulation from pre-existing functions such as mycorrhizal symbiosis, and how polyploidy could have played a role in the evolution of nodulation. The assumed (though still uncertain) correlation of the distribution of polyploidy and nodulation in papilionoids led to competing hypotheses: 1) polyploidy enabled or facilitated the origin of nodulation; vs. 2) polyploidy refined or “enhanced” pre-existing nodulation. The first hypothesis is untestable, but we are testing the second by studying the effects of polyploidy on nodulation in recently formed (<500 K years) allopolyploids in wild perennial members of *Glycine*, the genus that includes soybean. Although the effects of genome merger and duplication are not always predictable, we do see a general enhancement of nodulation in allopolyploid *Glycine* species. Specifically, *G. dolichocarpa* appears to have enhanced nodulation responses when compared to its diploid progenitors. We also have evidence that the allopolyploid exudes reduced levels of formononetin, an isoflavone that inhibits symbiotic signaling, compared to one of its diploid progenitors, and that it has an enhanced nodulation-related hormone signaling response, and reduced defense response, compared to the other progenitor, when treated with rhizobia.

T2-59-05

Phylogenetic and biogeographical implication of *Caragana, Calophaca* and *Chesneya* to the relation of Central Asia and East Asia flora

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Caragana, Calophaca and *Chesneya,* have a distribution concentration pattern of Central Asia and East Asia, especially occur in Himalayas-Tibet and Central Asian Mountains and desert. Based on our recent molecular phylogeny and biogeography of these legume genera, the perspectives of relation of Central Asian and East Asian flora are concluded as following floristic aspects. Most taxa at generic level, have the temporal origin and diversification in late Oligocene to early Miocene. Mountains chairs, such as Himalayas, Tianshan, Pamir-Alai, Hindu Kush, are hypothesized as the origin, diversification and evolutionary center, as well as the dispersal and migration routes. Geological and climate events occurring late Tertiary, such as QTP uplift, Tethys retreat, Central Asian aridification, and East Asian monsoon, are thought as evolutionary driving force.

T2-59-06

The legume genus dussia as a model to understand the complexity of taxonomic tree diversity, biogeography and origin of the neotropical flora

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Dussia is a genus of trees, described in 1892 from Martinique Island, confined to tropical rainforests and distributed from S. Mexico and Antilles to Bolivia and Amazon basin of Brazil. *Dussia* is poorly known, both taxonomically and biologically because its species are rare trees that have been seldom collected due to their large size. The most recent monographic revision (1963), stated that delimitation of taxa was “tentative” because of inadequate material and recognized 10 species; this revision involved no field study. Due to variation patterns in *Dussia* are highly complex and not amenable to simple delimitation of species. It appeared clear that to resolve problems of species delimitation in *Dussia* required extensive field study to gather more specimens, and to evaluate whether any “field” characters could provide extra feature for species delimitation. A forthcoming taxonomic revision based upon extensive fieldwork and upon many more specimens is likely to recognize up to eight new species, the highest diversity for the genus is found in Costa Rica. The phylogeny of *Dussia* clearly demonstrates stronger affinities of the Chocó with Central America than with Amazonia because the Pacific Ecuador accessions are nested in a Central American clade. In contrast, the phylogeny suggests that there have been no trans-Andean migrations between Amazonia and the Chocó region. The Chocó clade is nested in the Central American clade. This suggests that subsequent to the south-north migration event between South and Central America, a north-south migration event must have occurred ~7 Ma. The earliest migration between South America and Central America on the *Dussia* tree is dated at ~15 Ma, which substantially pre-dates the widely accepted date (ca. 3 Ma) of the formation of the Panamanian land bridge, so over-water dispersal seems possible. This dated phylogeny for *Dussia* shows there were multiple migrations between South and Central America. Three main biogeographical influences were found, the North Pacific Ocean, the Chocó region and the Andes.

T2-60: Use of target capture and high-throughput sequencing as a tool for plant systematics

T2-60-01

Data assembly and post-processing in aTRAM for museum phylogenomics

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NGS methods have quickly become a central resource for phylogenomics, enabling robust phylogenetic inference in a wide variety of taxa and greatly facilitating the use of historical DNAs. Methods for data collection and multilocus phylogenetic analysis are now well-trodden territory, but in the middle ground there is a conspicuous literature gap of identifying appropriate methodologies for data assembly. Much has been proposed in the way of alternative methods, but little exists in the way of comparative methodological studies. Several pipelines have appeared, among which aTRAM is particularly promising. aTRAM is a hybrid reference-based and reduced *de novo* method (that is, a *reference-guided* method). Reads matching a reference by BLAST are pulled and assembled as small *de novo* subproblems; this process is iterative in the sense that assembly results are the new reference for each successive round of BLASTing and assembly. aTRAM is highly parallelizable within each assembly problem and among loci and taxa, and is being actively optimized for performance and ported to python; the *de novo* portion integrates multiple methods with highly flexible parameters. Here, we report aTRAM results for (1) a deep divergence, comprising exon-capture data collected in the Saxifragales. This late Cretaceous radiation presents an assembly challenge due to deep nucleotide divergence and our reliance on primarily museum DNAs. We also show (2) results for a shallow divergence in *Heuchera,* a ~4 my divergence where low nucleotide variation and the acute need for gene-tree based analyses present the primary challenges. We compare these to *reference-based* short read aligning approaches and present new work in post-processing exonic data in a protein- and paralog-aware manner, complementary to alternative consensus-based approaches. We advocate a pluralistic approach to NGS assembly; much like *de novo* assembly, researchers should begin by testing several methods, and perhaps report comparative results among assemblies, which could shed light on methodological or biological issues that would be hidden in isolation.

T2-60-02

Using target-enriched high-throughput sequencing to gain robust nuclear genetic evidence to reconstruct the phylogeography of the Ponderosae, a recalcitrant clade of conifers

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Species relationships (and even species delineations) have been elusive in subsection *Ponderosae* (Pinaceae; *Pinus*; subgenus *Pinus*; section *Trifoliae*). The identification of morphological synapomorphies in this group was hindered by plasticity, within-population variability, and convergent evolution as populations migrated into similar ecological niches. The relatively recent origin of the clade, incomplete lineage sorting, and some instances of plastid transfer have complicated previous attempts at creating molecular phylogenies. The *Pinus* (pine) nuclear genome is huge, even compared to other plant species. At 22 billion base pairs, the recently published *P. taeda* genome is 163 times the size of *Arabidopsis* and the *P. ponderosa* genome is estimated to be 24 billion base pairs. As retrotransposon activity repeatedly copied genomic regions, many pseudogenes and larger gene families were created, making isolation of orthologous low-copy nuclear genes for phylogenetic evidence a challenging endeavor even though pines are all diploids. Nonetheless, the economic and ecological importance of these forest trees has inspired a substantial amount of molecular genetic research. Over 14 thousand unigenes were published for *P. taeda* that were successful targets for solution-based enrichment (retrieving complementary biotin-labeled oligonucleotides with streptavidin beads) prior to high-throughput short-read sequencing (Illumina). We selected 700 of these genes for an array of bait probes (MYcroarray MYbaits) and successfully multiplexed 48 target-enriched *Ponderosae* samples per lane. After a series of filtering steps to exclude putative paralogs, we aligned over 300 putative nuclear orthologs from 75 individuals. By including some raw DNA along with the target-enriched DNA in the sequencing lane, we were able to assemble nearly-complete plastomes as well. Coalescence-based species trees inferred from this dramatically larger data set provided a substantial step forward in our understanding of these species relationships.

T2-60-03

Dealing with phylogenetic noise in phylogenomic data: A pilot study applying the plant anchored hybrid enrichment method to new world sages (*Salvia* Subgenus *Calosphace*; Lamiaceae)

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Large-scale phylogenomic data has substantially increased our understanding about relationships within several lineages across the tree of life. These data can be obtained through varied genomic partitioning strategies (GPS), one of them is the Anchored Hybrid Enrichment (AHE) method. AHE was originally developed for vertebrates and only recently applied to flowering plants. This method uses the Angiosperm V. 1 kit, that contains short RNA probes, designed from 25 angiosperm genomes, to target up to 517 low-copy nuclear genes, potentially informative at a range of phylogenetic levels. We conducted a pilot study using AHE to resolve relationships among a mostly Neotropical sage lineage that may have undergone a recent evolutionary radiation. *Calosphace* is one of the two monophyletic subgenera of *Salvia.* It includes ca. 600 species, most of them endemic to the Neotropics, being the most diverse lineage in the genus. Four diversity centers have been postulated for *Calosphace*: Mexico, the Andean Region, Eastern Brazil and the Antilles. Conventional markers (ITS, *trnL-trnF* and *trnH-psbA*) have not been able to resolve the relationships among species nor along portions of the backbone of the clade. The aims of this study were to test: 1) the effectiveness of gene capture and massive sequencing in obtain quality data for this group; 2) the performance of the captured genes in resolving and supporting internal relationships; 3) the effect of noise reduction strategies on phylogenetic estimation; and 4) the potential of the enrichment by-catch of the two GPS in recovering plastid genomes for the study group. For the AHE method we sampled 12 representative species of subgenus *Calosphace* and included one species of *Salvia* s.l.'s closest relative, *Lepechinia*, as outgroup. Gene capture and sequencing yielded 448 alignments of individual loci with an average length of 704 bp, 64% of them containing all the taxa sampled. The performance of the AHE data in phylogenetic estimation was superior to that of conventional markers, increasing both support and resolution. Captured loci vary in the amount of net phylogenetic informativeness at different phylogenetic depths, making these data promising in phylogenetic estimation for this group, and possibly for other lineages within Lamiales. Noise reduction through the exclusion of sites with unusually high substitution rates resulted in increased support and resolution for shallow nodes in maximum likelihood phylogenetic trees resulting from concatenated analyses of all the loci. Additionally, it is expected that an increase in sampling (preferably taxa) will aid in resolving weakly supported, short internal branches at deep phylogenetic levels. From the 25 reference genomes used to design the probes from the Angiosperm v. 1 kit, the closest relative to *Salvia* is *Mimulus* (Phrymaceae), also from Lamiales. The genetic distance between these lineages allowed the hybridization of some of the probes with chloroplast DNA, resulting in the recovery of complete to nearly complete chloroplast genomes using by-catch from off-target reads.

T2-60-04

Phylogenetic analyses at different evolutionary levels using hybridization-based target enrichment

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Using a high number of independent and putatively orthologous low-copy loci is a large benefit for phylogenetics. Species trees reconstructed from big phylogenomic datasets are often completely resolved and well supported. Hybridization-based target enrichment (Hyb-Seq) is one of the methods for effective sampling of thousands of exons from hundreds of genes. Because hybridization enrichment is not 100% efficient nearly complete chloroplast genomes can be obtained from off-target reads. Huge amount of data generated by the Hyb-Seq NGS method need an appropriate bioinformatic pipeline for processing raw reads up to the reconstruction of species tree. We developed HybPhyloMaker, a modular set of BASH scripts for UNIX-like environment that is designed for compact and easy-to-use processing of raw Illumina paired-end reads, selecting suitable loci and constructing gene and species trees using different methods. Probe set covering 1,180 genes was designed for the genus *Curcuma* (Zingiberaceae) comparing *C. longa* transcriptome and *C. ecomata* genome-skimming data. The probes were successfully applied to enrich sequencing libraries not only within the genus *Curcuma* but also at the level of the whole family Zingiberaceae and the order Zingiberales. More than 1,000 loci were usable for the genus *Curcuma* and around 700 loci were used to establish the phylogeny of Zingiberaceae and Zingiberales. With our work we demonstrated really broad evolutionary applicability of the set of orthologous genes initially designed for generic level phylogeny.

T2-60-05

The potential of target enrichment and hybrid capture for improving our understanding of complex diversity patterns within the lettuce alliance (Asteraceae)

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The lettuce alliance (*Lactucinae*, *Cichorieae*, Asteraceae) contains ca. 200 species of varying ploidy level, distributed in Europe, Africa, Asia and North America. Phylogenetic studies to date have relied mostly on ITS and chloroplast markers that have highlighted the non-monophyletic status of currently recognized taxonomic groups within the alliance and revealed complex patterns of reticulation and incongruence between nuclear and chloroplast regions. In order to gain greater phylogenetic resolution and to unravel such complex patterns of diversity, we aimed to capture a greater portion of the nuclear genome compared to previous studies, via target enrichment and hybrid capture. We made use of already available probes that previous studies had shown to capture highly informative and potentially low copy nuclear genes for a number of Asteraceae taxa. The resulting dataset consisted of >800 nuclear loci for a range of taxa from across the lettuce alliance. The bioinformatics for preparation and analyses of such large datasets is continually being developed. We present the results of different phylogenomic analyses of our hybrid capture data including PHYLUCE, HybPiper and clustering. We discuss the application and potential of these different analytical approaches for understanding diversity within complex flowering plant groups such as the lettuce alliance.

T2-60-06

An angiosperm-wide bait kit for targeted sequencing of 354 low-copy nuclear genes across flowering plants

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3. MYcroarray

Utilizing high-throughput sequencing efficiently for phylogenomics requires methods that reduce genomic complexity whilst maximizing the number of taxa/samples. The use of RNA baits to pull out genomic regions of interest (a.k.a. bait hybridization, sequence capture, targeted enrichment) is now becoming widespread as one of the best methodologies to achieve this goal.We have developed a new angiosperm-wide probe kit from existing transcriptomes (1 KP) and genomes (Phytozome), in collaboration with the Chicago Botanic Garden and MYcroarray, and in the context of

Kew's Plant and Fungal Trees of Life (PAFTOL) project. Our angiosperm-wide kit targets 354 low-copy nuclear genes that are shared across land plants and sister algal lineages. Our bait design included up to 15 variants for each of the 354 genes, such that we could capture 95% of sequence diversity across angiosperms with no target sequences being more than 25% divergent from the bait sequence. This resulted in 75,151,120-mer probes, with 3x tiling density. To check the breadth of our kit we have tested the baits on all angiosperm orders and all angiosperm families that were not included in 1KP. Additionally, we tested the potential of these loci for shallower level systematics, by using several small sets of ~10 species within genera belonging to major flowering plant lineages. We have also tested the kit on a variety of samples, including herbarium specimens of varied quality, which were dried in different ways (e.g. air vs. ethanol), and those that are over a century old. Here we present results of the efficiency of capture across angiosperms and sample types, and at different phylogenetic levels. We also present the most up-to-date angiosperm phylogenetic tree at the family level, by combining our initial data with 1KP alignments.

T2-61: Diversification, evolution, biogeography and conservation of the family Magnoliaceae (two sessions)

T2-61-01

Magnolia genome: An additional reference for understanding angiosperm diversification

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Magnolia has received keen interest from botanists because it is one of the early-diverging angiosperms in the evolutionary history of angiosperms. Recent molecular phylogenetic studies show that Magnoliaceae is placed at the "Magnoliids" clade after the *Amborella*, Nympheaceae, and Austrobailales. Genomic study of magnolia, a member of basal angiosperm grade, highlights the importance of understanding the early diversification of angiosperms. We obtained more than 190 Gbp of filtered NGS sequences from *Magnolia kobus*, a popular garden tree in Magnoliaceae and an endangered species in Korea, with six different libraries (2 pair-end and 4 mate-pair libraries). The current draft of *M. kobus* genome assembly (ver. 0.4) consists of 1.91 Gbp with 26,553 bp and 9,852 bp of N20 and N50, respectively, which is slightly smaller than that from k-mer analysis (2.09 Gbp) but larger than that estimated from flow cytometry (1.44 Gbp). Gene prediction by AUGUSTUS showed 371,292 putative genes, of which 84,663 (22.80%) genes have known functional domains defined by InterProScan. *Magnolia* genome shares 5,282 functional domains (79.05%) with the *Amborella* genome, and 1,088 domains (16.28%) are *Magnolia*-specific. In the gene family analyses, 39 MADS-box genes were identified in the *M. kobus* genome, showing a similar number as in the *Amborella* genome (34 genes). However, members of AP2 (95 genes) and cytochrome P450 (567 genes) gene families identified in the *Magnolia* genome were almost two-fold higher in number than that that from the *Amborella* genome. For the improvement of gene annotation as well as for understanding the floral development of *M. Kobus*, we sequenced transcriptomes from nine different tissues. To find lineage-specific traits in Magnoliaceae, five additional species (*M. coco*, *M. sieboldii*, *M. grandiflora*, *M. portorensis*, and *Liriodendron chinense*) were sequenced (8.0~9.1 Gbp, respectively). We detected 120,334 simple sequence repeats (SSRs) throughout the genome of *M. kobus* and 171,317 SSRs from five other species of Magnoliaceae. Sequences and analyses results of magnolia species are accessible in the Magnolia Genome Database (http://www.magnoliagenome.net/), which is a web-based integrated platform for comparative genomics of Magnoliaceae.

T2-61-02

Genome-wide approach on the phylogeny of Magnoliaceae using a target-enrichment NGS sequencing

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Reconstruction of the phylogeny of Magnoliaceae, a member of early-diverging angiosperms, plays a key role in understanding the diversification of angiosperms. With the development of new molecular techniques, various phylogenetic studies on Magnoliaceae have been conducted such as studies based on chloroplast RFLP, and sequencing of single gene, multiple genes, and chloroplast whole genome. However, the evolution of Magnoliaceae remains unclear in many parts, especially the relationships among major sublineages in the family because overall base substitution rate in Magnoliaceae is very low, as compared to other angiosperm groups. In this study, we used a genome-wide approach on the phylogeny of Magnoliaceae with high throughput target-enrichment NGS sequencing. We identified 504 putative single/low copy gene regions (443 kbp) as targets for captured NGS sequencing. These regions are 1) expressed single-copy genes extracted from a preliminary genome assembly of *Magnolia kobus* (ver. 0.4; see talk 1401) using gene clustering (Tribe-Mcl) with its transcriptomes and 2) shared single/low copy genes among *Arabidopsis, Polulus, Oryza, Vitis*, and *M. kobus* . Probes of these regions were designed and hybridized with 130 taxa representing all previously reported sublineages (sections and tribus) of Magnoliaceae. Index sequences were added in the captured fragments and sequenced altogether using the Illumina HiSeq platform. Phylogenetic trees based on exons of targeted nuclear genes as well as adjacent intergenic sequences (including introns) were compared with those based on chloroplast genome data. The results provide a basis for the evolutionary diversification in the family as well as new classification system of Magnoliaceae.

T2-61-03

From investigation to implementation: BGCI's integrated conservation action to save threatened plants in China – the example of Magnoliaceae

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The family Magnoliaceae is among the most ancient groups of angiosperms. Comprising over 300 species in 2 genera – *Magnolia* L. and *Liriodendron* L., Magnoliaceae are found in the temperate and tropical regions of East and Southeast Asia and the Americas. Some 109 taxa are native to China of which more than half are endemic. Southwest and South China including Yunnan, Guangxi, Guangdong, Hainan and Guizhou are the centre of extant Magnoliaceae diversity of the Old World flora. 50% of China's magnolias are threatened with extinction in the wild (*The Red List of Magnoliaceae,* 2016). As in other areas of high magnolia diversity, forest habitat conversion, logging, over-exploitation and limited, natural reproduction, present major threat factors. Under the umbrella of the Global Trees Campaign (GTC) and working with Chinese partners including botanic gardens, academia and local authorities and communities, BGCI has been developing and implementing integrated conservation projects for various species of Magnoliaceae over the past 10 years. Activities range from establishing distribution maps to plant material sampling for propagation at botanic gardens aimed at *ex situ* conservation collections as well as population reinforcement and reintroduction in the wild. Public outreach and educational activities form an integral part of all the projects. Over the years, more than 10 rare and/or threatened species including *M. sinostellata, M. omeiensis, M. longipedunculata, M. coriacea*, *M. phanerophlebia, M. angustioblonga*, *M. hebecarpa*, *M. ingrata, M. odoratissima* and *M. aromatica* were included in practical conservation work. As an immediate insurance policy against their extinction in the wild, *ex situ* collections for a number of these species have been established at Chinese botanic gardens and local forestry research stations. BGCI's most recent magnolia conservation work focusses on the Critically Endangered *Magnolia omeiensis* as well as the Endangered *Magnolia sinostellata.* Known from only two locations in temperate forest on Mount Emei, southern Sichuan, less than 75 individuals of *M. omeiensis* have been recorded. Trees display low seed production and germination rates. Intensive logging is the single-most important threat to the species and no special protection is yet in place to ensure that the population remains intact. *Magnolia sinostellata* is known from 5 locations in southern Zhejiang province with some 500 individuals remaining in the wild. As with *M. omeiensis*, all populations are exhibiting limited reproductive performance. As a high-value ornamental plant, over-collection in the wild as well as ongoing deforestation are responsible for the continuing decline of *M. sinostellata* populations. BGCI is working with Emeishan Botanical Garden and Shenzhen Fairy Lake Botanical Garden to develop *ex situ* conservation collections and to reinforce populations of the species in the wild. Over the past two years, saplings of *M. omeiensis* and *M. sinostellata* have been established, generated from seed, cuttings and grafts. Overall survival rates of saplings planted *in situ* in 2016 to reinforce existing populations are at 95.4% for *M. omeiensis.* Reinforcement for *M. sinostellata* was initiated in March 2017. Conservation work continues in 2017 and beyond to consolidate and scale up the propagation and species recovery work. While grafting has been employed as a last resort to establish sizable stocks of plants in the short-term, major emphasis will be given to generating seedlings for use in future population reinforcement programmes. Representatives from local communities and authorities will be offered advanced training in propagation techniques, whilst a series of public outreach campaigns aims to enhance awareness of the needs and values to conserve China's unique magnolia diversity as a matter of national pride.

T2-61-04

Abundance, rarity and geographical range size in neotropical species of *Magnolia*: implications for conservation efforts in Mexico, Colombia and Ecuador

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The species abundance curves of trees in neotropical forests, at local, regional and continental scales, typically exhibit a "reverse-J" distribution with a few very abundant species, a larger number of species that are moderately abundant to uncommon, and a long "right-hand tail" of rare to very rare species. For instance, a recent floristic analysis of 1,170 one-hectare tree inventory plots from throughout the 6 million km2 landscape of lowland Amazonia, estimated 16,000 tree species for the region, but just 227 "hyper-dominant" species account for half of all the trees in Amazonia, and the most abundant species have total population sizes upwards of 10^9 individuals. In contrast, this study estimated 6,000 very rare tree species in the Amazon with a total population < 1,000 individuals – a very long "tail" of "hyper-rare" species. Although the quantitative data is scanty, evidence suggests that many *Magnolia* species in neotropical forests are within that rightward tail of species that exhibit the most restricted of the "seven forms of rarity" – small local population size, small geographic range, and narrow habitat breadth. For example, *Magnolia canandeana* is known from just two adult trees, each over 30 m with trunks over 100 cm DBH, within a 1300-hectare private nature reserve in the Pacific coastal region of Ecuador, in a region of active logging and conversion of the forest to African oil plantations. *Magnolia yantzazana* is known from just 13 adults in a 5 km^2 of forest in the Cordillera del Cóndor region of southeast Ecuador, and the entire population is within an area of a gold mining concession; in this case the mining company, within its environmental management plan, is taking steps for *in situ* and *ex situ* conservation of this and other locally endemic plants. The extreme rarity of many neotropical *Magnolia* species poses considerable challenges for conservation efforts. At least 115 of a total of 160 neotropical species of *Magnolia* are considered threatened according to the IUCN Red List Criteria. Mexico, Colombia and Ecuador harbor the greatest number of Neotropical Magnolias, with 40, 34 and 25 species, respectively, reported for these three countries. Conservation programs for threatened *Magnolia* species including *in situ* and *ex situ* components, are currently in progress in these three countries. Methods to overcome the challenges of *ex situ* conservation, including difficulties of obtaining seed, and difficulties in propagation of recalcitrant seeds, are being addressed through various methods including micro-propagaion *in vitro* of vegetative meristems. Reintroduction of *Magnolia* plants into appropriate native habitats is in initial stages in several areas of these three countries.

T2-61-05

The Lirianthe Spach (Gwillimia + Blumiana clade) as the basal lineage of Magnoliaceae

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Evolutionary relationships among members of the family Magnoliaceae have been difficult to clarify due to the lack of informative morphological and molecular characters together with unbalanced sampling. Thus, several hypotheses have been proposed regarding the position and taxonomic delimitation of the groups that conform the family. We aimed to clarify the evolutionary relationships within Magnoliaceae using DNA sequence data from three chloroplast (*mat*K, *psb*A, ORF 350) and two nuclear (GAI, PHYA) genomic regions obtained from GenBank and 15 newly sequenced Neotropical species for a total of 80 taxa, from both Asia and America. Phylogenetic analyses were conducted for a concatenated alignment of chloroplast and nuclear sequences using Bayesian inference (BI) and Maximum likelihood (ML). Substitution models for each genomic region were selected with the Akaike Information Criterion. The nomenclature used mostly followed Figlar and Nooteboom´s classification. Results from the BI and ML analyses of the dataset were largely in agreement, except for some differences in the tree topology and bootstrap support values for the inferred clades. Except for subgenera and the basal lineage of the family, several sections or subsections were recovered as monophyletic with high support values. Phylogenetic relationships among clades were recovered in general agreement with previous analyses, yet improved in terms of resolution. Results from the BI show that the base of subfamily Magnolioideae is composed of the lineage that originated extant *Gwillimia* + *Blumiana* species, recognized as the genus *Lirianthe* Spach by some authors. This basal configuration differs from those of previous phylogenetic hypotheses resulting in either a polytomy or placing *Macrophylla* Figlar & Noot. or *Talauma* Juss. as the basal clade. Besides, our results provide a well-supported hypothesis with improved resolution of the phylogenetic relationships within Magnoliaceae. Many subsections were recovered as monophyletic with high support values except for the clades *Gwillimia* + *Blumiana*, *Dugandiodendron*, *Manglietiastrum* + *Gymnopodium* and the complex *Aromadendron* + *Maingola* + *Michelia* + *Ermerillia*. Subsect. *Auriculata* was placed as a sister clade to *Oyama* + *Rhytidospermum* + *Manglietia*), sharing a common ancestor; its similarity with *Macrophylla* is a convergence, given their placement in independent clades; the latter more related to the *Magnolia* clade. The largest and well supported clade consisted of the subgenera *Yulania* + *Gynopodium* + section *Kmeria* of subgenus Magnolia. In relation to Figlar & Nooteboon´s Classification of Magnoliaceae, 12 taxa were supported in our results: six sections (*Gwillimia*, *Auriculata* Figlar & Noot., *Manglietia* Blume, *Macrophylla*, *Magnolia* L., and *Kmeria* (Pierre) Figlar & Noot.); and six subsections (*Talauma* Baill., *Cubenses* Imkhan., *Oyama* (Nakai) Figlar & Noot., *Rhytidospermum* Spach, *Tulipastrum* Spach) Figlar & Noot. and *Yulania* Spach.). Alternatively, ten genera could here be recognized as monophyletic in this study: *Houpoëa* N.H.Xia & C.Y.Wu, *Kmeria* (Pierre) Dandy, *Lirianthe*, *Magnolia*, *Manglietia* Blume, *Metamagnolia* Sima & S.G.Lu, *Oyama* (Nakai) N.H.Xia & C.Y.Wu, *Paramagnolia* Sima & S.G.Lu, and *Talauma*. In order to facilitate our comprehension of the evolution of biogeography of Magnoliaceae, an updated classification system is urgently needed, one that integrates the evolutionary relationships presented here with those reported in previous efforts.

T2-61-06

Tempo and mode of diversification in Magnoliaceae

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Diversification, the interplay between speciation and extinction, has recently rise a profound interest among researchers due to the advent of new analytical methods developed since the seminal paper of Nee *et al.*, (1994). These macroevolutionary metrics have been increasingly used in trying to explain why some lineages are more diverse than others and shed light on the factors and processes that drive such diversity imbalance. In flowering plants, many species-rich groups of relatively recent divergence have been subjected to these kind of analyses, while groups near to the base of the angiosperm phylogenetic tree have been overlooked, thus missing the opportunity to study interesting plant lineages with long evolutionary histories.The family Magnoliaceae represents a basal group of angiosperms composed of c. 400 species and a temporal range of evolution spanning c. 100 My. The family exhibits a strong disparity in species richness among its constituent lineages with almost half of the total diversity contained in two groups; the Neotropical basal lineage *Talauma* and the Paleotropical *Michelia*. Using a phylogenetic approach, the temporal pattern of diversification in Magnoliaceae was investigated with lineage through time (LTT) plots and the constant-rates test (gamma statistic). The likelihood-based method MEDUSA was employed to identify shits in the diversification rate across lineages in the phylogeny of Magnoliaceae. The LTT plot and the gamma statistic failed to reject a model of variable rate of diversification in time while the MEDUSA analyses favored a model with three diversification rate shifts. Our results indicate that the mode and tempo of diversification in Magnoliaceae started at a slow steady pace that has been maintained until the present with a high turnover, probably due to extinction. The relatively high diversity of *Michelia* can be explained by a shift in diversification rates recovered at the crown node of the group while no shift in rates was suggested for *Talauma* nor for any other lineage within the family. This result suggests that other mechanisms of origination and maintenance of diversity should operate for *Talauma* while the emergence of key innovations may be argued for *Michelia*.

T2-61-07

Historical biogeography and ancestral areas of Neotropical Magnoliaceae

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The improved understanding of patterns in species diversity and relationships within Neotropical clades of *Magnolia* could provide valuable information for understanding the global and regional biogeography of Magnoliaceae. In this study, we analyze the historical biogeography of Magnoliaceae with the complex Dispersal–Extinction–Cladogenesis model, using newly available information on Neotropical taxa and molecular phylogenetic reconstruction based on cpDNA loci from 74 species. The availability of fossil record for Magnoliaceae, as well as known phylogenetic positions of fossil taxa allowed us to test and interpret the results of the Dispersal–Extinction–Cladogenesis model. The probable ancestral area of the whole Magnoliaceae has been identified as a combination of temperate and subtropical regions in Eurasia and North America, as the connected mid-latitude zone of the Northern Hemisphere during the early Eocene. This ancestral area for Magnoliaceae is consistent with previously proposed hypothesis of fragmentation of Eocene 'Arcto-Tertiary flora' or more recent 'Boreo-Tropical flora'. During the early Tertiary, Magnoliaceae included two lineages that persisted until modern time: *Liriodendron* ancestors and species of *Magnolia* clade. In *Liriodendron*, the integrity of the wide distribution range shared between Eurasia and North America persisted until the Miocene, while in *Magnolia* the geographical separation between America and Eurasia started the middle Eocene with evolving of geographically independent sections *Gwillimia*, *Manglietia*, *Kmeria*, *Michelia* in Eurasia, and *Magnolia*, *Macrophylla*, *Auriculata*, *Talauma* in North America. However, in the case of the subsections *Yulania* and *Tulipastrum*, the interchange between Eurasia and North America persisted until the Oligocene, and in the section *Rytidospermum* until the middle Miocene. We revealed a possible general migration pathway of a Neotropical *Magnolia* ancestor from eastern North America through Greater Antilles that resulted in the establishment of the modern *Talauma* distribution. *Talauma* as one of the first modern sections to evolve in the middle Eocene within *Magnolia*, has its ancestral area shared between the subtropical zone of North America and the subtropical zone of Eurasia, but evidently went extinct in the latter one. We interpreted the historical habitat availability data in relation to the actual and inferred ancestral distribution of *Talauma* to reveal the possible historical migration pattern. The ancestor group for modern Neotropical section *Talauma* as well as related *Dugandiodendron* and *Splendentens* taxa had started migration southwards from the southeastern North America during middle Eocene, and experimented diversification initially in Greater Antilles, later in Oligocene penetrated to northern South America, northern Andes and Central America. The section *Macrophylla* actually known form Mexico and USA had the ancestral area in subtropical and temperate North America in middle or late Eocene, unrelated to distribution of *Talauma* clade. The detailed biogeographical scenario for neotropical Magnolia will require extended representative sampling within *Talauma*, *Dugandiodendron* and *Splendentens*.

T2-61-08

Genetic population structure and diversity of *Magnolia pacifica A.*Vázquez species complex in southwestern Mexico Inferred from ISSR Markers

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The delimitation of species, the fundamental units of evolutionary biology, is an essential task of current systematic studies. Magnoliaceae is still considered a relatively primitive group of angiosperms, comprising ca. 350 species of trees and shrubs native to Asia and America. Although several molecular phylogenetic studies based on DNA sequence data have been conducted on the Magnoliaceae family, several major and minor clades have not been completely resolved. *Magnolia pacifica s.s.*, *M. pugana* and *M. vallartensis* are recently described species grouped in the *Magnolia pacifica* A. Vázquez species complex. These species are endemic, endangered, and distributed in isolated fragments of tropical montane cloud forest and gallery forest in southwestern Mexico. In this study, we used inter-simple sequence repeats (ISSR) in order to: (1) to analyze species boundaries within the M. pacifica complex under a population genetics approach, (2) evaluate genetic diversity and (3) provide information for species conservation. Six ISSR primers amplified a total of 76 loci from 278 individuals sampled in four localities of *M. pugana*, three of *M. pacifica s.s.* and three of *M. vallartensis*. Sixty five percent of loci were polymorphic among all samples. Bayesian cluster analysis, the Exact Test for population differentiation and Unweighted Pair-Group Mean Average (UPGMA) cluster analysis were consistent with the morphological recognition of *M. pugana* and *M. pacifica* at the species level. Two subpopulations of M. pugana were identified, they are separated mainly by the Santiago river canyon (500-700 m depth and 3-15 km width). ISSR data suggests that *M. vallartensis* is in a process of incipient speciation with a recent partial divergence from *M. pacifica s.s.* Positive and significant correlation between geographic and genetic distances (Mantel Test $r = 0.8$, $p = 0.001$) indicates isolation by geographic distance. The Analysis of Molecular Variance (AMOVA) showed that most of the genetic variation was within populations (83%), lower proportion among taxa (8%) and among populations of the same taxon (8%). G_{ST}, θ and D_{EST} indices revealed significant differentiation among localities within each taxon, but differentiation was higher in *M. pugana* ($G_{ST} = 0.168$, $\theta = 0.2$, $D_{EST} = 0.028$) than in *M. pacifica* ($G_{ST} = 0.102$, $\theta = 0.131$, $D_{EST} = 0.018$) and *M. vallartensis* ($GST = 0.127$, $\theta = 0.159$, $DEST = 0.021$). Diversity indices were lower for M. pugana HT = 0.158, HS = 0.134 than for *M. pacifica* and *M. vallartensis* ($H_T = 0.175$, $H_S = 0.159$ and $H_T = 0.171$, $H_S = 0.153$, respectively). Findings of the present study bring out the usefulness of molecular data and a population genetics approach to help delineate species boundaries within species complexes. The two species, *M. pacifica* and *M. vallartensis*, and the two subpopulations of *M. pugana*, should be considered as separate units of conservation. Concerted efforts must be made to protect these three taxa, but a greater focus on *M. pugana* is needed.

T2-61-09

Taxonomic revision of Sect.*Yulania* of *Magnolia*

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Magnolia Sect. *Yulania* Spach. is an important taxa in Magnoliaceae which with comparatively evolved features such as deciduous leaves, pollen lateral cracking. This section contains 16 general recognized species (including two varieties) which are mainly distributed in north subtropical and warm temperate zone. Among these species, *M. acuminata* L. and *M. acuminata* var. *subcordata* L. distributed in southeast of America, *M. kobus* (DC.) Spach, *M. stellata* M. and M. *salicifolia* (Sieb. & Zucc.) Maxim.distributed in Japan, the other 11 species are all occurred in China. The systematic position and internal classification of this group has been controversial. More than 28 new species were published recently in China. In this study, 267 individuals from 45 populations representing 17 taxa in sect.*Yulania* were studied for the taxonomic revision of sect.*Yulania*. The morphology variation and geographical distribution, as well as molecule data analysis on the combine data of three universal DNA regions (*mat*K, *rbc*L, *trn*H-*psb*A) and three self-screening chloroplast gene spacer sequences (*pet*A-*psb*J, *psb*E-*pet*L, *trn*T-*psb*D), were conducted to detect the phylogenetic relationships. The results revealed that sect. *Yulania* was divided into two subsections (Subsection *Yulania* and subsection *Tulipastrum*) with four gourp, 11 speceis 8 varieties: Accuminata group (*M. accuminata* L., *M. acuminata* var. *subcordata* (Spach) Dandy), Binodii group (*M. biondii* Pampan., *M. biondii* Pamp. var. *purpurascens* Y.L.Wang & S.Z.Zhang, *M. biondii* var. *zenii* (Cheng) Y.L.Wang & S.Z.Zhang, *M. kobus* DC, *M. salicifolia* (Sieb. & Zucc.) Maxim., *M. sinostellata* P.L.Chiu et Z.H.Chen, *M. stellata* (Sieb. & Zucc.) Maxim.), Sprengeri group (*M.campbellii* Hook f. &Thous, *M. sprengeri* Pampan., *M. sprengeri* var. *sargentiana* (Rehder & Wilson) Y.L.Wang & S.Z.Zhang), Denudata group (*M. cylindrica* Wils., *M. cylindrica* E.H.Wilson var. *purpurascens* Y.L.Wang & S.Z.Zhang,, *M. denudata* Desr., *M. denudata* var. *glabrata* Y. L. Wang et S. Z. Zhang, *M. denudata* var. *pilocarpa* (Z. Z. Zhao & Z. W. Xie) Y. L. Wang et S. Z. Zhang, *M. liliiflora* Desr., *M. liliiflora* Desr. var. *polytepala* (Law, R.Z. Zhou & R.J. Zhang) Y. L. Wang et S. Z. Zhang).

T2-61-10

An overview of species richness, conservation and ecology of Neotropical *Magnolia*

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The classification of Magnoliaceae has been controversial, especially at the genus level including infrageneric categories. In the Neotropics, there are approximately 130 species of Magnoliaceae belonging to subgenus *Magnolia*, sections *Macrophylla, Magnolia* and *Talauma*. This last section comprises four subsections: *Cubensis* (West Indian species), *Dugandiodendron* (Species from North of South America including Colombia and Venezuela), *Talauma* (from Mexico to Bolivia) and a new one recently described based on morphological characters: *Chocotalauma* (species distributed throughout the Choco Biogeographical Region). Mexico, with 40 species, is the country with the highest diversity of *Magnolia* species in the Neotropics, followed by Colombia with 36 species reported up to date. Several Neotropical species of *Magnolia* have been recently described, especially from Mexico, Ecuador and Peru. In the Neotropics, *Magnolia* species are characterized by a restricted geographical distribution in highly disturbed forests, populations with few individuals and in some cases, their trees have been overharvested. Consequently, most species are in some risk of extinction and are currently considered as a priority for conservation. The very low seedling recruitment observed in natural populations suggests that forest fragmentation could affect tree reproduction by altering pollination interactions, increasing seed predation and herbivory on juveniles. In some species, fruit formation and seed production are extremely low despite the availability of flowers. This aspect may be associated to specific plant-pollinator interactions that could be affected by high levels of forest fragmentation. Frugivory could also affect the reproductive success of these species. However, little is known about their ecology. The goal of this work is to show general aspects on species richness, conservation and ecology of Magnolias from the Neotropics, specifically those related to reproductive biology as a key factor to be considered for successful conservation initiatives and future research programs.

T2-61-11

Early evolution and dispersal of the magnoliaceae—an alternate hypothesis

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The Magnoliaceae is an ancient family that dates from the early radiation of flowering plants. The Magnoliales and related taxa are among the Basal Angiosperms that were diversifying 127 mya, even before the emergence of the Basal Eudicotyledons. During the mid-Cretaceous and into the Paleogene, the Tethys Seaway served as a tropical corridor connecting Asia with the islands of Europe to the north, Africa to the south and the Americas to the far west. Welwitschia, restricted to southern Africa today, was present in the Lower Cretaceous of Brazil as well as in some angiosperm remains found in lower Cretaceous sediments in North Africa and Portugal, which demonstrates potential ancient land connections and plant migration patterns. This low latitude east-west movement of tropical plants can also be seen in the relationships of some floristic elements occurring in Paleocene and Eocene plant fossils of southeastern North America to plants currently living in tropical Asia, tropical South America and Africa today. Common elements are also shared in the Paleocene and Eocene pollen floras of these regions. Also found in the fossil record of Cenozoic sediments in southeastern North America are remains of *Dugetia* in the Annoniaceae, now found in South America and Africa; *Gordonia* in the Theaceae, now found in South America and tropical Asia; and *Nypa*, a palm now found only in tropical southern Asia. These are some tropical floristic elements shared between Asia and the Americas and were components of floras that migrated along the low latitude lands bordering the Tethys Seaway rather than moving over a high latitude route. The extant taxa of the Magnolialiales are predominately distributed today in the Asian and American tropics and to some extent in the African tropics. These plants could have easily traversed the low latitude tropical islands of the Tethys Seaway extending between Asia and North America. This could explain the disjunct distributions of many tropical American and Asian plants today. Here I propose the hypothesis of a *Low Latitude Migration Pattern* for many of the disjunct truly tropical genera including those of the Magnoliaceae found in the American and Asian tropics today rather than by the migrations of these taxa over a temperate to cool temperate Bering Sea or cooler North Atlantic land bridges. Currently much of the published research seems to support of high latitude migrations of both temperate and tropical plant taxa. This should be reconsidered in relation to the ecological tolerances of the various taxa involved and the various climates of the migration routes proposed through time. Because of the extreme tropical nature of many of these plants, it is questionable that so many tropical taxa would have migrated between the tropical Americas and tropical Asia even during the most extreme Paleocene Eocene Thermal Maximum (PETM). During the Late Cretaceous the major taxa of many angiosperms, including the Magnoliaceae, had already migrated and were similar to extant taxa.

T2-61-12

Molecular phylogeny of Magnoliaceae based on cpDNA sequence data

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A molecular phylogenetic study of Magnoliaceae was conducted using sequences of coding and noncoding chloroplast DNA regions (total ca. 9,000 bp) to reveal the phylogenetic positions of about 100 Magnolia taxa covering all subgenera (Magnolia, Yulania, and Gynopodium), sections (Magnolia, Gwillimia, Talauma, Manglietia, Kmeria, Rhtidospermum, Auriculata, Macrophylla, Yulania, Michelia, Gynododium, and Manglietiastrum), and subsections (total 20). In this study, several tropical Asian Neotropical species are newly sequenced and added to the sequence data matrix previously conducted. Phylogenetic analysis of the data matrix indicates that subsect. Dugandiodendron is sister to subsect. Cubenses, and the clade is sister to subsect. Talauma. That is, the three Neotropical groups (Dugandiodendron, Cubenses, and Talauma) are closely related and formed a monophyletic clade. However, close relationship between the Neotropical clade and southeast Asian groups (subsects. Gwillimia and Blumiana) is still controversial. Phylogenetic relationships among these tropical Asian species are also still unclear. Although I am still obtaining additional sequence data from Asian and American species, the tree will be helpful for understanding the taxonomy and the evolution of morphological characters in Magnoliaceae.

T2-62: Evolutionary and developmental morphology of basal monocots

T2-62-01

Implications and importance of morphological and developmental diversity of Aponogentanaceae in Indian subcontinent with special emphasis on embryo diversity

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Family Aponogetonaceae with a single genus *Aponogeton* L.f. of about 58 aquatic tuberiferous species is distributed in tropical and subtropical regions of the world. The genus is well studied from phylogeny and biogeography point of view. Three new species have been added to Aponogetons of India and presently there are about nine species viz. *A. appendiculatus, A. bruggenii, A. crispus, Aponogeton* species*, A. lakhonensis, A. natans, A. nateshii, A. satarensis* and *A.undulatus* in India. Among the species, *Aponogeton appendiculatus, A. bruggeni, A. nateshii* and *A. satarensis* endemic to India are of special significance either from their phytogeography, reproductive biology or embryo morphology point of veiw. Similarly, *Aponogeton* species similar and close to *A. jacobsenii* from Sri Lanka is reported to occur in India. In present investigation implications of newly described species of Aponogetons, interrelationships among Indian species of the genus and unique embryo morphology have been presented and discussed. *Aponogeton satarensis* is the only Indian dioecious relict endemic species with biforked spike whose close relative viz. *A. decaryi* occurs in Madagascar. This has great phytogeographical significance. *Aponogeton bruggenii* is narrow endemic with barriers of unknown nature in sexual reproduction and seldom sets seeds. It propagates through formation of daughter tubers on mother tuber. *Aponogeton nateshii* is a unique in its embryo morphology. The embryo is globular and possesses 15-22 appendages all around the embryo. Such embryo is so far not reported in any other angiosperm except *A. appendiculatus* which has 3-5 tortuous appendages. Three population of *A. nateshii* showed variation in number of appendages per embryo and their fusion with tuberous part of embryo. All Indian species are closely allied to each other except for *A. satarensis*. The size, shape, appendages (cotyledons), position of plumule and emergence of radical root varies with species. Attempts have been made to understand morphological and developmental diversity of Aponogetonaceae in Indian subcontinent with special reference to embryo diversity and diversification of the genus.

T2-62-02

Pollen diversity and parallel evolution in Alismatales

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The order Alismatales comprises Tofieldiaceae, Araceae and core Alismatales (including 'tepaloid' and 'petaloid' clades). Some core Alismatales demonstrate adaptations for hydrophilous pollination accompanied with severe modifications and reductions in sporoderm structure (Furness & Banks, 2010). The early-divergent member of the tepaloid clade, Aponogetonaceae, is characterized by monosulcate pollen that may represent a plesiomorphic feature in Alismatales and monocots in general. Many other Alismatales have inaperturate pollen, but pantoporate (Alismataceae, petaloid clade) pollen is also known. Diaperturate pollen is not common but remarkably diverse in Alismatales. In *Tofieldia* (Tofieldiaceae), one aperture is distal and another is proximal, which is confirmed by our developmental data. In contrast, *Calla* (Araceae) has two equatorial sulci or a ring-like aperture (Ulrich *et al.,* 2013). Thus the increase in number of apertures has been realized in different ways in different lineages. *Scheuchzeria* occupies a key position in the tepaloid clade. Scheuchzeriaceae, Juncaginaceae and Maundiaceae all possess inaperturate pollen and are wind-pollinated. These families are the three successive members of the grade leading to a more derived monophyletic group of tepaloid clade that includes several seagrass lineages with filiform pollen, but also some members with aperturate or tenuitate pollen (*Ruppia* and *Althenia* spp.). This pattern suggests evolutionary loss and re-gains of apertures in the 'tepaloid clade'. In the light of this scenario, a question arises whether any rudimentary traces of an aperture can be seen at ultrastructural and developmental levels in the inaperturate members of the tepaloid clade. Our data on pollen development in *Scheuchzeria* does not reveal any possible aperture sites in the process of microsporogenesis that favours the hypothesis of the complete evolutionary loss and subsequent regain of the apertures (Volkova *et al.,* 2016). The unusual permanent pollen dyads of *Scheuchzeria* can be considered as adaptation to fertilization of both ovules typically present in carpels. *Althenia* s.l. (Potamogetonaceae) includes two dissimilar species with inaperturate (omniaperturate) and several species with diaperturate pollen. Species with diaperturate pollen fall in two groups based on structure of sporoderm revealed by scanning and transmission electron microscopy. Fine structure of sporoderm in the apertural region in each group is similar to the sporoderm along the entire surface of pollen grain in one of the two omniaperturate species. Along with molecular phylogenetic evidence (Ito *et al.,* 2016), this suggests that the omniaperturate condition appeared twice in course of evolution of *Althenia*, each time by expansion of the apertural area onto entire surface of the pollen grain. We speculate that the transition from inaperturate to diaperturate pollen in Potamogetonaceae should be viewed as an evolutionary step towards a complete loss of interapertural type of sporoderm. Thus the evolution of spherical omniaperturate pollen was homoplastic, like the origin of the filiform omniaperturate pollen in different families of tepaloid Alismatales. More data on pollination biology are needed to understand the biological significance of different types of pollen grains with reduced sporoderm. The work on pollen morphology and development was performed by EES and OAV with funding from the Russian Science Foundation (project 14-14-00250).

T2-62-03

Morphological diversity of core Alismatales

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The core Alismatales includes all families of the order except Tofieldiaceae and Araceae. This is a rare example of a group of angiosperm families commonly recognized in pre-molecular era (as subclass Alismatidae) and then fully supported by molecular data in the same circumscription. Aquatic or semi-aquatic habit is of course the most remarkable feature of the group (hence the historical name Helobiae), but in terms of diagnostic characters, the presence of intravaginal squamules and the large storage embryo are the most remarkable. These two features are present in all members of core Alismatales (the squamules of Scheuchzeria are transformed in hairs) and are otherwise very rare among monocots. Both features are present in some Araceae being homoplastic within the family, and the squamules are present in Acorus. Morphological nature of intravaginal squamules is enigmatic. Arber (1923) highlighted that the squamules are attached above the leaf to the base of stem internode. We hypothesize that the squamules are homologous to collateral buds that are characteristic to monocots in general but not to the core Alismatales. The number and morphology of intravaginal squamules are of taxonomic significance in the core Alismatales, for example, distinguishing *Potamogeton* and former Zannichelliaceae. Leaves of the core Alismatales are diverse in terms of external morphology and anatomy. It is unclear whether they can be considered unifacial in any of the core Alismatales other than *Tetroncium* (Juncaginaceae). Leaves or leaf petioles have three-dimensional venation in several families, normally with a system of small peripheral vascular bundles. As in angiosperm succulents and halophytes (Ogburn & Edwards, 2013), the three-dimensional leaf venation in thick aquatic and helophyte leaves of Alismatales serves to reduce transport distances between veins and photosynthetic cells (Platonova *et al.,* 2016). Like in the eudicot order Caryophyllales (e.g., Melo-de-Pinna *et al.,* 2014), the patterns of orientation of peripheral collateral bundles (with inverted adaxial or abaxial bundles) are unstable in Alismatales. These slender bundles cannot be used for the identification of unifacial leaves. *Maundia* (Maundiaceae) is remarkable in having similar anatomical structure of foliage leaves and inflorescence peduncles, both with xylem of small peripheral bundles oriented towards periphery of the organ. The similarity is due to their shared function as photosynthetic organs, though functional significance of particular types of bundle orientation remains unclear (Platonova *et al.,* 2016). Compared to many other monocot lineages, the core Alismatales are remarkably diverse in terms of number and position of floral organs and degree and mode of intercarpellary fusion. Floral characters are homoplastic in Alismatales and inferring directions of their transformation is problematic. Patterns of floral diversity in Alismatales can be only partially explained by adaptations to aquatic habitats. In most cases, evolutionary reductions in number of floral organs left no rudiments in the core Alismatales, with notable exceptions in the family Juncaginaceae. The study is supported by the Russian Science Foundation (project 14-14-00250).

T2-62-04

Floral development of petaloid Alismatales as a clue to the origin of the trimerous pentacyclic flower of the Monocots

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The monocots are remarkably homogeneous in sharing a common trimerous pentacyclic floral Bauplan. A major factor affecting monocot evolution is the unique origin of the clade as an offshoot of the basal angiosperms. The origin of the floral Bauplan of monocots is still controversial, as no immediate sister groups with similar structure can be identified among basal angiosperms, and there are several possibilities for an ancestral floral structure. Either more complex flowers with a higher stamen and carpel number, or strongly reduced flowers could be ancestral, while a stable Bauplan is only established beyond the divergence of Alismatales. We revisited the floral development of members of the three 'petaloid' Alismatales families Butomaceae, Hydrocharitaceae, and Alismataceae. Outer stamen pairs are present in Alismataceae and Butomaceae, It is demonstrated that paired stamens always arise independently, and are either shifted opposite the sepals or close to the petals. The position of stamen pairs is a matter of development of the petals. In Butomaceae the perianth is undifferentiated and not delayed; the larger petals push the stamens in antesepalous pairs. Alismataceae have delayed petals and stamens are shifted in close proximity to the petals, leading to a close association of pairs with the petals in so-called stamen-petal complexes. In the studied Hydrocharitaceae paired stamens are replaced by larger single stamens and petals are not delayed. Despite the fact that the petaloid Alismatales are not at the base of monocot divergence, the floral Bauplan of Butomaceae and some members of Tofieldiaceae (e.g. Pleea) are probably close to the ancestral flowers of monocots. Among basal angiosperms, the comparative Bauplan is only retrieved in members of Cabombaceae, such as *Brasenia*, and may indicate that Monocots evolved as an offshoot from aquatic basal angiosperms, with variable stamen and carpel number.

T2-62-05

Taxonomy and evolutionary morphology of Australian *Althenia* (Potamogetonaceae, Alismatales)

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Traditionally *Althenia*, with two species in Eurasia and Africa, was regarded as one of four genera of submerged aquatics comprising family Zannichelliaceae, related to Cymodoceaceae. Molecular phylogenetic data confirmed monophyly of Zannichelliaceae but placed the clade with *Potamogeton* and its segregates, so that current classifications include Zannichelliaceae in Potamogetonaceae. A recent study (Ito *et al.,* 2016) using several molecular markers demonstrated that *Althenia* is nested within the Australian and New Zealand *Lepilaena*, most closely related to *L. preissii*. Consequently the two genera were combined under the name *Althenia*. According to Ito *et al.,* (2016), the genus comprises seven species (five in Australia). However, many aspects of morphological diversity, diagnostic characters, taxonomic composition and geographical distribution of *Althenia* remain unresolved. Our current data shows *Althenia* comprises at least eight species in Australia. This first comprehensive study of Australian herbarium collections showed up to 30 percent were incorrectly identified. Re-identification of all available material resulted in much better understanding of the ecological requirements and distribution patterns of Australian species of *Althenia*. One species is restricted to marine habitats or estuaries. *Althenia* is of interest with respect to several aspects of morphological evolution. The genus comprises both dioecious and monoecious species (one species probably possessing monoecious and dioecious populations). Monoecy is likely plesiomorphic in *A. australis*, which was shown to be sister to the rest of the genus (Ito *et al.,* 2016). In other cases, monoecy is possibly derived from dioecy as a reversal. In some dioecious species, foliage leaves associated with male inflorescences are pronouncedly ligulate while those associated with female inflorescences lack ligules. The sheathing leaf bases and ligules are apparently involved in the mechanism of release of pollen grains because they may enclose anthetic stamens. Traditionally, *Althenia* s.str. was distinguished from *Lepilaena* mainly by male flowers with 2-sporangiate (vs. 4-12-sporangiate) anthers and funnel-shaped, circular, polysymmetric (vs. monosymmetric) stigmas. The discovery of a new dioecious species from Western Australia challenges these criteria and supports molecular phylogenetic evidence for lumping the two genera. The species has polysymmetric circular stigmas like *Althenia* s.str. but 12-sporangiate anthers like *Lepilaena*. Like some other Australian species, the new taxon has strongly dimorphic male and female inflorescences. Scanning electron microscopic study of developmental series of male and female inflorescences of the new species showed that in spite of habit differences, major structural and developmental features of floral units are identical in both sexes. Among interesting features is the possession in male floral units of very long filamentous (distally flattened) terminal structures that resemble tubular and filamentous terminal structures that sporadically develop in inflorescences of *Potamogeton*. However in the case of our *Althenia* species their regular occurrence in only one of the two sexes indicates that they cannot be treated as a developmental abnormality and must have a certain function, unknown as yet. Phylogenetic studies of *Althenia* using molecular data are being expanded with additional geographic sampling and new species. This work was supported with funding from the Russian Science Foundation (project 14-14-00250).

T2-62-06

Secondary carpel margin in ascidiate carpels of Alismatales

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Completely ascidiate carpels (often with funnel-shaped stigmas) are especially common among tepaloid alismatids (basal monocot order Alismatales). Along all their length, these carpels represent a tubular structure with ventrally attached single pendent ovule. There is a considerable piece of tissue between ovule and carpel margin. Developmentally, the ovule originates on the ventral placenta which is located in the cross-zone. Usually, ovule initiation happens early in the development when the carpel is represented by low meristematic rim. As the carpel walls grow, the ovule attachment becomes lifted above the receptacle. Thus, the ovary is formed by circular zonal growth below the level of placenta. The most intriguing process is style development which proceeds via circular growth above the level of placenta. We speculate that directly above the cross-zone a new, secondary carpel margin appears. The cross-zone itself is a marginal meristem to build up the ventral carpel wall and to produce the ovule. Technically, the cross-zone becomes exhausted by ovule initiation and the growth of carpel wall ceases ventrally after ovule initiation in many angiosperms. To continue with tubular style, a new meristematic activity is needed above the ovule. The secondary ventral carpel margin as a result of such an activity develops more slowly than lateral flanks. This leads to slit appearance of carpel orifice which faces ventrally. During further development, the carpel orifice becomes more or less horizontal. The work was performed with funding from the Russian Science Foundation (project 14-14-00250).

T2-63: Ecological and biogeographic implications of Asian Oligocene and Neogene fossil floras

T2-63-01

Cenozoic plant diversity of Yunnan: A review

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Yunnan in southwestern China is renowned for its high plant diversity. To understand how this modern botanical richness formed, it is critical to investigate the past biodiversity throughout the geological time. In this review, we present a summary on plant diversity, floristics and climates in the Cenozoic of Yunnan and document their changes, by compiling published palaeobotanical sources. Our review demonstrates that thus far a total of 386 fossil species of ferns, gymnosperms and angiosperms belonging to 170 genera within 66 families have been reported from the Cenozoic, particularly the Neogene, of Yunnan. Angiosperms display the highest richness represented by 353 species grouped into 155 genera within 60 families, with Fagaceae, Fabaceae, Lauraceae and Juglandaceae being the most diversified. Most of the families and genera recorded as fossils still occur in Yunnan, but seven genera have disappeared, including *Berryophyllum*, *Cedrelospermum*, *Cedrus*, *Palaeocarya*, *Podocarpium*, *Sequoia* and *Wataria*. The regional extinction of these genera is commonly referred to an aridification of the dry season associated with Asian monsoon development. Floristic analyses indicate that in the late Miocene, Yunnan had three floristic regions: a northern subtropical floristic region in the northeast, a subtropical floristic region in the east, and a tropical floristic region in the southwest. In the late Pliocene, Yunnan saw two kinds of floristic regions: a subalpine floristic region in the northwest, and two subtropical floristic regions separately in the southwest and the eastern center. These floristic concepts are verified by results from our areal type analyses which suggest that in the Miocene southwestern Yunnan supported the most Pantropic elements, while in the Pliocene southwestern Yunnan had abundant Tropical Asia (Indo-Malaysia) type and East Asia and North America disjunct type that were absent from northwestern Yunnan. From the late Miocene to late Pliocene through to the present, floristic composition and vegetation types changed markedly, presumably in response to altitude changes and coeval global cooling. An integration of palaeoclimate data suggests that during the Neogene Yunnan was warmer and wetter than today. Moreover, northern Yunnan witnessed a pronounced temperature decline, while southern Yunnan experienced only moderate temperature changes. Summer precipitation was consistently higher than winter precipitation, suggesting a rainfall seasonality. This summary on palaeoclimates helps us to understand under what conditions plant diversity occurred and evolved in Yunnan throughout the Cenozoic.

T2-63-02

Cenozoic floras in the Qinghai-Tibetan Plateau: Were they isolated?

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The Qinghai-Tibetan Plateau (QTP) and adjacent areas are well known for the high biodiversity worldwide at the present day. Plant fossils are pivotal to understand their histories of biodiversity and biogeography under dramatically paleoenvironmental changes in this large and fascinating region; however, fossil floras there have been far from fully investigated. During recent five years, we found several paleofloras ranging from Eocene to Pliocene in high altitudes of the QTP. For the early Eocene Liuxiang flora (~ 55 Ma) in southern Tibet, Moraceae are the most abundant; the late Eocene Kajun flora (~34.6 Ma) in eastern Tibet is dominated by *Cyclobalanopsis* and *Betula*, representing evergreen-deciduous broadleaf forest; For the early Miocene Lunpola flora (~23 Ma) in central Tibet, grasses firstly occurred together with Arecaceae and *Koelreuteria*. The Pliocene Zhada flora (~ 4 Ma) in western Tibet is represented by small-sized leaves. The floristic history proves dramatically environmental changes throughout geological time in the QTP. Meanwhile, it suggests that the floristic modernization in the QTP occurred no later than the late Eocene. Some extinct taxa, e.g., *Hemitrapa*, *Limbophyllum*, and *Cedrelospermum*, as well as many taxa being still widely distributed in the QTP, e.g., *Betula*, *Cyclobalanopsis*, *Excentrodendron*, *Koelreuteria*, and *Rosa*, indicate that there were frequently floristic exchanges between the QTP and other parts of the North Hemisphere. This work is supported by National Natural Science Foundation of China (41661134049,31470325, U1502231), Excellent Young Scientists Foundation-CAS (QYZDB-SSW-SMC016), and Youth Innovation Promotion Association, CAS (2017439).

T2-63-03

Oligocene and Neogene vegetation change in Siberia and the northeast of Russia reconstructed from palaeocarpological

data

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The Cenozoic continental deposits of Siberia are reknowned for their carpological record. P. Nikitin, V. Nikitin and P. Dorofeev carried out comprehensive studies there throughout the 20th century. Based on the flora-bearing horizons four main evolutionary stages were outlined by V. Nikitin. In the first phase, the Pre-Turgayan, a subtropical flora existed (Late Cretaceous – Eocene). The second, Turgayan phase is characterized by the expansion of a boreal, warm temperate flora. This flora evolved during the Early Oligocene and was replaced by diverse mesophilous mixed coniferous-broad-leaved forests during the Late Oligocene to Early Miocene. The last phase, the Post-Turgayan (Middle Miocene to Early Pliocene), mainly shows the dominance of forested steppe. Steppe landscapes evolving since the end of the Pliocene represent the modern stage of the vegetation in the southern part of Western Siberia (WS). The analysis of the diversity of plant functional types (PFTs) is proven to be very useful for quantifying spatial vegetation patterns and gradients and their evolution during the Cenozoic. Here we use the diversity of 27 herbaceous to woody PFTs to classify 148 carpofloras from WS and the northeast of Russia (NER) covering the time-span from the OLigocene to the late Pliocene. Our novel PFT classification scheme, designed for use in biome modeling, comprises 26 herbaceous to arboreal PFTs based on physiognomic characters and bioclimatic tolerances of plants, completed by an aquatic PFT. Using multivariate statistics, localities with similar PFT spectra are grouped and interpreted in terms of biomes. The results are visualized on palaeovegetation maps. The PFT technique facilitates interregional comparisons and unravels differentiations in biome type that are hardly quantifiable in classical vegetation reconstruction from the palaeobotanical record. Moreover, the use of diversity of single plant functional types instead of specimen abundance minimizes taphonomic bias, which may be considerable when carpofloras are regarded. In a number of cases it can be shown that PFT diversity gradients in WS and NER follow climatic patterns. Our study reveals a longitudinal gradient between WS and NER existing in Eurasia already in the early Miocene but getting more significant along with the Neogene continental cooling. In WS, late Cenozoic vegetation change relates to a decline of arboreal components, together with a strong diversity increase in herbaceous plants. This process already began in the late Palaeogene, with the onset of a global step-wise cooling, and intensified from the middle Miocene on. In the later Neogene, increasing diversity of xerophytes in the southern part of WS indicates drying of the continental interior. At the same time, the declining diversity of thermophilous PFTs in the northern part of WS testifies the cooling of the higher latitudes. According to our findings, NER was persistently covered by forests until the Pliocene, including the High Arctic. The humid and thermophilous aspect of late Miocene to Pliocene palaeofloras from the western part of NER contravenes the global cooling trend and could mirror a later Neogene intensification of the East Asian Monsoon System.

T2-63-04

Oligocene-Miocene floral changes in the circum-Japan Sea areas- climate changes and floristic separation

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Oligocene–Miocene floral changes in the circum-Japan Sea areas were reviewed to understand the floristic response to global and local climates as well as to the effects of the opening of Sea of Japan during the early–middle Miocene. We recognized two floral stages in the Oligocene and four in the Miocene, based on detailed stratigraphic correlations. The early Oligocene, here referred to as Stage O-1, is represented by the Wakamatsuzawa flora in Hokkaido and the Kobe flora in Central Honshu, Japan. Some assemblages in western Primorye (Kraskino, Rettikhovka) and North Korea (Kogeonwon, Kungshim) can be correlated to this stage. Floristic composition of each assemblage differs one to the other possibly due to latitudinal temperature changes; however, most of them typically contain lobed-oaks, Palaeocarya and show similar physiognomic characters. The next Stage O-2 of late Oligocene is recorded only in western Japan. Two assemblages represent temperate forest vegetation with few evergreen species. Stage M-1 in the earliest early Miocene in Japan is called Aniai-type flora. Comparable assemblages have been recorded in the Korean Peninsula (Janggi, Yongdong) and western Primorye (Sinij Utes). All these assemblages contain deciduous temperate elements like Fagus and various species of Betulaceae, Ulmaceae, and Sapindaceae with no or few evergreen angiosperms. Stages M-2 and 3, denoted here as late early and middle Miocene, are comparable with the Daijima-type floras of Japan, which are distributed throughout the Japanese Islands. They are composed of a mixture of evergreen and deciduous species of dicotyledons in Honshu, but represented more by deciduous elements in Hokkaido. Sinjeongri (South Korea) and Tongcheon (North Korea) floras are compared with M-2, while Yeonil and Hamjindong floras of South and North Korea are compared with M-3 assemblages. Marine organisms that indicate tropical–subtropical oceanic conditions have been recorded from the strata dated between Stages M-2 and 3, in which pollen grains of mangrove plants have been reported from over ten sites in central to northern Honshu. Assemblages from the Nezhino and Novokachalinsk horizons in Primorye are considered contemporaneous with Stages M-2 and 3. However, floristic composition of the Russian assemblages differ considerably from those in Honshu and southern Korean Peninsula. Stage M-4 assemblage, denoted as the late Miocene, is known as the Mitoku-type floras in Japan. The Ust Suifun flora in western Primorye is compared stratigraphically. In Japan, a number of inland basins started to develop at this stage. There is a strong difference between those from inland basins and coastal lowlands. The former is represented by deciduous temperate species, while the latter consists of mixture of evergreen and deciduous species. Quantitative climate analysis of these assemblages from lowland vegetation indicates a trend roughly parallel to the global climate pattern. However, after the separation of Japanese Islands from the continent, the extent of temperature increase from stages M-1 and 3 evidently differed between assemblages on the continent and those on the island, which is probably due to the influence of cold and warm water currents both toward the north and south of the Sea of Japan.

T2-63-05

Elaeocarpus L. (Elaeocarpaceae J.) fossil fruits from Oligocene-Miocene of Guangxi, South China

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Elaeocarpus as a genus of tropical and subtropical evergreen trees and shrubs is widespread in the Indo-Pacific region from India, Southeast Asia, southern China, and Japan, Malaysia, New Guinea, Australia to the outliers including Madagascar, New Caledonia and New Zealand. Centers of high diversity include northeastern Australia, New Caledonia and the Malesiana region. In China, there are approximately 39 extant species in the genus, which are mainly distributed in the South and Southwest China. The fossil record of *Elaeocarpus* is rare, including some pollen, leaves and fruits from several Oligocene and younger deposits in Australia and India. However, the fossil pollen and leaves attributed to the genus are questionable. *Elaeocarpus* fruits, by contrast, are recognizable from other genera by their unique woody and highly variable morphology and ornamentation types of mesocarps, which are extremely important in understanding systematic relationships within the genus. Therefore, the most reliable and the earliest fossil record of *Elaeocarpus* is the fruit stones of *E. spackmaniorum* Rozefelds, *E. cunningii* Rozefelds and *E. rozefeldsii* Dettmann and Clifford from the early-late Oligocene of central Queensland, Australia. In this article, well-preserved *Elaeocarpus* fruit stones recovered from the Oligocene of the Nanning Basin and Miocene of the Guiping and Nanliu Basin, Guangxi Province, South China were studied. Micro-CT was used in reconstructing three dimensions and sections of the fossil and extant specimens, six species types are confirmed based the highly variable morphology and ornamentation of mesocarps. They are similar to extant species of *Elaeocarpus glabripetalus* Merrill, *E. rugosus* Roxburgh, *E. lacunosus* Wallich ex Kurz, *E. serratus* L., *E. prunifolioides* Hu, and *E. sikkimensis* Masters. The origin for the genus was inferred as southern hemisphere origination and migrated to the northern hemisphere via birds, based on the fossil records and modern distribution pattern of the genus. This is the first fossil record for the occurrence of the genus in the lowest latitude of Asia, indicating that the genus has been spread into South China as early as Oligocene and diversified in the Miocene, and providing new and significance evidence for understanding the origin and evolution of the genus and family.

T2-63-06

Earliest fossil fruit record of *Canarium* (Burseraceae) in eastern asia and its implications for phytogeographical history

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As a distinctive genus within the Burseraceae, *Canarium* contains approximately 78 species of predominantly medium to large sized rainforest trees that are naturally distributed in low to middle altitudes of tropical or subtropical regions. It is characterized by distinctive drupaceous fruit with a trilocular endocarp derived from three fused pyrenes. Recently some new *Canarium* fossil fruits were collected from the late Oligocene of Yongning Formation, the Miocene of Erzitang Formation and the late Miocene of Foluo Formation in Guangxi Province, South China, providing the first confirmed fossil occurrences of *Canarium* in eastern Asia. The fruits of *Canarium guangxiensis* Han, Manchester, Wu, Jin et Quan sp. nov. are ovoid to spindle shaped, 22.8-34.3 mm long, 10.7-14.6 mm wide. Computed tomography (CT) scan was used to study the morphological and anatomical characters of fossil and modern *Canarium*, which facilitates identification of the fossil fruits. This new occurrence supplements other megafossil records of *Canarium* fruits from the Early Eocene in North America, the Middle Eocene to Oligocene in Europe, the Oligocene to Miocene in Asia and from the Pleistocene in Australia and the Pacific islands. The fossil records indicate a wide dispersal of *Canarium* over the Northern Hemisphere during the Eocene and Oligocene, followed by a phytogeographical contraction during the Miocene as the result of its extinction from North America and Europe. The origin and migratory routes of this genus are not clearly resolved, but based on the fossil records known so far, we hypothesize the *Canarium* may have had a North American Eocene origin, with subsequent spread to Eurasia and followed by dispersal to the Southern Hemisphere continents.

T2-64: Floral adaptation and mating efficiency

T2-64-01

Quantum dots—a new tool for studying components of male fitness in plants

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Apart from the advent of molecular tools, pollination biology has been a “bucket and spade science”, and the basic toolkit has remained largely unchanged for more than a century. Perhaps the reason why pollination biology has had such a long and distinguished history, is that with only a few simple and inexpensive tools (rulers, mesh bags, sticky labels, and permanent markers), biologists have made spectacular advances in understanding natural selection and evolution-primarily through counting seeds. However, easy as it is to count seeds, every pollination biologist at some point must confront an uncomfortable truth-seed counts only quantify the female aspect of fitness. What about pollen export and components of male fitness? The responses of male and female fitness components to selection may not always be similar, and many floral traits are thought to have evolved primarily through male components of fitness. Despite this, male fitness is very rarely quantified. This is because our tool box is missing an important tool: one which allows us to track pollen grains and quantify different avenues of pollen export from individual plants. Here, in the grand tradition of buckets and spades, I introduce a long overdue addition to the pollination biologist's basic collection of rulers, bags and sticky labels: a simple method to directly tag individual pollen grains and track their subsequent fates. The method, developed in our lab over three years, attaches different coloured fluorescent nanocrystals (q-dots) directly to pollen grains. This allows us to uniquely identify their origins upon recovery from pollinators, stigmas, or any other surface they may end up on and enables us to quantify all potential avenues of pollen movement. Unique q-dot labels can be applied directly to dehisced anthers in the field and attached to pollen grains within minutes, allowing for rapid assessment of pollen movement *in situ*. I will demonstrate details of the method with examples of systems in which we have employed q-dots for pollen tracking. Our method makes tracking pollen grains and quantifying male fitness nearly as easy as quantifying female fitness. We hope that this method will usher in a new era in pollination biology, where tracking pollen movement, and easily quantifying male fitness, is finally possible.

T2-64-02

The impact of pollinator behaviour on the adaptive function of heterostyly exposed by quantum dots

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Intraspecific variation in stigma placement, or heterostyly, has been a staple of evolutionary inquiry ever since Darwin first considered it a century and a half ago. Tristyly is a subset of heterostyly characterised by reciprocal herkogamy of anthers and stigmas at three levels and self/intra morph incompatibility. Traditional theory posits that tristyly evolved in order to promote disassortative mating between floral morphs exhibiting anthers and stigmas at the same level. Other explanations hold that it evolved to limit pollen wastage and opportunities for selfing. These theories have been largely untested, mostly due to an inability to track pollen from anther to stigma. The genus *Oxalis* represents an excellent system to elucidate the evolutionary mechanisms behind tristyly as all South African species exhibit two anther rings and one stigma ring contained within a central column inside actinomorphic flowers. Here we investigate pollen fates of self-incompatible *Oxalis purpurea* visited by either nectar or pollen foraging pollinators by employing a novel technique for tracking individual pollen grains. Using fluorescent quantum dots, we label pollen grains from each anther level in each stylar morph with distinct colours before allowing honeybees to forage in a controlled laboratory setup. Our results reveal that opportunities for selfing are rampant despite a well-developed tristylous syndrome. Of pollen exported, disassortative mating was more frequent relative to random expectations for all three morphs. Of pollen imported, long styled flowers in particular received predominantly legitimate pollen. We further discuss the influence of different pollinator behaviours on male and female plant fitness in heterostylous species.

T2-64-03

Comparative biomechanics of pollen dispersal in wind- and animal-pollinated plants

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Aerodynamic forces have shaped the structure and function of many plant organs, including flowers and inflorescences. Reproductive organs are frequently exposed to some amount of airflow which can dislodge pollen from anthers. Approximately 10% of flowering plant species rely on wind for pollination, and aerodynamic pollen release is the first critical step in successful reproduction. Conversely, releasing pollen into airflows is costly to plants which rely solely on animal pollinators. The contrasting demands of these two pollination modes suggest that wind- and animal-pollinated species will differ with respect to their adaptation to aerodynamic forces. Convergent evolution of floral traits in wind-pollinated species supports this hypothesis as it is thought to result from biophysical adaptation for fluid dynamic processes. However, some species do not exhibit the classical wind pollination syndrome and yet may still rely on wind pollination or a mix of animal and wind pollination (ambophily). Biophysical analysis at the wind-flower interface is needed to interpret the structural characteristics of flowers controlling pollen release, and yet such analyses are rare. We investigated pollen release mechanisms in wind- and animal-pollinated species of *Thalictrum* (Ranuculaceae), a moderately large genus with multiple independent transitions between animal- and wind-pollination syndromes. Using high speed video of pollen release in a wind tunnel, a laser particle counter and computer vision algorithms, we compared the vibration response of stamens to mechanical perturbation and measured pollen release probability. We characterized stamen vibrations because turbulence-induced shaking of reproductive structures has been shown in species of angiosperms, gymnosperms, bryophytes and fungi to initiate the dispersal of pollen or spores by wind. We also investigated the effects of stamen vibration on pollen release rate and export in field arrays of the putatively ambophilous *T. pubescens*. Arrays exhibiting variation in stamen vibration were either open pollinated or placed in insect exclusion cages to determine the relation between stamen vibration, aerodynamic pollen release rate and the amount of pollen exported by pollinators. Our studies identified novel biomechanical floral traits that are related to pollen release probability including the fundamental frequency of vibration and stamen stiffness. In many cases these traits confound conventional descriptions of discrete pollination syndromes. Consequently, pollen release may be more common among species exhibiting an animal-pollinated syndrome than previously thought and factors other than aerodynamic efficiency may contribute to shaping the wind-pollination syndrome. Moreover, aerodynamic pollen release may play contrasting roles in the evolution of specific pollination strategies in environments that differ with respect to atmospheric conditions (wind speed and turbulence intensity) and pollinator availability. We discuss the potential costs and benefits of atmospheric pollen release and the likely evolutionary outcomes for floral structures.

T2-64-04

The function and adaptive significance of morphological

polymorphisms characterizing the heterostyly syndrome

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Animal-pollinated plants display a remarkable diversity of structural adaptations that function to promote cross-pollen dispersal and reduce pollen wastage. Heterostyly is a convergent floral syndrome that has evolved in ~28 angiosperm families and is one of the most striking examples of the functional fit between flowers and pollinators. Most heterostylous species possess a reciprocal arrangement of stigmas and anthers (reciprocal herkogamy), diallelic self-incompatibility and ancillary polymorphisms of pollen and stigma. Here, we investigate the function of the morphological polymorphisms that characterize the heterostyly syndrome in two unrelated taxa of heteromorphic species through experimental approaches. We used tristylous *Lythrum salicaria* (Lythraceae) to (1) evaluate Darwin's hypothesis that reciprocal herkogamy promotes disassortative pollination, and to (2) examine the extent to which floral design, specifically the location of sex-organs within a flower, influences disassortative pollination. Using experimental arrays we detected varying degrees of disassortative pollination among the three morphs with sex-organ height playing an important role in determining compatible and incompatible pollen transfer and capture. Our studies provide support for the Darwinian hypothesis that heterostyly functions to promote disassortative pollination, with floral design playing a critical role in determining its intensity. To investigate the functional significance of ancillary polymorphisms, we conducted field experiments on three species of Plumbaginaceae to determine the extent to which pollen and stigma polymorphisms influence the composition of pollen loads. Specifically, we evaluated Dulberger's topographical complementarity hypothesis, which proposes that ancillary polymorphisms function in the rejection of incompatible pollen thus promoting disassortative pollination. To test this hypothesis (1) we investigated patterns of pollen transfer and capture in natural populations of two pollen-stigma dimorphic species lacking reciprocal herkogamy (*Armeria maritima* and *A. pubigera*) and a distylous species with pollen stigma dimorphism (*Limonium vulgare*) and (2) we examined pollen adherence and germination patterns in *A. maritima* following controlled hand-pollinations. We detected disassortative pollination in populations of each species, and no difference in compatible pollen capture between mating types was observed, although 'cob stigmas' captured more incompatible pollen. Our controlled hand-pollination experiment revealed the failure of incompatible pollen to adhere and germinate on stigmas. These results demonstrate that pollen-stigma dimorphisms serve to promote disassortative pollination by limiting incompatible pollen deposition through an intimate combination of structural and physiological mechanisms. Our studies provide novel insights into the functioning of key components of the heterostylous syndrome, and demonstrate that both reciprocal herkogamy and ancillary polymorphisms promote disassortative pollination and reduce pollen wastage.

T2-64-05

Quantum dots reveal the mechanics behind handedness in *Wachendorfia* flowers

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Stylar polymorphisms such as enantiostyly (handedness) in plants are underpinned by a classic body of compelling theory, much of which remains poorly tested. A key part of that theory is that the spatial separation of male and female reproductive parts (herkogamy) to reduce selfing, presents a problem to plants: The spatial separation also reduces the efficiency of pollen transfer. In theory, this problem was elegantly solved by the evolution of stylar polymorphisms like enantiostyly and heterostyly because they retain the spatial separation of the sexes but facilitate pollen transfer between reciprocally matching stylar morphs. Biologists have been unable to test whether this solution holds up to scrutiny because until recently it has been almost impossible to evaluate the efficiency of pollen transfer due to the difficulty in marking and tracking granular pollen grains. Using fluorescent nanocrystals (quantum dots) which attach to the surface of pollen, we were able to identify the origins of pollen grains on the bodies of pollinators and on the stigmas of flowers. We show that pollen from left versus right facing anthers separate perfectly on either side of pollinating insects. This results in exceptionally strong assortative pollen movement, where most pollen moves between morphs rather than within morphs. This suggests that a shift from herkogamy to enantiostyly would indeed be accompanied by more efficient pollen transfer.

T2-64-06

Effect of floral phenotype on mating patterns and male and female fitness in experimental populations of *Narcissus papyraceus*

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The floral phenotype and its variation is the result of natural selection affecting the pollination success of flowering plants. In single hermaphroditic plants, reproductive success is divided into its female and male components; while the measurement of the former is straightforward through seed production and quality, a direct measure of the latter requires paternity analyses in the population to determine siring success of particular plants. The floral phenotype may also determine affinities between particular phenotypes, which might be disposed to cross with each other more likely, as it occurs in heterostylous and other reciprocal polymorphic plants. *Narcissus papyraceus* (Amaryllidaceae) is a self-incompatible style-dimorphic species with individuals presenting either long-styled or short-styled flowers in a single population, a condition which is similar and evolutionarily ancestral to heterostyly. Dimorphic and long-styled monomorphic populations are associated with different pollinator faunas in the central and north regions of its range, respectively. Previous work on experimental populations of this species (Simón-Porcar, Meagher & Arroyo 2015) demonstrated the effect of the style morph in the mating success of individuals, but nothing is known about the possible influence of other floral traits. Here we explore the effect of the floral phenotype in mating and reproductive success of individuals of *Narcissus papyraceus* under different natural conditions. We present new results from the experimental populations of Simón-Porcar *et al.,* (2015), which were exposed to ambient pollinators in the central and marginal regions of the range of the species and subjected to genetic paternity analysis. We characterize the whole floral phenotypes of the individuals and relate them to their maternal and paternal reproductive success, and investigate their influence on mating patterns. Our comprehensive approach allows to prove the existence of natural selection on the floral phenotype, and to correlate male and female components of fitness. Thus, we advocate that experiments and observations set in natural conditions should always account for both maternal and paternal fitness when aiming to assess similar questions.

T2-65: Using fossil evidence to explore the plant evolution, diversity, and their response to global changes

T2-65-01

Paleobotanical evidence of environmental constrains of early hominid expansion into southern Europe

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The earliest hominid expansions from Africa into Eurasia date to about 2 Ma based on the Dmanisi record of *Homo erectus* in Georgia. In South Europe however records (notably from Spain and Italy) are much younger and extend back to 1.2 and possibly 1.4 Ma. The scarce records may represent a single or several out-of-Africa events, and questions remain as to what extent climate and environmental change facilitated or hindered the occupation of Europe by early *Homo*. Climate change is considered a major driving factor of hominin evolution and dispersal patterns. In fact directly or indirectly by its severe influence on vegetation, physiography of landscape, and animal distribution, climate modulates the availability of resources. Several researchers suggest that orbital cycles (especially the obliquity 41 ka cycle) were driving regional climate providing short time windows of favorable landscape and vegetation conditions for hominine expansions in Europe. Early Pleistocene vegetation successions with more and less favorable conditions for hominid occupations at the end of each glacial period (warm, but still dry enough to enable open landscapes) have been documented from Southern Europe. However, this is not the case throughout the possible expansion routes. Based on own results from the Caucasus region, we extrapolate the maximum extend of forests and mosaic landscapes for different climatic phases during Early Pleistocene as a prerequisite for the reconstruction of early human environments. In comparison with existing paleobotanical records from Southern Europe possible climatic constraints of early hominid expansions into Europe become evident and imply restricted expansion corridors during some very warm and humid climatic phases.

T2-65-02

A review of three Eocene floras from southeast China and southeast United States: Implications for understanding the extant floristic disjunction between eastern Asia and eastern north America

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The floristic similarity between temperate eastern Asia (EA) and eastern North America (ENA) was first recognized by Linnaeus in 1750. This disjunct distribution of plants has intrigued both neobotanists and paleobotanists for more than 260 years. An early interpretation, often referred to as Gray's hypothesis, is that the floras of eastern North America (ENA) and eastern Asia (EA) were more similar to each other than to the flora of western North America (WNA). Different approaches, focusing on disjunct groups of plants at generic, familial, or community levels, have been employed to interpret this relationship and to hypothesize the processes that shaped this distribution pattern. The geological records indicate that Eurasia and North American were geographically connected throughout most of the Cenozoic or during the Paleogene by two major land bridges, i.e., the Bering land bridge and the North Atlantic land bridge. It is widely accepted that these land bridges significantly contributed to the floristic similarities between eastern Asia and North America. Paleoclimatic and molecular analyses of some groups of angiosperms from the two areas seem to support the hypothesis that most taxa with this disjunct pattern are the result of vicariance. With the application of new techniques such as molecular phylogenetics and the molecular clock, the origin, time of disjunctions, and relationships of some lineages are now better understood. Recent molecular phylogenetic analyses have yielded two important results. One is that, contradictory to the traditional view of the floristic similarity between eastern Asia (EA) and eastern North America (ENA), the examination of this disjunct pattern in the broader context of Northern Hemisphere biogeography suggests a closer relationship between eastern North America (ENA) and western North America (WNA) than between eastern North America (ENA) and eastern Asia (EA). The disjunction between eastern Asia (EA) and eastern North America (ENA) mostly represents a relic of a wider distribution in the Cenozoic and is resulted from complex processes of dispersal/migration, extinction, speciation, vicariance, and morphological convergence and stasis. The second result of the use of new techniques is that most intercontinental species pairs studied are not sister species and that some taxa, traditionally treated as disjunct, are now considered paraphyletic and polyphyletic. We present our preliminary results based on observations of thousands of fossil specimens of reproductive material from three Eocene floras, the Claiborne Flora from southeastern United States and the Changchang and Maoming floras from southeastern China. After comparing the floristic components of these Eocene floras at the generic and familial levels, we discuss the floristic patterns and their possible causes. We then compare the distribution of the Eocene geofloras with that of the extant floras of eastern Asia and eastern North America. The result will provide some insights for interpreting the modern floristic relationship between these two regions.

T2-65-03

Impact of Mid-Miocene to Late Holocene climate changes on the development of the eastern Himalayan flora: evidence from plant mega and microfossils

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Indian monsoon system characterised by seasonally reversing winds and precipitation changes associated with asymmetric heating of land and sea is a substantial component of the global atmospheric circulation that brings about 80% rainfall to the Indian subcontinent. In order to understand the evolution, dynamics and response of the plant communities to these changes of Indian monsoon, long term palaeoclimate records are essential. A synthesis of the quantitative palaeoclimate records to throw light on the evolution of the Indian monsoon since the mid-Miocene time and climate driven vegetation dynamics in the Darjeeling Himalaya is focused here. CLAMP (Climate Leaf Analysis Multivariate Program) analysis of a mid-Miocene fossil flora from the Darjeeling Himalayan Siwalik suggests a warm (tropical to subtropical) humid climate with a distinctive monsoon signature. A moderately high rainfall with over 2000 mm mean annual precipitation (MAP) and an average annual humidity of 80% might have harboured an evergreen to moist deciduous forest in the mid- Miocene Darjeeling Himalaya. Darjeeling fossil flora estimating a ratio of wet and drier months as 3.8:1 is indicative of a more even distribution of rainfall throughout the year, apparently due to a wetter dry season. A mean annual temperature (MAT) of 25.4 ± 2.8 °C (all uncertainties ±2 sigma) with warm month mean temperature (WMMT) of 28.4 ± 3.39 °C and cold month mean temperature (CMMT) of 17.9 ± 4 °C also further suggests a conducive environment for sustenance of a dense evergreen to moist deciduous forest. A further analysis of palynofossils through Coexistence approach (CA) and other proxy data portray palaeomonsoon oscillations in the Darjeeling Himalaya over the last ca. 50 ka. CA estimates coupled with other proxies indicate significant oscillations in rainfall during 46.4 and 25.9 ka, 25.9−15.6 ka, and 5.4 to 3.5 ka. Span between ca 46.4−31 ka is characterized by a comparatively low monsoonal activity and slightly higher temperature than that during ca 31 ka onwards. Simultaneous expansion of deciduous trees and C4 grasses also imply a drier and warmer phase. Between 31 and 22.3 ka, higher precipitation and a slightly cooler temperature led to an increase in evergreen elements over deciduous taxa and wet-loving mixed C3-C4 grasses over dry-loving C4 grasses than earlier. After ca 22.3 ka, shrinking of forest cover, expansion of C4 grasses and dry-loving heliophytic elements in the vegetation with lowering of temperature and precipitation characterized the onset of the LGM that persisted till 18.3 ka. A restoration in the forest cover and in the temperature and precipitation regime marks the end of the LGM. Later, a strong monsoonal activity during 5.4 to 4.3 ka supported a dense moist evergreen forest cover that subsequently showed a thinning during 4.3 to 3.5 ka with an increase in deciduous elements and non-arboreals. A reduction in rainfall with rise in temperature might be responsible for a shrink in the forest cover during this phase. A multiproxy comparison of the global data of the ~50 ka implies that these fluctuations in plant succession were mainly driven by monsoonal variations.

T2-65-04

Early cretaceous angiosperm reproductive diversity

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Angiosperm origins and earliest radiations can be traced back most consistently to the early Lower Cretaceous with some reports of Jurassic and even Triassic remains. Angiosperm evolution is centered upon a wide variety of reproductive strategies to encourage and/or require outcrossing. Evidences of Angiosperms are commonly found in Early Cretaceous sediments from which their diversity rapidly radiates. The early records include reproductive axes that contain both ovule-bearing organs subtended by pollen-bearing organs. The homologies of these organs may be interpreted as representing individual female and male flowers on an axis or alternatively as one unit as a whole, similar to a compound inflorescence. In either case, the *Archaefructus* axis appears to represent an early attempt to achieve a successful outcrossing system in the evolution of the bisexual flower. The active engagement of insects, co-evolving with early angiosperms, during the Late Jurassic and Early Cretaceous, shaped the evolution of both the insects and the angiosperms. The co-evolution of insect pollinators had and continues to have a profound influence on angiosperm evolution. This is joined by the evolution of wind pollination that became a common feature of genetic exchange in many angiosperm lineages. At the same time, some lines of angiosperm evolution were using other systems to manage their reproductive biology. Fossils similar to the extant *Ceratophyllum* were widespread and diverse. These angiosperms managed outcrossing in aquatic environments. Water was the abiotic agent that moved genetic material within and between populations. This is a syndrome used by diverse land plants since the Paleozoic to manage outcrossing. This syndrome seems to have persisted in the earliest angiosperms as well. While the first flower in the world remains a mythical concept, we need to focus our research upon the evidence of the steps of change preserved in the fossil record. Then perhaps we can understand the complex reproductive biology of these plants as they functioned in their biotic and abiotic environments.

T2-65-05

The fossil history of *Mesocyparis* (Cupressaceae) and its response to the global cooling across Cretaceous-Paleogene boundary

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The fifth largest mass extinction on the earth was occurred across the Cretaceous-Paleogene Boundary (K-Pg Boundary, ca. 66.0 million years ago), which accompanied by a dramatic global climate cooling in both oceans and landmasses. There are some zoological cases to show how animal taxa responded to climate changes across the K-Pg boundary in richness and morphological changes, but few in plant taxa. *Mesocyparis*, an extinct genus, was distributed from Eastern Asia to Western North America across this time interval. Here we report a new species of *Mesocyparis* with seed cones and foliage in the early Paleocene Wuyun Coalmine from NE China, which has ovate seed cones, 4-decussate cone bracts and flattened foliage. Phylogenetic analysis show that *Mesocyparis* is a member of Cupressoideae and is sister to the clade containing *Juniperus*, *Cupressus*, *Hesperocyparis*, and *Xanthocyparis*. Within *Mesocyparis*, two Western North American species formed a clade, whereas three Asian species formed anther. The paleogeographic distribution of this genus, together with phylogenetic analysis reveal it originated at high latitudes in the North Hemisphere, and divided into an Eastern Asiatic clade and Western North American clade. Morphological comparison shows the genus changed markedly across K-Pg boundary, i.e. the seed cones became larger, the umbo was erect (vs. reflexed) and the position of the umbo varied from middle part of cone scale to the apex. The northern limit of *Mesocyparis*, with those of two other arboreal taxa *Metasequoia* (gymnosperm) and *Nordenskioeldia* (angiosperm), migrated 4 - 5° paleolatitude southward, which might be driven by the global mean surface cooling across K-Pg boundary. These findings give a case on how a specific plant taxon responded to the K-Pg climate change.

T2-65-06

Mesozoic fossil wood diversity variations and paleoclimate implications in China

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As an important component of plant remains, fossil wood plays a significant role in understanding the floral composition and evolution of plants in the geological past. Fossil wood is also one of the significant proxies for terrestrial palaeoclimate and palaeogeographical reconstruction in earth history. Diversified fossil wood has been documented from the Mesozoic deposits in China after a long time of investigations. During the past few years, many new fossil wood materials were reported from a variety of horizons in some fossil localities, including Sichuan, Chongqing, Yunnan, Xinjiang and Liaoning provinces, ranging from Triassic to the Cretaceous, providing significant references for reconstructing the Mesozoic palaeoclimate. In this paper, we summarize the recent advances in Mesozoic fossil wood studies in China. Particularly, the new discoveries of fossil wood from the Upper Triassic in Sichuan of southern China, the Jurassic Yanliao Biota and the Early Cretaceous Jehol Biota in western Liaoning are reviewed with emphasis on the diversity variations, palaeoclimate perturbations and tempo-spatial distribution of the Mesozoic wood in China. The future research directions of Mesozoic fossil wood in China are further discussed.

T2-66: Evolution of intercontinental disjunctions in ferns and lycophytes

T2-66-01

The biogeographic history of hairy tree ferns (Dicksoniaceae-Cyatheales): A parting with different pathways and paces

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The tree fern family Dicksoniaceae, encompassing the genera *Calochlaena* (5 spp. paleotropical), *Lophosoria* (3–4 spp. neotropical), and *Dicksonia* (26 spp., widespread), dates back to the early Cretaceous (ca. 125–140 Mya) and is considered a typical Gondwana element. A phylogeny of cp marker (*rpl16, trnL-F, trnG-R* and *matK*) confirmed the monopyhly of the genera and found three monophyletic groups within *Dicksonia*, whose stem ages are younger than the separation of the tectonic units of Gondwana to which they seem restricted today. Other geographical disjunctions, e.g. between Australia and Malesia, are even more recent and thus more likely to be the result of long-distance dispersal. The different scenarios are discussed in the context of paleoclimate and geologic events.

T2-66-02

Biogeography of the brake fern genus *Pteris* (Pteridaceae)

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The brake fern genus *Pteris* contains about 200–250 species, some of which are widely used as ornamental plants or arsenic hyperaccumulators. The global distribution of the genus makes it an ideal system to test biogeographical hypotheses. Earlier analyses were largely based on unresolved phylogenies using chloroplast markers. In this study, analyses of five chloroplast markers and one nuclear marker of 160 species (60–80% of the all species in the genus) resulted in a robust phylogeny and confirmed the monophyly of *Pteris* s.l. Our main results include: (i) The genus might have originated from tropical Asia-Australasia; (ii) The early diverging lineages of *Pteris* underwent accent range expansions and diversified in Africa, Eurasia, and Neotropics; (iii) The Southern Hemisphere endemics are closely related to one another than to the Northern Hemisphere endemics; (iv) The Neotropical endemics form two independent clades suggesting that they originated independently; (v) The island endemics are all resolved within the 15 clades defined by our previous study and long-distance dispersals contributed to the successful colonization from Mainland Asia to Taiwan, Japan, and Hawaii, and from Mainland Africa to the near archipelagos.

T2-66-03

From Asia to Africa: Biogeography of the fern genus *Pyrrosia* (Polypodiaceae)

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The Old World fern genus *Pyrrosia* (Polypodiaceae) has representatives in Africa and Asia, but its highest species diversity is clearly found in southern Asia and Malesia. Earlier phylogenetic analyses based on small sampling did not confirm the monophyly of the genus and the the relationships within the genus were not well resolved, and therefore it was not possible to understand the biogeography of the genus. In the present study, DNA sequences of five plastid markers of 220 accessions of Polypodiaceae representing two species of *Drymoglossum*, 14 species of *Platycerium*, 47 species of *Pyrrosia*, and the only species of *Saxiglossum* (subfamily Platycerioideae), and 12 species of other Polypodiaceae representing the remaining four subfamilies are used to infer a phylogeny of *Pyrrosia*. Our major results include: (1) *Pyrrosia* is supported as a monophyletic group; (2) The Himalayan *P. costata* and the Southeast Asian *P. stigmosa* together are sister to the remaining species in the genus; (3) The genus apprears to have originated in Asia, while the African species appear to have arrived via long-distance dispersal from southern China/Southeast Asia; and (4) Divergence time estimation suggests that the first occurrence of *Pyrrosia* species was at ca. 25 Ma in the Himalaya-Southeast Asia.

T2-66-04

Climatic niche evolution in polyploid and diploid vascular plants

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The dynamics of ecological niche evolution has long been hypothesized to be a critical determinant of polyploid success and establishment. In general, newly formed polyploids are reproductively isolated from their parental taxa, yet initially remain in the same geographic area and/or niche as their parents. Polyploids also typically begin with a small population size and face a number of short term and long-term ecological and evolutionary challenges. Previously, it was thought that niche divergence was a prerequisite for polyploid success and comparative studies from microhabitat associations and regional floras show mixed support for this hypothesis. Here, we developed a broad comparison of polyploid and diploid niche evolution to ask three key questions: 1) Is climatic niche divergence necessary for polyploid success and establishment, 2) Is the rate of climatic niche evolution different for polyploids than congeneric diploids, and 3) Are polyploids with divergent ecological niches associated with higher net diversification rates? Using a manually curated dataset of known polyploids, their diversification rates, and their parental taxa, we analyze data across vascular plants and compare and contrast patterns and processes between pteridophytes and seed plants.

T2-66-05

Understanding the odd man out pattern in African plant: New insights from ferns

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Africa has long been recognized as the “odd man out” in terms of global plant distribution patterns. The continent has a whole has between three and nine times fewer species per square kilometer than neighboring continent-scale areas to the west and east (South America and the Indo-Malaysian region, respectively). This pattern has been documented across plant groups, including in palms, orchids, and ferns and lycophytes. Differences in species richness can be caused by different rates of speciation, extinction, and migration, which in turn are driven by myriad factors specific to a given place and time, such as climate, proximity to other areas, geologic history, and time available for speciation, among other things. In ferns, data collected to date for three genera (*Dryopteris*, *Marsilea*, and *Cheilanthes*) suggest that ferns may only have undergone significant radiation in Africa over the last 20 million years, despite the fact that Africa has existed in largely its current location and orientation for far longer than that (at least 300 million years). In addition, the last 20 million years have largely corresponded with a period of aridification in sub-Saharan Africa, and if ferns in general have indeed diversified in Africa during this time, it would be striking given that we tend to think of ferns as moisture-loving organisms dependent on water for their survival. I am using a combination of phylogenetic, biogeographic, and diversification analyses to try and understand patterns of fern diversity and species richness in Africa, and to shed light on the “odd man out” phenomenon in African plants.

T2-66-06

Biogeography of Tectariaceae

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Tectariaceae are typically characterized by the presence of multicellular and uniseriate (ctenitoid) hairs and costae that are non-sulcate adaxially. They contain seven genera and have pantropical distribution with *Arthropteris*, *Tectaria*, and *Triplophyllum* being in the Old and New Worlds, *Draconopteris* and *Hypoderris* in the New World, and *Malaifilix* and *Pteridrys* in the Old World. Earlier pre-molecular works or molecular works with small sampling were not able to understand how the family acquired today’s intercontinental distribution The current study included the largest taxon and character (plastid and nuclear markers) sampling so far. Based on the phylogeny reconstructed and the extant geographical distributions of taxa in the family, it is postulated that: (1) the family originated in the Old World; (2) none of the genera were involved in more than one intercontinental dispersal between the Old and New Worlds; (3) there might have been five independent dispersals from the Old World to the New World: single dispersal of *Draconopteris* and *Tectaria* each from Asia to the New World, single dispersal of *Arthropteris* and *Triplophyllum* each from Africa to the New World, and single dispersal of *Hypoderris* from the Old World to the New World; (4) within the Old World there were multiple dispersals among Africa, Asia, and the Pacific islands within *Tectaria*; and (5) African species of *Tectaria* evolved from Asian ancestors.

T2-68: The evolution of Plant Diversity in the "third pole", Qinghai-Tibetan Plateau

T2-68-01

Relationships through time between the orography of Tibet, the Himalaya, Asian monsoon systems, and the vegetation of southern Asia

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The mechanisms underlying Asian monsoon dynamics are poorly understood but the Tibetan plateau and the Himalaya modify climate systems associated with seasonal latitudinal migrations of the Inter-Tropical Convergence Zone (ITCZ). ITCZ migrations must have generated monsoonal precipitation regimes at low latitudes (~10°- ~30°) throughout Earth history so much of southern Asia must have experienced monsoonal regimes since at least the early Cretaceous. Abundant evidence shows that a high (>3 km) proto-Tibetan edifice existed prior to the onset of the India - Eurasia collision at around 55 ± 10 Ma and if an elevated Tibet in any way amplifies monsoon systems over southern Asia the rich biota of the region must have evolved under strong monsoonal influences. However, these Paleogene monsoons were different from those of today. A monsoon climate is more than just strong seasonal variations in precipitation. Charting the evolutionary history of Asian monsoon systems has been hampered by the lack of deep time proxies compatible with meteorological monsoon definitions. However, the leaves of woody dicots have to be particularly well adapted to the seasonal extremes they experience across all climate variables (thermal and hydrological) simultaneously. These adaptations give rise to unique monsoon 'fingerprints' encoded in fossil leaf architecture. Multivariate analysis reveals early Eocene (~55 Ma) to latest Oligocene (~23 Ma) leaf form across southern Asia was adapted to climates characteristic of ITCZ type monsoons and not those of the modern South Asia Monsoon (SAM), which is influenced by a high Himalaya. Recent analysis of Tibetan plant fossils suggests that the Himalaya formed against a pre-existing Gangdese Highland (palaeoelevation ~4.5 km) soon after the India-Eurasia collision began. By the end of the Oligocene the proto-Himalaya had reached an elevation of ~2.3 km, and then rose to ~5 km by 15 Ma. Only later did the Himalaya project above the average elevation of the Tibetan plateau to generate the modern SAM circulation.

T2-68-02

Out-of-Tibet: Taxonomy and spatio-temporal evolution of Gentiana (Gentianaceae)

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The origin and evolution of species richness in Asian mountainous hotspots of biodiversity is complex and probably caused by non-mutually exclusive factors such as explosive radiations, lineage persistence and biological interchange. Whereas a plethora of studies has claimed that orogenesis alone triggered the radiation of alpine plants around the world, the role of climate oscillations, niche evolution, and the immigration of pre-adapted lineages are usually overlooked (or underestimated) as contributors to local biodiversity. After revising the taxonomy within Gentianinae and *Gentiana*, we investigated the evolutionary history of *Gentiana* with regard to the uplift of the Qinghai-Tibetan Plateau (QTP) with phylogenetic reconstructions, molecular dating, ancestral area reconstructions, and diversification rate analyses. The common ancestor of *Gentiana* and subtribe Gentianinae lived in the QTP region at around 34 (25–45) million years ago (Ma), and 40 (29–52) Ma, respectively. From the surroundings of the QTP, *Gentiana* lineages dispersed to eastern China, Taiwan, Europe, North and South America, Australia and New Guinea, from mid-Miocene onward (c. 15 Ma–present), with only one older dispersal event to Europe (c. 37–21 Ma). Diversification rates gradually increased over time, and one switch of diversification rates (c. 7 Ma) was identified in the lineage encompassing *Gentiana* sections *Pneumonanthe* and *Cruciata*, following a niche shift to lower elevations. Our results confirm the general role of the QTP in promoting the adaptation of tropical lineages to temperate habitats, and underline its status as a source area for alpine plants worldwide. However, because a slow and steady increase in diversification rates coincides with the extension of the QTP, we argue that the museum theory rather than the explosive radiation theory prevails for gentians in this region, and that the sharp increase of diversification rates is unrelated to the uplift, but rather corresponds to niche shifts and key innovations.

T2-68-03

Diversity of *Arisaema* (Araceae) in the Himalayas, Hengduan Mountains and Tibet inferred from an updated molecular phylogeny

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In 2016, we presented an updated genus-wide phylogenetic tree of *Arisaema* based on a large data set of four plastid non-coding regions (a total 2,858 bp after alignment) from 138 taxa representing the diversity of the genus. It shows eight major clades and one branch with unique sequence variation, although the relationships were unclear due to a polytomy and weak support. These nine clades mostly correspond to the sections recognized in recent classifications. Four sections are distributed mainly in the Himalayan region. Three of them, sections *Dochafa*, *Tortuosa* and *Tenuipistillata* are included in the first clade. Most of the species are distributed in and around Himalayan region but *Arisaema flavum* subsp. *flavum* is subendemic to Tibet and *A. wardii* is endemic to Tibetan-Qinghai plateau. Section *Arisaema* corresponding to the second clade is diversified both in Himalayan region and Hengduan Mounatains. It have been well known that *A. costatum* of this section is sister to an African group. Section *Sinarisaema* is distributed widely throughout China to South India and Sri Lanka. This section is included in the final large clade together with other four sections. Of them sect. *Sinarisaema* and sect. *Pistillata* share a spilodistichous leaf arrangement and are considered to be more recent origin than the sections which are distributed in Himalayan region mentioned above.

T2-68-04

Phylogeographic perspectives on plant evolution in the Qinghai-Tibetan Plateau (QTP) region and adjacent areas

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Over the last decade, molecular phylogeographic studies have led to an improved understanding of the origins of exceptionally high plant diversity and endemicity of the Qinghai-Tibetan Plateau (QTP) itself and the forested southern and eastern margins of the Plateau, i.e. the Himalaya-Hengduan Mountains (HHM). Here we review recent spatial genetic and palaeo-modelling analyses of QTP–HHM plant diversity related to the importance of these mountain ranges and deep valley systems as floristic hotspots, in which high topographic and climatic complexity not only promoted strong population structure, multiple and independent refugial isolation, and (incipient) allopatric species formation, but probably also buffered against lineage extinction. Moreover, molecular dating studies spanning the flora of the QTP region – and especially within the southwest – continue to implicate a major influence of palaeo-geological and/or -climatic events on intraspecific lineage divergence and demographic histories from the Late Pliocene/Early Pleistocene onwards, thus being largely consistent with the most recent and rapid episodes of uplift of the eastern part of the QTP. Finally, by using the perennial understory herb genus *Dysosma* Woodson (Berberidaceae, Podophylloideae; *c.* 7 spp.) as a model system, we demonstrate how the establishment of (sub)alpine plant diversity in the QTP region might have contributed to the origin of high levels of species diversity and endemism in the warm-temperate deciduous and evergreen forest biomes of subtropical China.

T2-68-05

Nectar guide change and it mechanism in *Arnebia szechenyi* Kanitz (Boraginaceae)

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Communications between plants and their pollinators involve a complex interplay of different sensory modalities. Nectar guides are the contrasting patterns on the corolla, and considered to be advertising the presence and indicating the location of floral rewards (nectar or pollen) to pollinators. However, the changes of nectar guides across a flower's lifetime are poorly understood, and the mechanisms of nectar guide changes remain unclear. In *Arnebia szechenyi* Kanitz (Boraginaceae), a montane perennial herb native to China, flowers typically display nectar guides on the first day of anthesis until they start fading out and then disappear altogether on the second day, with no apparent link to the longevity of individual flowers (ca. 4 days). We explore the functions of the nectar guides and their changes by designing different treatments to the nectar guides and by performing field observations, and examine the different gene expressions by next generation sequencing. Our results illustrate that nectar guides disappear in netted flowers, suggesting that these subtle color changes are intrinsic, although pollination could facilitate fading of nectar guide in *A. szechenyi*. Control experiments indicated that nectar guides increase male but not female reproductive fitness. Plants with more flowers attracted more pollinators and received more visits, but the visitation rates to single flower with nectar guides did not increase with enlarged floral display size. Transcriptome analysis show that 154,021 genes are differentially expressed in different stages of anthesis, and among these transcripts, low expression levels of key genes in the anthocyanin biosynthesis during flowering process are *CHS*, *F3H*, *UF3GT*, *ANS* and *DFR*. Notably, except for these vital genes, there is one gene encoding subgroup 6 R2R3-MYB transcription factor greatly show down-regulated expression, which are known to activate the structural genes in anthocyanin biosynthesis. Conclusively, we propose that nectar guides and their changes have developed via selection on the male function of *A. szechenyi*, and older, nectar-depleted flowers increase the floral display size and are therefore likely to be maintained by selection through the enhanced pollen export rates to flowers with nectar guides on the same plant. The set of down-regulated genes indicates the crucial role of anthocyanin regulation in the nectar guides disappearance, and the modulation of R2R3-MYB transcription factor may play an important role in regulating the nectar guide changes in *A. szechenyi*.

T2-68-06

Tempo and mode of floristic assembly in the Hengduan Mountains

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The Hengduan Mountains region is one of the world's richest hotspots of plant diversity, and is unusual in being neither tropical nor Mediterranean in climate. How was its flora assembled, in terms of tempo (rate of species accumulation) and mode (*in situ* diversification vs colonization) through time? A recent biogeographic analysis of 19 plant clades shows a remarkable increase in the rate of in situ diversification around 8 Ma, corresponding to independent estimates of when Hengduan Mountains were tectonically uplifted. This supports the idea of uplift-driven diversification. However, many Hengduan clades show signatures of older radiations that may have coincided with uplift of the Himalayas.

Many outstanding questions about assembly of the Hengduan flora remain, such as the conspicuous lack of polyploidy and the role of biotic interactions.

THEME III: ECOLOGY, ENVIRONMENT & GLOBAL CHANGE

T3-01: Plant climatic adaptation in the new genomic era

T3-01-01

Metabolomic changes of stomatal responses to CO_2 in the changing climate

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Stomatal movement in respond to various stimuli is an important process that balances plant carbon dioxide (CO_2) uptake and water transpiration. Elevated CO_2 induces stomatal closure, while low CO_2 induces stomatal opening. Here we report the metabolomic responses of *Brassica napus* guard cells to elevated and low CO_2 using three hyphenated metabolomics platforms: gas chromatography (GC)-mass spectrometry (MS), liquid chromatography (LC)-multiple reaction monitoring (MRM)-MS, and ultra high-performance LC (UHPLC)-quadrupole time-of-flight (QToF)-MS. A total of 358 and 411 metabolites were quantified in a time-course response of guard cells to elevated CO_2 and low CO_2, respectively. Major alterations in fatty acid, flavonoid, organic acid, sugar, phenylpropanoid, and amino acid metabolic pathways indicated changes in both primary and specialized metabolic pathways in guard cells. Most interestingly, multiple hormones including jasmonic acid (JA) were altered in the course of the CO_2 treatments, indicating hormone involvement in guard cell CO_2 responses.

T3-01-02

Omics in wild soybean (*Glycine soja*) – insights into the plant climate adaptation and stress management

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Climate change has generated various critical challenges to agriculture susceptibility and food security. These challenges may be met by the development of novel crop varieties with increased biotic or abiotic resistance that enable them to thrive in changing environments. Crop wild relatives (CWRs) harbor a much higher level of genetic diversity than cultivated crops and have the potential to meet these challenges. The wild soybean (*Glycine soja*), from which domesticated soybeans (*Glycine max*) were derived, is widely distributed throughout diverse habitats in East Asia. We use *G. soja* as our study system to investigate its genomic diversity, population structure, climate adaptation, and biotic stress resistance, integrating genomic, transcriptomic, and metabolomic approaches. Using ~42,000 genome-wide single nucleotide polymorphism (SNP) data, we estimated that environmental factors contributed 23.6% to population differentiation, much more than that for geographic factors (6.6%). Precipitation variables largely explained the divergence of the groups along longitudinal axes whereas temperature variables contributed more to latitudinal divergence. We also used a genome scan of outlier loci to identify candidate loci involved in climate adaptation. This scan uncovered a total of 32 known genes with diverse functions, seven of which are involved in abiotic stress responses. We also integrated genome-wide association studies, RNA-seq comparisons, and metabolomic comparisons to dissect the genetic basis of wild soybean resistance to soybean cyst nematode (SCN), the most devastating pest of soybean. We identified some candidate genes involved in SCN type-specific resistance, as well as others exhibiting broad-spectrum resistance to multiple types of SCN. These candidate genes will not only shed light on the molecular mechanisms of plant and SCN interactions, but also will facilitate the molecular breeding and development of soybean varieties with improved SCN resistance.

T3-01-03

How plants adapt to harsh environments on the Qinghai-Tibet Plateau at genomic level

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Interpretation of genetic variation at the whole genome level can contribute to understanding how organisms adapt to changing environments. The Qinghai-Tibet Plateau (QTP) has the highest biodiversity for an extreme environment worldwide, and provides an ideal natural laboratory to study adaptive evolution. Little genome/transcriptome based study has been conducted on how plants adapt to the environments of QTP compared to numerous studies on vertebrates. 1) We generated a draft genome sequence of cyanobacteria *Trichormus* sp. NMC-1 in the QTP and performed whole transcriptome sequencing under low temperature to investigate the genetic mechanism by which *T.* sp. NMC-1 adapted to the extreme environment. The genome sequence of *T.* sp. NMC-1 was 5.9 Mb with a G + C content of 39.2% and encompassed a total of 5362 CDS. Genome comparison between *T.* sp. NMC-1 and six closely related species showed that the functions of unique, significant positively selected, expanded orthogroups, and differentially expressed genes were related to specific adaptation traits, such as signal transduction mechanisms, cell wall/membrane biogenesis, secondary metabolite biosynthesis, defense

mechanisms, and energy production and conversion. 2) *Crucihimalaya himalaica* is a close relative of *Arabidopsis* with typical QTP distribution, and hoped to be a new model system to study speciation and ecological adaptation in extreme environment. We de novo generated a transcriptome sequence of *C. himalaica*, with a total of 49,438 unigenes. Compared to five relatives, we have identified 487 extremely significantly positively selected genes (PSGs) in *C. himalaica* transcriptome. Theses PSGs were enriched in functions related to specific adaptation traits, such as response to radiation, DNA repair, nitrogen metabolism, and stabilization of membrane. These functions are responsible for the adaptation of *C. himalaica* to the high radiation, soil depletion and low temperature environments on QTP. 3) *Thlaspi arvense* is an emerging model wild plant distributed across an elevation range of nearly 4,500 m, for which a complete genome sequence has been released. We generated ~150 Gb of data using a Pool-seq genome resequencing approach in two high and two low altitude populations, and identified more than three million single nucleotide polymorphisms. Using sliding-window ZFst (rms) approach, we identified 135 candidate naturally selected genes associated with altitude variation. We also measured the transcriptome expression level of different altitude *T. arvense* seedlings. Combining these analyses, we identified several genes related to cell wall biogenesis and polysaccharide metabolism that might be responsible for adaptation to distinct environments. In conclusion, differ from previous studies on animals, our results provide novel insights into genetic mechanisms of highland adaptation in plants.

T3-01-04

Quaternary niche dynamics and range dynamics in alpine plants

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The ecological niche is a central issue to understand ecology, evolution and biogeography of species. In this context, two hypotheses have been proposed to explain range dynamics, niche conservatism and niche evolution. Under the first hypothesis, species are tracking their ancestral climatic niche, whereas under the second hypothesis range shifts are achieved by adaptation to new niches. The recurrent (re-)colonization history of alpine plants due to Quaternary climate fluctuations renders the European Alps a suitable system to study range dynamics in the context of climate change. In this study, we employed RAD sequencing and climate modelling to compare intraspecific niche dynamics and phylogeographic patterns of eight alpine species. We address (i) whether (postglacial) range shifts in alpine plants are characterised by niche evolution or by niche conservatism and (ii) whether ecologically similar species have similar biogeographic histories.

T3-01-05

Local adaptation in the context of climate change: Insights from field studies of the subalpine mustard plant, *Boechera stricta*

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Abiotic and biotic environments vary across the landscape. Over evolutionary time, divergent selection regimes can favor the evolution of local adaptation, wherein ecotypes have elevated fitness in their home environment and depressed fitness in contrasting environments. Human activities are simultaneously modifying multiple abiotic and biotic agents of selection, likely leading to growing discrepancies between current and optimal phenotypes. We hypothesize that novel selection imposed by climate change shifts fitness landscapes, disrupting local adaptation. As a consequence, local populations could experience diminished growth rates. Climatic variation across mountains affords the opportunity to test hypotheses about the evolution local adaptation to continuous environmental variation. We predict that in future climates, local ecotypes will have reduced fitness in their home sites relative to low elevation families in that same site, whose modern climates are similar to projections for higher elevations. We test this prediction in the mustard *Boechera stricta,* which is native to the Rocky Mountains and inhabits elevations as low as 1,500 m and as high as 3,500 m. In this region, warming winter temperatures reduce snowpack and warming spring temperatures cause the remaining snow to melt early. We simulated these climate change dynamics via snow removal experiments in five common gardens (elevations: 2,530 m, 2,710 m, 2,890 m, 3,133 m, 3,340 m) near the Rocky Mountain Biological Lab including transplants of 28,490 seeds and 17,832 juveniles (N = 270 families from 68 populations). We have found clear evidence for adaptation to local environments over short geographic distances in *Boechera stricta*. We have also found consistent genetic clines in heritable life history traits: high elevation families flower early, at a small size, and for a short duration, likely representing an evolutionary response to short growing seasons. Foliar herbivory increases with provenance elevation in all gardens, revealing limited herbivore resistance of high elevation families. Short cool summers could limit herbivore numbers and reduce selection for defense at high elevations. The rapid and favorable responses of herbivores to climate change could disproportionately diminish the future fitness of high elevation plants. Poor performance of high elevation families at lower sites foreshadows future maladaptation as temperatures warm. Across sites in 2014, fecundity declined by 15% for every 100 m difference between source and garden elevations ($p < 0.0001$) and snow removal depressed fecundity by 10% relative to ambient snowmelt ($p = 0.0026$), as expected. Furthermore, lower elevation families appear to outperform local families even under control conditions, suggesting that climate warming may have already disrupted local adaptation.

T3-01-06

Evolution of wild *Arabidopsis thaliana* populations over the last 150 years

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While major phenotypic changes, especially in phenology, over the course of anthropogenic climate change have been observed, the role of evolution in this is unclear. We resequenced whole genomes of ~100 herbarium specimens of *Arabidopsis thaliana*, with densest sampling of three populations. We tested for evidence of polygenic adaptation, asking whether changes in allele frequency over time corresponded to alleles associated with particular phenotypes or environments. We also tested whether alleles that changed frequency in association with temporal climate fluctuations' were also associated with certain phenotypes. We included multiple phenotypes likely important to climate adaptation: dormancy, flowering time, $d^{13}C$, and stomatal and trichome density.

T3-02: Patterns and processes of the subtropical/ warm-temperate vegetation of East Asia

T3-02-01

Flora of subtropical China: Ancient, rich, and threatened

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China is one of the richest countries in the world in terms of plant diversity. The estimated number of vascular plant species is about 33,000 (30,000 angiosperms, 250 gymnosperms, and 2,600 pteridophytes). In agreement with this, four of the world biodiversity hotspots are located totally or partially within China. The endemic component of the Chinese flora is considerable: ~240 genera and over 17,000 plant species are probably endemic to China, a substantial part of them being relict elements. Most of these relict taxa belong to lineages that were widely distributed along the Northern Hemisphere at the Miocene but that at present are restricted to small and isolated ranges along the mountains of central and southern China; conspicuous examples include *Cathaya argyrophylla*, *Eucommia ulmoides*, *Ginkgo biloba*, *Glyptostrobus pensilis*, *Metasequoia glyptostroboides*, and *Taiwania cryptomerioides*. Although multiple reasons are responsible for this relict, highly endemic, and rich flora, reduced extinction rates during the late Cenozoic global cooling—thanks to the existence of large refugia—is certainly the main driving factor. Since early 2000s, a growing body of research (including studies aimed to identify centres of endemism, studies on phylogeography/genetic diversity, and ecological niche modelling) has revealed the existence of large refuge areas in China, which were mostly concentrated in the current subtropical region. Instead of a single refugium, the mountains of subtropical China followed a pattern of "refugia-within-refugia" (that is, plant species often had multiple refugia, sometimes even within the same mountain range). Unfortunately, subtropical China, like the rest of the country, is facing enormous threats directly related to the global change and China's burgeoning economic and population growth (some of the major concentrations of population of China are within the subtropical area, such as the Sichuan Basin, Wuhan's region and the Yangtze Delta region). At national level, nearly 4,000 plant species have been listed as endangered, and at least 200 taxa having already become extinct since the 1950s. To avoid further losses, a series of conservation measures should be urgently implemented, which may include: (1) increasing the research in any aspect of plant biology, especially for rare and threatened species, (2) expanding the lists of protected plants to all the endangered species of China, (3) improving the efficiency of the already established protected areas, (4) setting up new protected areas in the floristically richest regions (hotspots), (5) ensuring that all endemic and threatened plants are well represented in botanical gardens and germplasm banks, (6) stressing upon enforcement of environmental laws, (7) promoting the sustainable use of wild plant resources, (9) strengthening the environmental awareness and education of Chinese society.

T3-02-02

Patterns and processes of the subtropical vegetation of Southwestern China

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Southwestern China (SW China, 21°08′-34°20′N, 97°30′-110°10′E, including Yunnan, Sichuan, Guizhou provinces and Chongqing Municipality) falls into latitudes classified as subtropical, except for the small tropical area of southernmost Yunnan. With that exception, altitudinal climates range from subtropical, to temperate, subalpine, alpine and frigid. I propose to elucidate the patterns and processes of the subtropical vegetation, in the context of historical events (e.g., geology and evolution), and with reference to ecological factors and human activities. Fossil evidence indicates that in the Early Tertiary period, the areas now including eastern Yunnan, Sichuan, Chongqing and Guizhou bore subtropical dry and semi-dry sparse woodlands, while most areas now comprising Yunnan had a warm-rainy winter and hot-dry summer climate supporting tropical and subtropical evergreen broad-leaved forests. In the Late Tertiary period, the vegetation of SW China became a subtropical mixed evergreen and deciduous broad-leaved forest, except in southern Yunnan, home to tropical and subtropical evergreen broad-leaved forests. In the Quaternary, vegetation changes were taking place at about 10,000 years BP (years before the present), and by 7,000 years BP the pattern of the mixed evergreen and deciduous forest had become more complex. Some parts of SW China had not been covered by the ice sheet during the Quaternary. In the region, the present subtropical vegetation, including the semi-humid, the monsoon, the mid-montane moist and the typical humid evergreen broad-leaved forests and several other forest types and minor plant communities, reflect variable environmental conditions and levels of disturbance. The monsoon and the mid-montane moist evergreen broad-leaved forests achieve maximum floristic diversity and structural complexity. Subtropical primary evergreen broad-leaved forests, dominated by species of *Castanopsis*, *Lithocarpus*, *Cyclobalanopsis*, *Machilus*, *Phoebe*, *Schima*, *etc.* are now restricted to less accessible steep slopes, remote mountains, temple grounds and nature reserves. Also within this subtropical zone, there are rare and precious Tertiary relict forests dominated by gymnosperms such as *Ginkgo*, *Metasequoia*, *Taiwania*, *Thuja*, or broad-leaved deciduous *Davidia*, *Tetracentron*, *Liriodendron* in specific habitats, mainly scree slopes, limestone areas and stream banks. In the past few centuries human activities have increasingly changed the subtropical vegetation ecosystem. Secondary stands now prevail over large areas on foothills and lower mountain slopes. In some cases bamboo communities appear after the original evergreen broad-leaved forests have been destroyed. Dramatic expansions of coniferous *Pinus*, *Cunninghamia*, *Cryptomeria* and exotic broad-leaved *Eucalyptus* appear. Complex mosaics of stands of varying ages, composition and structure intermingle with agricultural fields or the mono-species of fast-growing plantations. SW China is a land of plant diversity, vegetation dynamics and succession in time and space.

T3-02-03

Altitudinal zonation of tree species in Philippine mountain forests, 1996-2016

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The paper discusses the pattern of vegetation structure and dynamics along altitudinal gradient in selected Philippine mountain forests from 1996-2016. It presents synthesis, insights and suggest future research directions and conservation measures. Many mountain forests are beset with anthropogenic disturbances affecting the vegetation structure and dynamics and consequently the ecosystem services for the surrounding human communities. Field investigations for more than 20 years revealed anomalous zonation patterns of vegetation, quite different from the normal trend in the tropics. In these mountain forests, lower elevation dominants encroach in upper elevations, replacing original dominants destroyed by farming. *Pinus kesiya* of northern Philippines (Mount Pulag and Mount Akiki), replaces the *Lithocarpus* and other oaks. The *Diplodiscus* of Mount Makiling in southern Luzon colonized the nich of *Dipterocarpus* destroyed during the extensive logging in the 1940s. The *Astronia* of the volcanic Mount Mayon in southern Luzon and the *Artocarpus* of Mount Tabunan on Cebu island, central Philippines and that of the Aborlan Guba System in Palawan island are encroaching higher altitudes. Multivariate regression analysis shows that elevation, temperature, pH, moisture, water holding capacity and human disturbance, significantly influenced the distribution of tree species. In northern Luzon island, *Pinus* becomes a topo-edaphic climax displacing the oaks. The case of *Diplodiscus*, *Artocarpus* and *Astronia* could be a temporary seral stage of a long succession process initiated by logging and swidden agriculture, drastically changing the edaphic and microclimatic conditions, favorable for the lower dominants. There should be a strong will in protecting core forest zones and in pursuing rehabilitation efforts such as establishment of biodiversity corridors. Additionally, however, there should be sustained community biodiversity education for kids, youth and adults.

T3-02-04

Climate change impact on the potential habitats of dominant evergreen broad-leaved tree species in the Taiwan-Japan archipelago

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Ecosystem vulnerability to climate change remains elusive in the species-rich Taiwan-Japan archipelago. We predicted potential habitats (PHs) of ten dominant evergreen broad-leaved tree species by using the current and twenty potential climate change scenarios using Generalised Additive Models. The presence/absence records of each species, extracted from vegetation database, were used as response variables. Four climatic and one spatial variable were used as explanatory variables. The results showed that the interaction terms of spatial variable, indicating historical range shifts or species interactions, restricted the distribution of all the target species as much as that by the each climatic variable. The PHs of all the target species were predicted to consistently increase, and in particular, to expand northward and upward to the cool temperate zone. However, the PHs were predicted to decrease within the range of 23.6–38.1% in the Ryukyu Islands for *Castanopsis sieboldii* and *Elaeocarpus japonica*, respectively, and within the range of 32.4–42.3% in Taiwan for *Camellia japonica* and *Distylium racemosum*, respectively. These findings suggest that the four species will be vulnerable at the southern range limits; however, the remaining six species will potentially increase within the PH areas in the future at all regions.

T3-02-05

Vegetation patterns of abandoned farms in Penghu southern islands

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Abandoned farms have a long history linking to the human cultivation and activities. The vegetation patterns on these sites are influenced by cultivation legacy such as biomass alternation, fertilization, *etc.* To understand the vegetation patterns influenced by abandoned farms and stocking goat, a succession scheme are applied to elucidate the ecological processess. The target area is the South Penghu National Park among 119º30’E-119º41’E and 23º14’N-23º16’N, which is located at the southern regions of Penghu archipelagos. These islands include four largest islands: Dong-Ji, Si-Ji, Dong-Yuping and Si-Yuping. We established 81 vegetation plots of standard size with 25 m^2 and collect environmental factors, such as soil properties, wind speed, wind directions and topographical factors for exploratory analysis. Succession index was used to construct the succession scheme to explain the process and seral stage among different vegetation types. The results showed that the vegetation was classified into seven types: I. *Tetragonia tetragonoides*-*Leucaena leucocephala* type, II. *Epaltes australis*-*Fimbristylis cymosa* type, III. *Portulaca pilosa*-*Evolvulus alsinoides* type, IV. *Imperata cylindrica* var. *major*-*Vitex rotundifolia* type, V. *Wedelia prostrata*-*Cassia sophora* var. *penghuana* type, VI. *Bothriochloa glabra*-*Sorghum nitidum* fo. *aristatum* type, VII. *Sporobolus virginicus*-*Peucedanum japonicum* type. The succession degree suggests that the vegetation type III in plateau would be the early stage of succession seres, and it would be transitioned into vegetation types IV, V and VI. The succession seres in coastal region are early seral stages (types II and VII). If the continuous expansion of *Leucaena lecocephala* proceeds, these two types would be transitioned into type I. For the broad view of the southern Penghu islands, major vegetation belongs to the early seral stage and disturbed by wild goats continuously. We also found that the species diversity would be affected by the wild goats, but it needs to be confirmed by further experiment.

T3-02-06

Climatic and human impact on Holocene vegetation change inferred from a pollen record in the northern edge of Wuyi Mountains, eastern subtropical China

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The study core was taken from a mountain peat bog at 900 m elevations in the northern edge of Wuyi Mountains, eastern subtropical China. Pollen and charcoal analyses were conducted to examine the vegetation changes caused by both climatic variation and anthropogenic activities over the past 8,200 years. Our results show a humid climatic phase between c. 8,200 and 5,600 cal. yr BP, marked by high proportion of pollen from evergreen broadleaved forests (e.g., *Quercus* and *Castanopsis*) and hygrophilous alder (*Alnus trabeculosa*), which is consistent with the Holocene moisture maximum in eastern China. After 5,600 cal. yr BP, the spread of the wooded swamp taxon, *Glyptostrobus*, suggests the formation of local swamp caused by gradual drying. The drying trend generally corresponds with the speleothem isotope records from this region, revealing a weakening East Asian summer monsoon due to a decrease in Northern Hemisphere summer insolation. Peaks in the abundance and concentration of *Glyptostrobus* pollen at c. 4,600–4,400 cal. yr BP and c. 3,300–3,000 cal. yr BP suggest two periods of swamp expansions, which coincide with the drought intervals revealed by the speleothem records. The pollen and charcoal record demonstrates that human-induced land cover change was negligible before 3,600 cal. yr BP. This may be resulted from the geographical factor that huge mountain ranges inhibit cultural expansion of agriculture civilization from lower Yangtze plain and Hangzhou Bay toward mountainous southern China. We consider the first signal of intensive human activity and landscape clearing to be the noticeable increase in charcoal particles at around 3,600 cal. yr BP, followed by dramatic decrease in arboreal pollen and sharp increase in Poaceae (including rice pollen) and pioneer fern (*Dicranopteris*) and trees (such as *Pinus*), likely reflecting a transition to rice-paddy agriculture in the study area (Grant No. 41230101).

T3-03: Nectaries and nectars: from molecules to ecosystems

T3-03-01

Floral nectar of the obligate outcrossing *Canavalia gladiata* (Jacq.) DC. (Fabaceae) contains only one predominant protein, a class III acidic chitinase

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Floral nectar can affect the fitness of insect-pollinated plants, through both attraction and manipulation of pollinators. Self-incompatible insect-pollinated plants receive more insect visits than their self-compatible relatives, and the nectar of such species might face increased risk of infestation by pathogens carried by pollinators than self-compatible plants. Proteins in nectar (nectarins) play an important role in protecting the nectar, but little is known regarding nectarins in self-incompatible species. The nectarins from a self-incompatible and insect-pollinated leguminous crop, *Canavalia gladiata*, were separated using two-dimensional electrophoresis and analysed using mass spectrometry. The predominant nectarin gene was cloned and the gene expression pattern investigated using quantitative real-time PCR. Chitinolytic activity in the nectar was tested with different substrates. The *C. gladiata* nectar proteome only has one predominant nectarin, an acidic class III chitinase (CaChi3). The full-length CaChi3 gene was cloned, coding for a protein of 298 amino acids with a predicted signal peptide. CaChi3 is very similar to members of the class III chitinase family, whose evolution is dominated by purifying selection. CaChi3 was expressed in both nectary and leaves. CaChi3 has thermostable chitinolytic activity according to glycol-chitin zymography or a fluorogenic substratem but has no lysozyme activity. Chitinase might be a critical protein component in nectar. The extremely simple nectar proteome in *C. gladiata* disproves the hypothesis that self-incompatible species always have more complex nectar proteomes. Accessibility of nectar might be a significant determinant of the evolutionary pressure to develop nectar defence mechanisms.

T3-03-02

Nectaries and nectar secretion in Cleomaceae

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The complex study of floral nectary development and functioning in 15 *Cleome* s. l. species and two closely related (outgroup) species was carried out. Three groups were distinguished according to the secretion circadian dynamics, duration of secretion and yield of nectar. The diversity of nectary morphology and anatomy alongside with ultrastructure of secretory cells was demonstrated. The genus was characterized by high diversity of the nectar composition which is not related to the nectary morphology and ultrastructure. Data on nectary vascular system and stomata formation, as well as starch storage dynamics confirm the passive role of phloem and stomata in nectar secretion. It was shown that secretion of sugars from secretory cells into the apoplasm is an active ATPase driven process regardless of the nectar composition, morphology of the nectary and ultrastructure of secretory cells. The data obtained give an evident support for the universal hypothesis of nectar secretion as a pressure-driven continuous mass flow. Analysis of nectary-related evolutionary traits in Cleomaceae lineages shows that the morphology of nectaries is one of the important characters that could be used for systematics of the genus *Cleome*. However neither the nectar composition nor the ultrastructure of secretory tissue can be used as systematic features. Results indicate the multiple transitions between different types of nectaries in Cleomaceae evolution.

T3-03-03

The diversity of substances produced by floral nectaries in Anacardiaceae and their functional and systematic significance

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Anacardiaceae includes approximately 800 species grouped in two subfamilies: Anacardioideae and Spondioideae. Most species present a floral nectariferous disk. However, its structure, nectar sugar composition and chemical nature of other secreted substances are poorly understood. In a previous study, the production of lipids, phenolic compounds and nectar by the nectariferous floral disk of *Tapirira guianensis* Aubl (Spondioideae) were detected. The ultrastructural study of *T. guianensis* showed that the cellular structure of the disk is more directed towards for the production of oil than sugars. At the same time, nectar sugar composition is also poorly explored in the family, showing some variations between sucrose and hexose dominant types. Based on this background, we examined the anatomy and histochemistry of the floral nectary in 13 different species belonged to both subfamilies of Anacardiaceae, but also the proportion of secreted sugars. Results include ultrastructural data to gain insights on the production and route of secretion of the different substances. The morpho-anatomical study was carried out through transmission, scanning electron and light microscopy. Histochemical tests were performed to detect different compounds. Nectar sugars were identified via spectrophotometry. The floral nectary in Anacardiaceae is a nectariferous disk or it can be trichomatous. The nectariferous disk comprises a single-layered epidermis, a nectariferous and subnectariferous parenchyma, with or without phloem supply. Our results showed evidence of a mixed secretion in all the studied species, composed by sugars, phenols, hydrophilic substances and lipids. The production of lipids by the floral nectary seems to be a synapomorphy of Anacardiaceae, evidenced mainly by the ultrastructural data. This set of species showed a great variability in the nectar sugar composition, with sucrose-dominant, sucrose-rich, hexose-rich and hexose-dominant species. This variability on nectar sugar composition, even among species belonging to the same genera, suggests the absence of a clear phylogenetic signal. The nectariferous cells showed dense cytoplasm, large nucleus, mitochondria, plastids with osmiophilic inclusion, oil drops scattered in the cytosol, dictyosome, and rough endoplasmic reticulum segments. Some species also presented abundant amyloplasts in pre-secretory phase, indicating that they function as a resource in the formation of nectar. Subcellular evidence suggests that the abundance of mitochondria contribute to eccrine mechanism of secretion, although dictyosomal vesicles may contribute to a granulocrine process. A symplastic pathway, via plasmodesmata, is the most likely to be occurring. The manner of nectar exudation varies according to the species. Some species that present nectarostomata, and it would be the most likely route; however, the cuticle may also play a role in this process. In the other species nectar is exuded exclusively thought the cuticle, without disruption. The lipids are exuded through the permeability of the cuticle and phenolics seem not to be secreted and remain stored within the vacuoles. We conclude the floral nectary of Anacardiaceae is responsible for the production of a mixed secretion, with lipids and sugars always present. This may be related with the attraction of pollinators, since lipids are substances that provide more energy than nectar.

T3-03-04

Comparative functional genomics of nectaries and nectars in the dicots

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Plants attract mutualistic animals by offering a reward of nectar. Specifically, floral nectar (FN) is produced to attract pollinators, whereas extrafloral nectar (EFN) mediates indirect defenses through the attraction of mutualist predatory insects to limit herbivory. Nearly 90% of all plant species, including 75% of domesticated crops, benefit from animal-mediated pollination, which is largely facilitated by FN. Moreover, EFN represents one of the few defense mechanisms for which stable effects on plant health and fitness have been demonstrated in multiple systems, and thus plays a crucial role in the resistance phenotype of plants producing it. In spite of its central role in plant-animal interactions, the molecular events involved in the development of both floral and extrafloral nectaries (the glands that produce nectar), as well as the synthesis and secretion of the nectar itself, have been poorly understood until recently. To date, a holistic and coordinated characterization of nectar secretion from a genomic and molecular perspective has been lacking. Toward this end, we have evaluated the transcriptomes of floral and extrafloral nectaries throughout development across twelve dicotyledonous species and identified core sets of genes involved in the synthesis and secretion of nectar across species, as well as its regulation. Similarly, metabolite profiling coupled with transcriptomic data has identified specific loci potentially responsible for different nectar characteristics that influence mutualist visitation.

T3-03-05

Nectar secretion dynamics in different flowering plants and its effect on honey bee foraging and proboscis extension responses

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Plants produce nectar and pollen, food of honey bees. The experiment was carried out to check nectar secretion dynamics and its role in attracting different pollinators. Nectar secretion dynamics were tested at different times of the day during flowering season. The honey bee foraging was also recorded in relation to nectar secretion. The nectar was collected from flowers and different races/subspecies of honey bees were tested against these nectars for Proboscis extension responses (PER). A significant positive correlation was recorded between nectar secretion and temperature in *Acacia* and *Zizuphus* species. The nectar secretion was peaked at noon time, whereas in morning and evening the secretion was significantly less. In some flowering plants the nectar secretion remained similar with temperature. A was positive correlation between nectar secretion and honey bee foraging was also observed. The nectar was then tested for Proboscis extension responses (PER) in three races/subspecies of *Apis mellifera.* The PER was tested at two daytimes, morning and afternoon. A significant difference was recorded in PER among the three races during morning and evening. PER is used to examine the quality and attractiveness of the bee forages, and to understand how the attractiveness of a specific bee forage varied according to bee genotype and weather.

T3-03-06

Genome editing tools meet plant chemical ecology

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Genome editing tools have been developed to manipulate genomic DNA in living organisms; in particular, the recent genome editing tool, CRISPR/Cas9, allows us to induce targeted mutagenesis in several plant species. CRISPR/Cas9 also allows the genetic bases of ecological traits of interest to be explored in plants and the qualities of crop plants to be improved. In this talk, I will talk about how we can apply CRISPR/Cas9 system for plant genome editing. Especially, I will introduce a DNA-free plant genome-editing tool with Cas9 protein mixed with a single guide RNA (ribonucleoprotein, RNP) and a simple vector system for *Agrobacterium*-mediated plant transformation. We are now generating targeted knock plants to understand nectary development and nectar chemistry.

T3-04: Rising atmospheric CO_2 and higher plants: physiology, genetics and biological diversity of plant responses

T3-04-01

The impact of elevated [CO_2] on the hydraulic traits and growth of seedlings of four evergreen and four deciduous trees in SW China

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Elevated [CO_2] can reduce plants stomatal conductance, and could further change the plants hydraulic traits. However, there was no agreement on the effect of elevated [CO_2] on plants hydraulics, some studies found elevated decreased plant hydraulic conductance, while other studies found the opposite results. We used four close top growth chambers, two chambers were ambient [CO_2] and two chambers were elevated [CO_2] (700 ppm), to investigate the impact of elevated [CO_2] on the leaf gas exchange, leaf and branch hydraulic traits and biomass of seedlings of four evergreen and four deciduous trees in a subtropical forest in SW China. We found that elevated [CO_2] increased leaf area-based photosynthesis rate for all the eight species, and all the tree species excerpt *Populus davidiana* decreased stomata conductance. However, there was no significant change of leaf hydraulic conductance for all the tree species excerpt for *Lithorcarpus hancei*. The branch hydraulic conductance increased significantly for *Populous davidiana,* while there was no significant change in the other tree species. The biomass increased for all plant tree species excerpt *Eriobotrya bengalensis*. The increase of biomass in the three evergreen tree species ranged from 38% to 128%, however, the increase of biomass in deciduous trees ranged from 21% to 51%. Our results indicated that elevated [CO_2] reduced plant stomatal conductance, but had little impact on either leaf or branch xylem water transport capacity. Evergreen trees had great variations than those of deciduous trees in their response to elevated [CO_2].

T3-04-02

Phosphorus limits photosynthesis in tropical trees on four continents

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Over a third of the world's soils have low plant-available phosphorus. Phosphorus might strongly constrain forest productivity and growth on these soils, with tropical forest growth processes particularly limited by phosphorus. If this is the case, then global modellers will need relationships between plant metabolism and leaf phosphorus to reproduce productivity in their models, but relatively few such relationships have been examined across a broad geographical swath and a wide number of plant families. With key data contributors, we compiled a large global dataset of leaf nutrient concentrations and photosynthetic function, comprising over 500 species from six continents from over 250 plant families to examine whether there were broad relationships between leaf photosynthesis and leaf phosphorus and explore the physiological phenomena underlying these relationships. We used the biochemical photosynthesis model of Farquhar *et al.* (1980) and subsequent revisions to parameterise relationships between photosynthetic function and leaf phosphorus, considering soils and site phosphorus status, leaf nitrogen and leaf mass per area as variables that could modulate this relationship. These factors were considered in direct analysis as well as a multiple regression framework designed to consider a principal driving variable with other explanatory variables held constant. Our dataset contains plants with some of the lowest leaf phosphorus concentrations measured (<1 mg/g), as well as plants from habitats where phosphorus was not considered limiting or where leaf N:P ratio was low. We found relatively strong overall relationships between both leaf photosynthesis and leaf phosphorus, and leaf RuBP regeneration capacity and leaf phosphorus across the four continents in the tropics. An Australian and Amazonian site showed some of the lowest leaf P concentrations (0.6 mg/g) and highest N:P ratios, yet maintained moderate light-saturated net photosynthesis (11 ± 3 μmol CO_2 m^{-2} s^{-1}) and RuBP regeneration capacity. The overall relationships we observed suggest that global plant productivity models should consider soil or plant phosphorus as a potential constraint to future climate change responses, and employ triose-phosphate limitations in simulating photosynthesis across the globe.

T3-04-03

Transgenerational exposure to elevated CO_2 attenuates the impact of drought on wheat

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Climate change scenarios predict more frequent drought spells along with an elevation in atmospheric CO_2 concentration ($e[CO_2]$). Despite the effects of drought or a single generation exposure to $e[CO_2]$ on the physiological and yield responses of winter wheat (*Triticum aestivum* L.) have been intensively studied, the transgenerational effect of $e[CO_2]$ on plant water use relations and nitrogen nutrition in response to drought stress remains largely unknown. Seeds harvested from plants after two generations (2014-2015) continuously grown in ambient CO_2 ($a[CO_2]$, 400 mmol l^{-1}) and $e[CO_2]$ (800 mmol l^{-1}) were sown in 4 L pots, and the plants were grown separately in greenhouse cells with either $a[CO_2]$ or $e[CO_2]$. At stem elongation stage, in each of the cells half of the plants were subjected to progressive drought stress until all the plant available soil water was depleted, and the other half were well-watered and served as controls. The results showed that transgenerational exposure of the winter wheat plants to $e[CO_2]$ could attenuate the negative impact of drought stress on dry biomass accumulation, nitrogen uptake, and water use efficiency. The modulations of multi-generational exposure to $e[CO_2]$ on stomatal control over plant water use and nitrogen uptake could have contributed to the improved water use efficiency and nitrogen nutrition. These findings provide new insights into the response of wheat plants to a future drier and CO_2-enriched environment.

T3-04-04

Simulation of carbon dynamics of *Tectona grandis* forest in Western Ghats of Kerala, India using Century model

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Century model can be used to simulate carbon dynamics and the effects of other components in ecosystems. Many studies have measured the carbon stocks of Teak (*Tectona grandis)* but those studies only captured a static view without taking into account the role of other components of the ecosystem. This research has simulated the carbon dynamics of *Tectona grandis* in Western Ghats of Kerala, India using Century model. The research stages included model parameterization, validation, and analysis of the pattern of carbon accumulation in a *Tectona grandis* stand grown in a reforestation setting. The parameterization was done by adjusting the model parameters to the characteristics of *Tectona grandis* and the environment condition of the study area. The validation was conducted by comparing the simulation results to analysed statistical data from the field measurements of carbon stocks in *Tectona grandis* stands 0-5, 06-10, 11-20, 21-30 and above 30 years old. The validation process demonstrated that the output of simulation approaches analysed statistical data. Pattern of the simulated dynamics in 50 years shows that the carbon accumulated in the forest system, *Tectona grandis* biomass, and necromass increase as the age of stand increases. However, the accumulation of soil carbon initially decreases until it reaches a relatively constant value.

T3-04-05

Physiological integration ameliorates effects of UV-B radiation in clonal herb *Duchesnea indica*

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Ultraviolet-B (UV-B) radiation can result in deleterious effects on many plant growth processes. In clonal plants, resources and hormones are often shared between connected ramets to deal with various environment. However, little comprehensive study is known about whether and how clonal integration influences the ability of clonal plants to respond to UV-B radiation. In this paper, the change in physiological integration intensity of water transportation rate and photosynthetic ability and survival and growth of *Duchesnea indica* under homogeneous and heterogeneous ultraviolet-B radiation (280–315 nm) were evaluated. Ramet pairs of *Duchesnea indica* were subjected to four treatments: (1) connected ramet pairs under a homogeneous condition, i.e. pairs of ramets were kept under no additional UV-B conditions and the stolon connection between the ramets remained intact; (2) disconnected ramet pairs under the same UV-B condition as in treatment (1); (3) connected ramet pairs under a heterogeneous condition, i.e. one of the ramets was exposed under additional UV-B radiation and the stolon connection between the ramets remained intact; (4) disconnected ramet pairs under the same UV-B condition as in treatment (3). When ramets were connected and exposed to heterogeneous UV-B radiation, the velocity of water transportation from the UV-B treated ramet to its connected sister ramet was markedly lower. Moreover, stolon connection resulted in an increase of biomass and ramet number in the UV-B stressed part of the clonal fragments at the expense of that in UV-B unstressed part, which meant that full compensatory growth effect was existed in *Duchesnea indica* under heterogeneous UV-B radiation. In addition, chlorophyll contents and UV-B absorbing compounds were shared between connected ramets under heterogeneous UV-B radiation to benefit for the whole clonal fragment. This inference extends the concept of physiological integration in clonal plants to include defensive responses. Net photosynthesis rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) were lower and transpiration rate (E) were higher in UV-B stressed part, and C_i in UV-B unstressed part were reversed. Moreover, in UV-B unstressed part, maximum photochemical efficiency of PSII (F_v/F_m) and photochemical quenching coefficient (q_P) had no significant difference, the electron transport rate (ETR) were lower and non-photochemical quenching (NPQ) were higher, and in UV-B stressed part, additive UV-B radiation resulted in a notable decrease of F_v/F_m, ETR and q_P and an increase of NPQ, stolon connection made F_v/F_m, ETR, q_P and NPQ changed more in the connected group. Clonal integration significantly reduced the stressful impact imposed by enhanced UV-B radiation with a cost, and the whole clone fragment will benefit, from survival and growth,

chlorophyll and UV-B-absorbing compounds concentrations, photosynthetic efficiency in heterogeneous UV-B micro-habitat.

T3-04-06

Dendrochronology of *Fagus grandifolia* subsp. *mexicana* in the state of Hidalgo, Mexico

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The analysis of tree rings growth in environmental history is a valuable source of information for the reconstruction of past events. In this research we used the information from historical archives, interviews and chronology of the growth rings of the species *Fagus grandifolia* subsp. *mexicana* to identify the main causes of disturbance. Sampling of the nuclei of growth was carried out in three localities where the species mentioned dominates the canopy, forming the beech forests. A total of 3,355 tree rings were measured and the standardization of the time series was elaborated. The annual averages of ring width were similar between localities, ranging from 0.98 to 1.08. The analysis of the percentage of change of growth allowed to recognize a multiple pattern of suppressions and releases, associated with local events and the natural development of the species. Although the correlation values between chronologies were not statistically significant, similar trends of variation were observed in the localities El Gosco and La Mojonera. The linear correlation values between the ring width index and the climatic variables (annual total precipitation, minimum, maximum and average temperature) of each locality were not statistically significant. In the Hidalgo state, there have been no major natural or human disturbances of at least 150 years, with the exception of El Gosco, where anthropogenic disturbance has increased dramatically in the last decade.

T3-05: Niche evolution and biome assembly through time (two sessions)

T3-05-01

Despite phylogenetic effects, C_3-C_4 lineages bridge the ecological gap to C_4 photosynthesis

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C_4 photosynthesis is a physiological innovation involving several anatomical and biochemical components that emerged recurrently in flowering plants. This complex trait evolved via a series of physiological intermediates, broadly termed 'C_3-C_4', which have been widely studied to understand C_4 origins. While this research program focused on biochemistry, physiology, and anatomy, the ecology of these intermediates remains largely unexplored. Here, we use global occurrence data and local habitat descriptions to characterize the niche of multiple C_3-C_4 lineages, as well as their close C_3 and C_4 relatives. While C_3-C_4 taxa tend to occur in warm climates, their abiotic niches are spread along other dimensions, making it impossible to define a universal C_3-C_4 niche. Phylogeny-based comparisons suggest that, despite shifts associated with photosynthetic types, the precipitation component of the C_3-C_4 niche is particularly lineage specific, being highly correlated with that of closely related C_3 and C_4 taxa. Our large-scale analyses suggest that C_3-C_4 lineages converged toward warm habitats, which may have facilitated the transition to C_4 photosynthesis, effectively bridging the ecological gap between C_3 and C_4 plants. The intermediates retained some precipitation aspects of their C_3 ancestor's habitat and likely transmitted them to their C_4 descendants, contributing to the diversity among C_4 lineages seen today.

T3-05-02

Australia: A dry continent with small terrestrial succulents, but not big ones

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Why are there no big terrestrial succulent plants native to Australia, a continent within which ca. 75% of the land area is arid or semi-arid? Why, in such a water-limited landscape, are only seven terrestrial Australian species known to exhibit crassulacean acid metabolism (CAM), a water-use efficient mode of photosynthesis that is globally associated with succulent plants? On the basis of the frequency of CAM in the planetary angiosperm flora one might expect ca. 1,300 CAM species in Australia. It has been suggested that the unpredictable rainfall in arid Australia cannot support massive water-storing succulents. Yet large succulents can do well in Australia. In the 1920s exotic *Opuntia* occupied 24 million hectares. A recent resurgence has seen opuntias infest ca. 2 million hectares. Although depauperate in large succulents, Australia does support a spatially-widespread terrestrial flora of endemic small leaf-succulents. Low level of CAM is known in species of *Anacampseros* (Anacampserotaceae), *Calandrinia* (Montiaceae), *Carpobrotus* (Aizoaceae), *Crassula* (Crassulaceae), *Disphyma* (Aizoaceae) and *Portulaca* (Portulacaceae). Substantial CAM is known only from *Cynanchum* (Apocynaceae), Australia's largest terrestrial succulent. A surprising observation is the preponderance in the small terrestrial succulents of drought-induced facultative (i.e. optional) CAM, a form of CAM considered uncommon, and previously known from ca. 20 species worldwide. Facultative CAM in Australian succulents is present in species in which the principal photosynthetic phenotype in well-watered plants is C_3 but is also present in Portulaca, in which the principal photosynthetic phenotype in well-watered plants is C_4. It is not yet clear whether the high incidence of facultative CAM seen in the succulents is solely an Australian phenomenon or a more widespread one that has been overlooked so far. As the Australian genera with facultative CAM have close relatives in the New World and Africa, more thorough surveys of small succulents in those regions may be instructive. It is not difficult to imagine how small annual succulents with a facultative water-use efficient mode of photosynthesis might be selected in warm, low-nutrient landscapes subject to low and unpredictable rainfall. However, the absence of large stem succulents in remains enigmatic. Environments that support large succulents may be uncommon in contemporary Australia, but they exist. There must be more to the absence of large stem-succulents than just the lack of dry environments with a predictable rainy season. Perhaps taxa with the evolutionary capacity to develop stem-succulence simply did not make it to Australia, or perhaps there was insufficient niche space in existing vegetation assemblages with their associated fire regimes. Deeper exploration of the Australian extant succulents will surely be informative.

T3-05-03

General features of the evolutionary assembly of biomes

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Major changes in climate over geological time have been responsible for the emergence and spread of modern biomes. Prolonged directional selection has resulted in the massive convergence of climate-related traits, perhaps through gradual in situ evolutionary change. The extent and the phylogenetic distribution of this convergence is also a function of the relative accessibility of particular adaptive "solutions" in different lineages. Consequently, we observe distinctly clumped patterns of convergence, with multiple shifts occurring within particular lineages. An additional factor in explaining the shifting of lineages among biomes is the extent (in both space and time) to which biome boundaries have existed. Evolutionary shifts between adjoining biomes are far more common than they are between disjunct biomes. Our research has focused on direct shifts between warm tropical forests and cold temperate forests. We argue that many key evolutionary changes that have accompanied this shift have been driven by changes in the rhythm of growth with the occupation of cold climates. This, we think, has driven the evolution of both the deciduous leaf habit and aspects of leaf form, including broadly ovate leaf shapes and toothed or lobed leaf margins. Although the specific drivers of evolutionary change will certainly differ with the occupation of different biomes, we suspect that changes in the rhythm of growth underlie many such changes, irrespective of biome. An evaluation of this proposition (and other such generalities about biome assembly) will require multiple integrative studies of well-sampled clades whose members straddle biome boundaries.

T3-05-04

Biogeography of Berberidaceae and biome shifts

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Berberidaceae is one of the most characteristic components of northern temperate environments, comprising about 17 genera within which mostly are herbaceous perennials. However, at the species level, more than two-thirds of species diversity are found in *Berberis* L., a shrubby group almost entirely restricted to montane to alpine habitats globally. Why is *Berberis* the most successful group among its perennial sisters and what are the evolutionary processes in shaping diversification rates and current distribution of the genus? Recent phylogenetic and biogeographic studies have reconstructed the evolutionary trajectory for the genus, unraveling an uncommon arid-adapted origin in North America with a key niche shift into global high-mountain systems. While molecular dating and diversification models indicate an increase of diversification rate from Oligocene onward, the successful transition into a cold temperate biome of *Berberis* may be triggered and influenced by the paleoclimate deterioration during Neogene. Here we further identified two more recent mountain radiations (Andes vs Himalayas-southwestern mountains of China) by using BAMM and BiSSE analyses. Preliminary investigation on leaf area (LA) from herbarium collections among *Berberis* and sister genera also suggested the habitat and LA had a positive interactive effect on the diversification. Together, *Berberis* may entail a unique case of biome shifts in the assembly of global temperate biota, and its high diversity is likely a result of adaptive radiation facilitated by leafing traits evolution

T3-05-05

Strong regional effects in Northern Hemisphere tree physiology and phenology

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Donoghue and Edwards (AREES 2014) stress the expected, but under-researched link between *biomes*, which are defined by climate and eco-physiology, and *biogeographic realms*, which reflect isolated centers of diversification for multiple groups of organisms. But into which conceptual framework do we fit the following experimental finding: trees and shrubs native to Eastern North America (ENA) on average have a three-week shorter vegetative season than woody species in East Asia and Europe because the leaf-out later and break down their chlorophyll 11 ± 4 days earlier (Zohner and Renner, in review)? Modern average temperatures cannot explain this difference because mean annual temperature and temperature seasonality experienced in species' natives ranges today have small effect sizes on experimentally investigated leaf-out and leaf senescence in ca. 400 woody species from throughout the Northern hemisphere. Nor can biogeography explain these regional effects because closely related species in different biogeographic realms exhibit the local strategy. Instead, we propose that the physiology and phenology of the modern ENA woody flora is the product of multiple pre-Pleistocene climate disruptions that selected for conservative growth strategies. These results highlight climate as a major driver of plant traits, such as the timing chlorophyll breakdown, and also underscore that physiological traits will remain 'invisible' unless we combine indirect comparative approaches with experiments.

T3-05-06

Geographic divergence and functional evolution of Magnoliaceae

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The early diverged Magnoliaceae shows a historical temperate-tropical distribution among lineages, indicating a divergent evolution. Analyse the relationship between phylogeny, environmental constraints and associated trait variances could reveal how species distributions were shaped. Phylogenetic comparative models can be used to disentangle the relative roles of spatial and phylogenetic distances in explaining trait variances, reveal phylogenetic signals in ecophysiological traits, and test whether these traits are involved in correlated evolution. We analyzed variances in 15 plant traits and 12 environmental variables of 183 Magnoliaceae species in China using four comparative models. We also measured 20 ecophysiological traits of 27 species of the four largest Magnoliaceae genera. Species richness gradually decreased from south to north, with phylogenetic clustering along the southern coast, and in north and central China. Variances of plant diameter, length and width of leaves and tepals, and fruit length were strongly associated with phylogenetic relatedness. In contrast, variances of plant height, flowering and fruiting time, and all of the 12 environmental variables were associated with both spatial proximity and phylogenetic distance among species. Strong phylogenetic signals in water-conducting and nutrient-use related traits were identified, and correlated evolution of several key functional traits was demonstrated. Among the three evergreen genera of tropical origin, *Lirianthe* had the lowest hydraulic-photosynthetic capacity and the highest drought tolerance compared with *Manglietia* and *Michelia*. Contrastingly, the temperate centred deciduous *Yulania*, showed high rates of hydraulic conductivity and photosynthesis at the cost of drought tolerance. Overall, the present tropical-temperate distribution of Magnoliaceae was associated with the divergent evolution of plant functional traits under both geographical separations and environmental constraints. Specifically, the regulation of hydraulic and photosynthetic processes during the adaptations to local environments, was the ecophysiological basis for phylogenetic niche conservatism in functional traits among divergent lineages of Magnoliaceae.

T3-05-07

The past is the key to the present: Using fossil plant to uncover the continent-specific assembly of grassland ecosystems

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Reconstructing how terrestrial ecosystems were assembled through time is vital for understanding the processes that affect them today—and in the future. The grassland biome currently covers nearly 40% of Earth land surface, and has a major influence on the climate system, the faunas that inhabit it, as well as global silica and carbon cycling. Documenting the Cenozoic rise of this important biome has preoccupied paleontologists and evolutionary biologists for nearly 140 years. The rich Cenozoic terrestrial record on many continents has allowed detailed study of functional morphology of animals, fossilized soils, and stable isotopes, documenting broad patterns of grassland evolution. In contrast, paleobotanical data have historically been scarce; however, in recent years, plant silica (phytoliths) has emerged as a highly suitable tool for tracking diversification of Poaceae lineages and the spread of grassland vegetation. Unlike pollen and most macrofossils, grass phytoliths are often diagnostic of ecologically distinct grass subclades, providing vital information about grass community composition. In addition, plant silica can be found in sediment types representing depositional environments that do not preserve other kinds of plant remains, extending the range of sampled habitats to include, for example, dry “uplands”. In combination with other lines of evidence, phytolith data collected so far in North and South America, Europe, Asia, and Africa have shown that the assembly of the grassland biome was substantially more complex than previously assumed. For instance, it now appears that open-habitat grasses (pooids, PACMADs) diversified long before becoming ecologically dominant in both North and South America, indicating that the traits associated with tolerance of open, dry habitats may have evolved in response to a different set of environmental triggers than those that promoted the expansion of open, grass-dominated habitats. It is also clear that the earliest Miocene grasslands were dominated by C_3, pooid grasses that were only later replaced by C_4 grasslands in at low-mid latitudes (e.g., North America, Asia). Furthermore, comparison between faunal and phytolith assemblage data shows that grazing herbivores did not evolve in tight coevolution with grasslands in North America and Eurasia, but instead appeared several millions years after vegetation changed. In contrast, in southern South America, grazer-like morphology originated independently of grass-dominated habitats, in the context of palm-dominated shrublands that seemed to have occupied this region for much of the Cenozoic. This open, dry-adapted, but nearly grass-free vegetation type, which lacks clear modern analogs, likely expanded as a result of increasingly arid regional climates during the middle-late Eocene. The exact ecology of the pooid and PACMAD grasses that formed a minor component of this vegetation remains unclear. Taken together these results suggest that non-analog conditions have fundamentally shaped the history of grasslands in many regions and point to essential differences in ecosystem assembly among continents.

T3-05-08

Insights into the historical construction of East Asian subtropical evergreen broadleaved forests revealed by the temporal history of Theaceae

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Subtropical evergreen broadleaved forests (SEBLFs) are among the most important vegetation types in East Asia, contributing fundamentally to both the biodiversity function and ecosystem service of this region. The East Asian SEBLFs were proposed as unique zonal vegetation that might have developed in concert with the Asian monsoon system, particularly the East Asian summer monsoon (EASM). Although paleovegetation reconstructions revealed that the SEBLFs have existed in Southwest China during the Miocene, the historical construction of the SEBLFs and its relationship with the dynamics of the EASM in East Asia are poorly known. We used the tea family (Theaceae), a characteristic component of the SEBLFs in East Asia, to gain insights into the assembly of this important biome. Fifty-three representatives from all three tribes and nine genera of Theaceae were represented, covering major distributional range of the family. Eighteen species from seven families within Ericales were used as outgroups. The phylogenetic relationships within Theaceae were reconstructed using plastome and nuclear ribosomal DNA sequence data. The temporal history of the tea family was estimated using eight fossils from Theaceae and related families in Ericales. Ages of other six structural genera of the SEBLFs from Fagaceae, Lauraceae and Magnoliaceae were also integrated based on previous studies. Analyses of speciation rate and potential rate shifts within Theaceae were conducted using a Bayesian approach. The ancestral states of vegetation and habit of Theaceae were inferred based on the maximum likelihood method. The recovered phylogenetic framework of Theaceae in our study is robust, providing well-resolved relationships among tribes and genera in the family. The phylogenetic backbone of Theaceae is in agreement with the evolution of the endosperm. The early-diverged Stewartieae contain the most copious endosperm, and the other two sister tribes (Theeae and Gordonieae) retain only a thin layer of or perhaps no endosperm. The reconstructed temporal history of the tea family suggests that the major lineages of the SEBLFs possibly originated around the Oligocene-Miocene (O-M) boundary. A consistent pattern was also found in four genera of other three characteristic families within the SEBLFs. In addition, the accelerated speciation rate and transition from deciduous to evergreen leaves within Theaceae were detected close to the O-M boundary. It seems that a rapid assembly of the SEBLFs in East Asia might have occurred around the O-M boundary. Moreover, two structural genera from Fagaceae and Lauraceae date back to the Eocene, suggesting that the establishment of the SEBLFs could be possibly traced back to the Eocene epoch. The initiation of the EASM in the Eocene and its subsequent intensification around the O-M boundary may have triggered the establishment and rapid assembly of the SEBLFs in East Asia, respectively. Our results indicate that the historical construction of the SEBLFs in East Asia was closely related to the dynamics of the EASM, which brought abundant rainfall and enhanced humidity within the region.

T3-05-09

Into the tropics: High tropical diversification not time-for-speciation explains latitudinal gradient in *Rhododendron* species richness

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The latitudinal gradient in species richness is a well-known biological phenomenon which has puzzled ecologists since the early 19th century. The mechanisms underlying this pattern have remained one of the most controversial issues in ecology and biogeography. Several hypotheses have been proposed to explain this diversity gradient, many of which can be broadly grouped into two categories; ones that regard contemporary environmental factors (e.g., water, energy, habitat heterogeneity, *etc*.) and others that consider evolutionary history as the major determinant. Although species diversity shows strong relation with the environment, large scale pattern of species diversity must ultimately result from the process of speciation, extinction, and dispersal that occur over evolutionary time scales. The time-for-speciation hypothesis (TSH) and diversification rate hypothesis combine both these ecological and evolutionary perspectives to provide an explanation for large-scale richness patterns. Here, we evaluate the relative effects of contemporary environment and evolutionary history on the diversity pattern of a species-rich plant genus *Rhododendron*, which comprises over 1,000 species globally. Using global climate, a complete distribution data of global species, and a comprehensive phylogeny of *Rhododendron* constructed herein, we reconstructed ancestral area, ancestral climate and traced biogeographical history of this group for the first time. Specifically we aim to 1) compare the relative contribution of contemporary environment, time-for-speciation and diversification rate on species diversity, and 2) evaluate the relationship between net diversification rates and environmental variables. Our findings suggest that *Rhododendron* likely originated in the temperate region of Northeast Asia in the early Paleocene, from where it dispersed southward to the Himalayas and south-western China. This dispersal history reflects a rare into-the-tropics phenomenon. Interestingly, despite being a temperate group, *Rhododendron* diversity is higher and the species are younger in the tropics, showing evidence inconsistence with the prediction of TSE. The accumulation of species diversity away from their origin centre suggests that longer time does not necessarily yield more species. Instead, the differences in net diversification rates across tropical and temperate latitudes provide a better explanation for uneven species richness. We conclude that high *Rhododendron* diversity in the tropics is, particularly, due to increased allopatric speciation and elevated net diversification rates facilitated by the formation of complex topography in the south-eastern fringe of the Tibet-Himalayan region and the Malay Archipelago.

T3-05-10

Niche divergence driven by range overlap and time in *Mimulus*

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Species' niches and distributions interplay across multiple spatial scales through evolutionary time. In particular, the ways that niches diverge between close relatives can reveal determinants of co-occurrence, while the degree of range overlap can determine when selection favours niche convergence or divergence. Quantitative analysis of niche divergence and range overlap in a phylogenetic framework can provide important insights into niche evolution and speciation modes. In areas of range overlap, competitive interactions may drive greater niche divergence. Alternatively, shared selective pressures due to environmental filtering may lead to greater niche conservatism. We tested these hypotheses in 16 pairs of western North American monkeyflowers (*Mimulus*), using data on various niche axes, geographic ranges and a robust phylogeny. We found that coarse-scaled environmental niche divergence decreased with increasing range overlap and phylogenetic distance, consistent with environmental filtering that operates as range overlap increases following allopatric speciation. No such relationship was detected for local-scaled microhabitat niche divergence, except a positive association with phylogenetic distance along one microhabitat niche axis (related to vegetation cover). Furthermore, greater microhabitat niche divergence was found in sympatry than in allopatry along at least one niche axis for each species pair with partially overlapping ranges. Together, these results reveal that different niche axes show different patterns of evolutionarily lability, perhaps because the relative importance of environmental filtering versus biotic interactions depends on spatial scale.

T3-05-11

Evolution along the C_3-CAM photosynthetic continuum in *Rumicastrum* (Montiaceae)

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Crassulacean Acid Metabolism (CAM) photosynthesis is a complex trait that has evolved many times from the more common C_3 photosynthesis and improves water use efficiency in plants under drought and temperature stress. In addition to strong CAM, there are recognized C_3-CAM intermediate phenotypes (constitutive low-level CAM and facultative CAM), which are hypothesized to represent transitional states along the CAM evolutionary trajectory; however there has been no explicit demonstration of how these potentially intermediate types relate to one another, or to the evolution of the full CAM syndrome. *Rumicastrum* (Montiaceae) is an ideal lineage in which to investigate the evolutionary assembly of CAM photosynthesis. It comprises ~70 species of annual and perennial succulent herbs endemic to Australia, and species within this group are known to span the C_3 to CAM phenotypic spectrum. Using a targeted gene enrichment approach, we sequenced 100s of loci from multiple gene families of interest across *Rumicastrum* and other Montiaceae. Here we present the first, complete phylogeny for *Rumicastrum*, its relationship to the rest of Montiaceae, and how the lineage likely radiated across the Australian continent. In addition, we explore the evolution of CAM within this group, integrating physiological, isotopic, and anatomical measurements to more fully understand the expression, evolution, and reversibility of the CAM photosynthetic syndrome.

T3-05-12

Plant mating system predicts island biogeography and range size

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Key life history traits, such as shifts from outcrossing to selfing, can have major impacts on the geographic properties of species. The reproductive assurance provided by selfing may allow selfers to successfully establish after long distance dispersal events (e.g., to oceanic islands), and to rapidly expand their ranges. Using phylogenies, geographic occurrence and mating system data, we conduct a large-scale test of this hypothesis in flowering plants. We find that 1) self-compatible species are significantly enriched on oceanic islands relative to self-incompatible species and 2) automatically selfing species tend to have larger geographic ranges than their outcrossing relatives, and that this tendency increases with time since a selfing species diverged from its outcrossing relative. Thus, traits that improve mating success may be at least as important as other traits, such as dispersal ability and environmental tolerance, in helping plants to establish new populations and increase their range size.

T3-06: Functional traits explaining plant responses to past and future climate changes

T3-06-01

Phenology constraints of plant growth under climatic change

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It is widely held that climatic warming will stimulate metabolism, growth, length of growing season, and thus, productivity of plants and ecosystems. This rationale does not account for the basic role of phenology in plant life. Plant phenology, that is, the progression of seasonal development, is controlled by plant-internal and external drivers. Phenology responses are under strong evolutionary selection because they (1) insure plants against biological activity at the wrong time (e.g., escaping from periods with risk of freezing damage in the temperate zone), and (2) they ensure gene transfer in sexual reproduction (synchrony of flowering). Both of these functions of phenology are so fundamental for successful plant life, that evolution selected against dominant influences of the curiosities of weather, that is temperature or moisture only. Yet, if controls were that simple, selection would have driven phenology to strictly follow an astronomic calendar (photoperiod) that minimizes the statistical risk of exposure to dangerous conditions. Since the photoperiod is the same in spring and autumn, it requires at least a second control, a certain chill-dose, signalling the passing of winter. Photoperiod thresholds are selected for by the regional long-term probability of freezing in the case of a pronounced temperature seasonality or other regularly occurring adverse events (drought season). These ‘escape’ mechanisms come into play in long-lived plants that cannot rely on a long-term seed bank to survive ‘misbehaviour’ (e.g., flushing or flowering at the wrong time). In long-lived temperate zone taxa (trees) thermal forcing within a safe window set by photoperiod comes in as a third driver. Because, what commonly is observed, is largely this temperature response, once other signals have ‘opened a window of perceptivity’, temperature has widely been held as the master-driver of phenology, which clearly is not the case in late successional, native tree species. It can safely be expected that evolution does not play lottery, but prevents plants from being active at the wrong time. Across the entire plant world and different climatic zones an immense number of combinations of these three phenology controls can be expected. There is also space for opportunistic behaviour. Hence responses of phenology to climatic change are species and region specific, which is severely constraining predictive models. As a rule of thumb, the control of phenology by temperature alone declines with (1) a species’ longevity, (2) the annual amplitude of natural habitat temperature, (3) unpredictability of temperature (including topography driven snow cover), and, (4) as a combination of (2) and (3), with latitude. Since most soil organisms exert immediate responses to climatic conditions, rapid climatic change will create a mismatch between soil activity (provision of nutrients) and plant activity (demand in nutrients) until micro-evolutionary selection has tracked those climatic changes, which may take centuries in the case of trees.

T3-06-02

Functional traits are good predictors of global change impacts on tree species abundance dynamics in a subtropical forest

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Significant changes in the composition of tree species have been observed in various forests worldwide. We hypothesized that these changes might result from variable sensitivities of species to global change, and species sensitivities might be quantified using functional traits. Employing long term (1978-2010) species abundance data of 48 tree species from a permanent subtropical forest plot, where multiple global change factors have been observed, including soil drying, we examined the relationships between temporal trends in abundance and suits of functional traits. We found that species with high photosynthesis rates, leaf phosphorus and nitrogen concentrations, specific leaf area, hydraulic conductivity, turgor loss point, and predawn leaf water potential had increased in abundance, while species with opposite trait patterns had decreased. Our results demonstrate that functional traits underlie tree species abundance dynamics in response to drought stress, thus linking traits to compositional shifts in this subtropical forest under global changes.

T3-06-03

Clonality-latitude relationship and the underlying mechanisms in Australia

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We provide a large-scale quantification of the relationship between latitude and the proportion of species with clonal reproduction. The parasite pressure is thought to be higher at lower latitudes, favoring sexual reproduction. The abiotic conditions is relatively harsh at high latitudes, which may harm sexual reproduction. Therefore, we predicte that there would be a higher proportion of clonal species at high latitudes than at low latitudes. We collected data of 4,386 native seed plant species from 446 genera and 99 families present in ABRS *Flora of Australia*. Species' occurrence records were taken from the *Atlas of Living Australia*, including 817,450 species-site combinations spanning 34.5° of latitude. Logistic regression showed that the proportion of clonal species significantly increased with latitude, rising from just 3.3% clonal species at 9.25°S to 26.7% clonal species at 43.75°S. The overall average proportion of clonal species in Australian seed plants was 9.4%. It reveals Australian vegetation contains a relatively low proportion of clonally reproducing species. In order to test which factor forcing the latitudinal gradient in clonality, we collected temperature, precipitation and net primary productivity (NPP) data from CRUTEM dataset, GPCC dataset and the MOD173A annual product, respectively. Growth form and plant height data were also collected from the species descriptions of ABRS, reliable websites (e.g., FloraBase, PlantNET) or from previous publications. We aim to investigate the main environmental factors that drive plant reproductive strategy, determine whether plant traits are related to reproductive strategy and detect which is more important to shape reproduction strategy selection, plant own traits or environmental factors. The results showed that annual average temperature, maximum temperature in hottest month, and the seasonal variation in precipitation were significantly negatively related to the proportion of species with clonal reproduction. The average monthly precipitation in driest month and NPP were significantly positively related to clonality. Among the environmental factors, annual average temperature explained the largest variation. Plant growth form and height significantly affect reproductive strategy. Herbaceous and short species tend to have clonal reproduction. This study indicated a positive relationship between latitude and the proportion of species with clonal reproduction and illustrates the forcing factors, which adds to our growing understanding of dramatic latitudinal gradients in the way plants grow and reproduce. This project was funded by China Scholarship Council, the National Natural Science Foundation of China (41571055), and the State Key Basic Research Development Program (2015CB150800) to HX Zhang and by an ARC discovery grant to SPB and ATM (DP110100210).

T3-06-04

Water relations are coupled with photosynthetic light- and water-response functions across 12 mosses

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Trade-offs between water conservation and carbon gain are well-studied in vascular plants, were stomata regulate gas-exchange rates. In bryophytes, water loss and access to CO_2 are not regulated but depend on moss morphology and moisture conditions. Although water is essential for photosynthetic activity, excess water hinders CO_2 diffusion to the chloroplasts and thus hinders photosynthesis. Bryophytes thus need to balance between being too wet and too dry. We hypothesize that species can follow two main strategies: 1) staying wet as long as possible, with a low photosynthetic capacity needed because CO_2 supply will be limiting, or 2) avoiding external water, with high photosynthetic rates needed to be very productive before drying out completely. Consequently, we hypothesis that the water relations and photosynthetic light- and water-response functions of bryophytes should be inter-dependent. In the current study, we aimed to find out 1. How does the maximum water content (WC_{max}) of moss species relate to their water loss rate? 2. How are the position and the 90%-range of the optimal water content for photosynthesis (WC_{opt} and $WC_{90\%\text{-}range}$) related? 3. How are water relations and photosynthetic light- and water-response traits of mosses related? We determined the light-compensation and -saturation points, dark respiration rates, mass-based light saturated assimilation rates (A_{max}), WC_{max}, 50% water loss time (50%time), water-loss decay constants (DC), WC_{opt} and $WC_{90\%\text{-}range}$ of 12 forest-floor mosses from central Germany. Principal component analysis and bivariate correlations were used to explore the associations between these traits and their distributions among the studied species. The first PCA axis was dominated by the extreme trait values of the two *Sphagnum* species, which had a very high WC_{max} and 50%time, a low DC, a high WC_{opt} and large $WC_{90\%\text{-range}}$, and a high A_{max}. *Sphagnum* species are known for having specialized water-holding cells, thereby avoiding external water while still remaining wet as long as possible. WC_{max}, 50%time, WC_{opt} and $WC_{90\%\text{-range}}$ were generally positively related, and negatively related to DC. A_{max} was positively related to WC_{max}, WC_{opt} and $WC_{90\%\text{-range}}$, but this relationship was strongly dominated by the two *Sphagnum* species. Excluding these species, A_{max} was negatively related to WC_{max} and had no signifiant relationships to the photosynthetic water-response traits. When also excluding the two Polytrichaceae, species with internal water-conducting tissues, no relationships between water relations and photosynthetic capacities could be detected. This was due to the lower variation in trait values and to the low remaining sample size (n = 8). Our study shows that water relations and photosynthetic water-response traits are inter-related across moss species, but these relationships depend strongly on the species selected for analysis. Thereby *Sphagnum* and Polytrichaceae having over-proportionally large effects due to their special water-related adaptations, allowing alternatives to the two hypothesized strategies. Further studies should test the generality of these relationships for larger sets of species, including other habitats and climate zones and at the level of plasticity within species. Modelling could help to understand the suggested constraints of the trait space by simulating moss carbon gain in different wetting and drying scenarios.

T3-06-05

Extremely thick cell walls and low mesophyll conductance: Welcome to the world of ancient living!

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Mesophyll conductance is thought to be an important photosynthetic limitation in gymnosperms, but they currently constitute the most understudied plant group in the extent to which photosynthesis and intrinsic water use efficiency (WUE_i) are limited by mesophyll conductance. However, it is necessary to study a variety of taxa to predict vegetation responses and the carbon-cycle in the changing climate. A comprehensive analysis of leaf gas-exchange, photosynthetic limitations, mesophyll conductance (g_m, calculated by three methods previously used for across-species comparisons), and the underlying ultra-anatomical, morphological and chemical traits in 11 gymnosperm species in varying evolutionary history was performed to gain insight into the evolution of structural and physiological controls on photosynthesis at the lower return end of the leaf economics spectrum. Two primitive herbaceous species were included in order to enhance evolutionary context. Low mesophyll conductance was the main limiting factor of photosynthesis in the majority of species. The strongest sources of limitation were extremely thick mesophyll cell walls (T_{cwm}), high chloroplast thickness and variation in its shape and size, and the exposed surface area of chloroplasts per unit leaf area. In gymnosperms, the negative relationship between net assimilation per mass and leaf mass per area (LMA) reflected an increased T_{cwm}, whereas the easy-to-measure integrative trait, LMA failed to predict the underlying ultrastructural traits limiting g_m. These adaptations may be relics from the atmospheric concentrations in which these genera evolved, still present due to evolutionary constraints, but may give evergreen gymnosperms an advantage over angiosperms in the rising CO_2 levels of this century.

T3-06-06

Intraspecific plasticity of leaf physiology in widely distributed eucalypts to combined water and heat stress varies under current and future climates

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The capacity of plant species to cope with rapid climate change will depend upon phenotypic plasticity. For woody species, populations originating from different environments usually show differentiation in response to warming or drought. However, few studies have examined intraspecific variation in woody plant response to simultaneously occurring climate extremes. Here, we investigated the effects of warming and co-varying climate extremes (i.e., drought and/or heat waves) on growth and physiology of two widely distributed *Eucalyptus* species (*E. grandis* and *E. tereticornis*), each of which contained a temperate (drier and cooler) and a tropical (wetter and warmer) provenances. The two species in general did not show interspecific differentiation in response to the same environmental changes, but we observed significant differences between the temperate and tropical provenances in plant growth response to warming and in photosynthetic response to heat waves, both of which depended on taxon temperature of origin. However, provenances of both species responded similarly in growth and physiology to drought. Heat stress alone generally had little effect on plant growth rates and photosynthesis, but the synergism between drought and heat imposed significantly greater impact on plants than each applied separately. Furthermore, two distinct strategies (senescence of older mature leaves *vs*. complete closure of stomata) were observed, and both proved to be effective, in coping with combined drought and heat stress. Taken together, our results suggest that plant populations of widespread tree species may differ in their response to climate warming and heat waves depending on the climate of origin, but may not necessarily show difference in response to drought. Drought is likely to be the dominant stressor during heat waves. Widespread tree species may possess different strategies to cope with climatic extremes and show interspecific or even intraspecific variation.

T3-07: Interspecific pollen transfer: ecological circumstances, behavioral mechanisms, and evolutionary consequences

T3-07-01

Temporal variation in pollinator network and pollen transfer network: How common is misplaced pollen and its causes?

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Studies of plant-pollinator interaction in diverse communities have shown that one pollinator species may visit multiple plant species in one foraging bout. Such interspecific visits could deposit heterospecific pollen on stigmas, causing deleterious effects on sexual reproduction in flowering plants. Heterospecific pollen transfer (HPT) is deleterious to both pollen donor and receipt species, suggesting that co-flowering species may have evolved adaptive strategies to reduce the effects of HPT. For example, heterospecific pollen carried by pollinators may not be picked up by stigmas of the next visiting plant species. To a better understanding pollen transfer network in natural communities, we need quantify the relationship between the three stages of pollination process, i.e., plant-pollinator interaction, pollen placement on the pollinator body and pollen receipt by stigmas. Our recent survey in an alpine meadow in the eastern Himalaya, southwest China, showed that plant species with more outgoing pollinator moves tended to disperse more of their own pollen to others, but plant species which received more pollinator interspecific moves received less heterospecific pollen. In the natural community, HPT varied from 0 to over 50% that misplaced pollen on stigmas was more than conspecific pollen in some species. We still know little about what factors affect the degree of HPT in natural communities and whether generalized and specialized flowers differ in donors and receipts of heterospecific pollen. We have quantified temporal variation of HPT in the alpine meadow involving over 30 co-flowering species for three years, to explore why some species could experience relatively higher HPT but other species do not. Pollen-transfer network was constructed by investigating pollen placement of the bumblebee body, which bees acted as the predominant pollinators. I will present recent results of pollination ecology at the community level from a field station in Shangri-La. These studies highlight the importance of understanding how co-flowering plants affect each other from multiple scales of pollination, improving our understanding of plant-plant interactions, interspecifc pollen flow, reproductive isolation and coexistence of diverse species.

T3-07-02

Tradeoffs of pollinator visitation rate and pollination quality

James Thomson
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I will review older literature that considered heterospecific pollen transfer as a component of the broader issue of plant competition for pollination service. In that view, the negative effects of competition would have a quantity component (visitation rate) and a quality component (including effects of heterospecific transfer). Both components are expected to vary spatially, depending on how much two plant species overlap and intermingle in space. However, much of the new literature on HPT has not explicitly considered spatial effects. Because it is hard to examine such effects in the field, I have modeled the process using artificial flowers that dispense and receive particles of food dye as analogues for pollen grains. Arrays of flowers are visited by bumble bees in flight cages. I will present data from experiments that simulate a species boundary between a nectar-rich plant species and a nectar-poor one. The nectar-poor species receives more visitation in the vicinity of the nectar-rich species, indicating a positive "magnet species effect, " but the increased visitation entails a cost of greater HPT.

T3-07-03

Ecological character displacement: Style length variation in *Pelargonium* driven by geographic mosaics of plant community structure.

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Geographically heterogeneous patterns of selection are thought to be important in generating intraspecific floral variation. Usually, geographic mosaics in the pollinator landscape are inferred as the primary agents of divergent selection on floral traits. However, it is also possible that geographic heterogeneity in plant community structure can give rise to divergent selection and consequent intraspecific floral variation. Here we investigate ecological character displacement as the possible driving force behind floral variation in *Pelargonium* communities. First, we demonstrate that the co-occurrence of *Pelargonium* species may have negative consequences in terms of interspecific pollen transfer. In particular, we show that the precise area of pollen placement on the body of the pollinator is determined by the amount of style exertion. Using visitation experiments, we demonstrate that individuals from different species experience high interspecific pollen transfer if they have similar style lengths. In contrast, individuals from different species experience less interspecific pollen transfer if they have very different style lengths. Using crossing experiments, we also show that interspecific pollen transfer has negative consequences on seed set, suggesting that co-occurring species should evolve style length differences in sympatry to reduce interspecific pollen transfer. Patterns of style length variation over 22 different *Pelargonium* communities indicate that style lengths are always very different when species co-occur, however style lengths can be very similar when the same species occur in allopatric populations. Given the apparently strong selection pressure for competition to drive style length divergence in sympatry, we suggest that character displacement is the most parsimonious explanation for the observed patterns of style length variation in a guild of long proboscid fly pollinated *Pelargonium*.

T3-07-04

Costs of interspecific pollen transfer for Neotropical bat-pollinated flowers

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Although competition for pollination is often invoked as a driver of broad-scale evolutionary and ecological patterns, we still lack a clear understanding of the mechanics of such competition. When flower visitors alternate between two species of flower, heterospecific pollen transfer takes place. The impact of these mixed loads on the female reproductive success of a recipient has received considerable attention, but the concomitant loss of male reproductive success—due to pollens grains being lost to foreign stigmas—has received less. Furthermore, pollen losses are not limited to grains that land on stigmas, but can also include deposition on non-stigmatic surfaces of the intervening flowers, or loss from the animal's body through passive detachment or active grooming. Here I quantify pollen transferred by nectar bats between focal flowers (*Aphelandra acanthus*) with and without intervening visits to one of two competitor species. One competitor (*Centropogon nigricans*) places its pollen in the same region of bats' heads as the focal species, while the other (*Burmeistera sodiroana*) places its pollen farther forward. I found that 1) any intervening visit caused some reduction in the number of pollen grains transferred, 2) competitor flowers with similar pollen-placement locations caused greater reductions in pollen transfer, and 3) of these competitors, those in male-phase (dispensing pollen) caused greater pollen loss than those in female-phase (without pollen). This study provides rare empirical support for the detrimental effects of competition for pollination on male fitness via pollen misplacement, and is the first to show an added cost imposed by male-phase competitors. Although this competition is especially strong when competitors overlap in pollen placement, diverging in pollen placement will not completely eliminate pollen loss during visits to foreign flowers simply because pollen sheds or is groomed from pollinator's bodies at some background rate over time. This suggests that any angiosperms that share pollinators face a pervasive selective pressure through male fitness to diverge in floral traits, alleviating competition by attracting different pollinators, altering floral phenology, or encouraging floral constancy.

T3-07-05

Heterospecific pollen transfer alters distribution of closely related species through reproductive interference

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Heterospecific pollen transfer may reduce fitness of a recipient species, and this phenomenon is known as Reproductive Interference (RI). The most conspicuous property of RI is its positive frequency dependence, which stimulates its self-reinforcing impact via positive feedback. For example, when pollen of one species exerts adverse effect on seed set of another species, it reduces the number of descendants of the latter. We have focused on RI from an alien species on a native congener, and have studied how the alien species has altered the distribution of native species in *Taraxacum*. *Taraxacum officinale* was introduced to Japan in the early 1900s and it is now widely distributed throughout the country. Most of the species populations, including hybrids between the species and native species, are triploid and reproduce asexually, although they produce pollen. Some of the native *Taraxacum* species in Japan, on the other hand, reproduce sexually and are self-incompatible. The alien and native species share flower visitors, allowing heterospecific pollen transfer. Through a field survey and a hand-pollination experiment, we showed that RI from the alien to the native species was moderate to strong in regions where the alien species outnumbered the native species, and marginal where it did not. A simulation model using the field data revealed that the native species could be eliminated much faster if the alien species exert RI, compared to the situation without the RI. We also observed pollen tube behaviour following the heterospecific pollination on the native species, and concluded that RI from the alien dandelion might occur through pre-emption of the native ovules by the heterospecific pollen. RI should be considered when devising strategies to protect native biodiversity from invasive species.

T3-07-06

Keep the old, attract the new: Floral color change by plants for a full exploitation of site-faithful pollinators

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Floral color change—the retention of old, nonreproductive, rewardless, but unwilted flowers in an altered color—has been suggested as an adaptive strategy for plants to attract pollinators from a distance with an increased display while minimizing pollinator visits to nonreproductive, rewardless flowers using distinct coloration. However, considering that most visually oriented pollinators would respond similarly to these aspects of floral display, the reason for low prevalence of floral color change in nature is unclear. Here we briefly review our recent studies aiming to provide evolutionary explanations for the sporadic distribution of floral color change in angiosperms. First, based on spectral data for 219 flower species, we show that pollinators with UV-sensitive eyes can see color changes of flowers more often than humans. We then present the results from phylogenetically independent analyses suggesting that the occurrence of floral color change is consistently correlated with bee pollination. A plausible explanation for the association between floral color change and bee pollination may lie in the behavioral characteristics of bees—their tendency to forage within small areas and learn to make frequent returns to profitable plants or patches. As our laboratory experiments with bumble bees illustrate, plants with discolored old flowers receive persistent return visits from resident foragers by presenting a useful color guide to rewarding flowers, as well as they could attract the passersby by increasing the size of an entire display. Thus, floral color change can be considered as a strategy to fully exploit site-faithful pollinators such as bumble bees, by maintaining experienced foragers while attracting inexperienced ones. Moreover, our field data on color-changing and non-color-changing species in *Weigela* suggest that these advantages of floral color change could be highly context dependent. In natural habitats of the non-color-changing species, most insects visit flowers in an opportunistic way, making little or no return visits to the same plants or patches. This pattern holds even for bumble bees because the non-color-changing species often grows in large monoculture stands. In contrast, bumble bees are more site-faithful in habitats of the color-changing species, where small number of plants are intermingled with other co-flowering species that offer different levels of reward; in such situations, bumble bees focus on small areas containing more rewarding plants to maintain a high foraging performance. Based on these observations, we suggest that the color-changing species gains advantage from site-faithful bumble bees only in such multi-species plant communities, where interspecific competition for pollinators is intense as well as heterospecific pollen receipt could impose substantial fitness costs. More studies are needed to clarify whether this hypothesis could be extended to other angiosperm species with color-changing flowers.

T3-08: Ecology and evolution of plant mating systems (two sessions)

T3-08-01

Mating system and density-dependent effects on fitness in *Noccaea caerulescens*

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We use *Noccaea caerulescens*, a heavy metal tolerant Brassicaceae that relies in part on pollinators to set seeds, to test theories we developed of the evolution of flowering phenologies and selfing rates under pollinator constrains. These theories predict intermediate selfing rates will emerge in *N. caerulescens* via geitonogamy generated by pollinator foraging behaviour among flowers of the same plant. A corollary predicts that different pollinators select for different floral displays and selfing rates. We also expect individual variance of flowering time to amount to that at the population level due to expected large stochasticity in pollinator visitation rates. At the community level and because of competition among plant species for shared pollinators, we predict staggered species flowering phenologies. Using common garden experiments in spring 2016 and a factorial design crossing ecotypes (from normal versus metal-polluted soils) and pollination modes, we excluded autonomous selfing in *N. caerulescens* and showed that its fruit set is pollen limited, results almost equally from pollinators and wind, and is affected by neighbouring plant density (~1m). Using weekly observations during the springs of 2015 and 2016 of four natural populations growing on either normal or metal-polluted soil in Southern France (Cévennes), we tested for the effect of pollinator communities on mean population selfing rates, and the effect of pollinator visitation rates on both temporal variation in fitness and individual selfing rates. We estimated in each population (1) *N. caerulescens* plant densities at several spatial scales, (2) individual flowering phenologies of 12 *N. caerulescens* random focal plants and of all their neighbouring conspecifics in a 0.8 m radius, (3) temporal variation of seed and fruit sets of the 12 focal plants across the seasons, and (4) the composition and flowering phenologies at the species level of the plant and pollinator communities. Two consecutive years of bad weather prevented the estimation of direct effects of pollinator visitation rates. Mean population selfing rates ranged from 10 to 50%, and contrary to previous results, ecotypes showed divergent changes between years. Fruit set was less variable among plants in denser populations found on metal-polluted soils. We are currently estimating the temporal variation of selfing rates at the plant level, genotyping seeds along the main inflorescence of each of the 12 focal plants in each population and each year, to correlate it to variation in fitness components, flowering phenology, plant density and plant/pollinator communities.

T3-08-02

Sexual mimicry promotes outcrossing and multiple paternity in sympatric orchid species

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Most flowering plants engage animals to carry out the essential service of pollination. The behaviour of animals therefore exerts a profound influence on important microevolutionary processes such as mating and gene flow. However, our knowledge of exactly how different kinds of pollinators control plant gene flow patterns remains poor. Specialist pollination systems offer the most tractable systems to explore this relationship because observed patterns of plant mating can safely be ascribed to a single species or functional group of pollinator. This multidisciplinary study examines the consequences of highly specific sexually deceptive pollination in the mating and pollen dispersal patterns of two sympatric Australian orchids. First I describe a detailed and innovative study of behaviour and movement ecology for a species of wasp pollinator. Genetic studies of mating and pollen flow then provide evidence that pollination via sexual deception results in near exclusive outcrossing despite clonality, as well as multiple paternity–a rarity for orchids. In summary, multiple lines of evidence support sexually deceptive pollination as an adaptive solution to the problem flowers face of simultaneously attracting pollinators while persuading them to leave quickly.

T3-08-03

Pollinator-mediated constraints on the evolution of selfing

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Hermaphroditic plants can produce both selfed and outcrossed progeny, termed mixed mating. Evolutionary models of mating systems classically predict two outcomes, either complete selfing or complete outcrossing, depending on the balance of two genetic factors: the automatic gene transition advantage of selfing and inbreeding depression. If inbreeding depression is less than 0.5, complete selfing is predicted to evolve, otherwise complete outcrossing should be favored. The abundance of species with mixed-mating systems has thus presented something of a paradox. A longstanding question posed by plant scientists who study mating system evolution is: Why do high levels of selfing persist within populations despite high levels of inbreeding depression? In general, the evolution of autonomous self-pollination and its role in providing reproductive assurance when mates or pollinators are scarce have been raised as answers to this question and to explain the maintenance of mixed mating systems more generally. Instead, we turn the original question around to ask: Why do high levels of outcrossing persist in some populations despite low levels of inbreeding depression? We propose a new functional hypothesis: the evolution of autonomous self-pollination effectively results in an asymmetry in the benefit of plant-pollinator mutualism such that plants may not be able to escape visitation by pollinators. That is, while autonomously selfing plants are less reliant on their pollinator mutualists for seed production, pollinators may still strongly rely on specific species for pollen and nectar. This results in continued outcross pollen delivery, halting or hindering the progression of the evolution of high selfing. We will discuss evidence supporting our hypothesis, including data from a recent analysis of inbreeding depression from mixed mating species and their inbreeding depression levels (Winn *et al.,* 2011) and the constraints that plants may face in circumventing pollination mutualism relative to other types of mutualisms. We conclude by offering future directions for testing predictions from this "persistent pollinators" hypothesis. This perspective can provide novel insight on the evolutionary transitions between outcrossing and selfing and the maintenance of mixed mating despite low inbreeding depression.

T3-08-04

Selfing as an evolutionary dead-end? Insights from a model of inbreeding depression due to stabilizing selection on quantitative characters

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Phylogenetic analyses suggest that self-fertilization is an evolutionary dead-end, characterized by higher rates of extinction than in predominantly outcrossing taxa, but maintained by frequent evolutionary transitions from outcrossing to selfing. Higher rates of extinction of highly selfing taxa are thought to be related to lower rates of adaptation, but the details of the mechanisms are still largely unknown. Here we explore an alternative explanation in a constant environment, by analyzing the maintenance of quantitative genetic variance in a mixed-mating system of self-fertilization and outcrossing. We consider two models, in which purely additive genetic variance is maintained by mutation and recombination under stabilizing selection on the phenotype of one or more quantitative characters. The two models have contrasting architectures of quantitative characters. Our approach differs from previous theory by distinguishing lineages according to the number of consecutive selfing events in their ancestry since their last outcross. We examine how equilibrium genetic variance, and the resulting inbreeding depression, varies across selfing rates. In both models, at low selfing rates the equilibrium genetic variance of characters remains almost the same regardless of the selfing rates, maintained by compensatory effects of (1) an increase in genic variance with increased selfing rates caused by increased homozygosity and (2) stabilizing selection, creating negative covariance among loci. Above a threshold selfing rate, the value of which depends on the number of characters under selection, the equilibrium genetic variance declines and reaches a "purged equilibrium". In one model, the purged equilibrium also exists at low selfing rates, and the purging of the genetic variance and inbreeding depression is associated with an accumulation of high genic variance, hidden by large negative covariance. This hidden variance is released in outcrossing events, which prevents a transition from selfing to outcrossing. The implications for evolution of selfing rates, and for adaptive evolution and persistence of predominantly selfing species, provide a theoretical basis for the classical view of Stebbins that predominant selfing constitutes an "evolutionary dead end."

T3-08-05

Population-genetic expectations for trait filtering of self-incompatibility on islands

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Environmental and genetic factors play a critical role in the ecology and evolution of reproductive traits in nature. A major goal of modern biology is to provide mechanistic explanations for non-random patterns. The most common evolutionary trend in flowering plants is the transition from obligate outcrossing to selfing. Indeed, about half of all angiosperm species have self-incompatible (SI) breeding systems but this condition is frequently and irreversibly lost, producing self-compatible (SC) lineages. The hypothesis of reproductive assurance attributes this pattern to the advantages of selfing when mates or pollinators are difficult to find. The colonization of oceanic islands has long been heralded as an iconic exemplar of this idea, since island plant communities maintain more SC plants. Baker was impressed by this pattern and suggested, "With self-compatible individuals a single propagule is sufficient to start a sexually reproducing colony.." (Baker 1955). However, it is theoretically unclear whether island breeding systems reflect extinction of colonists or adaptive evolution after arrival. To address these hypotheses, it is necessary to model island colonization and explicitly study the costs of self-incompatibility: extinction and breeding system evolution. We consider an island-mainland model with evolution at the S-locus and dynamic inbreeding depression at many loci throughout the genome. First, we treat the probability of extinction by reproductive failure in purely SI island colonists, assuming they originate from mainland populations at equilibrium. Analytical results show that unless the number of colonists and S-alleles are both very low, immediate extinction of SI colonists is unlikely given adequate pollinator availability. However, with continued sampling in recently founded SI island populations, extinction rates increase when population growth is slow. Second, we consider polymorphism at the S-locus and show that SC mutations are often maintained at low frequencies in mainland populations. While the presence of SC mutations at the S-locus slightly reduces the chances of reproductive failure immediately after colonization, this advantage is offset by the low probability of their inclusion during founding. These results highlight several important points. With few island colonists, SC mutations are often not sampled, and extinction eliminates SI populations in short order. With more colonists, extinction rates decline but the spread of SC mutations is favored unless population growth is rapid and sufficient diversity at the S-locus is present. This work clarifies the spectrum of costs that self-incompatible lineages face on islands and quantifies their importance beyond the verbal appeal of Baker's rule.

T3-08-06

Interactions between drift and selection in the evolution of plant mating systems

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Typically, mating system variation is explained by hypotheses focusing on the benefits of selfing, such as reproductive assurance, that may vary with ecological context. However, mating system evolution may also be driven by historical changes in genetic load. For example, colonization from glacial refugia to current distributions is expected to shape population genetic structure. Increases in homozygosity will reduce cost of inbreeding and create conditions permissive for the evolution of selfing. Here we bring together studies of the contemporary pollination environment as well as an evaluation of genetic structure due historical colonization events to explain range-wide variation in mating system in the insect pollinated herb, *Campanula americana*. 24 populations sampled from throughout the range from putative southern refugia north to the range edge were grown in a pollinator-free environment. Autonomous selfing increased in populations that were further north and west in the range. Decreased duration of male phase (dichogamy) in this protandrous species was associated with increased selfing while herkogamy was not. The same 24 populations were evaluated for pollen limitation and reproductive assurance in each of two years. Pollen limitation was greater for populations located in northern regions of the range and these populations had fewer bumblebee pollinators. Despite substantial levels of autonomous fruit set under pollinator-free conditions, no reproductive assurance was found in natural populations. These results will be combined with ongoing work in the same focal populations estimating selfing rates in nature, inbreeding depression following one generation of self fertilization, and patterns of genetic diversity and genetic load across the range. Data to date indicate variation in mating system among *C. americana* populations is more tightly associated with geography and genetic structure due to post-glacial migration than pollinator abundance and pollen limitation. Such historic processes are likely to be common across many temperate taxa, and create broad expectations for range-wide patterns in mating system.

T3-08-07

Reinforcement and speciation between outcrossing and selfing *Clarkia* species

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Studies of reproductive isolation often find that prezygotic barriers evolve more rapidly than postzygotic barriers between incipient species. However, it has been challenging to determine whether selection has directly caused elevated isolation (reinforcement) or whether is has occurred as a by-product of adaptation to alternative environments. In plants, pollinator isolation has commonly been found to influence reproductive isolation. However, little is known about how mating system divergence may contribute to the speciation process. In both cases, few studies have established whether natural selection can directly enhance reproductive barriers via reinforcement. In *Clarkia xantiana*, there are two subspecies that differ in mating system despite both being self-compatible. One subspecies, *xantiana*, is primarily outcrossing and bee pollinated, with pronounced dichogamy and herkogamy. The other, *parviflora*, primarily autonomously self-fertilizes, with minimal dichogamy and protandry. The self-fertilizing taxon is monophyletic and recently derived from the primarily outcrossing taxon (~65,000 yrs) and phylogeographic studies show that they have come into secondary sympatry in a narrow zone. Field experiments assessing male and female mating success under pollen limitation have shown evidence of disruptive selection where divergent mating systems are favored by selection, whereas intermediate phenotypes suffer reduced fitness. Moreover, there is a partial postzygotic barrier to the production of F1 hybrids. Across the range of the two subspecies, there is a pronounced pattern of reproductive character displacement (RCD) where floral traits are most strongly diverged in sympatry and only minimally diverged between the most allopatric populations. This is a key signature of reinforcement. We used field experiments pairing sympatric and allopatric genotypes to test whether reinforcement selection has directly caused the evolution of RCD in floral traits. Our results indicate that hybridization between incipient species is strongly reduced between sympatric genotypes but is approximately twice as likely when allopatric genotypes of the two taxa are paired, consistent with the reinforcement hypothesis. By contrast, we found no evidence that floral evolution has occurred as by-product of adaptation to contrasting pollination environments in the allopatric and sympatric regions. These results are novel in demonstrating a contribution of both reinforcement selection and mating system divergence to the speciation process.

T3-08-08

Why do plant mating systems often vary widely among populations?

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Classical genetic models predict that disruptive selection on outcrossing rate will cause mixed-mating to be rare and transitory. To more fully characterize the extent of among-population variation in outcrossing, we surveyed all published microsatellite and allozyme studies reporting multilocus outcrossing rates (t_m) for three or more natural populations of hermaphroditic Angiosperms. Our dataset includes t_m values for 753 populations from 105 species. These taxa are distributed across 43 families and 80 genera. Many of the species in our survey exhibit substantial among-population variation in outcrossing rate. For 32/105 species the range between the lowest and highest t_m was > 0.3. Nearly two-thirds of all species have at least one mixed-mating population. Given this marked variation among populations, there is a critical need for studies that explore how both ecological context and heritable differences in floral traits influence mating system phenotypes. For mating systems to evolve, there must be heritable variation for mating system traits within natural populations. Nearly all studies estimate outcrossing rates for entire populations, rather than for individuals, because estimates for individuals often have large error due to violations of assumptions about the local pollen pool and statistical limitations of the maximum likelihood procedure. However, if there is sufficient genetic variation at marker loci to facilitate unambiguous paternity assignment to all sampled offspring, it is possible to quantify individual outcrossing rates, and to explore relationships between outcrossing rate, siring success, and pollen discounting. We are using this approach to characterize individual variation in outcrossing rates within and among populations of *Mimulus ringens*, a wetland perennial native to eastern North America. By clonally replicating genets and exposing them to different ecological contexts, we can explore how changes in the pollination environment influence the pattern of selection on the mating system. We are complementing these experimental studies with studies of natural populations that address whether rates of selfing and levels of inbreeding depression correlate with natural variation in ecological context and floral display.

T3-08-09

Consequences of clonal growth on plant mating systems

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Clonal growth is widespread among angiosperm species, so many plants have a mixed reproductive strategy of both sexual and asexual reproduction. Clonality has been suggested to negatively impact aspects of sexual reproduction, especially mating patterns. Importantly clonality could reduce female fitness, owing to among-ramet self-pollination (geitonogamy), especially in species with clumped ramets that display many flowers simultaneously. However, most previous studies compared the effects of variation in clone size, rather than between clonal and non-clonal individuals with the same resource investment in floral display. We investigated this issue experimentally with bumble bees foraging on artificial flowers and through computer simulations. Individual bees visited fewer flowers on plants with multiple inflorescences than on plants with a single inflorescence with the same total number of flowers. In simulations with all flowers receiving and donating pollen, this visitation behavior caused less geitonogamy for plants with clonal growth. Thus, when flowers simultaneously receive and donate pollen clonality can promote pollination quality without increasing geitonogamy. However, a single large inflorescence was more advantageous if segregation of sex roles among flowers (dichogamy or monoecy) reduced geitonogamy. To examine the influence of clonality on pollinator foraging and mating we investigated natural populations of *Aconitum kusnezoffii* and *Delphinium glaucum*, both of which produce clumped clones with protandrous flowers that are visited by bumble bees. On *Aconitum kusnezoffii*, bumble bees primarily moved between flowers within ramets. As a result, autogamy, within-ramet, and between-ramet geitonogamy accounted for 12%, 68%, and 20% of the selfing rate, respectively. In *Delphinium glaucum*, unmanipulated genets with two ramets outcrossed less than those for which we tied the ramets together to produce a single large inflorescence. The results from natural population of *Aconitum kusnezoffii* and *Delphinium glaucum* were consistent with the results of our simulations and demonstrated that a single large inflorescence can be more advantageous than multiple flowering ramets for dichogamous plants. Future research on the mating consequences of clonality should address the combined effects on female and male function and the influence of clonal architecture. Comparison between clonal and non-clonal sister taxa and manipulative experiments of display characteristics would be of particular value in identifying the influence of the distribution of floral resources within and among ramets on plant mating patterns.

T3-08-10

The evolutionary breakdown of heterostyly to homostyly in *Primula chungensis* (Primulaceae)

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The evolutionary pathway from predominant outcrossing to high levels of self-fertilization has been followed in numerous taxa of herbaceous flowering plants. A classic example of this mating system transition involves the evolutionary breakdown of the sexual polymorphism heterostyly to a monomorphic condition. This evolutionary change is of particular value for studies of mating system transitions because the origin of selfing in heterostylous groups generally has a simple genetic basis and is usually followed by a characteristic sequence of changes to the floral morphology and morph structure of populations. Here we address three questions on this topic involving the population genetics and floral biology of the breakdown of distyly to selfing in *Primula chungensis* (Primulaceae). This species of primrose is native to SW China and is unusual among heterostylous plants in possessing both rare distylous and common homostylous populations. The specific questions addressed in our study are: 1) Is there evidence that homostylous populations are derived from distylous populations, and if so has the transition occurred more than once in different parts of the geographical range? 2) What are the patterns of floral variation following transitions from outcrossing (distyly) to selfing (homostyly) and how are these likely to influence mating patterns? We genotyped plants from 20 populations throughout the entire range, using the chloroplast intergenic spacer region (*trn*L-*trn*F), nuclear ribosomal ITS, and 10 nuclear microsatellite loci and determined genetic relationships among populations and variation in floral traits associated with homostyle evolution. The marker data consistently identified two multi-population lineages (Tibet and Sichuan) and one single-population lineage (Yunnan), a pattern consistent with at least two independent origins of homostyly. Evidence from flower and pollen size variation indicated that transitions to selfing have likely arisen by the same genetic mechanism, although the two main lineages have followed divergent evolutionary pathways resulting in populations with both approach and reverse herkogamy. The evolution of herkogamy may limit opportunities for predominant selfing and small flowers to evolve in homostylous populations. Our results provide several new insights into the historical processes governing the breakdown of distyly to selfing and on the trajectories of floral evolution in selfing populations.

T3-08-11

Variation in mating system, life history and floral traits of *Incarvillea sinensis*, a species with annual and perennial populations

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Flowering plants display considerable variation in mating system, specifically the frequency of cross- and self-fertilization. This diversity is distributed non-randomly with respect to life history. Annual species commonly exhibit high selfing rates whereas perennial species are more often outcrossing. Few studies have explicitly investigated the correlated evolution of life history, floral biology and mating system because species that maintain variation among populations in these characteristics are rare. Here, we present results of investigations on variation in mating system and floral traits in *Incarvillea sinensis* (Bignoniaceae), a bee-pollinated species with annual and perennial populations. We also examined seasonal variation in the mating system of *I. sinensis* to investigate the influence of abiotic and biotic environmental factors on day-to-day variation. *Incarvillea sinensis* has a broad geographical range in China and we surveyed 16 populations across seven provinces spanning 2,000 km of the range. In each population, we measured five floral (corolla length and width, corolla tube depth, distance between stigmas and anthers, daily floral display) and two life-history traits (plant height and branch number) and collected open-pollinated seed families and data on population size and density. Of the 16 populations seven were annual, eight were perennial, and one contained annual and perennial plants. Annual populations were distributed in more northern localities and perennial populations were distributed in southern locations. In the mixed population, daily floral display, corolla tube length, stigma-anther separation and corolla length and width were greater in perennial than annual plants. Among remaining populations, corolla length, corolla tube length, stigma-anther separation and branch number in perennial populations were greater than annual populations, whereas plant height and daily flower number were greater in annual populations. Our studies of mating patterns are in progress but we predict, based on theory and empirical evidence from other species, that annual populations are likely to exhibit higher selfing rates than perennial populations. Our investigation of seasonal variation in mating patterns in an annual desert population from Mu Us Sand Land, Inner Mongolia, revealed the dynamic nature of mating. Flower density increased significantly over most of the 50-day flowering season, but was associated with a decline in pollinator service by bees, particularly on windy days. Multilocus estimates of outcrossing rate indicated high selfing rates of ~80%. There was evidence for a significant increase in selfing as the flowering season progressed and pollinator visitation declined. Biparental inbreeding also declined significantly as the flowering season progressed. Delayed selfing by corolla dragging largely explains the occurrence of mixed mating in this population of *I. sinensis,* and this mode of self-fertilization probably functions to promote reproductive assurance when pollinator service is limited by windy environmental conditions and competition from co-occurring flowering plants. The striking geographical variation in life history and floral traits in *I. sinensis* provides outstanding opportunities to examine the demographic and environmental factors causing mating system variation and provides a suitable model system for investigating the correlated evolution of life history and mating patterns.

T3-08-12

Pollen dispersal distance within a population is influenced by the frequency of females in the gynodioecious geranium, *Geranium maculatum*

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Breeding systems in the flowering plants are highly diverse – species ranges from being entirely hermaphroditic with perfect flowers as the only floral form to being dioecious such that every plant has either pistillate or staminate flowers only with many different combinations of floral forms within or between individuals existing between these two extremes. A key evolutionary question is, thus, to understand how such vast diversity in the flower sexual expression (breeding systems) has evolved and been maintained in nature. A powerful approach to address this question is through studying systems that are polymorphic in floral sexual expression - such as gynodioecy, a system considered to be the most important intermediate evolutionary stage from hermaphroditism to dioecy. In gynodioecious species, male-sterile (i.e. Female; with pistillate flowers only) genotypes are found to coexist with hermaphrodites (with perfect flowers) in some natural populations. We chose to study the interactions between the gynodioecious wild geraniums, *Geranium maculatum,* and their pollinators. Specifically, we are testing the hypothesis that because pistillate flowers do not produce pollen rewards and are much smaller than hermaphroditic flowers, their presence in a population will influence the flight pattern of the pollinators and, hence, influence the pollen dispersal distance. To test this hypothesis, we sampled seeds of 10-20 maternal plants from 10 populations that range from 0-35% females. We genotyped both seeds and maternal plants for 10 microsatellite loci in each population. Using the software POLDISP, we estimated the average pollen dispersal distance in each population to range from 1.2 m to 1.7 m. In addition, we found that the presence of females in a population significantly reduces average pollen dispersal distance. We will discuss this result in the context of how the presence of female individuals may change not only pollinator behavior but also the patterns of population genetic structures – both at the within-population fine-scale and across a larger geographical region.

T3-09: Seeds and climate change

T3-09-01

Climate warming and regeneration from seeds in cold adapted plants

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Climate has a large influence on plant recruitment. Understanding the impacts of climate change on regeneration from seedlings is therefore urgently needed to predict future plant population dynamics and migration. Moreover, because of the substantial variation in both genetic and phenotypic plasticity of seed dormancy, germination and longevity regeneration from seeds is expecting to play a crucial role in plant adaptation to climate change. In this context, arctic and alpine ecosystems are considered highly threatened by climate change because they are predicted to experience above-average warming and because they are characterized by species adapted to low temperatures. Consequently, an increasing number of studies have been addressing the effects of climate warming on different life-history traits and functions of plants in these ecosystems, including seed germination and longevity responses. Here we present the results of several studies investigating the effects of pre- and post-dispersal climate warming on seed dormancy release, germination requirements and seed longevity across alpine plants. We show that climate warming could change the germination phenology and the longevity of seeds of alpine plants both directly (i.e. after dispersal) and indirectly (i.e. via parental effects), by shifting the timing of emergence from spring to autumn, by increasing the proportion of emerged seedlings and by extending the seed resistance to heat stress. Strictly cold-adapted chorotypes were more affected by warming, the extent to which was linked to seed dormancy traits: with deeply dormant species showing major changes in response to germination temperature and less dormant species in response to winter vernalization. The most significant aspect relating to warming on alpine ecosystems dealt with the extension of the snow-free period in spring that enhanced recruitment success, at least in the short term. We conclude that range shift and/or changes of local dominance might occur in response to changes in recruitment success. The overall success will be dependent on interactions with range shifts in new species (i.e. more thermophilous plants). In this context, transgenerational plastic adjustments of seed traits might play a crucial role in future plant adaptation to climate change.

T3-09-02

Thermal sensitivity of seed germination to a changing climate

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Seed germination is a critical step in the natural regeneration of the majority of the world's flora. Germination is highly regulated by environmental temperature, yet the sensitivity of germination to climate change has been quantified for relatively few species. Thermal time models are one tool to quantify the response of species to temperature. These seed population based models use germination progress over a range of thermal environments to establish thresholds for germination. This approach enables comparative studies of germination responses between species and habitats, and when related to the growing environment, are fundamental to understanding germination niche in the context of climate change. Examples are given where thermal time approaches have provided an overview of plasticity in the thermal germination phenotype, including for the Cactaceae family, and for a range of other species including crop wild relatives. The limitations of seed population based models are also discussed. Revealing the extent of sensitivity or resilience to environmental change has identified descriptors of germination plasticity that are critical for assessing how well species may cope in the future. This information is currently being used to identify species most at risk and to predict the proportion of species that will be "winners" and "losers" as a result of climate change.

T3-09-03

Climatic sensitivity of early life stages: Plant traits as indicators of environmental tolerance range

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Plant sensitivity to climate change will be dictacted in part by the range of conditions a group of organisms can tolerate for any given environmental variable (e.g., temperature, water availability). Those with a broad environmental tolerance range (TR) are expected to be buffered against changing climate–at least in the short term–while those with a narrow TR are predicted to be more sensitive. However, TR varies across life stages, species, and presumably even populations. Recently, early plant life stages (e.g., seed dormancy break, germination, and seedling establishment) were identified as more sensitive to climate change than adult stages. However, traditional approaches to forecasting plant responses to climate change are largely based on the geographic distribution of a species, which does not explicitly represent the TR of early life stages, potentially overlooking a large bottleneck to plant recruitment. I will present results from two germination studies investigating variation in TR both among species and, importantly, populations. All study species possess physiologically dormant seeds, but differ in key traits expected to influence genetic neighborhood size, and thus environmental TR. One study examined three common forb species (an *Asclepias*, *Physalis*, and *Penstemon*) with differing seed dispersal mechanisms (wind, animal, gravity). The second study examined three congeners (*Asclepias* spp.) with wind-dispersed seed that vary in multiple other traits relevant to gene flow, including pollination syndrome, breeding system, habitat specificity, and degree of clonality. Multiple populations of each study species were collected along a latitudinal gradient throughout the Midwest United States. A subset of populations included in the first study were collected over two subsequent years. Tolerance ranges were calculated based on germination proportion across a range of treatments manipulating cold stratification length, cold stratification temperature, and incubation temperature. Consistent with our hypotheses, both studies found average TR breadth reflected presumed genetic neighborhood size for each species based on known plant traits; however, there was relatively little difference in TR between wind and animal dispersed species. In the first study, TR shifted, but not consistently between collection years: TR of *Asclepias* populations decreased from Year 1 to Year 2, while TR of *Physalis* populations increased. In the second study, two of the three *Asclepias* taxa exhibited a negative linear relationship between TR breadth and collection latitude, while one showed no relationship. Interestingly, this trend was not present in the *Asclepias* populations included in the first study. Taken together, our findings suggest that known plant traits associated with gene flow via seed or pollen dispersal may serve as effective indicators of germination tolerance range; however, TR is readily influenced by local climate, interannual climatic variation, and presumably other factors. Results from this research have direct implications for forecasting plant responses to climate change, substantially improving current predictive, species distribution models by incorporating environmental limitations of early life stages, as well as population-level variation.

T3-09-04

Are seeds better physically defended towards the equator?

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There have been heated debates on the existence and generality of the latitudinal gradient in biotic interactions. Empirical studies and data syntheses have shown that seed predation is not more intense towards the tropics. One possible explanation for this finding is that seeds may be better defended at low latitudes than at high latitudes. Our goal was to provide the first broad-scale, quantitative analysis of the latitudinal gradient in physical investment in tissues surrounding seed reserve. We measured the biomass ratio of protective tissue to seed reserve for 250 species-site combinations ranging from 15°30'S to 43°35'S along the east coast of Australia. Contrary to expectations, we found that the proportional investment in tissues surrounding seed reserve was greater towards high latitudes in seeds exposed to pre-dispersal seed predators, while seeds exposed to post-dispersal seed predators did not show a latitudinal gradient in the proportional investment in tissues surrounding seed reserve. The physical investment in tissues surrounding seed reserve was not correlated with the level of seed predation, at either the pre-dispersal or the post-dispersal stage. Fleshy-fruited species invested proportionally less biomass in tissues surrounding seed reserve while suffering less pre-dispersal seed predation. Our findings indicate that natural seeds in their natural habitats are not better defended at lower latitudes. Together with recent studies of latitudinal gradients in seed predation, leaf herbivory and defence, this study adds to the growing evidences that challenge the idea of a declined latitudinal gradient in biotic interactions, and therefore cast doubt on our current understanding of the factors as plant-pest interactions in driving the latitudinal gradient in plant diversity. Our findings also demonstrate a defensive role of fleshy fruits in deterring pre-dispersal seed predators.

T3-09-05

Climate change on seedling germination and growth of *Larix principis-rupprechtii* and *Pinus tabulaeformis*

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Early development stages of trees are expected to be more sensitive to climate change, and represent a major bottleneck to plant community recruitment. In North China, drought index had increased dramatically from 1990s, understanding on seedling's response to anticipated climate changes is urgently needed. *Larix principis-rupprechtii* and *Pinus tabulaeformis* are two dominant coniferous tree species in North China, and play significant ecological roles in water conservation. We collected seeds of these two species from Guandi Mountain, Shanxi Province, and conducted a controlled greenhouse experiment to investigate how climate changes (warming and precipitation change) would affect their seedling emergence and growth. Three levels were set for both temperature and water supply. Temperature levels were monthly mean temperature (T_0) over the past 15 years (1999-2013), increased by 2 °C (T_1) and increased by 6 °C (T_2). Water supply levels were monthly mean precipitation (W) over past the 15 years (1999-2013), reduction by 20% (W^-) and addition by 20% (W^+). A two-way ANOVA was used to compare seedling growth (including seedling germination percentage, needle length, height, length of main root, number of lateral root, and length of lateral root) between the two treatments to the species. If the interaction was tested to be significant ($P < 0.05$), one-way ANOVA was used to test the effects of water treatments on seedlings indexes, and multiple comparison was used otherwise. The least significant difference method (*LSD*) was used to test the significant differences of all data at level of 0.05. Seed germination of two species was affected by temperature and precipitation changes. Seedling stable germination time reduced and seedling germination percentage improved by increased temperature of 2°C and water addition. However in the condition of increased temperature of 6 °C or water reduction, seedling stable germination time delayed and seedling germination percentage decreased. Seedlings of the two species presented different responses to the climate change. *L. principis-rupprechtii* was affected more greatly by warming and drying climate than *P. tabulaeformis*. Seedling morality of *L. principis-rupprechtii* increased sharply and seedling height decreased by higher temperature and water reduction. The changes from increased temperature or precipitation showed no significant effects on seedling survival and aboveground-growth of *P. tabulaeformis*. Underground growth of both *L. principis-rupprechtii* and *P. tabulaeformis* increased, indicating by longer primary and lateral roots. Soil drought was induced by dramatically increased temperature and reduced precipitation, which resulted in adaptive strategy of the two species by reducing aboveground growth and giving priority to root growth. Seedlings showed an interspecific variation of response to water deficit. *L. principis-rupprechtii* was more sensitive to climate change than *P. tabulaeformis*. The regeneration of *L. principis-rupprechtii* was inhibited by warming and drying climate. The seedling growth was negatively affected by highly increased temperature in growing period, and it could not be improved by water addition.

T3-09-06

Project Baseline: A time capsule of seeds to study evolution

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Project Baseline is a new research-grade seed bank established in 2011-2015 that offers an unprecedented opportunity to study spatial and temporal dimensions of microevolution during an era of rapid environmental change. This seed bank is specifically designed to be used over the upcoming 50 years for research that employs the "resurrection approach". This kind of research entails withdrawing ancestral seed from the seed bank, recollecting contemporary seed from the same locations, and then growing antecedent and successor plants side-by-side to directly observe evolutionary change. Project Baseline includes seeds from 65 native and nonnative species collected from an average of 15 populations across their respective species' ranges in the continental USA in sites that are likely to be preserved into the future (see http://baselineseedbank.org/ for more detail). More than half of the species include collections from sister taxa. Seeds were collected from multiple species at 100 sites, providing rich resource for testing evolutionary hypotheses. The seeds are stored by maternal line (100-200 lines per population) under conditions that maximize seed longevity at the ARS USDA National Center for Genetic Resources Preservation in Ft. Collins, CO and are accessioned with site and population data. This open-access resource will be available to researchers at regular intervals to evaluate contemporary evolution with the first call for proposals anticipated in 2019. Our expectation is that research using the Project Baseline collection will vastly improve our understanding of temporal and spatial changes in phenotype and the evolutionary processes underlying it.

T3-10: Plant traits under global change: from traits-environment-ecosystem function relationships to trait-based vegetation modelling (two sessions)

T3-10-01

Towards a trait-based universal model of terrestrial primary production

Han Wang[1,2], *Colin Prentice*[1,2, 3], *Ian Wright*[2], *Trevor Keenan*[2,4], *Tyler Davis*[3,5], *William Cornwell*[6], *Changhui Peng*[1]
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3. Imperial College
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Based on well-established ecophysiological principles and consideration of the adaptive significance of traits, we propose universal relationships between photosynthetic traits (carbon fixation capacity, and the ratio of electron transport capacity to carbon fixation capacity) and primary environmental variables, which capture observed trait variations both within and between plant functional types. Moreover, incorporating these traits into the standard model of C_3 photosynthesis allows gross primary production (GPP) of natural vegetation to be predicted by a single equation with just two free parameters, which can be estimated from independent observations. The resulting model performs as well as much more complex models. Our results provide a fresh perspective with potentially high reward: the possibility of a deeper understanding of the relationships between plant traits and environment, simpler and more robust and reliable representation of land processes in Earth system models, and thus improved predictability for biosphere-atmosphere interactions and climate feedbacks.

T3-10-02

Sources and ecological consequences of variation in bark traits

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The study of variation in plant traits, as a tool for understanding and predicting vegetation composition and ecosystem functions, is still hot. However, compared to other plant organs, bark traits have not received the research attention they deserve based on the important functions of bark for woody plants. I will first focus my talk on key aspects of variation in bark traits between and within species, including the role of ontogeny. Then I will discuss the various pathways by which variation in bark traits affects carbon cycling, from the role of bark in carbon gain and transport, in protecting carbon-rich tissues in the xylem against biotic attack and abiotic stress, and from its roles in carbon release. I will give empirical examples of how bark trait variation, through afterlife effects on bark litter, can be important for the two main carbon release pathways, i.e. decomposition and fire regimes. These bark trait afterlife effects can be direct, via the bark decomposability and flammability of different species, and indirect, by influencing the decomposition and flammability of the wood inside the bark. I hope to convince the audience that the study of bark traits should be high on the agenda for various woody ecosystems in the world.

T3-10-03

Quantifying and modelling the responses of leaf gas exchange to drought: From traits-drought function relationships to trait-based vegetation modelling

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Global climate change is expected to increase drought duration and intensity in certain regions while increasing rainfall in others. The quantitative consequences of increased drought for ecosystems are not easy to predict. Process-based models must be informed by experiments to determine the resilience of plants and ecosystems from different climates. This study provides quantitative information aimed at improving land surface models (LSMs). It includes four papers. (1) Responses of leaf-atmosphere gas exchange to short-term drought were analysed, across plant functional types and climates, based on a synthesis of previous experiments. Explicit and consistent definitions of stomatal *versus* non-stomatal responses were adopted. Both types of response were shown to be important, and plants adapted to arid climates responded very differently from others. (2) Parallel responses of stomatal conductance, mesophyll conductance, and photosynthetic capacity were found in two glasshouse experiments with tree species from Australia and Europe, revealing a common, co-ordinated pattern of increasing tolerance in plants from drier environments. (3) Xeric and riparian species of *Eucalyptus* were subjected to short- and long-term drought. The species were found to differ not only in their tolerance for short-term drought, but also in the extent to which they could acclimate to long-term drought through morphological adjustments. (4) Experimentally based drought responses were used to define new, plant type-dependent relationships of stomatal sensitivity and photosynthetic capacity to soil water potential in the Community Atmosphere Biosphere Land Exchange (CABLE) LSM. Comparison with CO_2 and latent heat flux measurements from eddy covariance flux measurement sites in Europe during the 'heatwave' year of 2003 showed that discrepancies between model results and observations were not substantially improved by the inclusion of the more realistic functions, due to a persistent positive bias in the model's simulation of evapotranspiration which overshadowed the differences between different representations of drought response functions.

T3-10-04

TRY – a global database of plant traits

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The global database initiative named TRY (www.try-db.org) was established in 2007 and since then has united a wide range of the plant trait research community worldwide. This talk will briefly introduce the TRY initiative and then present recent key developments based on the unprecedented buy-in of plant trait data, i.e. ongoing efforts to characterize the global spectrum of plant form and function, to better understand trait-environment relationships and to provide global maps of traits relevant for ecosystem function.

T3-10-05

Trait-based reconstructions of palaeoclimate

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Leaf size and margin dissection have been used to reconstruct palaeotemperature from fossil leaf assemblages from many regions of the world, but reconstruction uncertainties are very large and show the confounding effects of other aspects of climate. We have used logistic regression to investigate the relationship between a suite of 25 leaf morphometric traits and summer temperature, plant-available moisture and temperature seasonality and the frequency of 25 leaf morphometric traits, based on data from 96 sites from the China Plant Trait Database sampling the range of climate and vegetation types found in China. Variations in the frequency of leaf morphometric traits can be explained as a response to gradients in summer temperature, plant-available moisture and temperature seasonality. These analyses identify distinct syndromes of morphometric traits associated with the carbon cost of leaf formation are recognized: warm, wet climates with low seasonality favour broad leaves that are evergreen, large, tough, thick, and dark in colour; dry, warm climates with high seasonality favour small, fleshy, glaucous leaves of intermediate thickness; cold, wet climates of high seasonality favour needle leaves that are evergreen. Leaf size and margin dissection are influenced by both temperature and moisture gradients. However, some individual traits (e.g., surface patterning, rugosity) show strong relationships with temperature but not moisture gradients, while others (e.g., hairs, drip tips) are related to moisture gradients but not temperature. Many specific relationships between traits and bioclimate variables are conservative across all woody life forms, and often between woody and non-woody life forms. Given that the observed variations in morphometric traits along climate gradients are both predictable and physically understandable, we then use the climate-leaf trait relationships established for the present day to build predictive models for estimating the temperature seasonality and moisture availability. We apply these models to predict the climate using fossil leaf floras from the Fushun Basin (Eocene), from Shandong Basin (early Miocene), from Xiaolongtan Basin (Miocene), and from Shengzhou/Zheijiang (Pliocene). These palaeoclimate reconstructions are well-constrained and produce estimates that are consistent with our physical understanding of climate changes during these past intervals when CO_2 was higher than today and regional climates warmer. These analyses suggest that exploiting known climate-trait relationships for a large set of morphometric traits simultaneously could offer a way to make robust reconstructions of pre-Quaternary climates.

T3-10-06

Plant traits, leaf economics and next-generation ecosystem models

Iain Colin Prentice

Imperial College London

It is now widely recognized that the "plant functional type" (PFT) concept, which was a valuable simplification in the early stages of global vegetation model development, has largely outlived its usefulness. This insight has come mainly from empirical studies of plant functional traits, which have revealed (a) that variation in most traits is continuous, (b) that most traits vary as much or more within conventionally defined PFTs as they do between PFTs, and (c) that a substantial component of this within-PFT variation is adaptive and systematically related to environmental gradients. Many traits also show (d) a component of similar magnitude that is related to phylogeny rather than environment, accounting for considerable variation that is expressed within the plant community. The data analysis techniques of generalized linear modelling, trait gradient analysis and constrained ordination have been deployed to quantify each of these aspects of trait variation and covariation and their relationships to environmental predictors. The leaf economics spectrum (LES) has provided an organizing principle for understanding a key leaf-level trade-off, between leaf longevity and specific leaf area. However, both leaf area – a trait showing strong, adaptive relationships to macro- and microclimate – and photosynthetic capacity, expressed on a per unit area basis, appear to show variation orthogonal to the LES. This finding suggests that models to account properly for functional trait diversity need to take account of at least three dimensions of trait variation. Variation in photosynthetic capacity, moreover, can be understood in terms of an optimality principle: the return on investment in photosynthetic capacity falls off abruptly at the point where light availability becomes limiting to photosynthesis, so the realized photosynthetic capacity is expected to be close to this point. Its actual value is also dependent on temperature, ambient CO_2 concentration, and the ratio of leaf-internal to ambient CO_2 – itself a key trait (indexed by the stable carbon isotope ratio) that depends in a predictable manner on temperature, vapour pressure deficit and atmospheric pressure, and appears to be perfectly plastic along environmental gradients. A growing body of empirical evidence supports the idea that ecophysiological traits, including leaf nitrogen content (through its relationship to photosynthetic capacity) and respiration rate, are tightly regulated at the leaf level following well-defined optimality principles. Indeed, these principles can be said to be the ultimate source of predictability in plant and ecosystem function. Next-generation ecosystem models will have to account for continuous variation in plant functional traits, both between and within local environments; and the huge expansion in the collection and availability of trait data can provide a sound empirical basis for such models. But data alone, however advanced the statistical analysis, cannot provide the key relationships and parameter values that will be required by models that are supposed to remain valid under altered environmental conditions. The necessary theoretical underpinning for models will be provided by optimality hypotheses that are explicitly testable with data. Achievement of this goal will require the adoption of a hypothesis-driven “strong inference” paradigm in ecosystem model development.

T3-10-07

How tightly tree form and function are integrated within a local forest community? A perspective from plant architectural, economics, hydraulic, anatomical and physiological traits

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The global plant economic spectrum provides a useful framework for improving models that predict future vegetation based on continuous variation in plant form and function. However, whether the global spectrum of plant form and function still holds in the local tree communities remains largely unknown. We measured five tree architectural, four economic, six physiological, eight hydraulic, three anatomical, and one stem thermal exchange traits for 56 individual trees (18 species) in an evergreen forest and 33 individual trees (8 species) in a deciduous forest in subtropical China. Principal component analysis (PCA) was employed to construct the local spectrum of tree form and function in each community types. We find that architectural, economic, physiological, hydraulic, anatomical, and stem thermal traits are highly intra- and inter correlated, thus formulating a local spectrum of plant form and function in two communities. Multi-trait variations in PCAs reveal that the structure of the local spectrum of plant form and function depends on community types. In evergreen forest, three notable dimensions of trait variation stand out. The first dimension is defined mainly by tree architecture and water transportation rate, i.e., short trees with large proportion of dispersed leaves and specific leaf area, but small and shallow tree crown, low light saturation point, stomatal density and vessel diameter, are capable of moving water and assimilating light and CO_2 slowly while exchanging stem temperature rapidly. The reverse is true for tall trees with larger proportion of clumped leaves. The second dimension is defined mainly by water transpiration rate and light capture ability, i.e., running from tall trees with high transpiration rate, stomatal conductance, light compensation point, leaf nitrogen concentration and proportion of vertical stretch branch, to short trees with high dark respiration rate, the apparent quantum efficiency, and proportion of horizontal stretch branch. The third dimension is defined mainly by hydraulic properties, i.e., trees with high wood density and Huber value of petiole tend to have low leaf water potential, vessel density, and hydraulic conductivity in both leaf and branch. In deciduous forest, there are two independent dimensions of trait variation. The first dimension is defined by tree architecture, water transportation and economics traits, almost showing the similar pattern compared to the first dimension in evergreen forest. The second dimension is structured by the coupling relationships between physiological, hydraulic, and architectural traits. Relative to short trees, tall trees with larger proportion of dispersed leaves and wider vessel diameter have low Huber value of petiole, dark respiration rate in leaves and vessel density, whereas higher hydraulic conductivity, transpiration rate, stomatal conductance, the maximum photosynthesis rate, light saturation point, and leaf nitrogen concentration. In conclusion, our results suggests that tree height induced variations in architectural, economics, hydraulic, anatomical and physiological traits that relate to water, carbon and nutrients are highly integrated at the local tree communities. The finding of this local spectrum of plant form and function would be beneficial for modelling carbon and water fluxes in the changing climate.

T3-10-08

Eco-evolutionary dynamics of plant community structure and function in Tibetan alpine grasslands

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The functional composition of plant communities responds to environmental change and disturbance through a combination of ecological and evolutionary adaptions involving the balance between intraspecific trait variability (ITV, resulting from both phenotypic plasticity of individuals and the adaptive evolution of populations) and changes in community composition (CCC, resulting from both species turnover and shifts in species abundance). Working in this conceptual framework, we analyze the eco-evolutionary dynamics of community structure and function in Tibetan alpine meadows distributed along soil resource gradients and in response to grazing. For every species within each plot in alpine meadows both at differing elevations and aspects on each of three Tibetan mountains and at grazed and ungrazed plots in five sites across the Qinghai–Tibetan Plateau, we measured two chemical traits (leaf nitrogen and leaf phosphorus concentrations) and two morphological traits (specific leaf area and leaf dry matter content) that characterize fundamental tradeoffs in resource uptake strategies associated with growth rate. We distinguished the relative importance of ITV and CCC in affecting shifts in the community-weighted means of these foliar traits in response to soil resource availability and to grazing. In resource limited environments, alpine plant communities on Tibetan mountains increasingly tend to be dominated by species with a conservative resource strategy, while subordinate and infrequent species have an exploitive resource strategy and fast growth rates that increase their relative abundance and buffer decline in trait diversity at the community level. In contrast, less resource limited environments favor dominance by fast-growing species with exploitive resource use, while subordinate and infrequent species have a conservative resource use strategy. In short, the eco-evolutionary interplay between CCC and ITV effects acts to stabilize community function along soil resource gradients, promoting variation in species composition among plots at alpine meadow sites on Tibetan mountains. This interplay between CCC and ITV could provide an important buffering mechanism maintaining the structure and function of alpine communities under global change. In response to continuous grazing, plants in Tibetan alpine meadows increase specific leaf area and foliar nutrients but tend to have lower leaf dry matter content, a response consistent with faster growth and regrowth under grazing. This ITV mediated response drives a shift in community function from conservative, slow-growing resource use in ungrazed meadows to exploitative resource use under grazing. This community-wide functional response enhances forage quality, in turn favoring the secondary productivity of small herbivorous mammal communities, but also contributes to accelerated depletion of soil available phosphorus.

T3-10-09

Assessing the spatiotemporal dynamics of vascular epiphytes on the forest canopy via a mechanistic forest-epiphyte simulation model

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Vascular epiphytes live in a three-dimensional, highly dynamic, and heterogeneous habitat that is the forest canopy. Knowledge about how forest structure and dynamics influence the spatio-temporal distribution of epiphytes is still limited owing to difficulties in accessing the forest canopy and a lack of long-term observations. In this study, we present a coupled forest-epiphyte model that is three-dimensional, spatially-explicit, niche-, trait-, and individual-based. The model considers species-specific light requirements and simulates individual dispersal, recruitment, growth, and mortality. By coupling spatiotemporal distribution of trunks and branches obtained from a functional-structural forest model with our epiphyte model, we first assessed whether the model results satisfactorily match real-world data. Thereafter, we addressed how differences in long-term natural forest dynamics, selective logging, and the size of forest fragment influence epiphyte assemblages. The model generated vertical distributions of individuals and species and rank-abundance distributions that were comparable with field data from lowland forests in Panama and Ecuador. Higher forest dynamics had complex effects on epiphyte assemblages, with stable forests having generally high abundances, low extinction rates and highest saturation. Selective logging of larger trees resulted in lower abundances, species numbers, and saturation levels of epiphytes. Strikingly, a slight reduction in minimum harvested tree size from 45 to 40 cm DBH had a catastrophic effect on the epiphyte assemblage and led to a near complete extinction of epiphytes. Increasing fragment size decreased species extinction rates. Our model demonstrated that the average abundance and biomass of epiphytes are strongly influenced by forest dynamics and human activities. We concluded that modeling approaches that explicitly account for forest dynamics and epiphyte demography provide valuable insights on epiphyte assemblages, with potential applications for assessing long-term impacts of human-induced changes.

T3-10-10

Macroscale patterns on the functional diversity of pollination modes among conserved, restored and secondary areas of a tropical forest

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Questions: (1) How does the functional diversity (FD) of tree pollination modes vary in relation to the species turnover within semi-deciduous forests at a regional scale? (2) In comparison with conserved forest areas, are there any significant difference in the FD among restored and secondary vegetation areas? Methods: an extensive literature review was carried out about the pollination modes of tree species obtained from 40 floristic surveys at 18 remnants, 11 restored and 11 secondary vegetation areas. Species and functional dissimilarities, species and abundance proportions of pollination modes and functional richness and evenness were calculated and compared within and among forest types. Results: (1) Species dissimilarity was much higher than functional dissimilarity within forest areas (mean Morisita-Horn distances > 80%, < 35% respectively). (2) Conserved tree communities were characterized by the predominance of generalized insect pollinated species (49%), followed by secondary proportions of bee (11%), wind (10%) and moth (8%) pollination. Other pollination modes were under-represented (< 4%). FD indices among restored and secondary forest were not significantly different from remnants. However, significant changes were found in the species and abundance proportions of several pollination modes. On restored forests, reductions were found in generalized insect, moth, wind, fly, pollen flowers and very small insect pollination, while the species pollinated by bees and bats were more than duplicated. On secondary vegetation areas, reductions included moth, fly and fig-wasp pollination, whereas bee, beetle, big animal and small insect pollination were increased. Such changes could not be related to the successional time on restored and secondary forests. Conclusions: Our results indicate a rather stable functional assemblage of pollination modes and also high ecological redundancy among trees regardless of the species replacement at a regional scale. Thus, major changes in FD between forest types would primarily rely on human disturbance. Tree communities showed a relatively low FD of pollination modes; hence, greater FD among plants with different growth forms is expected. Efforts in recovering such patterns on FD should be encouraged in order to avoid possible negative effects in plant-pollinator interactions.

T3-10-11

Facilitation as a driver of plant assemblages in Caatinga

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Facilitation is a positive interaction in which a nurse plant reduces the environmental severity experienced by neighboring plants by providing shade, enabling nutrient accumulation or repelling herbivores within patches of vegetation. Nurse plants within facilitated patches should preferentially promote the coexistence of ecologically dissimilar species with little niche overlap, and if ecological traits are conserved within evolutionary lineages (i.e., niche conservatism), this should result in phylogenetic overdispersion. In contrast to competition, facilitation is expected to increase species richness. Therefore, to examine the role of facilitation as a driver of plant assemblages in Caatinga, we quantified the functional traits of nurse species and compared species richness, phylogenetic diversity, phylogenetic structure of the tree layer and of the herbaceous layer between patchy Caatinga and Caatinga with segregated plants. Furthermore, we determined the phylogenetic community structure (whole community) of Caatinga patches. Results show that functional traits related to resilience (resprouting) and resistance against herbivory (spines, thorns, urticancy and toxicity) seem to be crucial for facilitation in Caatinga. Autochory occurs at a higher frequency in nurse plants than in Caatinga in general. The herbaceous layer of patchy Caatinga is richer in species than Caatinga with segregated plants and facilitation is the suggested cause. As the whole community of the 196 patches is phylogenetically overdispersed compared to the null expectations for the metacommunity, facilitation seems to predominantly promote the coexistence of dissimilar species with little niche overlap. Therefore, niche diversification promoted by facilitation in patchy Caatinga likely caused its increased herbaceous richness, its increased phylogenetic diversity and its phylogenetic overdispersion.

T3-10-12

Transpiration is more important for leaf temperature regulation of the plants from a hot dry habitat than the plants from a hot wet habitat

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Leaf temperature has important impact on the micro-environment of plants and physiological processes in the leaves. Plants from different habitats may show different leaf temperatures even under the same environment. However, the mechanism that causes temperature differences among plant groups is unclear because of the coupling of transpiration and morphological traits. We investigated 21 morphological leaf traits and thermal properties of 38 species in the common garden. Among them, 18 species were dominant species from the hot wet habitat (HW) and 20 species were dominant species from the hot dry habitat (HD). To separate the impact of transpiration and morphological traits on leaf temperature regulation, we measured the diurnal course of leaf temperature with and without transpiration. The temperature of a reference leaf beside each individual was measured simultaneously to exclude the diurnal thermal variance of environment. Generally, the species from HD showed lower leaf temperatures than the species from HW under the same condition. Plants from HD dissipate heat more efficiently by developing higher transpiration capacity and synergic leaf morphological traits. Moreover, leaf temperature was more dependent on transpiration than leaf morphological traits for the plants from HD. Among the leaf morphological traits, leaf length and the thickness of the abaxial epidermis were significantly correlated with the temperature of the leaf without transpiration. Transpiration is efficient to cool leaves, which provides the possibility of fast growth when water was available. Leaf morphological traits can damp heat stress to some extent, but they are not enough to provide a safe temperature for photosynthesis for the species in present study. Our results complemented the strategies of plants adaptation to hot environment with the regard to thermal regulation.

T3-11: Interaction between plants and insects

T3-11-01

Accumulation, spillover and spillback: An introduced thistle as a reservoir for insect and pathogen enemies of native plants

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Invading plants often escape their native-range natural enemies, as described by the Enemy Release Hypothesis. However, once in a new region, exotic invaders acquire new enemies recruited from among invaded-range species. As well, enemies from the invader's original range may be accidentally or deliberately co-introduced; these species also may attack local natives. As a result, many invaders soon accumulate a new community of natural enemies, many of which are shared with co-occurring natives. Such shared enemies may participate in feedbacks on natives through spillover (spread of introduced enemies from exotic hosts to natives) and spillback (spread of native enemies to exotics followed by spread back onto their original hosts). Here, we present results from ongoing research into the potential of a common non-native thistle, *Cirsium arvense*, to act as a reservoir for insects and diseases attacking co-occurring native plants in its invaded range in Ontario, Canada. We documented the potential of *C. arvense* to host herbivores and pathogens by sampling plants over a 800 km transect from agricultural southern to boreal northern Ontario. We also monitored colonization of artificial populations, and investigated soil feedback using inocula sampled at both local (inside/outside existing populations) and geographic scales. Results indicate that thistle populations host a diverse set of herbivores and pathogens, but their impacts depend upon location. Leaf damage declined strongly with latitude. Different seed predators showed contrasting latitudinal patterns, while damage by the bacterial pathogen *Pseudomonas syringae* varied irregularly. Plants inoculated with northern soils outperformed those inoculated with southern soils, suggesting an accumulation of pathogens at southern sites. Artificial populations were colonized rapidly by herbivores, but even short (< 100 m) separation from other populations significantly slowed this process. Similarly, plants grown in soils inoculated with samples from outside thistle populations performed better than plants inoculated with soil from invaded areas only 50-100 metres away. A second set of experiments was then established to assess impacts of these enemies on co-occurring native plants. First, we compared insect damage and populations of shared herbivores on native plants in proximity to *vs*. isolated from populations of *C. arvense*. Second, we conducted an experiment in which we compared the effects of fungicide on survival of seeds of native competitors buried inside *vs*. outside thistle populations. Third, we inoculated root systems of co-occurring natives with soil biota sampled from inside *vs*. outside thistle populations. Finally, we inoculated native competitors with a bacterium, *Pseudomonas syringae*, sampled from *C. arvense*. Results indicate that spillover and spillback of some, but not all, of the insects and microbial enemies commonly attacking *C. arvense* might increase the ecological impacts of this invader on co-occurring native species. However, the magnitude of these interactions likely depends on the location and isolation of the host thistle population, the host-specificity of the enemy in question, and the phylogenetic relationship between *C. arvense* and co-occurring natives.

T3-11-02

Impact of pollen grain properties on pollen collectability by *Bombus terrestris* (Apidae)

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The pollination of plants via bees faces a dilemma due to the fact that bees do not only passively transport pollen grains between conspecific flowers, but instead actively collect pollen to provision their larvae. Therefore, several kinds of protection against pollen collection by bees have evolved in bee-pollinated plants, e.g., toxic pollen, pollen hidden in floral tubes. Here we report about a new mechanism of protection against collection: Spines of large pollen grains impede the storage of pollen grains in the corbiculas of bees. Micrographs show that the spines of pollen grains of the the Malvaceae family are not covered with pollenkitt. Nevertheless, the spiny pollen grains adhere very well to the bristles of the bees' body. We analysed the influence of pollen grain properties – diameter, spine length, and spine density – on the collectability of echinate pollen by bumblebees. Buff-tailed bumblebees (*Bombus terrestris*, Apidae) were kept in a flight cage to forage on plants of the families Malvaceae, Cucurbitaceae, Dipsacaceae, and Campanulaceae which feature pollen with an echinate exine structure. The bumblebees foraged pollen significantly less on the Malvaceae and the Dipsacaceae species. Both the number of visited flowers and the weight of collected pollen are positively correlated with the handling time of the flowers, which in turn is negatively correlated with the pollen grains' spine length. To further test the influence of pollen structure on the collection behaviour of bumblebees, five plant species (*Alcea ficifolia*, *Lavatera thuringiaca*, *Cucurbita pepo*, *Centaurea jacea*, and *Syringa* spec.) were offered progressively over the course of five days. The results show that bumblebees stopped collection of pollen following the failure to groom pollen grains into their corbiculas. Melittophilous plants benefit from exine structures that render pollen uncollectable for polylectic bees which are seldom the most efficient pollinators. This does not deter nectar foragers from visiting the flowers and contributing to pollination. Consequently, mechanical defence of pollen grains against collection by non-specialist bees proves to be an effective solution to the pollen dilemma.

T3-11-03

Extrafloral nectaries: Helps in behavioural defences for Plant-Herbivore interaction between ant-plant communities

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Extrafloral nectar (EFN) (or extranuptial nectar) is secreted on the vegetative and less commonly the reproductive parts of a plant and does not contribute to its pollination. Indeed, ants attracted to nectar that is secreted around flowers can even repel flower visitors, which can have a significant ecological cost for the plant. Most, if not all, extrafloral nectary bearing plants secrete EFN to attract ants, wasps, and parasitoids as the enemies of their enemies to enhance top-down control of the herbivores and thereby their defense against herbivores. Recent study was conducted on some members of Malvaceae family to see the ant-plant interactions and that's depend on extrafloral nectary secretion. In the present study we observed and recorded EFN visiting ants and other insect activity in *Gossypium herbaccum, Hibiscus mutabilis, Thespesia populnea, Urena lobata, Urena rigida* over a period of one year and compiled all the data on a large statistical database SPSS version 11 using contingency tables and Kruskal-Wallis test. Besides this we also monitored the taxonomic composition of EFN, abundance, frequency and aggressiveness of the EFN associated ant species. To study the protective role of the observed ant assemblage, we analyzed the data by Spearman rank correlation coefficients, and analyzed the relationships between both EFN presence and abundance and ant defense behaviors against (1) stimulated herbivores and (2) natural nectar thieving butterflies.

T3-11-04

A survey of Queen Anne's Lace on Nantucket Island, Massachusetts, USA and an assessment of its effect on native pollination

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Queen Anne's Lace (*Daucus carota*, QAL) is a non-native plant species that was introduced to North America from Europe during the early colonial period, and its presence on Nantucket Island, Massachusetts has been documented since at least the nineteenth century. Research in other plant systems has investigated whether native pollination was affected by the presence of a non-native, and the results have been mixed. Depending on the system studied a native may be negatively affected, may not be affected or may be positively affected by the presence of the non-native. As yet, there has been no survey of QAL on Nantucket, and no study known to the authors has investigated the effect of QAL on a native species. In summer 2015 and 2016 a survey of QAL populations was performed on properties owned and managed by the Nantucket Conservation Foundation, Linda Loring Foundation and Nantucket Islands Land Bank, as well as other, public properties. Following this, a pollinator observation study was performed on QAL and a native species, Toothed White-top Aster (Sericocarpus asteroides, TWTA). Pollinators were observed for three hours per day and five days each for the 2 species individually and both species cohabitating together. Additionally, in summer 2016 a removal treatment was performed on sites where both species cohabitate. The sites were observed as described for one day. After observation was over QAL umbels were removed from the site and pollinators were observed on TWTA as described the following day. Finally, TWTA populations from a range of distances from QAL were assayed for the presence and magnitude of heterospecific pollen. We had four hypotheses: 1) QAL would be present across Nantucket Island; 2) The presence of QAL would increase pollinator visitation rates and diversity indices on TWTA; 3) The removal of QAL would restore pollinator visitation rates and diversity indices to those found on sites with only TWTA; 4) Higher levels of heterospecific pollination would be found on TWTA in populations located close to QAL. We found QAL to be abundant across the island, with an average density of 12.2 plants/m^2 and a high density of 109.4 plants/m^2. While both plant species are generalist pollinated, QAL attracted 15 insect families and TWTA attracted only 8, the majority of each were flies and bees. Each removal treatment received at least 50% fewer pollinator visits after QAL was removed.

T3-11-05

Islands in the trees: A biogeographic exploration of epiphytes and the spider communities inhabiting them.

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According with the equilibrium theory of island biogeography, within oceanic and habitat islands, the species number of a given taxa is determined by the interaction of two antagonist factors: islands size and isolation. It has been proposed that epiphyte plants in the tropics function like habitat islands for canopy arthropods. The base for this statement is the positive relationship found between the epiphytes' size and the species richness of the arthropod communities inhabiting them. Despite this observation, it stills remains unknown if there is an isolation effect produced by the spatial-arrangement of epiphyte plants. Epiphytes spatial distribution reflects the conjugation of intrinsic (plant microclimatic and physiological traits) and extrinsic factors (host tree phenology and morphology), but the isolation effect is related to the dispersal and locomotion abilities of each singular arthropod taxa. In the nature epiphyte islands are occurring in two different ways, like single plants or like plant mats. The abundance and diversity of epiphyte plants conforming an epiphyte-island is an approximation to habitat heterogeneity of oceanic islands, and recently this has been proposed like a third factor driving island's species richness. In this work we used spider communities inhabiting epiphytes to test if there is an isolation effect produced by the plants' spatial distribution. We collected all epiphytes and their companion spiders from tree threes in a shade coffee plantation of Veracruz, Mexico. Epiphytes tridimensional position was recorded at one by one centimeter scale, then we calculated the distance between all epiphytes within each tree. Spiders are capable of navigate through their environment by means of both cursorial and aerial way, therefore we used two different distance systems (Euclidian and Topological) in order to better understand the implications of this divergence. We found that spiders' diversity increased with epiphyte-islands' size and that this effect was negatively related to isolation. In the other hand, the effect of epiphyte-islands' heterogeneity was at most marginal and inconsistent. We also found that crown position and isolation across epiphyte islands affected spider species similarity. Regarding spiders' locomotion, walking distances (Topological) explained more of the diversity patterns than flying distances (Euclidean). This is the first study presenting evidence of species-isolation processes driving the diversity of epiphyte-dwelling spider communities. The three-dimensional structure, abundance and high biodiversity of epiphyte communities present an excellent opportunity for further testing the generality of island-biogeographic theory predictions.

T3-11-06

Diaspore dispersal by carnivorous wasps

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The California-endemic spicebush (*Calycanthus occidentalis*; Calycanthaceae) produces achenes with an auxiliary organ that is attractive to carnivorous wasps of the genus *Vespula* (Vespidae). Studies on the presentation, dispersal, and chemistry of the achenes suggest that these wasps may be a major agent of dispersal of spicebush diaspores, and that a protein reward is involved (the auxiliary organ is more than 22% protein by dry weight). Foraging worker wasps appear to be strongly attracted to an unidentified volatile compound that probably mimics the high-protein items (e.g., carrion and other insects) that are the typical targets of these wasps. Studies are currently underway to determine if a similar dispersal system exists in the other members of the Calycanthaceae, found in the southwestern United States and China. In addition, there is preliminary evidence that seeds of the California endemic pipevine (*Aristolochia californica*) are also dispersed by these wasps.

T3-12: Ecological Genetics

T3-12-01

Clonal extent of a temperate canopy tree species (*Tilia cordata* Mill.) across the UK geographic range

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Vegetative asexual propagation (clonality) is a common mode of plant reproduction. However the extent of clonality, how it is combined with sexual reproduction and the factors influencing the relative balance of the two is rarely explored. The genus *Tilia* is a group of woody tree species that have mixed mating systems. Within the UK, *T. cordata* is a native species of ancient woodlands that often grows gregariously. This study uses molecular markers to identify the extent of clonal growth within the species across its UK range. Results from an examination of the proportion of clonality, the size and spatial arrangement of clonal groups, fine-scale spatial genetic structure and the potential relationship of asexual reproduction to location/climate in several populations are considered.

T3-12-02

Uplifting of the Qinghai –Tibetan Plateau led to speciation of shrubs *Spiraea alpina* and *S. mongolica* (Rosaceae)

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The phylogeographic history of two closely related species, *Spiraea alpina* and *S. mongolica*, were analysed across the Qinghai–Tibetan Plateau (QTP) before, during, and after the most recent glaciation or orogenic events using chloroplast DNA (cpDNA) and microsatellite data sets. Data were obtained by generating and analysing data sets for 851 individuals from 63 natural populations. Three cpDNA markers were used to infer phylogenetic relationships using Network and PAUP*. Microsatellite data were analysed in STRUCTURE 2.2.3. Patterns of variation among and within populations were analysed in Arlequin 3.5. Divergence time was calibrated in BEAST 1.7.5 with *Sorbaria kirilowii* fossils. Phylogenetic relationships of the two species revealed two main groups and three subgroups. Alternatively, the microsatellite data revealed three main clusters. Demographic analysis based on chlorotypes revealed population recolonization in the late Pleistocene after the most recent glaciation and high genetic diversity in both species. Our results indicate that the high genetic diversity of these shrubs is attributable to Pliocene and Pleistocene climatic changes, combined with orogenic events of the QTP. The distribution of these spiroides species on the QTP resulted from the combine effects of climatic instability during quaternary glacial–interglacial periods and uplift of the QTP.

T3-12-03

Introduction of Sahara mustard (*Brassica tournefortii*) in North America: Evolutionary and environmental mechanisms promoting invasive species success

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Sahara mustard (*Brassica tournefortii*) has successfully colonized semi-arid regions eastward from California to Texas and northward into Nevada and Utah since its introduction in California in the early 20th century. The dispersal and genetic mechanisms that have enabled its success remain unexplored and are a major hurdle in the successful control of the species. The recent, rapid invasion of this species provides an opportunity to address hotly debated questions in invasion biology and natural selection. We used a next-gen genotyping by sequencing technique to generate reduced representation genomics for 943 individuals from 55 locations and six states across the species' invaded range to identify population structure and invasion history. Further, we hypothesized that human-mediated dispersal is driving colonization in the US. To address this, we tested for roadways as dispersal corridors as an alternative to isolation by distance models. We inferred discrete populations and calculated the hierarchical genetic structure within and among locations. Further, we identified a unique pattern of private alleles that helps explain structure. Last, we identified introduction, expansion, and admixture events in the species' history. We followed our genetic analyses with a multi-generational common garden experiment to test for trait variation and identify adaptive traits across the range. We identified 1,164 single nucleotide polymorphisms across the 55 locations sampled. We found no evidence of isolation by distance among locations, revealed a significant amount of genetic variation across and within locations, and found evidence for distinct genetic clusters of Sahara mustard in the US. Overall genetic diversity was low across sites, perhaps due to the species dispersal modality and natural history. Surprisingly high heterozygosity across the species range likely indicates admixture of multiple introduction sites after initial invasions. Overall, we found moderate to low levels of genetic structure supporting human-mediated migration along roadways. Additionally, we found genetically-controlled trait differences including phenological, morphological, and physiological traits that may explain the species success in local environments. We present Sahara mustard as an eminently-suited study system to understand invasion evolutionary biology, and discuss our next steps aimed at genomic analyses in the species native range.

T3-12-04

The battle between historical bottleneck and gene flow during population expansion in natural forest of *Cyclobalanopsis neglecta* (Fagaceae)

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The anthropogenic disturbance causes massive forest decline, and the ensuing forest conservation would make forest expanding rapidly. However, it is still uncertainty how populations genetically respond to the demographic change. Hong Kong has current expanding forest which was mostly developed after severe habitat disturbance in 1940's during the wars. We took an oak species *Cyclobalanopsis neglecta* as an example to study the demographic genetics of young natural forest in Hong Kong. Genetically diverse compositions were detected in old generations and increasing genetic compositions in young generations which indicated the genetically diverse founder populations. Historical bottleneck effect was shown by the low population allelic diversity, high among-population genetic differentiation and high within-population spatial genetic structure (SGS) in old generations compared to young generations. Gene flow could erase the negative consequence of bottleneck effect. In conclusion, both historical bottleneck effect and current gene flow determined the demographic genetics, and rapid population expansion would speed up the process of genetic recovery. Understanding the dynamics of genetic structure and genetic diversity could determine the relative role of stochastic and deterministic ecological and evolutionary forces, which can guide the practice of forest restoration and conservation.

T3-12-05

The immediate capacity for adaptation and its realization in natural plant populations

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Adaptation results from evolutionary response to natural selection operating across all life-history stages of an organism's existence. The rate of this adaptation, as predicted by the Fundamental Theorem of Natural Selection, depends on the additive genetic variance for fitness, σ_A^2 (*w*). Explicit quantitative predictions regarding the rate of adaptation can be made from estimates of σ_A^2 (*w*) and compared with measured, realized adaptation in order to fully evaluate the dynamics of the evolutionary response to selection. Whereas this prospective, quantitative approach to estimating the capacity for adaptation has been available for over 80 years, studies have tended to focus retrospectively on qualitative changes in fitness. Such qualitative assessments of the changes in fitness demonstrate adaptation, but do not directly illuminate the adaptive process. In contrast, prediction of the change in mean fitness via σ_A^2 (*w*), together with evaluation of the realized change in mean fitness, elucidates ongoing adaptation from standing additive genetic variation in natural populations. This talk describes results from a multi-generation study of the annual legume *Chamaecrista fasciculata* to evaluate the immediate capacity for adaptation, and the degree to which that capacity is realized under contemporary natural selection. Pedigreed seeds generated from greenhouse crosses within three populations (1,737-3,593 seeds) were planted into restored prairie near the seed source for each population (Minnesota and Iowa, USA). Each individual was followed throughout its complete lifespan with fitness outcomes recorded at each life-cycle stage used cumulatively to estimate σ_A^2 (*w*). For the following year, progeny resulting from the reproduction of the initial pedigreed populations were planted into each respective site. In addition, individuals from the initial pedigreed greenhouse population were planted again so that mean lifetime fitness of the two generations could be evaluated in common, contemporaneous environments. The use of aster modeling permitted inclusion of all components of fitness (e.g., seeds that did not germinate) that traditional statistical treatments omit to accommodate statistical assumptions. Further, explicit modeling of the dependence of components of fitness measures on those expressed at earlier life-history stages (e.g., number of seeds produced given total number of fruits set) produces more precise assessments of fitness. This talk will report on a large-scale experiment to empirically predict the rate of adaptation, and evaluate the accuracy of this prediction with realized measures of adaptation over two generations in three natural populations. These results will lay the foundation for the interpretation of natural selection and adaptive evolutionary responses. Finally, these results also directly relate to assessing the potential for evolutionary rescue in the face of ongoing climate change.

T3-12-06

Genomic, epigenomic and metagenomic analyses reveal parallel ecological divergence in *Heliosperma pusillum* (Caryophyllaceae)

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Mosaic distribution of interbreeding taxa with contrasting ecology and morphology constitute an ideal opportunity to study the pace and mode of neutral and adaptive processes during repeated evolution of ecotypes. Applying a combination of population genetic, epigenomic, transcriptomic, ecologic and metagenomic tools we investigate here the evolutionary history of an alpine and a montane ecotype of *Heliosperma pusillum* (Caryophyllaceae) in the south-eastern Alps. From six pairs of geographically close populations of the two ecotypes we obtained a high-coverage RADseq dataset that was further used for demographic inference to test the hypothesis of parallel evolution of the two ecotypes. The data demonstrate repeated ecological divergence in *H. pusillum*, uncovering up to five polytopic origins of one ecotype from the other. Coalescent-based demographic simulations evidence a complex evolutionary history, with local isolation-with-migration in two population pairs and intra-ecotype migration in two others. In all cases, the time of divergence or secondary contact was inferred as postglacial. A metagenomic analysis on exogenous contaminant RAD sequences demonstrates divergent microbial communities between the ecotypes, whereas measured abiotic conditions are significantly divergent between the two types of localities, but consistent within each ecotype. The similar set of biotic interactions is involved, together with abiotic factors, in shaping common selective regimes at different growing sites of each species. However, a lack of shared genomic regions of high divergence across population pairs illustrates the power of drift and/or local selection in shaping genetic divergence across repeated cases of ecological divergence, together with the complexity of molecular basis of ecologically-relevant traits. Epigenetic alterations can be expected to be particularly important in the early phases of adaptation and divergence. Employing a novel approach (bisulfite-converted RAD sequencing–bsRADseq), we screened DNA methylation patterns across more than 200,000 nucleotide positions in the six population pairs of the two ecotypes. Comparing by sequencing DNA methylation state and context (CpG, CHG, CHH) in 120 individuals, we demonstrate that epigenetic patterns are much more conserved across representatives of the two ecotypes than predictions given the distinct native environments and that several candidate loci for convergent epigenetic modification during parallel adaptation to a similar environment are present. From progeny screens across three generations we infer high heritability of DNA methylation, that is however, context dependent. Altogether, our

work supports the hypothesis that repeated ecological divergence, observed here at an early stage, may be a common process of species diversification.

T3-13: Halophyte physiology

T3-13-01

How do recretohalophytes secrete salt? From structures to genes

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To survive in a saline environment, halophytes have evolved many strategies to resist salt stress. The salt glands of recretohalophytes are unique features for directly secreting salt out of a plant. Knowledge of the pathways of salt secretion in relation to the function of salt glands may help us to change the salt-tolerance of crops and to cultivate the extensive saline lands that are available. Recently, ultrastructural studies of salt glands and the mechanism of salt secretion, particularly the candidate genes involved in salt secretion, have been illustrated in detail. In this report, we summarize current researches on salt gland structure, salt secretion mechanism and candidate genes involved, and provide an overview of the salt secretion pathway and the asymmetric ion transport of the salt gland. (1) Salt gland structure. To date, eleven families (65 species) have been discovered to have salt gland structures. The number of component cells has been used to separate multi-cellular salt gland and bi-cellular salt gland. Typical characteristics were identified in salt gland: the cuticles surrounding the salt gland, plasmodesmata between the mesophyll cells and the salt gland, and accumulated vesicles, which were considered to play a significant role in salt secretion combined with both apoplastic and symplastic transport. (2) Salt secretion mechanism. Three hypotheses of salt secretion were also supported by the recent studies: the role of the osmotic potential in salt secretion, a transfer system similar to liquid flow in animals and salt solution excretion by vesicles in the plasma membrane (exocytosis). In the last few years, more and more evidence has supported the third one. In addition, the membrane-bound translocating proteins are likely to be involved in salt secretion and these transporters mainly distributed asymmetrically in the other side of plasma membranes of salt gland cells adjacent to the collecting chamber. (3) As a typical recretohalophyte possessing salt glands, *Limonium bicolor* is a potential model plant for investigating the salt secretion mechanism by salt glands in the future. 102 genes high likely to be involved in salt secretion of *L. bicolor* have been screened by high-throughput RNA sequencing. The roles of these genes are being identified in our laboratory. For a decade, various ion transporters have been shown to participate in salt resistance as verified in *Arabidopsis*. However, the in-depth mechanism of halophyte salt tolerance, in particular the molecular mechanism of salt gland secretion, is largely unknown. More and more scientists have begun to use halophytes as materials to study the salt tolerance mechanism. Acknowledgements: This work has been supported by the NSFC (National Natural Science Research Foundation of China, project No. 31570251), Programs Foundation of Ministry of Education of China (20123704130001) and Natural Science Research Foundation of Shandong Province (ZR2014CZ002).

T3-13-02

ZxAKT1 is essential for K^+ uptake and K^+/Na^+ homeostasis in the succulent xerophyte *Zygophyllum xanthoxylum*

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The inward rectifying K^+ channel AKT1 constitutes an important pathway for K^+ acquisition in plant roots. In glycophytes, excessive accumulation of Na^+ is accompanied by K^+ deficiency under salt stress. However, in the succulent xerophyte *Zygophyllum xanthoxylum*, which exhibits excellent adaptability to adverse environments, K^+ concentration remains at a relatively constant level despite increased levels of Na^+ under salinity and drought conditions. In this study, the contribution of *ZxAKT1* to maintaining K^+ and Na^+ homeostasis in *Z. xanthoxylum* was investigated. Expression of *ZxAKT1* rescued the K^+-uptake-defective phenotype of yeast strain CY162, suppressed the salt-sensitive phenotype of yeast strain G19, and complemented the low-K^+-sensitive phenotype of *Arabidopsis akt1* mutant, indicating that *ZxAKT1* functions as an inward-rectifying K^+ channel. *ZxAKT1* was predominantly expressed in roots, and was induced under high concentrations of either KCl or NaCl. By using RNA interference technique, we found that *ZxAKT1*-silenced plants exhibited stunted growth compared to wild-type *Z. xanthoxylum*. Further experiments showed that *ZxAKT1*-silenced plants exhibited a significant decline in net uptake of K^+ and Na^+, resulting in decreased concentrations of K^+ and Na^+, as compared to wild-type *Z. xanthoxylum* grown under 50 mM NaCl. Compared with wild-type, the expression levels of genes encoding several transporters/channels related to K^+/Na^+ homeostasis, including *ZxSKOR*, *ZxNHX*, *ZxSOS1* and *ZxHKT1;1,* were reduced in various tissues of a *ZxAKT1*-silenced line. These findings suggest that *ZxAKT1* not only plays a crucial role in K^+ uptake but also functions in modulating Na^+ uptake and transport systems in *Z. xanthoxylum*, thereby affecting its normal growth.

T3-13-03

Short and long term effects of salinity on photosynthesis and biomass of *Phragmites karka*: A biofuel candidate

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Phragmites karka (tall reed) considered a potential biofuel crop due to high productivity and ligno-cellulosic contents. Whereas, photosynthesis is a growth controlling mechanism and affected by salinity particularly in C_3 grasses. However, plant exhibit different strategies to deals with the time-course effects of salinity. Knowledge about plant response to short and long term exposure of salinity could help in better manage our crop in saline areas. Therefore, the aim of this study was to investigate the short (0-7 days) and long (15-30 days) term effects of salinity on growth and photosynthetic responses of *Phragmites karka.* Plant growth, gas exchange, chlorophyll fluorescence, soluble sugar (SS), stomatal stoichiometry, photosynthetic pigments, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content were measured after different time periods (7,15 and 30 days) in 0 (control), 100 (moderate) and 300 (high) mM NaCl treated plants. In short term exposure of moderate salinity, plant maintain biomass and photosynthetic performance but in high salinity reduced growth link with the decline in whole plant photosynthesis (A_W). Reduction in net photosynthetic rate (A_{NET}) at 300 mM NaCl could be accounted by lower stomatal conductance (gs), transpiration (E) and internal carbon dioxide concentration (Ci). During long term salinity treatment, *P. karka* reduced growth about 35% and 80% at 100 and 300 mM NaCl, respectively. Similarly, inhibition of A_W was more severe under high salinity. In moderate salinity plant maintained A_{NET} similar to control by improve water use efficiency (WUE) and instantaneous carboxylation efficiency (COE), with no change in relative excess of light energy (EXC), maximum and effective quantum yield of PS II (Fv/Fm, YII). Moderate salinity reduced the stomatal size but increase number of stomata, photosynthetic pigments, dark respiration (Rd) and sugar content. In long term exposure with 300 mM NaCl, A_{NET} decreased along with gs, E, stomatal size and density while Ci was maintained. High salinity reduced COE and YII, whereas Fv/Fm declined at noon but not at predawn time. Moreover, higher H_2O_2 and MDA content in plant of 300 mM NaCl were linked with the increase in photosynthetic pigments and EXC. In conclusion, *P. karka* maintained photosynthesis via alter stomatal stoichiometry and increase the efficiency of CO_2 fixation while reduce growth for long term survival in moderately saline areas. Whereas high salinity severely decreased biomass production and photosynthesis due to stomatal limitation (in short term effect) and lower efficiency of PSII and CO_2 fixation (in long term effect). Our results indicate that *P. karka* could cultivate under moderately saline areas or with brackish water irrigation for source of energy.

T3-13-04

Coping with extremes: Molecular and physiological basis of salinity stress tolerance in halophytes

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Sustainability of world agriculture is critically dependent on the development of new generation crops that will be more resilient to drought and capable of coping with increasing concentration of salt in the soil, without yield penalties. Recently we have argued (Shabala *et al* (2014) Trends Plant Sci) that salinity tolerance of traditional crops may be improved by incorporating some specific traits found in halophytes. In this talk, I will focus on two major mechanisms conferring salinity tolerance in halophytes that can be targeted in crop breeding programs. The first one is effective Na^+ sequestration in either internal (vacuoles) or external (epidermal bladder cells; EBC) structures. The second mechanism is plant's ability to maintain efficient photosynthetic gas exchange via stomata under hyperosmotic stress conditions. Working along the first lines, we show that internal (vacuolar) Na^+ sequestration in halophytes is achieved by the orchestrated regulation of at least four major traits: (1) higher expression levels and operation of tonoplast NHX Na^+/H^+ exchangers; (2) higher tonoplast H^+-pump activity; (3) better cytosolic K^+ retention originating from intrinsically higher plasma membrane H^+ pumping ability; and (4) efficient control of SV and FV tonoplast channels to prevent futile Na^+ cycling between the cytosol and vacuole. I then analyse mechanism underlying sequestration of toxic Na^+ and Cl^- species in external structures such as epidermal bladder cells. I describe the essentiality of the tissue-specific expression of specific transporters and show experimental evidence for the functional expression and operation of HKT, SOS1, and HAK transporters in epidermis-stalk cell- EBC continuum. In the final part of my presentation I discuss peculiarities of stomata operation in halophytes. The questions asked are: (1) is there a requirement for Na^+ as an osmoticum for stomatal movements in halophytes? (2) is there any difference in guard cell sensitivity to abscicic acid (ABA) and hydrogen peroxide (H_2O_2) between glycophyte and halophyte species? (3) what is the molecular identity and regulation of key membrane transporters mediating stomatal movement in halophytes? Once molecular identity of key genes and control modes of the above processes are understood, plant breeders would be able to devise breeding strategies to incorporate desirable features into traditional crops via molecular or traditional breeding tools thus expanding the sustainable limits for productive use of soil and water resources.

T3-13-05

Comparative study of salt tolerance mechanisms in *Oryza coarctata* and *Spartina anglica*

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Oryza (=Porteresia) coarctata is a wild rice species which has high tolerance to salt with traits which are of interest for improving tolerance of rice growing under saline conditions; while, *Spartina anglica* is a serious invasive species in natural saltmarsh ecosystems. In this study mechanisms of tolerance to salinity were analyzed. The leaf anatomy and ultrastructure of salt glands, pattern of salt excretion, features of gas exchange, accumulation of key photosynthetic enzymes, leaf water content, Na^+ and K^+ accumulation, and osmolality along with levels of some osmolytes and betaine aldehyde dehydrogenase expression, were compared in the two species grown without salt, with 200 mM NaCl, and with 200 mM KCl. Measurements of gas exchange showed photosynthesis was not affected by salt treatments. Despite differences in the type of photosynthesis (*S. anglica* is C_4 while *O. coarctata* is a C_3 plant), the two species have a very similar leaf anatomy; in both the abaxial side is flat while the adaxial side has distinctive ridges. Both species have salt glands which are located deep in the leaf ridges; however they are very different in their anatomy and ultrastructure. *S. anglica* has bicellular glands with basal and cap cells. However, *O. coarctata* has unicellular glands with wall labyrinths near the apical part of the cell which are more developed than in the basal gland cell in *S. anglica*. In contrast to a previous report, our results show that the multiple unicellular hairs which occur in *O. coarctata* are not responsible for salt secretion. Both species accumulate compatible solutes with increasing osmolality but they differ in osmolyte composition. In summary, *Spartina anglica* and *Oryza coarctata* show high tolerance to salt in function of photosynthesis, maintenance of water content, and in excretion of salt from leaves; but, they have distinct differences in the structure of salt glands and accumulation of osmolytes.

T3-13-06

Genetic improvement of alfalfa by transferring genes from xero-halophyte *Zygophyllum xanthoxylum*

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Alfalfa is an important legume forage containing a variety of vitamins and high protein content. In recent years, the demand for alfalfa has been shown a dramatic increase in China, since it is the vital foundation for Chinese dairy industry. Most of the existing alfalfa cultivars show high water consumption, weak tolerance to salinity and nutrient (such as P and N) deficiency. Therefore, under the background of limited arable land, developing alfalfa cultivars suitable for growing on the marginal lands is necessary for alfalfa production in northern China. However, it is hard to significantly enhance the stress tolerance of alfalfa only relying on the existing alfalfa resources and using intraspecific hybridization. *Zygophyllum xanthoxylum* is a xero-halophyte distributing in desert regions of northern China with excellent adaptability to adverse environments. Our previous studies showed that the transporters/channels involved in uptake, transport and compartmentation of Na^+, K^+ and other important nutrient elements play vital roles for *Z. xanthoxylum* to adapt to arid and saline environments. Tonoplast Na^+/H^+ antiporter (ZxNHX) is essential for controlling Na^+ and K^+ uptake, long-distance transport and their homeostasis at whole-plant level through feedback regulating the expression of genes involved in Na^+ and K^+ transport, thereby determining the salt tolerance of *Z. xanthoxylum*. On the other hand, the surface of *Z. xanthoxylum* is covered by thick cuticle, which can prevent the water loss via leaf in dry and heat conditions. In the present study, we co-expressed *Z. xanthoxylum ZxNHX* and *ZxVP1-1* genes in alfalfa. The transgenic alfalfa grew faster than wild-type under either greenhouse or field conditions by developing a larger root system, and showed enhanced salt and drought tolerance with higher leaf relative water content and greater photosynthesis capacity, which was associated with more Na^+, K^+ and Ca^{2+} accumulation in plants. As a consequence of increased vacuolar compartmentation, the addition of 50 mM NaCl significantly alleviated the deleterious impact of osmotic stress on the growth of transgenic alfalfa. Interestingly, transgenic alfalfa showed enhanced tolerance to P deficiency, which was consistent with more P accumulation, and improved adaptation to low nitrogen condition with a higher nodule formation ability after inoculated with *Sinorhizobium meliloti*. Compared with wild-type, transgenic alfalfa developed more and bigger multiple-branched nodules. Finally, the assessment of forage quality indicated that transgenic plants accumulated more crude protein, crude fat, and have higher relative feeding value (RFV) than wild-type, especially under stress conditions and in the field. To further fortify the stress tolerance of transgenic alfalfa, we also introduced another gene *ZxABCG11* of *Z. xanthoxylum* into alfalfa and are developing the transgenic lines with thick cuticle on leaf surface, since ABCG11 was considered as playing a vital role in cuticular lipids secretion. This study provides a solid foundation for creating the alfalfa cultivars with high yield, good quality and wide adaptability on saline, dry and nutrient-deprived marginal lands of northern China.

T3-14: Harnessing new genetic approaches to improve restoration success

T3-14-01

Restore & renew: Providing evolutionary, environmental and ecological information on a large scale to support restoration practices

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National Herbarium of NSW

The flagship research project Restore & Renew, is a world-first initiative that will create a comprehensive and easy to use website readily accessible by the community and supporting effective and sustainable land restoration. Utilising novel whole-genome sequencing and environmental modelling techniques, the project will uncover an unparalleled level of information to understand how genetic diversity is partitioned across the landscape, and if genetic provenances are associated with climatic and environmental variables. The project will collect, analyse and share genetic, adaptive, environmental, and ecological data for over 200 plant species commonly used in restoration projects across Australia's Eastern seaboard and representing the regional floristic, ecological and phylogenetic diversity. No other project on this scale and scope exists worldwide and I will present the concept and preliminary outcomes of this large-scale study. Restore & Renew will deliver a readily accessible resource of comprehensive restoration and management guidelines on an unprecedented scale. This enduring community resource will improve the success and long-term viability of land restoration projects, as well as improve predictive capacity to respond to climate change. Beside supporting restoration practices, the project will also provide significant information from species to landscape levels, discover regions of high genetic diversity, identify commonalities among taxonomic and functional groups that will improve our ability to generalise beyond the 200 species, and enable us to explore how species and assemblages are likely to respond through time. Understanding and predicting how plants will grow and interact in changing ecosystems is vital to conserving, or restoring, resilient natural habitats.

T3-14-03

Revegetation rewilds the soil bacterial microbiome of an old field

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Ecological restoration is a globally important and well-financed management intervention used to combat biodiversity declines and land degradation. Most restoration aims to increase biodiversity towards a reference state, but there are concerns that intended outcomes are not reached due to unsuccessful interventions and land

use legacy issues. Monitoring biodiversity recovery is essential to measure success, however most projects remain insufficiently monitored. Current field-based methods are hard to standardise and are limited in their ability to assess important components of ecosystems, such as bacteria. High-throughput amplicon sequencing of environmental DNA (metabarcoding of eDNA) has been proposed as a cost-effective, scalable and uniform ecological monitoring solution, but its application in restoration remains largely untested. Here we show that metabarcoding of soil eDNA is effective at demonstrating the return of the native bacterial and fungal communities in an old field following native plant revegetation. Microbiome composition shifted significantly after 8 years of revegetation, where younger sites were more similar to cleared sites and older sites were more similar to remnant stands. Revegetation of the native plant community strongly impacted on the belowground microbiome, despite the revegetated sites having a long and dramatically altered land use history (i.e. > 100 years grazing). We demonstrate that metabarcoding of eDNA provides an effective way of monitoring changes in microbiomes that would otherwise go unchecked with conventional monitoring of restoration projects. With further development, awareness of microbial diversity in restoration has significant scope for improving the efficacy of restoration interventions more broadly.

T3-14-04

Metabarcoding reveals soil microbial community succession patterns over a 30-year chronosequence of post-mining forest ecosystem restoration

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Advances in next generation sequencing (NGS) methods are promising to revolutionise the practical contribution of genetics to ecological restoration. Novel applications include an enhanced capacity to measure adaptive genetic variation for seed sourcing, gene expression analysis as a measure of genetic resilience of restored populations, and high-throughput assessment and monitoring of natural and restored biological communities aboveground and belowground. Soil microbial community characterization through high throughput meta-barcoding provides a powerful tool to assess responses and trajectories of restoration site soils post-disturbance. This study assesses changes in bacterial and fungal communities at 36 sites through a 30-year-old chronosequence of highly diverse forest-community rehabilitation after bauxite mining in south-west Australia. Bacterial and fungal communities were analysed using Illumina MISEQ sequencing of the 16S rRNA and ITS1 gene regions respectively. These were assessed against changing restoration practises, above ground biodiversity, adjacent intact remnant communities, and physical/chemical properties of the soil. Measuring below-ground richness using NGS considerably alters perceptions of biodiversity in restored and natural landscapes, and helps to optimise reconstructed substrates for aboveground and belowground biodiversity.

T3-14-05

A test of climate-informed restoration in forests adjacent to the north shore of Lake Superior, MN, USA

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Great Lakes forests will likely experience some of the most dramatic effects of climate change in the continental U.S.A. Simulation modeling indicates that temperate tree species that currently occur in the region at low abundance levels (e.g., *Quercus rubra*, *Q. macrocarpa* and *Pinus strobus*) will be well-adapted to future climate conditions, but also demonstrates that the natural colonization rates of these species cannot keep pace with the rapidly changing climate leaving many suitable niches unrealized. In the first climate-informed restoration project in this region, we tested the efficacy of within-range planting of climate adapted species sampled from more than one seed transfer zone. In 2013 and 2014 we planted a total of 4,400 seedlings in sixteen sites using both local and seed sources from further south or west where climate conditions are warmer. For three years (2014-2016), we assessed survival, growth, and spring and fall phenology. We compared the fit of the data using four models that featured contrasting factors: 1) seed transfer zones, 2) forest types, 3) geographical position, and 4) climate variables. Overall, the geographical model fit the data best for most traits according to DAICc. Differences between plants sampled from contrasting seed zones was found for both oak species and in some cases for the pine. Interestingly, the plant ecotype that performed best in northern conditions differed between species. In sum, early results suggest climate-informed restoration within the geographic range of species may be an effective strategy for maintaining key forest functions in a changing climate.

T3-14-06

The genomic basis of climate adaptation in lodgepole pine and interior spruce

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Local adaptation is common in widespread conifer species and current reforestation policy reflects this through local seed sourcing and breeding programs. However, as the climate changes local tree populations may become mismatched to their local environments. Our goal was to identify the genes responsible for climatic adaptation in western Canada's two most economically important conifers, lodgepole pine (*Pinus contorta*) and interior spruce (*Picea glauca*, *P. engelmannnii*, and their hybrids). To do this we conducted a population genomics study of local adaptation for both these species across similar climatic gradients. Using a sequence capture approach that targeted the exome, we used environment allele associations, and phenotype allele associations to identify candidate regions of the genome important for temperature adaptation. Our comparative analysis of these regions between the two species revealed that adaptation to temperature has a polygenic signature of convergence at the genomic level. This is despite the fact that these species have been diverged for more than 140 million years. This suggests that adaptation to climate is somewhat genetically constrained, with key genes, particularly transcription factors, playing non-redundant roles. Our results will be important for designing reforestation policies that consider future climates, and for understanding the genetic capacity of natural populations to adapt to new climatic conditions.

T3-15: Effects of thermophilisation on alpine vegetation

T3-15-01

No more late bloomers: Warming induces late-season flower deficits in bumble bee host plants

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Climate-induced changes contribute to recent pollinator declines by disrupting pollination mutualisms through phenological, functional or behavioral mismatches. Social insects, such as bumble bees, have complex life histories that require flower resources during three key stages: queen emergence, worker accrual, and mating. Bumble bees are dominant pollinators in climate-sensitive high altitude environments which forecast the effects of climate change in other ecosystems and where abundance of bumble bee host flowers has declined with warming temperatures. The seasonal spacing of these declines will shed light on the implications for bumble bee life cycles. In one montane habitat, a mid-season flower deficit has emerged with recent climate change, potentially restricting colony growth but not queen overwintering provisions. Here, we determine the impact of temperature and snowpack on the onset, peak and duration of flowering for historical bumble bee host plants on three mountains in Colorado: Pennsylvania Mountain, Niwot Ridge Long-Term Ecological Research station, and Mt. Evans Wilderness. Using phenology data collected across a 300 m altitudinal gradient on Pennsylvania Mountain from 1977-1980 and at all three sites from 2012-2014, we tested for climate-induced shifts in flowering onset, peak and duration. We asked whether temperature and snowpack variation among sites gives rise to geographical variation in flowering phenology, congruent with historical trends on Pennsylvania Mountain. Taken together, these data elucidate whether flower availability aligns with key bumble bee life history stages at the scale of bumble bee foraging. In comparison to historical norms on Pennsylvania Mountain, bumble bee host plants flowered for 11 fewer days, on average, in 2012-2014. Late flowering plants have advanced more in both onset and peak flowering with warming than early flowering species, resulting in a compression of the flowering season. Similarly, warmer temperatures were associated with compressed flowering on three geographically distinct mountains with flowering duration declining on average by 3.15 days per 1°C warming. While the flowering season was compressed, flowering overlap did not increase. Indeed, co-blooming species richness decreased by 0.81 species per 1°C warming. Plots with higher species richness bloomed for longer, indicating that fewer species bloom in each plot when it is warmer. Overall, these results suggest that warming-induced flower deficits will be most severe at end of the colony cycle when new reproductive bees are developing, mating, and provisioning for over-winter torpor. Contrary to mid-season deficits seen at lower altitude, the late-season phenological mismatch between flowering and colony needs could weaken colony production and queen overwintering success in high-altitude bumble bees.

T3-15-02

The genus *Saxifraga* as a model to study drivers of alpine diversification and global climate change risks

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Mountains can harbour high and unique plant diversity, but in spite of long standing scientific interest the fundamental principles responsible for the diverse mountain biota are not fully understood. The species-rich genus *Saxifraga* L. (ca. 440 spp.) has a predominantly alpine distribution and is emerging as a model system to study the eco-evolutionary assembly of mountain floras. We are assembling a high-resolution phylogenetic tree of *Saxifraga* and to this end use next generation sequencing (HybSeq) to obtain genome-wide data to overcome problems due to a lack of genetic variation among recently diverged species. In addition, a global database will be built of *Saxifraga* distribution, habitat and functional traits. Combined with the species-level phylogeny this database can be used to infer historic dispersal events and radiations, and the role of habitat- and trait evolution therein. Furthermore, many *Saxifraga* taxa are narrow endemics in sensitive alpine habitats. Our study also aims to unveil to what extent species diversity and functional diversity are at risk due to global climate change.

T3-15-03

Range shifts in response to climate change of *Ophiocordyceps sinensis*, a fungus endemic to the Tibetan Plateau

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Recent climate change has been widely recognized to influence the distribution of many plants and animals, while its impacts on the distribution of fungi remain largely unknown. Here, we used *Ophiocordyceps sinensis*, an entomopathogenic fungus and important traditional Chinese medicine whose distribution range was reported as decreased on the Tibetan Plateau in recent decades, as an example to predict the current potential distribution and the possible range shifts in response to climate change of a fungus by using extensive field records and an ensemble species distribution modeling method. It is demonstrated that the distribution range of the fungus wound decrease significantly, shifting upward in altitude and toward the central part of the Plateau. In an unlimited dispersal scenario, net habitat losses of 19% for both years 2050 and 2070 under representative concentration pathways (RCP) 2.6 and of 8% and 4% under RCP8.5 for the years 2050 and 2070, respectively, were predicted. If a non-dispersal scenario was considered, 36–39% of the current habitats would be lost in the future. The results presented here will not only provide useful information for the conservation of *O. sinensis*, but also provide a representative case of evaluating impacts of climate change on fungal distribution using species distribution modeling method.

T3-15-04

Plant geographic range size and climate stability in China: Growth form matters

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Geographic variation of range size is often proposed to be linked to climate seasonality and Quaternary glacial-interglacial oscillations. In addition, the importance of long-term climate stability is thought to be modulated by species' dispersal abilities. For plants, growth forms integrate numerous traits and likely also dispersal ability. Here, we assessed the relative importance of short- and long-term climate stability for range size patterns and whether the importance of the latter is greater for more poorly dispersed growth forms. Location: China. A comprehensive botanical dataset including over 30,000 vascular plant species was used to quantify the geographic patterns of range size across China for all species and eight growth forms separately. Then, spatial and non-spatial regressions with information-theoretic multi-models selection were performed to estimate explanatory importance of short- and long-term climate stability as well as land area and contemporary average climate conditions. The proportion of endemic species for each growth forms was also calculated. Geographic range sizes were significantly associated with both short- and long-term climate stability, with large range sizes occurred in areas with strong climate seasonality and historical climate change. The association between range size and climate change velocity was stronger for perennial herbs and shrubs than ferns, annual herbs, climbers and trees and stronger for small trees than large trees, i.e., more important for the growth forms with the lowest general dispersal capacities. In line with this interpretation, growth forms strongly influenced by velocity also tend have high proportions of endemism, which further confirm their generally low dispersal abilities. Our results suggest that range size patterns of vascular plants in China are shaped by both climate seasonality and long-term climate stability, despite that the region was relatively mildly influenced by the Pleistocene glaciations, in supplement of other factors, with a stronger influence of paleoclimatic stability on more poorly dispersed groups. The differential influences of paleoclimatic stability across growth forms suggest that biodiversity conservation needs consider plants with multiple growth forms to respond further climate changes.

T3-15-05

From the great plain to the Carpathians – How the global climate change affects the roses and hawthorns

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The affects of the global climate change can be felt specially in the Carpathian-Pannon region due to its geological conditions. The problem is complex since the behaviour and reactions of the species are determined by human activities, incorrect habitat management, forest and hunting management and also by extreme weather conditions. The efforts done locally to protect the sepcies might fail if the negative global trends would not change. The *Crataegus nigra* Waldst. et Kit. grows in the flood plains of the River Danube and Dráva (which closest relative is the *Crataegus chlorosarca* Maxim - which can be found in the Far-East, so in China also). It has has lost 50% of its habitat in the last century. One of the reasons might be the global warming, the drying of the wetlands beside the river and forest regulations. The *Curvisepala*-group: *Crataegus lindmanii* Hrab.-Uhr., *C. ovalis* Kit., *C. rosaeformis* Janka are prety tight tolerance (steoecicus) species. They grow from mid-mountains till high mountains and have long sepals. Beside the incorrect forest regulations the other reason of their desappearing is the negative change in the climate of the forests (extremely rainy weather in May and extremely hot and dry weather during the summer). As a first step they can only bloom (produce pollen), but are unable to produce fruit, as a result of this the populations can not renew. As a second step, through producing pollen they hibridize with euroecicus species. These hibrids are more viable. Its effect is easy to notice: these species are desappearing from the Hungarian mid-mountains but the hibrids can be found in great amounts. From these processes we can conclude the following: due to the global climate change the same will happen in the high mountains of the Carpathians. The Mediterranean *C. brevispina* Kunze was very rare in the Carpathian Basin hundred years ago, but it grows now in the submediterranean part of midmountains of Carpathian Basin. The most sensitive roses for the global warming are the *Villosa*-group (*R. villosa* L., *R. ciliato-petala* Besser, *R. velebitica*, *R. coziae*) and the *Tomentosa*-group (*R. tomentosa*, *R. kitaibelii*, *R. sherardii*, *R. floccida*, *R. umbelliflora*). These species can be found only in mid- and high mountains. These two groups have been retracting since the Ice age, the populations in Northern-Europe are disjunct, towards the south they become desperse. The split off-populations are on a unique evolution way, local small species have formed. The herbarium revision of the *R. villosa* showed that the number of speciments which were collected during the last cca 200 years has decreased, in spite of looking for the known-habitats the population was not found because the vast majority become extinct even from the high mountains. The Hungarian populations of the *R. ciliato-petala* partly have extincted, they remained only on the dolomit vegetation in the north side. The *Rosa velebitica* and *R. coziae* grows on the top of a few high mountains, the global climatechange risks mainly these populations. The *R. tomentosa* és *R. sherardii* behaves like the *R. villosa* but its populations only grow thinner, big populations can be found only in the high mountains. Several taxa (*R. kitaibelii*, *R. floccida*, *R. umbelliflora*) show termophil nature due to the global warming. They are trapped on the "islands" of the mid- and highmountains southern, warmer parts. It is not known how these micropopulations will deal with any further climate change. They might survive or disappear. The *Rosa pendulina* L. grows in the Transdanubian mountains in the XVIII. century, but it is extinct from there now and this species grows in the North midmontains and the Carpathians. The *R. glauca* Pourr. grow in the highmountains in Europe, it is common species in the Pyrenees, Alps and Velebit mountains, but this species is extinct from the Carpathian Basin during in the last century.

T3-15-06

Effects of climate change on Eurasian and North American alpine vegetation – a general overview based on field studies

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Despite broad evidence that climate change continuously effects alpine vegetation at different levels, the mechanistic understanding of these changes is still missing. A rapid warming observed in Eurasian and North American mountains is predicted to increase plant upper distributional limits, vegetation cover and abundance of species adapted to warmer climate. Plant community composition analysis throughout arctic and alpine North American and European sites show, that increases in the relative abundance of species with a warmer thermal niche were observed in response to warmer summer temperatures. However, effect sizes were greater over broad-scale spatial gradients relative to either temporal variability in summer temperature within a site or summer temperature increases induced by experimental warming. A pan-European survey showed that thermal differences caused by solar radiation determine plant species diversity on temperate mountains. The field studies indicate that the direction and magnitude of the changes differed considerably among the studied communities and were dependent on initial species composition, vegetation structure and geographical context of individual vegetation belts. It seems that plant species responses to ongoing climate change will not be unidirectional upward range shifts but rather multi-dimensional, species-specific and spatially variable.

T3-16: Floral chemistry and plant-pollinator diversification

T3-16-01

Pollinator-mediated hybrid and introgression under the diffuse coevolution may improve *Ficus* diversification greatly

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Whether and how coevolution between plant and insect pollinator improved plant diversification is one of the central question for both ecology and evolution researching. The extreme diversity of figs (*Ficus*) and their obligate pollinators, the fig wasps (Agaonidae), provides a nice opportunity to target that question. Here, following the diffuse coevolution mode, in which, a group of figs species coevolution with a group of pollinating fig wasps and the hybridization and introgression among fig species mediated with pollinator sharing or pollinator shifts are allowed, we try to test whether the diffuse coevolution is common in this obligate pollination system and how it contribute to the species diversification of the system. Using five sympatric dioecious fig taxa and their pollinators, we examine the degree of pollinator sharing and inter-taxa gene flow. We experimentally test pollinator preference for floral volatiles, the main host recognition signal, from different figs. All five fig taxa shared pollinators with other taxa and gene flow occurred between fig taxa within and between sections. Floral volatiles of each taxon attracted more than one pollinator species. Floral volatiles were more similar between closely related figs, which experienced higher levels of pollinator sharing and inter-taxa gene flow. This study demonstrates that pollinator sharing and inter-taxa gene flow occurs among closely related sympatric dioecious fig taxa and that pollinators choose the floral volatiles of multiple fig taxa. The diffuse coevolution should be common in this system. We further tested how many "good" fig species may speciation with hybridization and introgression via pollinator sharing or shifts as above diffuse coevolution. A chloroplast *Ficus* phylogeny with de novo assembled full cp genome sequences of 27 species of 16 sections of 6 subgenus was established and compared with recent nuclear *Ficus* phylogeny. Primary result shows chloroplast genome *Ficus* phylogeny get very high support in almost all deep and tip nodes. However, the cyto-nuclear discordance was repeatedly detected in both phylogeny of specie and section level, which may suggest the role of hybridization speciation via above diffuse coevolution should be one of important way for *Ficus* diversification.

T3-16-02

Floral chemistry and plant-pollinator diversification: Introduction to the symposium

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Plant evolutionary biologists have long appreciated the role of key innovations, such as nectar spurs, in the diversification of flowering plants. Recent evidence suggests that chemical features of flowers – their scent, nectar, pollen and oils – play important roles in the full spectrum of generalized to specialized, and mutualistic to deceptive relationships with pollinators. These roles include the structuring of plant-pollinator community networks through pollinator attraction and repellence, innate pollinator preference in specialized reward-based systems, partner choice in obligate mutualism and sensory exploitation in deceptive pollination. Improved methods in chemical analysis combined with new statistical, genomic and phylogenetic approaches make it possible to combine chemical ecological methods with the study of diversification in species-rich angiosperm lineages (e.g., orchids, figs, aroids). In this symposium, we showcase the state of the art of these combined chemical ecological and comparative approaches to explore the potential for floral chemical traits to function as key innovations in the diversification of angiosperm lineages. Southern China is an inspiring place to discuss such a topic, with botanical diversity across its biomes and emerging local research on chemical aspects of pollination biology.

T3-16-03

The chemistry and evolution of pollination by sexual deception in Australian orchids

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In highly specialised pollination systems, floral volatiles, floral morphology, or a combination of both likely underpin pollinator specificity. Floral volatiles in particular frequently play a key role in long-distance attraction, yet we often know very little about the chemistry involved. When pollinator specificity is strongly controlled by chemistry, we expect to find one of two main patterns: Either specificity is achieved using unusual blends of commonly occurring volatile compounds, or alternatively via novel or unusual compounds that are rare in nature. The former is predicted to be the most likely evolutionary scenario, since the semiochemicals can be readily produced by the existing biosynthetic machinery. However, testing this prediction is challenging since elucidation of functional floral volatiles is likely be strongly biased towards known and common compounds. Semiochemicals are of paramount importance in sexually deceptive orchids as they are used to lure male insects through mimicry of the female's sex pheromone. The strategy has evolved repeatedly in orchids, with a wide diversity of insect groups exploited. In Australian terrestrial orchids that attract male thynnine wasps by sexual deception, several different chemically unrelated sex pheromone systems are now known. These include: chiloglottones, representing a new class of floral volatiles when first discovered, new (hydroxymethyl) pyrazines, and blends of novel (methylthio) phenols not seen before in plants. Critically, confirmation of these compounds as semiochemicals is fully backed by bioassays in the field. We have also confirmed an unusual dual semiochemical system requiring a specific 1:4 blend of the widespread monoterpene plant volatile, citronellol, with an unusual disubstituted acetophenone not previously known as a floral volatile. Further work on the biosynthesis of these two compounds in the orchid has fully elucidated the biochemical pathway for citronellol production for the first time in any plant, and points strongly to a polyketide based biosynthesis of the acetophenone. Pollinator attraction in this case thus requires the coordination of not one, but two unrelated biosynthetic pathways for semiochemical production. An overlay of these semiochemicals onto the phylogeny of Australian terrestrial orchids reveals that there have been multiple independent origins of sexual deception in Australian orchids, with major switches in chemistry occurring even within orchid clades. Contrary to expectation, a diversity of floral volatiles new to science, and unusual combinations of known volatiles, underpin specific pollinator attraction in Australian sexually deceptive orchid systems. Furthermore, it is evident that we have barely scratched the surface of our understanding of the chemistry of sexual deception in Australian orchids, with our focus so far limited to male thynnine wasp pollinated systems, to the neglect of other groups of sexually exploited pollinators involving other groups of Hymenoptera, and Diptera. Our finding that both novel compounds, and unusual combinations of compounds, are frequent in our study system lead us to predict that similar discoveries await both other sexually deceptive systems, and other specialised pollination systems. Indeed, the chemistry of pollinator specificity may yet prove to be more often based on novel and unusual compounds, than on unusual blends of common floral volatiles.

T3-16-04

Herbivory, pollination and the diversification of floral scent in evening primroses (Onagraceae)

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Floral trait evolution is frequently attributed to pollinator-mediated selection but herbivores can play a key role in shaping plant reproductive biology, especially if the same floral traits mediate both mutualistic and antagonistic interactions. Due to their opposing effects on plant fitness, herbivores and pollinators have the potential to influence the evolution of floral phenotypes across geographic landscapes with implications for speciation and diversification at higher taxonomic scales. Here, we examine the role of herbivory in the diversification of floral scent chemistry in evening primroses, Onagraceae. Evening primroses represents the most diverse group of hawkmoth-pollinated plants in North America with remarkable divergence in floral morphology and chemistry. Preliminary analyses suggest that inter- and intraspecific variation in floral scent is pervasive, especially for two enantiomers of the monoterpene alcohol, linalool. In *Oenothera harringtonii*, a geographic (R) (-) linalool polymorphism is maintained in spite of high levels of gene flow, suggesting strong selection on floral scent. In many *Oenothera* species, (R) (-) linalool is negatively correlated with the presence of *Mompha*, a specialist flower and fruit-feeding moth genus. These data suggest that (R) (-) linalool might function as an herbivore deterrent, and that geographic variation in the strength of plant-herbivore interactions might diversify floral scent phenotypes with implications for plant-pollinator interactions. We examined the role of floral chemistry in mediating pollinator and herbivore interactions and in driving the diversification of evening primroses at multiple evolutionary timescales. We performed a manipulative field experiment, adding (R) (-) linalool to flowers of *Oenothera harringtonii* in a population lacking linalool, and measured pollinator visitation, herbivory and seed fitness. Controlled laboratory assays were used to determine whether hawkmoth pollinators preferentially oviposit on plants that vary in linalool presence/chirality. Finally, we surveyed the presence (and enantiomeric composition) of linalool and other floral volatiles in 61 populations of 16 species of Onagraceae (*Oeno-*

thera and *Clarkia*) across western North America, in conjunction with measurements of pollinator visitation and herbivore damage.

T3-16-05

Pollination of sexually deceptive genus *Lepanthes* (Orchidaceae: Pleurothallidinae)

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Pleurothallidinae is the largest Neotropical orchid subtribe encompassing > 5,000 species that are mainly dipteran pollinated. Of the few pollination systems documented in detail for the subtribe, the most interesting one occurs in the genus *Lepanthes*. Species of this genus are pollinated by sexual deception through genitalic pseudocopulation involving male fungus gnats (*Bradysia*, Sciaridae), probably attracted by a female pheromone-mimicking strategy. The pseudocopulation strategy of *Lepanthes* has not yet been fully investigated and remains largely hypothetical. Our objective was to investigate the pheromone-mimicking strategy in *Lepanthes*. We studied the ultrastructure of *Lepanthes* flowers focusing on their adaptations to pollinators through the integration of various microscopy methods (LM, SEM, TEM and micro-CT), histochemistry and chemical analyses of floral fragrances by GC/MS. The sepals do not react with any histochemical tests but show secretory trichomes likely involved in fragrance synthesis. The petals and lip are papillose and react with various staining tests for carbohydrates and lipids. Micro-CT scans show a close 3D fit between flowers and pollinators. Our results suggest that *Lepanthes* flowers do not produce obvious rewards. We further found that fragrances are composed of long chain hydrocarbons (alkanes, alkenes, esters and acetates). Some of these compounds act as pheromone precursors or are pheromone mimicing compounds. For example, Z-13-Octadecen-1-yl acetate, detected in *Lepanthes turialvae*, was reported as the pheromone mimic compound of the rice leafroller moth, *Cnaphalocrocis medinalis*, an economic pest of rice in China and Korea. Our data support the theory of a sex pheromone-mimicking strategy in *Lepanthes*. Floral morphology of *Lepanthes* varies astonishingly but is always based on similar (micro)morphology and chemistry. Thus, it is likely that a female pheromone-mimicking strategy is widespread in the genus. Variation in micromorphology and fragrance composition among species might act as isolation barrier thus promoting speciation.

T3-16-06

Landscape perspectives on pollinator-driven evolution of floral scent signals

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There is a general consensus that most phenotypic diversification of plants occurs in geographically separated populations and that this eventually translates into character differences between species. It is therefore important to understand the basis of phenotypic divergence among populations, particularly the roles of neutral versus adaptive processes. Floral volatile emissions have been shown in many cases to be correlated with pollination systems at the macro-evolutionary level and to be functional for pollinator attraction, but can we also detect a population level process of adaptation to local pollinator assemblages? The study of geographical variation in floral scent emissions is still in its infancy given the serious analytical challenges involved in collecting and analysing such data. However, available evidence shows that volatile emissions are often highly variable among populations, presenting an example of phenotypic variation which is usually cryptic to the human observer. Some recent studies based on reciprocal translocation experiments and laboratory choice tests have provided some tantalizing evidence for local adaptation of plants in terms of their volatile emissions.

T3-17: Increasing the sustainability of agricultural ecosystems

T3-17-01

Applying plant ecological knowledge to increase agricultural sustainability

Jacob Weiner
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Plant ecological knowledge accumulated over the past 150 years has enormous implications for agriculture, but most of these implications have not been appreciated by ecologists or agriculturalists. Here, I present several of the most salient examples: Agriculturalists refer to "improvements", but plant ecologists know that "trade-offs" represent a better conceptual framework for agricultural production. There is much evidence for trade-offs between yield and resource use efficiency, and between individual fitness and populations yield. I argue that there is also a "limiting trade-off" between short term yield and sustainability, and it is important to take this into consideration if we are serious about increasing sustainability. At the local level, agricultural sustainability is about maintaining or improving soil fertility, but this is not a priority in most agricultural systems worldwide. Natural plant communities with the highest productivity do not resemble the very earliest stages of succession with extremely low standing biomass, suggesting that it is possible to develop highly productive, more sustainable, plant productions systems, which share many properties of somewhat later successional stages. I present such a vision, which I call "High Biomass Cropping Systems". Plant ecology tells us that rotation of crops with very different nutritional needs and very different pests, diseases and weeds can make a major contribution to sustainability. The very limited crop rotations practiced in most modern plant production systems is a clear indication that farming practices are based on short-term economic and regulatory factors, without much if any consideration for sustainability. The modern scientific method tells us how we should test hypotheses, but it says nothing about how hypotheses are generated. We need to address the agricultural research agenda if it is to serve the interests of farmers, consumers and society as a whole, rather than narrow but powerful economic interests.

T3-17-02

Investigating past domestication to improve sustainable agriculture in future croplands

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Securing sustainable food provision in response to current global change and demographic growth poses challenges that must be addressed from diverse perspectives. Bringing better agronomic practices contributes to that endeavour. However, not only management affects food provision. Crop traits also play significant roles in modulating the ecosystem services (ESs) that secure sustainable yields in the face of global change. Here we advocate that understanding how crop traits evolved under domestication and impacted ESs delivery should help breed future crops. Thus, we address the effects of crop evolution (from initial domestication to current times) on relevant ESs, including crop yield and its stability over time, soil carbon (C) sequestration, and soil nitrogen (N) retention. There is initial evidence that crop domestication affects the delivery of ESs. However, most of those evolutionary effects are understudied. Therefore we propose research gaps that merit attention, including (1) assessing whether performance in polyculture is lower for crop mixtures than for mixtures of wild plants, and breeding for more efficient crop polycultures; and (2) investigating how crop evolution impacted plant effects on C storage and N cycling, and how such effects contribute to the stability of yields. The sustainable provision of cropland ESs, and thus food security, will benefit from exploring those avenues

T3-17-03

Using positive interactions between species to help sustain agricultural ecosystem

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Facilitative interactions between species (one species makes conditions more favorable for another) are receiving increasing attention in Ecology. It is also been recognizing that exploiting facilitative interactions between species play an important in the restoration of degraded ecoysytems and in the development of sustainable agricuture with biodiversity. In agro-ecosystem, although multiple-species cropping has showed over-yielding, however, additional species might not always increase agricultural productivity of the main crop due to competition (for nutrients, light, *etc*.). Thus in the utilization of species diversity in agriculture, it is critical to understand the characters of species interactions and to exploit the positive interaction (facilitation) between species. Here, we synthesize the current advances in our understanding of how agriculture systems with co-cultured species can ensure sustainable food production while minimizing environmental damage by a meta-analysis and a field survey. Crop yield in legume-cereal intercropping or rice-fish co-culture or agroforestry, and the corresponding monoculture was used in the meta-analysis. Papers published in the past twenty-years (1990-2012) were search in the public database using key words legume-cereal intercropping, or rice-fish, or agroforestry. A data set of 276 paired-field survey (rice-fish co-culture vs monoculture) was also used to do the meta-analysis. The effect size was calculated as the natural log of the response ratio (R, the mean of intercropping, or rice-fish, or agroforestry divided by the mean of monoculture). A positive effect size with confidential intervals (CIs) that do not overlap zero would indicate that the crop yield in intercropping, or rice-fish, co-culture or agroforestry than with monoculture. Our results from meta-analysis indicated that legume–cereal intercropping significantly increased cereal yield but did not affect legume yield. The positive effect size on cereal yield was greater with pesticide use but was not influenced by the other five factors (climate, planting pattern, irrigation, fertilization, or weed control). For legume yield, the effect size was not influenced by any of the six factors. For rice-fish co-culture system, meta-analysis indicates a positive effect size of rice–fish co-culture on rice yield regardless of fish culture type (single fish species or mixed fish species) in the co-culture system. For intercropped agroforestry systems, however, crop yields significantly increased only in some cases (41 studies among 115 studies). For most cases (74 studies among 115 studies), crop yields significantly decreased compared to monoculture. These yield reductions were mainly due to shading and root competition. Results from field survey on rice-fish co-culture showed that utilizing species diversity can sustain rice yield with less chemical fertilizer and pesticide inputs when the coexisting-species generate facilitative effects. This study indicates that co-cultured species in agriculture can increase crop yield, but these enhancements are sensitive to environmental conditions, planting techniques, and management.

T3-17-04

Crop diversity enhances agroecosystem sustainability via improving soil fertility in north-western China

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We face a major challenge feeding an expanding world population with highly degraded soils. Reversing the trend of soil degradation and ensuring food security and a sustainable future have therefore become essential. Although multispecies systems often are productive, the sustainability has been questioned in longer temporal and wider spatial variations as continuous overyielding generally removes more nutrients from soils. In order to address these issues, two field experiments were conducted initially in 2009 at the soils which were reclaimed for 3 years at Hongsipu in Ningxia (Exp. 1) and for 20 years at Jinyuan in Gansu (Exp. 2), China. The Exp. 1 was split-split-plot design, where main plot treatments included inoculation with rhizobium or not, and the sub-plot treatments included five N-application rates (0, 75, 150, 225 and 300 kg N ha^{-1}), and the sub-sub-plot treatments were sole faba bean, maize, and faba bean/maize intercropping. The Exp. 2 was two-factorial design, where main factor was P application rates (0, 40, 80 kg ha^{-1}) and the second factor was cropping systems, including canola/maize, chickpea/maize, faba bean/maize, and soybean/maize intercropping, and their corresponding monocropping. Each treatment has three replications in both field experiments. Productivity, soil physical and chemical properties (bulk density, water-stable aggregates, infiltraction, compactivity, soil organic matter, total N, Olsen-P, Exchangable K, CEC, and pH), and some enzyme activities were measured in 2012 and 2013 in both experiments. Results showed that overyielding were observed in faba bean/maize and chickpea/maize intercropping, whose productivity stability was maintained or increased compared with corresponding monocropping. The percentage of water-stable macro-aggregate (> 2.0 mm) was increased by intercropping compared to monocropped faba bean (67.9% vs 56.0%). Soil organic matter and total N were increased significantly by intercropping compared to monocropped maize 6.86 g/kg and 0.27 g/kg vs 6.03 g/kg and 0.22 g/kg) in the Exp. 1. In the Exp. 2, similarly, percentages of soil water-stable macro-aggregate were significantly increased by faba bean/maize, chickpea/maize, canola/maize intercropping, but not by soybean/maize intercropping. Soil organic and total N presented similar trends to Exp. 1. In summary, soil fertility was significantly improved after 4-5 years of intercropping compared with monocropping, especially in rewly-reclaimed desert soil. In other words, crop diversity can enhance agroecosystem sustainability via increasing crop productivity as well as inproving soil fertility in a long-term scale.

T3-17-05

Soil organic carbon accumulation, climate variability and crop production in Tanzania's semi-arid agro-ecological zone: A case study of the Kongwa District

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Various ecological and environmental indicators including climate change, soil fertility, water availability and proper agronomic practices that form optimal agricultural systems are needed to be integrated for increasing agricultural productivity in the Tanzanian semi-arid agro-ecological zone. Among these indicators, climate change and soil fertility are the major limiting factors to affect crop yields in this semi-arid agro-ecological zone. To improve crop productivity, this study assessed the accumulation of soil organic carbon (SOC), the trend of climate variability and crop production in the Kongwa District, a semi-arid zone in central Tanzania. In doing so, climate data and soil samples were collected from two representative villages of Mnyakongo and Ugogoni. Household surveys, informative interviews, physical observations, group discussions and literature reviews were employed for data collection and crosschecking. The Walkley-Black Method was used for SOC analyses while the SPSS v. 20, Mann-Kendall and Sen's Slope Test for climate, crop yields and climate-smart agriculture analyses. Qualitative data were analyzed using the theme content analysis and Community-based Risk Screening Tool-Adaptation and Livelihoods (CRiSTAL). Results showed that the accumulation of SOC was significantly greater in soils under organic fertilization (1.15 and 0.80 MgC ha^{-1} at soil 0-20 cm and 20-30 cm depth) than under no-fertilization (0.35 and 0.30 MgC ha^{-1} at 0-20 cm and 20-30 cm) and decreased with increasing soil depths. So did the crops yields (2 tn ha^{-1} under organic fertilization vs. 0.87 tn ha^{-1} under no-fertilization). The mean annual rainfall or temperature (1980–2015) fluctuated at a decreasing ($R^2 = 0.21$) or an increasing trend ($R^2 = 0.30$). Meanwhile, crop yields of maize, sorghum or millet fluctuated at a decreasing trend at $R^2 = 0.07$, 0.05 or 0.85, respectively. In whole, rainfall variability positively correlated with SOC or crop yields. Thus, to increase crop production, there is an immediate need to apply manure, irrigation and drought-tolerant crop seed. These results confirm that the studied semi-arid areas are among the most vulnerable regions to climate change impacts. Given to this vulnerability, this study proposes a district adaptation plan to increase the resilience of smallholder farmers. Similarly, it calls for more proactive practices to intervene the authentic and potential consequences in the country. A serious action to improve agronomic practices, mitigate and adapt to climate change impacts should concurrently be a priority in semi-arid areas to limit the level of vulnerability.

T3-17-06

Sustainability and management of water resources in *Hordeum vulgare* agro-ecosystems in relation to the allelopathic effect of *Retama raetam*

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The main objective of the present study was to manage water as a natural resource in *Hordeum vulgare* agro-ecosystems depending on the allelopathic potential of *Retama raetam* shoots aqueous extract (RRSAE). Germination percentage (GP), coleoptile (CL) and radicle lengths (RL) of the recipient species significantly increased under the allelopathic effect of RRSAE relate to control. The highest values (99%, 12.8 cm and 12.4 cm) were achieved at 3% concentrations level relative to the three parameters respectively compared to control. Furthermore, the effect of *R. raetam* shoots crude powder (RRSCP) on some growth parameters (number of tillers, spikes, grains spike^{-1} and grains plant^{-1}) as well as shoot dry weight, grains yield and grain harvest index of *H. vulgare* was evaluated. Results showed that the highest values were achieved at 3% concentration levels of RRSCP and 25% field capacity (low water field capacity) compared to the control. Phenolic derivatives (mg/kg DW) (especially catechin) of *H. vulgare* seedling was increased under the lowest water field capacity (25%) at 3% concentration level of RRSCP. Such increase initiatives stimulation in all studied growth parameters with adequate amount of water that help in conservation and management of water as a natural resource in agro-ecosystems.

T3-18: Plant invasion risk, control and ecosystem security

T3-18-01

Predicting potential benefits and risks of the ragweed leaf beetle as a biological control agent: From ecological to evolutionary studies

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An important but largely unknown outcome of biological control involves evolution, e.g., will a biocontrol herbivore over time be able to feed on a new non-target host, will the target host populations become tolerant, and what will be the outcome of the plant-antagonist interaction under climate change. Common ragweed *Ambrosia artemisiifolia* is native to North America and was accidentally introduced to many parts of the world, including Europe, Asia, South America, Australia and New Zealand. Like no other plant common ragweed has raised the awareness of invasive plants in Europe, causing great damage to our society due to its highly allergenic pollen, and as an important and hard-to-control crop weed. Both its spread and impact are likely to increase with changing climate, posing a significant risk even in countries presently not yet affected. The recently and accidentally introduced ragweed leaf beetle *Ophraella communa* (Col.: Chrysomelidae), is native to North America and presently used as a successful biological control agent in Asia and Australia. This beetle has the potential to reduce the population density and pollen production of ragweed in Europe and to halt its further spread, but it also bears the risk to attack taxonomically closely related sunflower and native endangered species. Following its first observation in Europe in 2013, we conducted extensive field surveys in Northern Italy to monitor the spread and target and non-target impact of *O. communa*, as well as experimental host specificity experiments under quarantine and field conditions. *Ophraella communa* expanded from early sites in S-Switzerland and N-Italy in 2013 by 500km both west- and eastwards up to 2016. Regression models showed that the exceptionally low amounts of airborne *Ambrosia* pollen observed in the Milan area since 2013 is most likely related to the presence of large numbers of *O. communa,* leading to yearly savings in human health costs of millions of Euros. Field survey and experimental biosafety studies indicate a certain risk to sunflower, particularly later in the season when most ragweed plants are defoliated by the beetle. We also initiated long-term studies to explore the evolvability of both the target ragweed populations to selection by *O. communa*, as well as of the *Ophraella* populations when kept on the sunflower as compared to ragweed. We present first results of a novel evolutionary approach to assessing the beetle's potential for genetically-based changes to novel biotic (host plants) and abiotic (future temperature) conditions, using next generation sequencing and bioassay approaches. Assessing evolvability of a biological control agent will greatly improve predicting its future benefits and risks.

T3-18-02

Adaptive genetic divergence and increased competitive ability in invasive *Ambrosia artemisiifolia*

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Invasive alien plant species face different abiotic and biotic conditions in their native and introduced range; furthermore, biological control programs will create an additional mosaic of species interaction in the introduced range. Together, these make them ideal study systems to address the basic question on rapid evolutionary changes of plant population. Differences in the selection and genetic variation in relevant traits can make adaptation difficult to predict. We used a common-garden experiment to assess patterns of adaptation in the invasive plant, *Ambrosia artemisiifolia*, sampled across 11 populations in its native (USA) and 10 invasive (China) ranges, with the 10 Chinese populations having a different history of biological control management. We tested for adaptive trait evolution by comparing differentiation at neutral genetic loci (F_{ST}) with differentiation at quantitative traits (Q_{ST}). We estimated multivariate Q_{ST}-F_{ST} tests to determine if phenotypic differences between countries are due to adaptation. Between countries, we found that two growth traits are distinguishable from genetic drift ($Q_{ST} > F_{ST}$), and interestingly those two traits are correlated with climatic variation in the USA, but not in China. Within countries, we found more traits under selection in the USA than that in China. Moreover, we found increased adaptive potential in the Chinese populations without biological control history ($Q_{ST} >> F_{ST}$) than with biological control history. Additionally, a pairwise greenhouse competition experiment using 10 invasive Chinese populations in competition with a native USA grass showed a reduced competitive ability for populations with than without biological control history, which might be due to a tradeoff between herbivore resistance and plant growth. Our results indicate that adaptation in invasive Chinese populations does not parallel patterns in native US populations due to differences in abiotic and biotic selection, genetic constraints, or both.

T3-18-03

Impact of plant invasions on biodiversity

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Plant invasions are one of the five major driving forces that lead to biodiversity loss worldwide. In this presentation, I will first explore how invasive plants affect native biodiversity through hybridization, disruption of ecological relations between species (e.g., native plants and pollinators), and alterations of ecosystem processes. I will then use exotic smooth cordgrass (*Spartina alterniflora*) as a case to show the effects of its invasion in the east coast of Chinaon biodiversity in saltmarshes.

T3-18-04

Higher resource sharing in invasive than in native clonal plants

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What confers invasive plants with competitive advantages over native ones remains an unresovlved question in invasion ecology. Many of the invasive plant species and many of the world's worst invasive plant species are capable of clonal growth. Clonal growth confers clonal plants with distinct traits such as clonal integration (resource sharing between connected ramets). While clonal integration has been shown to benefit the growth of some clonal invasive species, it is still unknown whether invasive clonal plants benefit more from clonal integration than native ones. We selected five pairs of invasive and native clonal plant species in China, and grew their disconnected (preventing clonal integration) or connected (allowing clonal integration) ramet pairs under heterogeneous light, soil nutrient and water conditions that are commonly encountered by alien plants during their invasion into new areas. To account for the phylogenetic relatedness, the invasive and native species of a pair were always from the same genus. Invasive clonal plant species grew better than native ones, and clonal integration increased the growth of both invasive and native clonal plants in all three types of heterogeneous environments. More interestingly, the benefits of clonal integration were significantly higher in invasive than in native clonal plants. Thus, clonal integration can confer invasive clonal plants with competitive advantages over native ones, and may partly explain the invasive success of alien clonal plants when invading new areas where light, soil water and/or nutrients are commonly heterogeneously distributed.

T3-18-05

A biocontrol agent may benefit a native plant in competing with an invasive plant under warming climate

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Climate warming may affect competition between native and non-native invasive plants directly or indirectly via herbivory. Here, we show that an introduced biocontrol beetle shifted competition between a native plant and an introduced plant under elevated temperature. In a 2-yr field experiment at 30.5°N, we found that experiment warming (+ 1.8°C) increased overwintering ability of the introduced beetle which prefers the invasive plant over the native plant, as a result, the beetle established sustainable population in the presence of the invasive plant throughout the trial. But under ambient temperature, the beetle failed to emerge in the second year. Warming also shifted life history of the native plant from annual to perennial. At the end of the second year, beetle suppressed the invasive plant and shifted the community from invasive plant dominated to native plant dominated under elevated temperature. Under ambient temperature, the invasive plant dominated the community under differing herbivore treatments. In addition, in a field survey from 21°N to 30.5°N where the beetle was present, the native populations were perennial < 25.8°N, but only annual populations were observed > 26.5°N, and the invasive plant only dominated the community > 26.5°N. Our results highlight that herbivore can play an important role in determining plant community structure and non-native plant invasions under warming climate. Thus, understanding how biotic interactions, herbivory in particular, shape responses of plant communities to climate change is crucial for predicting the risk of plant invasions under climate change scenarios.

T3-18-06

Changes in competitive ability, defense and allelopathic effect between invasive plants and conspecifics in their native ranges

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The Evolution of Increased Competitive Ability (EICA) hypothesis predicts that after liberation from herbivores, invasive plants evolve increased vigor and decreased defense by reallocating resources from costly defensive traits to growth or reproduction. However, invasive plants may not escape from generalists and may even experience increased damage in introduced ranges, although they generally release from specialists. Shifting Defense Hypothesis (SDH) predicts that invasive plants may evolve to decrease defense against specialist herbivores but maintain or even increase defense (increase toxic chemicals) against generalist herbivores in introduced ranges. It has been documented that plant toxins may also function as allelopathic agents. If so, the increased toxins may also contribute to the increased competitive ability of invasive plants by increasing allelopathic inhibition on co-occurring native plants. The Novel Weapons Hypothesis (NWH) predicts that unique chemicals of invasive plants may provide disproportionately allelopathic, defense, or anti-microbial advantages in their nonnative ranges. With respect to allelopathy, novel chemicals may have stronger effects on naïve native plants from nonnative ranges of the invaders, which have not adapted to the unique chemicals and therefore are more vulnerable to them than adapted plants from native ranges of the invaders. If novel plant chemicals conferred competitive advantages in the nonnative ranges of invaders, then selection pressure for the chemicals may be greater on the genotypes in the invaded regions than on the conspecific genotypes remaining at home. If so, the evolution of competitive ability might derive in some cases from selection for increased production of allelopathic compounds. The EICA hypothesis and the NWH are not mutually exclusive; plants can evolve larger size, greater production of novel allelochemicals, or changes in quantitative defense. However, little effort has been made to explore how these processes might be integrated in invasion success. In this presentation I will compare biogeographical differences in competitive ability, defense, and allelopathic effects of noxious invasive plants in China, and discuss how increased vigor and production of novel allelochemicals contribute to increased competitive ability of the invasive plants.

T3-19: Species interaction and community structure across environmental conditions ecosystem (two sessions)

T3-19-01

Life in the canopy: Comparative and community phylogenetic analyses of epiphytic growth in ferns

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Epiphytic growth is an important and recurring theme in fern evolution: transitions from terrestrial to epiphytic growth have occurred in at least five large fern clades, and may be associated with increased rates of diversification coinciding with the widespread appearance of angiosperm-dominated forests during the late Cretaceous and early Cenozoic. Despite the significance of epiphytic growth for fern evolution, however, few studies have investigated traits related to epiphytism in ferns while accounting for phylogeny. Here, we use the ferns of Moorea, French Polynesia, which include representatives of all five Cretaceous epiphytic fern radiations, as a study system to investigate evolutionary patterns in traits related to epiphytic growth in ferns. We analyze traits at both the species and community level, and test for degree of phylogenetic conservatism as well as phenotypic clustering. Our results are consistent with the canopy environment acting as a strong filter to restrict both phylogenetic and functional diversity of epiphytic fern communities. Our study provides an important phylogenetic perspective on the role of functional traits in ferns, and paves the way for more detailed studies examining how niche space is filled within terrestrial and epiphytic habitats.

T3-19-02

Dune stabilization and shrub size together regulate shrub-understorey herbs interactions in a semi-arid sandy ecosystem

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Questions: (i) do the stabilization stage of dunes and individual size of shrubs influence the direction and intensity of interactions between shrubs and their understorey species? (ii) do understorey species with different life-histories respond differently to dune stabilization and shrub size? (iii) does facilitation vary depending on the understorey species performance (i.e. growth or richness)? Location: Sand-fixing communities of the Mu Us Desert, China. Methods: We randomly selected shrubs of different sizes (large and small) below shrubs and in adjacent open areas along a dune stabilization gradient. We measured shrub crown size, environmental characteristics and the height, abundance, biomass and richness of subordinate species. Relative interaction index (RII) was calculated at the community-level and for annuals and perennials separately in all paired plots and analysed with ANOVAs. Understorey species composition was analyzed with Principal Component Analysis (PCA). Results: *A. ordosica* had dominant positive effects on understorey species in this dune system. However, we found contrasting variations in community-level facilitative effects across the gradient of dune stabilization and with shrub size depending on understorey species performances. Overall, facilitation for growth and in particular biomass decreased with increasing disturbance and shrub size, whereas the converse was observed for species richness. Notably, dune's stabilization and shrub's size were not only functioned independently but interacted to regulate the shrub-grass interactions. In addition, perennials were strongly affected by the shrub-grass interactions than annuals on both growth and recruitment. Species composition was changed due to the interactions, causing the invasion of perennials and reduction of annuals under the process of dune stabilization. Conclusions: Our observational study showed that community-level increase in facilitation for growth with increasing dune stability and shrub size were mostly explained by the turnover of abundance and biomass of perennials vs. annuals and the higher sensitivity of perennials to the effects of the shrubs, which were positive until a threshold of size. In contrast, the community-level increase in facilitation for richness with increasing disturbance and shrub size was mostly explained by the high recruitment strain of perennials in the disturbed open patches of the semi-fixed dunes, which was more likely to be ameliorated below large than small shrubs. Our study provides additional support to the theory arguing that communities are set of hidden interaction groups which might have contrasting responses to dominant neighbours in a single community depending on their functional strategies.

T3-19-03

Species associations in a subtropical forest in South China

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The spatial pattern of plants may provide significant insights into processes and mechanisms that maintain species richness. We applied data from a fully mapped 50-ha subtropical forest plot in Heishiding (HSD) Nature Reserve, China to conduct a community-wide assessment of the type and frequency of spatial association of species pairs. By analyzing the spatial association and attributes of the species pairs, we found that 55% of all species pairs showed negative bivariate association, which occurred less frequently among species sharing the same habitat association. In contrast, positive associations occurred more frequently among species with similar habitat requirements. The observation that species with similar habitat preference tend to aggregate suggests the vital role of environmental filtering in shaping community structure of this subtropical forest.

T3-19-04

Phenotypic effects of the nurse *Thylacospermum caespitosum* on dependent plant species along regional climate stress gradients

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Contrasting phenotypes of alpine cushion species have been recurrently described in several mountain ranges along small-scale topography gradients, with tight competitive phenotypes in stressful convex topography and loose facilitative phenotypes in sheltered concave topography. The consistency of phenotypic effects along large-scale climate stress gradients have been proposed as a test of the likely genetic bases of the differences observed at small-scale. Inversely, plastic phenotypic effects are more likely to vanish at some points along climate stress gradients. We tested this hypothesis for two phenotypes of the alpine cushion species *Thylacospermum caespitosum* at four points along regional gradients of cold and drought stress in northwest China. We measured the traits of the two cushion phenotypes and quantified their associated plant communities and environmental variables along the regional temperature and aridity gradients. Cushion height, convexity and stem density overall showed significant effect of phenotypes. Difference in tightness of cushions between phenotypes was consistent across climate conditions, whereas differences in cushion convexity and height between phenotypes increased with increasing cold stress. Phenotypic effects on species richness and abundance were consistent along both climate gradients but not effects on species composition, while there were no phenotypic effects on environmental variables. Additionally, RII curves were linear along the drought gradient but unimodal along the temperature gradients, likely due to the occurrence of contrasting species pools at the different sites. We conclude that the consistency of phenotypic effects of *T. caespitosum* was high for species richness and abundance and mainly explained by differences in interference mediated by likely heritable differences in cushion tightness. Additionally, our study shows that the shapes of the relationship between plant responses to neighbours and environmental stresses are not necessarily driven by niche-based deterministic factors.

T3-19-05

Changes in species diversity and community structure of the steppes along climate gradients on the Mongolian Plateau: Implications for conservation and adaptive management

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Understanding vegetation patterns along climate gradients are useful to predict vegetation dynamics and develop strategies for vegetation conservation and adaptive management under the global change. We investigated the steppe vegetation patterns on four transects along climate gradients radiating from the desert region in the central towards the relative humid areas in the north, northeast, east and south parts of the Mongolian Plateau. We found that (i) plant species richness and evenness in steppe vegetation declined, whereas the richness and dominance of C_4-species increased, with increasing climate aridity; (ii) the species-area curves showed a plateau at a lower level in relatively arid than humid regions, whereas the curve of species saturation (ratio of the number of species in a certain area to total number of species at a site) showed no difference on climate gradients; and (iii) the gradational replacement of the species of the same genera (ecological vicariance) were common along the climate gradients. These findings suggest that C_4-speceis are more adaptable to the environment of high temperature and low humidity, and that it is necessary to conserve plant communities in different sections on the climate gradient for biodiversity conservation. The results also suggest it appropriate to use the quadrat of the same size to survey plant diversity in different types of steppe vegetation.

T3-19-06

The extent to which mutualists affect the geographical range of plants in a changing environment

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Understanding the factors that underlie species distributions is a fundamental goal of ecology. Geographically disparate populations of species are likely to adapt to local biotic and abiotic conditions, potentially forming ecologically adapted populations (ecotypes), which may determine the range limits of species. However, we still do not fully understand the relative importance of environmental factors that lead to population differentiation. Despite having the potential to tolerate a broad range of environmental conditions, populations of species often only exploit a narrow range of resources. As their ranges are determined by abiotic factors (e.g., temperature, precipitation), and also by biotic factors (e.g., pollinators, mycorrhizal fungi), orchids are particularly sensitive to the local availability of environmental resources. As orchid seeds rely entirely on fungi to provide nutrients in order to germinate and establish, mycorrhizal fungi may determine the population occurrence of orchids. Hence, availability of mycorrhizas may lead to population differentiation according to the composition of the local mycorrhizal community. Sampling 50 populations over a 2,500 km range of the broadly distributed European orchid *Spiranthes spiralis*, I identified mycorrhizal communities using Illumina Miseq sequencing with the mycorrhizal specific primer pair, ITS3/ITS4OF. In addition, niche models were constructed to quantify the extent to which key abiotic environmental variables determine the distribution of *S. spiralis* and its mycorrhizas under current conditions and future climate scenarios. Results indicate that *S. spiralis* associates with a broad range of mycorrhizas, but that there is turnover in mycorrhizal communities. Temperature and precipitation play important roles in determining the current geographical range of both *S. spiralis* and its mycorrhizas, but its range will contract and shift under climate change scenarios. Results will be discussed in light of how such broad scale studies can help inform us to ameliorate plant declines due to anthropogenic manipulation of the environment, and shifting distributions due to climate change.

T3-19-07

Experience of inundation or drought alters the responses of plants to subsequent water conditions

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The availability of water is often highly variable over the life of a plant in nature, and most plants experience episodic extremes in water scarcity and abundance. The importance of plant plasticity in coping with such experiences is widely recognized, but little is known about how plastic responses to current conditions are affected by prior environmental experiences. Our objectives were to investigate the effects of early inundation or drought on the subsequent responses of plant species to the same, opposite or more favourable conditions. To address these questions, we subjected four invasive and four native herbaceous perennial species from different habitats (xeric, mesic, hydric) to two rounds of hydrological treatments (drought, moderate water, inundation) and analysed the effects of the early treatments on survival and performance (total biomass and relative growth) of individuals in the later treatments. In general, (i) early drought reduced the performance of more species than did early inundation, and decreased the final total mass of all species; (ii) early inundation and early drought did not lead to lower survival immediately or later, but improved the relative growth of survivors across all late conditions; (iii) late drought resulted in the highest mortality and lowest performance after any early treatment. With respect to habitat of origin: (i) early inundation was more beneficial for species from wet habitats than for other species; (ii) species from xeric habitats had the strongest drought tolerance after early drought; (iii) mesic species were more likely to suffer reduced later growth after either inundation or drought experience. Invasive species benefitted more from early inundation than did native species, but native species grew better after experiencing early drought. Results indicate that early exposure to inundation or drought conditions alters how plants respond to later conditions and suggest that exposure to extreme events can induce physiological or morphological changes that improve tolerance for either extreme conditions later. This increased tolerance can be at the cost of poorer performance under more benign conditions. Early inundation or drought experience may be harmful immediately, but can be beneficial for the later growth of plants. The ability of species to utilize early hydrological experiences was associated with the water range of their habitats and whether the species is invasive or native. The ability to modulate future plastic responses may be as important as short-term plasticity in adapting to temporal environmental heterogeneity. Such 'metaplasticity' can optimize current performance, while avoiding the potential costs of maintaining a high degree of plasticity throughout life.

T3-19-08

Trait-based adaptability of *Phragmites australis* to effects of soil water and salinity in the Yellow River Delta

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Ecological responses to soil properties of the *Phragmites australis* community were experimentally investigated through 96 field studies along the coastal-inland regions in the Yellow River Delta of China. The aim was to evaluate the relationship between phenotypic plasticity and environmental variables and we asked whether the selected functional traits could better respond to changes in electrical conductivity and soil water content. Within the range of soil water content (9.39–36.92%) and electrical conductivity (0.14–13.29 ms/cm), strong phenotypic plasticity of morphological and physiological based functional traits were observed. The effects of soil water content and salinity were not equally important for characterization of the morphological and physiological variability. When compared with soil water content, the response of salinity on plant traits, leaf traits and stem traits are more remarkable. The relationship between functional traits and electrical conductivity were generally linear and logarithmic, indicating that suitable electrical conductivity permits a wider range of functional variations in *P. australis*. Based on these results, salinity leads to reduced average height, specific leaf area, leaf area and base stem diameter, but increased leaf water content and leaf thickness. Overall, lower relative growth rate, longer leaf longevity, and preservation of internal resources were found to be the responses to salt stress to enable highly efficient utilization of resources. However, further studies, including underground traits and genetics, are also needed to clearly understand different responses to related ecotypes.

T3-19-09

Progresses and challenges on algal inhibition potential of hydrophyte allelopathy in shallow eutrophic Chaohu Lake

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Besides purifying and restoring the polluted water body by metabolizing, stabilizing, and transforming pollutants, aquatic macrophytes will kill harmful algae by producing active allelochemicals including phenols, terpenoids, fatty acids, esters and heterocycle group. Moreover, bio-diversity can promote algal suppression of typical hydrophytes by some formats such as positive interaction of many allelochemicals, the reasonable mixing culture of different ecotypes of macrophytes, and the efficient combination of hydrophytes and predatory zooplankton or benthic fauna. We have verified synergistic interactions among five allelochemicals [coumarin, ρ-hydroxybenzoic acid, protocatechuic acid, stearic acid, ρ-aminobenzene-sulfonic acid] with antialgal effects on bloom-forming *Microcystis aeruginosa*. Meanwhile, in the watershed of Chaohu Lake, through both field survey and lab experiment, three typical aquatic macrophytes (*Alternanthera philoxeroides*, *Ranunculus sceleratus* and *Trapa incise*) showed combined enhanced-effect on algal suppression. Interestingly, four predatory zooplankton, i.e. *Stentor polymorphus* Müller, *Brachionus calyciflorus* Pallas, *Acartia spinicauda*, and *Moina macrocopa* will help the macrophytes fighting with the noxious algae. Similar findings also occurred for some dominant zoobenthos such as *Limnodrilus hoffmeisteri* (oligochaete), *Chironomus plumosus* (chironomid larva), *Palaemonetes sinensis* (crustacean), and *Corbicula fluminea* (mollusk). Especially, the pollution conditions will influence algal inhibition potential by representative hydrophytes. Low level pollution reduced the allelopathic inhibition of microalgae by allelochemicals. However, many challenges need be solved. For example, how shall the exotic invasion of aquatic macrophytes like *A.*

philoxeroides (Mart.) Griseb, *Eichhornia crassipes* (Mart.) Solms, and, *Spartina anglica*, *etc.* affect the interaction between hydrophytes and algae, and ecosystem health and safety. Of course, the development of local travel, wading birds, and human interfering activities such as mowing and agricultural chemicals application also deserve more attentions.

T3-19-10

Effect of frugivore *Ocycerous gingalensis* on seed dispersal and seed germination of four threatened plant species in Mihintale sanctuary

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Frugivory is a common interaction mechanism between plants and animals. Sri Lankan grey hornbill is one of the frugivore as well as considered as omnivore which plays considerable dispersal task in dry zone forest ecology as old word endemic bird to Sri Lanka. Main goal was to determine the contribution to the dispersal of threatened plants by Sri Lankan Grey Hornbill. Study was conducted within Mihintale sanctuary (Latitude. 8.3333°, Longitude. 80.5167°) at Anuradhapura district. To determine whether SLGH could be legitimate disperser of threatened plants, fecal samples were collected during breeding season and focal sample analysis was done during non-breeding within 2014-2016. It was concluded that SLGH swallow the whole fruit and regurgitate the intact seeds. In final experiment germination trials were conducted for germination percentages and germination success of the seeds. The study shows the great inter specific variation in the effect of frugivore and seed germination percentage. As threatened plant species *Diospyros ebenum*, *Stychnos nux vomica*, *Strychnos potatorum* and *Manilkara hexandra* are considerably endangered and vulnerable plants which SLGH contribute the dispersal and enhance the germination among other dominant dry zone fruit bearing plant species such as *Locxoccocus* sp, *Azarichta indica*, *Drypetes separia*, *Filicium dicipiens* and *Ficus* sp. However pulp removal by frugivore was an important factor for germination success specially the enhancing ability to conserve threatened plants.

T3-19-11

Seed rain pattern in a subtropical evergreen broad-leaved forest in southwestern China

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Seed-rain pattern is a crucial process of natural forest regeneration. We conducted a three years field study on seed rain in subtropical evergreen broad-leaved forest in Chongqing, southwestern China. 172 seed traps (0.5 m^2) were erected throughout a 1ha permanent plot in Oct .2013. Seeds were collected monthly. More than 34,000 seeds from 26 species was found in the study period. There were highly differences in seed production between mass (147 seeds seeds m^{-2} yr^{-1} and 202 seeds seeds m^{-2} yr^{-1} in 2014 and 2016, respectively) and few (53 seeds seeds m^{-2} yr^{-1} in 2015) on community level. Seven species represent more than 90% of total seed rain in the community. The seed rain of dominant species *Castanopsis fargesii* represents different pattern compared to community seed rain (54 seeds seeds m^{-2} yr^{-1} and 45 seeds seeds m^{-2} yr^{-1} in 2014 and 2015, respectively, 22 seeds seeds m^{-2} yr^{-1} in 2016). The seed rain of sub dominant species *Elaeocarpus japonicas, Adenandra bockiana, Syraplocos setchuanensis, Engelhardtia roxburghiana* represent same pattern with community level. Our result suggested that longer time study should be conducted to better understand the seed rain in evergreen broad-leaved forest.

T3-19-12

Heritability and plasticity of competitive ability of invasive species

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Introduced exotic species can rapidly adapt to conditions in non-native ranges, including changes in size and competitive ability, which result in different invasive success for each individual species. Increased competitive ability generally increases invasive success, but the role of plasticity of competitive effects and responses on invasive success is unclear. Additionally, little is known about the heritability of such changes, and on the importance of hypothetical reintroductions in the maintenance of locally adapted traits. To test for this, we experimentally produced cohorts of the invasive *Centaurea solstitialis*, and the non-invasive *C. calcitrapa* and *C. sulphurea*, of ancestry from either the native range in Spain, the invasive range in California, or (for the invasive *C. solstitialis* only) inter-regional hybrids from both ranges. We then set up one-to-one competition experiments with the grass *Bromus hordeaceus*. The invasive *C. solstitialis* was the only species presenting significantly larger competitive effects and responses in the non-native range of California, but also a significantly lower plasticity for this trait. Additionally, while descendants of two Californian parents had almost twice the competitive effect on *B. hordeaceus* than descendants of two Spanish parents, inter-regional hybrids between Spain and California showed intermediate values. These results suggest that, at this stage of invasion, homogenizing gene flow resulting from hypothetical re-introductions from the native range could reduce their competitive effects on invaded plant communities.

T3-20: Interactions of plants and herbivores under global change

T3-20-01

Quantifying coexistence mechanisms contribution's to diversity maintenance in a species-rich semiarid grassland

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Despite the remarkable advances in ecological theory during the last two decades, the evidence for the mechanisms that maintain species diversity is still lacking. Coexisting species that compete for the same set of resources challenge classic theory, as the number of species in natural communities seems to exceed the limits imposed by competition. Modern coexistence theory recognizes two different mechanisms for coexistence. Equalizing mechanisms arise when the fitness differences between species are small, so no one species is capable of excluding rapidly the others in the community, leading to long-term coexistence. Such mechanisms are unstable as nothing prevents extinctions. In contrast, stabilizing mechanisms grant indefinite coexistence as the species' population growth rates increase when they are rare, averting extinction. Stabilizing mechanisms can be fluctuation-independent or fluctuation-dependent. The storage effect and the relative nonlinearity are fluctuation-dependent stabilizing mechanisms because they depend critically on fluctuations in population densities and environmental factors. Studies of the maintenance of species diversity have traditionally examined individual coexistence mechanisms. However, in nature, multiple mechanisms of coexistence are likely to operate simultaneously. Quantitative measurements are required to assess the relative importance of each mechanism, how they relate to each other, and if they depend on species traits such as their life history. Our aim was to determine the roles of different coexistence mechanisms in maintaining species diversity in a species-rich semiarid grassland. We quantify the contributions of equalizing and stabilizing mechanisms to the coexistence of 19 species, and, because of the large interannual variations in climate and species abundances in the study site, we place special emphasis in the two fluctuation-dependent mechanisms: relative nonlinearity and storage effect. Our analysis showed that 17 of the 19 species have the potential to coexist stably with the others in community. We found strong evidence that species diversity of this semiarid grassland is maintained by stabilizing mechanisms. Our results confirm recent findings that fitness differences between species are very small, and that niche differences are much larger than needed to stabilize the community. The extremely small fitness differences points toward a strong effect of equalizing mechanisms in maintaining diversity. Fluctuation-independent mechanisms were most important for species coexistence as a result of strong intraspecific competition compared to interspecific competition. Relative non-linearity has been usually considered to be an unlikely mechanism for coexistence in temporally fluctuating environments, but our results indicate that it may be far more important than storage effect, although secondary to fluctuation-independent mechanisms.

T3-20-02

Effects of climate change in Mediterranean and semiarid rangelands: Known and unknown in time of uncertainty

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Climate change is expected to reduce annual precipitation and increase its standard deviation in eastern Mediterranean rangelands. We examined how these expected changes may affect herbaceous aboveground net primary production (ANPP), and its inter-annual coefficient of variation (CV), in natural rangelands along a desert–Mediterranean precipitation gradient. We examined sites representing Arid, Semi-Arid and Mediterranean ecosystems, which exhibited a positive linear relationship between herbaceous ANPP and annual precipitation. Scenarios of reduced annual precipitation and increased inter-annual precipitation variability were defined by manipulating mean annual precipitation (MAP) and its standard deviation. For each scenario we simulated precipitation and calculated ANPP using current ANPP–precipitation relationships. Our model predicts that reduced precipitation will strongly reduce ANPP in arid and semi-arid sites. Moreover, the effect of reduced precipitation on CV of ANPP along the entire gradient may be modified by a change in inter-annual variability in MAP. In accordance with previous research in the region, reduced precipitation combined with decreased precipitation variability had greater relevance at the arid end of the gradient due to the strong effect on mean ANPP. In contrast, soil seed banks showed high resistance to rainfall reduction and increasing variability along the aridity gradient. The results will be discussed under different climate change scenarios combining known and unknown facts of these particular ecosystems under increasing uncertainty.

T3-20-03

Global change effects on ecosystem functions and services provided by African dryland rangelands

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In Africa's drylands, plant growth is mainly limited by low and variable rainfall, which constrains agricultural activities to livestock production. Thus, livelihood security relies heavily on forage provision from rangeland vegetation. A sustainable rangeland management requires a profound understanding of how ecosystem structure and functioning will respond to changing climate and land-use. However, this ecological understanding is still surprisingly poor. In my talk I will give an overview on my various research activities in Africa's savannas and grasslands that aim at improving this critical understanding. I will also discuss how my research contributes to help farmers cope with future climatic conditions.

T3-20-04

Overgrazing and carrying capacity: A new insight into rangeland management

Avi Perevolotsky

Agricultural Research Organization

A prudent ecological management of rangeland resources is strongly related to proper determination of the carrying capacity (CC) of the pasture and avoidance from overgrazing (OG). Even though these concepts have been extensively discussed in the scientific and professional literature we believe that they require further and more realistic analysis. Hereby we discuss these issues in relation to different vegetation types: herbaceous vs. woody plants and annual vs. perennial species. We urge to interpret CC and OG according to the specific vegetation formation which is embedded in a specific a-biotic envelop. We suggest to distinguishing between overgrazing and overutilization, each requiring a different managerial approach. The outcome of long-term field experiments in which grazing treatments are imposed on Mediterranean ecosystem helps to demonstrate our claims.

T3-20-05

Effect of endophyte infection and clipping treatment on growth and locust resistance of *Achnatherum sibiricum*

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Endophytes live asymptomatically within many cool season grasses for at least a portion of their life cycle. Thus far, the best-studied endophytes are *Neotyphodium lolii* and *Neotyphodium coenophialum*, which colonize perennial ryegrass (*Lolium perenne*) and tall fescue (*Lolium arundinaceum*), respectively. It is well documented that endophytes are able to enhance the resistance of host grasses to herbivory, and the anti-herbivore properties of endophyte infection are largely attributable to the production of a variety of alkaloids. Up to now, four classes of alkaloids associated with infected host grasses have been detected, including saturated aminopyrrolizidines (lolines), pyrrolopyrazines (peramines), ergot alkaloids and indolditerpenes (lolitrems). Endophytes not only exist in cultivated grasses such as tall fescue and perennial ryegrass but are also widely distributed in native grasses. Tall fescue and perennial ryegrass may produce three to four different classes of alkaloids. In contrast, endophytes in native grasses usually produce fewer classes and a lower concentration of alkaloids, and some endophytes do not even produce alkaloids. Can endophyte infection in native grasses, which do not produce alkaloids or produce a low concentration of alkaloids, enhance the herbivore resistance of host plants? The limited interactions reported are highly variable. *Achnatherum sibiricum* (L.) Keng is a caespitose perennial grass that is widely distributed in northern China and commonly infected by *Epichloë* endophyte. In this study, endophyte-infected (EI) and endophyte-free (EF) *A. sibiricum* were adopted as plant material. *Locusta migratoria*, a common herbivore of grasses, was adopted as the feeding herbivore. Clipping is a common practice in our sampling grassland; thus, we use clipping as the interference. We wondered whether endophyte infection has a positive effect on the insect resistance of *A. sibiricum* and whether clipping can influence insect resistance of infected *A. sibiricum*. We found that locusts preferred EF plants to EI plants, and the weight of locusts fed on EI plants was significantly lower than those fed on EF plants. Hence, endophyte infection significantly enhanced the resistance of the host to *L. migratoria*. Endophyte infection significantly decreased the concentration of soluble sugar and amino acids while significantly increased the concentration of total phenolic content, and these metabolites may contribute to herbivore resistance of the host. The clipping treatment further strengthened the locust resistance advantage of EI over EF plants. After clipping, the weight of the locusts fed on EI plants significantly decreased compared with those fed on unclipped plants, whereas the weight of the locusts fed on EF plants increased significantly. The results suggested that endophyte infection could increase herbivore resistance while decreasing the tolerance of the host grass by mechanisms apart from endophyte-conferred alkaloid defense.

T3-20-06

The effect of warming on root dynamics and soil microbial community in alpine meadow

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Studies have substantially explored the effects of global change on above-ground vegetation in grassland ecosystems. However, few studies have investigated the responses of root dynamics and soil microbes to simulated warming in an alpine meadow of the Qinghai-Tibet Plateau using a field OTC experiment. Root production, mortality, and turnover were observed in situ using minirhizotron tubes, while Soil microbial community composition and diversity were assessed by using Phospholipid-derived fatty acids (PLFA) and Biolog-Eco plates. The results showed clearly that 1) warming shifted the root standing crop distribution to deeper soil while increased the accumulated root mortality and root production at 0–10 cm soil depth; 2) warming increased the abundance of fungal PLFAs while decreased the abundance of other functional groups; 3) warming decreased the soil microbial functional diversity at the different soil layer; 4) bacterial and fungal PLFA contents were positively correlated with root mortality and turnover but was negatively correlated with root standing crop; 5) root dynamics significantly affect carbon utilization of soil microbes community by warming; and warming induced changes in root dynamics could associate with soil microbial community structure and function. Overall, these findings indicate that warming alters root production, mortality, and turnover and the relative abundance of different microorganisms in different soil layers. As a result, warming tended to shift microbial communities from bacteria towards fungi, leading to changes in the microbial community structure in different soil layers.

T3-21: Ecological processes of grassland ecosystems in a changing world (two sessions)

T3-21-01

Diversity patterns in and historical biogeography of Pannonian and western Pontic steppe grasslands

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Biodiversity and biodiversity loss are important topics in grassland ecosystem conservation. Beside recent (negative) impacts on biodiversity due to anthropogenic activities and global environmental change, diversity patterns are also significantly influenced by the biogeographical history of species and communities. Eurasian steppe grassland ecosystems are the largest on earth; they extend westwards to Central Europe forming there one of the most species-rich vegetation types. Their historical establishment and recent decline occurred both probably under (major) human impact. However, in our research we focus on Pannonian and western Pontic dry grasslands covering the steppe zone within the European part of Russia towards Eastern and Central Europe. We are aiming at analysing diversity patterns and biogeographical history in time and space using different sources of data: one the one hand, we use phytosociological relevés not only for classification of vegetation types, but also for extracting species richness within different communities. On the other hand, we exemplarily investigate characteristic plant species of different grassland communities, intraspecifically, using genetic data. Both data types will jointly allow reconstruction of possible historical biogeography of dry and semi-dry grasslands. More specifically, we ask whether and to what extent patterns and processes are similar across different grassland communities. Therefore, we study plant species representing differing, but typical plant communities in parallel, i.e. diagnostic species from the the grassland orders *Brometalia erecti, Festucetalia valesiacae* and *Stipo-Festucetalia pallentis*, respectively. Phylogeographical molecular analyses will focus on cpDNA sequence variation within the different diagnostic species sampled across the Pannonian area and adjacent regions, including potential long-term refugial regions. Aligning different biodiversity patterns across the large geographical range of Pannonian and western Pontic steppe grasslands will be achieved by a rigorous sampling design using grid cells for both vegetation (i.e. phytosociological community) data and intraspecific genetic diversity measures. This will not only provide insights into the biogeographical history of species and vegetation, but will also help defining important diversity regions in grassland ecosystems.

T3-21-02

Correlation between genome size and altitude in purple false brome

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Over many years there have been challenging questions in plant evolutionary biology that study correlations between genome size (GS) and changing ecological parameters or climatic parameters such as latitude, longitude, or altitude. It is still a contradictory question to give an idea about genome size and altitude above sea level or geographical latitude. Altitudinal gradients are excellent models for ecological and evolutionary studies to test the responses of climatic factors such as temperature, precipitation, wind, and sunshine on plant growth and development. Purple false brome (*Brachypodium distachyon* L.) is a model crop for temperature grasses. It belongs to the Poaceae family and is related to the cereals such as wheat and barley.We have We used 541 individuals belonging to 91 accessions distributed throughout Turkey ranging from sea level up to 1,219 m to answer how much correlation is there in genome size under various altitudinal habitats? We have used flow cytometry to determine DNA content (pg). We analysed data using statistical procedures for ANOVA, regression analysis, and principal component analysis, factor analysis, discriminant analysis in Minitab 8 to understand how change genome size among different altitudes. GS values varied widely among individuals, from 0.54 to 0.92 pg. The mean 2C DNA content of *B. distachyon* was determined to be 0.736 ± 0.02 pg, ($P < 0.001$). The scatter plot of all individuals shows that there was no correlation between altitude and genome size. However, altitudinal gradients have been grouped from 0 to 600 meters and 601 to 1,219 meters for testing the level of altitude to see differences in genome size. With these two group altitudinal levels we found a positive correlation between altitude and genome size. In the first altitudinal gradient (from 0 to 600 m) belonging to 409 individuals, the mean 2C DNA content was 0.734 ± 0.001 pg, ($P < 0.005$) and the second group (from 601 to1,219 m) belonging to 132 individuals, the mean 2C DNA content was 0,742 ± 0.002, ($P < 0.005$) pg genome size. Consequently, although gradual elevation gradient (every 100 meters) changes have not caused a correlation between genome size and altitude for individuals of the species, in parallel to higher groups of altitude (0 to 600 m and 601 to 1,219 m) the genetic answer is changed.

T3-21-03

Degradation of Tibetan grasslands: Consequences for soil organic carbon and nutrients losses

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The *Kobresia* pastures, commonly known as “alpine meadow”, cover the southeastern quarter of the Tibetan Highlands (~450,000 km^2). They host important grazing ground for livestock (i.e. yaks, sheep and goats) and thus ensure the livelihood of the Tibetan herders. The *Kobresia* pastures also store huge amount of soil organic carbon (SOC) and nutrients (e.g., nitrogen (N) and phosphorus (P)), which are required for sufficient forage production. In recent decades, the *Kobresia* pastures have experienced severe degradation due to anthropogenic activities and climate change, which has initiated high losses of SOC and nutrients and threatened the functioning of this ecosystem. Plenty studies have been implemented showing the response of degradation on SOC and nutrients levels on local scale. They classify these alpine pastures into various degradation stages that are mainly based on vegetation characteristics (e.g., vegetation coverage, proportion of edible plants). Within this study we synthesized their results in a review for a better understanding of SOC and nutrients losses following pasture degradation across the whole ecosystem. We aggregated the degraded *Kobresia* pastures into five degradation stages: Non-degraded, Light degradation, Moderate degradation, Heavy degradation and Extreme degradation. Results show that degradation from light to extreme stages has lost on average 42 ± 2% SOC, 33 ± 6% N and 17 ± 4% P as compared to the non-degraded pastures. This implies strong reduction of soil fertility and an exacerbation prevailing N and P limitations. Concurrently, degradation has decreased aboveground and belowground biomass by ~42 ± 3% and ~45 ± 6%, which reflects (a) decreasing photosynthetic C input and (b) less available forage for livestock. Besides, the declining vegetation promotes wind and water erosion. In conclusion, our results provide an overview and a quantification of degradation impacts on plant characteristics and soil properties that improve estimations regarding SOC and nutrients losses across the whole ecosystem. This highly matters because large amounts of SOC have been lost due to erosion and mineralization. Most likely this has polluted the Tibetan headwaters and contributed to climate change, respectively. Further, the decreasing N and P losses have reduced soil fertility lowering forage production. Therefore, it endangers the livelihood of the Tibetan herders, which highly rely on forage to feed their livestock. Despite plenty of ameliorations (e.g., fertilization, grazing enclosure, reseeding) have been proposed and implemented at many locations, their impacts on pasture ecosystems (especially on soil fertility) are still subtle and thus require further investigations.

T3-21-04

Wind erosion caused by land use changes significantly reduces ecosystem carbon storage and carbon sequestration potentials in grassland in Northern China

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Wind erosion and deposition of soil could greatly alter soil C pool and ecosystem C balance in arid and semi-arid ecosystems. Land use change is one of the most important driving forces influencing the intensity of wind erosion in those regions. However, how wind erosion under different land use scenarios will affect ecosystem C balance and its capacity for future C sequestration are poorly quantified. Here, we established an experiment in Xinlinhot, Inner Mongolia, and simulated different intensity of land use in grassland: control, 50% of aboveground vegetation removal (50R), 100% vegetation removal (100R) and tillage (TI). We monitored lateral and vertical carbon flux components from 2013 to 2016. We found that wind deposition resulted in net C gain during growing seasons, whereas wind erosion induced net C loss. Adding up the lateral C flux across the whole year, ecosystems under control behaved as a C sink of 44.85 g C m^{-2} yr^{-1}. However, under relative degradation ecosystems, the disturbance managements resulted in a net C loss, and the loss strength increased from 3.35 g C m^{-2} yr^{-1} under 50R to 135.86 g C m^{-2} yr^{-1} under TI and the erosion intensity increased rapidly with experimental duration. Land use also significantly altered the biological vertical carbon flux. The net ecosystem exchange (NEE) shifted from a net uptake of 86.85 g C m^{-2} yr^{-1} under control to a net emission of 35.12 g C m^{-2} yr^{-1} under TI treatment. With the increase in land use intensity, the contribution of lateral C flux to ecosystem C balance increased from 34% under control to 79% under TI. Wind erosion caused by land use changes not only result in dramatically surface soil C loss, but also significantly decrease soil C sequestration potential by altering soil texture. Permanent losses of organic carbon sequestration potential were 0.10 kg C m^{-2},0.12 kg C m^{-2} and 0.31 kg C m^{-2} in 50R, 100R, and TI, respectively. Overall, our study demonstrated that wind erosion could result in irreversible soil degradation and shape the landscapes of arid and semi-arid regions in the long term. Appropriate land use is critical to protect grassland ecosystems from being crashed.

T3-21-05

The underlying basis for the tradeoff between leaf size and leafing intensity

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Recent studies have reported a consistent tradeoff between leaf size (mass) and leafing intensity (the number of leaves produced per unit of supporting stem tissue volume), however, a theoretical basis for this tradeoff has not been described. We explore the mechanistic basis for this tradeoff and assess the relationship in light of other prominent theories for allometric biomass partitioning. We show algebraically how the allocation of mass to leaves versus stems and the density of stem tissue can potentially influence this tradeoff. To assess these possible effects, we compared the relationship between leaf size and leafing intensity, expressed on both mass and volume basis, at the level of a single branch as well as the entire above-ground plant in 61 forbs over a 3-year period. Our results support the idea that the tradeoff between leaf size and volume-based leafing intensity depends on both biomass investment (leaves vs. stems) and stem bulk density (mass vs. volume), whereas the tradeoff between leaf size and mass-based leafing intensity only depends on the biomass investment. Similar exponents in the scaling of leaf mass vs. stem mass and stem mass vs. stem volume at branch- and whole plant-level lead to similar tradeoffs. An isometric tradeoff between leaf size and volume-based leafing intensity is consistent with a constant biomass partitioning between leaves and stems as well as constant stem tissue density. Conversely, an allometric tradeoff between leaf size and volume-based leafing intensity arises when biomass allocation is allometric and stem bulk density varies with plant size.

T3-21-06

Long-term dynamics of flowering shoots in alpine lichen heath and *Festuca varia* grassland (Teberda Reserve, the North-western Caucasus)

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In alpine environment, flowering strongly depends on weather conditions of the previous growing season, when the buds of the most of the perennial species develop. The dynamics of reproductive shoots shows higher variability comparing with vegetative shoots. Under current climate changes it may influence not only plant species regeneration, but also populations of other organisms, such as pollinators and herbivorous insects feeding in flowers. I analysed long-term flowering shoot dynamics in alpine lichen heath (ALH, 2,800 m a.s.l.) and *Festuca varia* grassland (FVG, 2,100 m a.s.l.) at the Malaya Khatipara Mountain, the Teberda reserve, the North-western Caucasus, Russia. These communities are typical in the alpine belt of the Caucasus and contain a number of species common in both communities. ALH occupy ridges and FVG south-eastern slopes with low snow accumulation. I tested (1) whether there are trends in interannual flowering shoot number dynamics, (2) which weather conditions are the most important in determining of their dynamics. The number of flowering shoots was counted on the permanent plots every year, from 1981 to 2016 in ALH, and from 1987 to 2016 in FVG. The total area of observations was 62 m^2 in ALH and 50 m^2 in FVG. Temperatures and precipitation during the growing season were derived from the nearby Teberda weather station. To reveal trends in flowering, I run Detrended Correspondence Analysis (DCA), where years were used as "plots" and number of shoots was a criterion of species abundance. Rare species were downweighted in the course of the analysis. Spearmen's correlation coefficients were calculated between axes scores and weather conditions of the previous and the current year. For both communities, the first DCA axis was significantly ($p < 0.001$) correlated with ordinal number of year and with the mean August temperatures of the previous year ($p < 0.001$). DCA also revealed groups of decreasing species (including, for example, *Carex umbrosa, Carex sempervirens, Anemone speciosa* in ALH and *Cruciata laevipes, Galium verum, Leontodon hispidus* in FVG) and increasing species (*Euphrasia ossica, Plantago saxatilis, Aster alpinus, Alchemilla caucasica, Ranunculus oreophilus* in ALH and *Campanula collina, Euphrasia ossica, Hedysarum caucasicum* in FVG). The observed patterns in the dynamics of flowering shoots are similar with those in the dynamics of the total shoot number in these communities. Despite the fluctuations, when the species composition and abundance of their flowering shoots are close to those observed in any previous years, our data suggest trends in the total dynamics of the studied communities. The field works were supported by RFBR (grants № 11-04-01215,14-04-00214). Data analysis was supported by RSF (grant № 14-50-00029).

T3-21-07

Climate modulates the effects of grazing on plant functional group diversity in *Stipa Krylovii* grassland in Inner Mongolia, China

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Grazing is one of the prevalent human activities effects on grassland ecosystems. There has been a rapidly increasing interest in the effect of grazing on species diversity or functional group diversity in grasslands. In this study we analysed the effects of grazing on plant species diversity, plant functional group (PFG) diversity of *Stipa krylovii* grassland in Inner Mongolia, China. We analysed plant diversity data from 60 sampling plots by three estimated classes of low, medium and high grazing intensity in 2014 (wet year) and 2015 (dry year). The sample site located in Kerulen basin in New Barag Right Banner. We also classified a priori all plant species in four PFGs: perennial grasses, perennial forbs, shrubs/subshrubs, and annual/biennial herbs. The results showed that the species richness was highest in medium grazing condition in both 2014 and 2015. The species richness in high grazing intensity was higher than in low grazing intensity. There was no significant difference in PFG richness with grazing intensity. However, each PFG showed different response to grazing intensity. The perennial forbs was the dominated PFG in community composition, and it contained more than 50% species richness in each grazing intensity. The species richness in PFG of annual/biennial herbs increased as grazing intensity increased, especially in wet year of 2014. There was little changed of the species richness in PFG of shrubs/subshrubs as grazing intensity increased. Both of the species richness and PFG richness in 2014 were significant higher than those in 2015 in each grazing intensity because of the dry weather in 2015. In 2014, the degree of dominance of perennial grasses was higher than other PFGs in each grazing intensity. In 2015, the degree of dominance of perennial grasses decreased as grazing intensity increased, and the degree of dominance of perennial forbs was increased as grazing intensity increased. This finding may imply that medium grazing promotes the species richness, it could be predicted by an intermediate disturbance hypothesis. The perennial forbs have strong resistance with high grazing intensity in dry year. The annual/biennial herbs was more sensitive to precipitation than grazing. So, climate modulates the effects of grazing on plant functional group diversity in *Stipa krylovii* grassland in Inner Mongolia, China.

T3-21-08

Vegetation pattern of grassland habitats on local and landscape-level an its changes in changing geographycal environment in the Danube-Tisza Interfluve's Sand-ridge, Hungary

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The Danube-Tisza's Interfluve's Sand-ridge, a Pleistocene alluvial cone of the river Danube is situated in the middle of the Carpathian-basin on the western edge of the forest steppe zone. The average annual precipitation income is very rare, rather significantly under average precipitation-income favoring for dry grasslands or above average precipitation-income favoring for wet fens, wet salt communities and forests are typical helping - together with soil, hydrogeographical, geomorphological features - the maintain of forest-grassland mosaics. Wet years are typical after dry years within 2-3 years compensating somewhat the affect of dry years. This climatic feature with temporary changes and shifts to more boreal, atantic, sub-mediterranean and continental conditions was typical here during the Holocene resulting high climatic adaptive capacity of plants and plant communities, special vegetation pattern, transitional and endemic communities (see Pannonian sand grasslands). The local and landscape-level vegetation pattern and its gradients were studied with habitat-mapping using the General National Habitat Classification System (ÁNÉR). In consecutive wet and dry years the shift of zonation, formation and change of transitional communities, their species composition and the coverage of species were studied with coenological plots alongside hydrogeographical gradients and with repeated habitat-mappings on the western edge of this mesoregion (Turján-land). In this sample area (around Kunpeszér, Kiskunság National Park) the natural grasslands remained on significant areas, where zonation-shifts, transitional communities with fast annual changing species-composition and coverage are typical in years with different precipitation income. The vegetation pattern is highly influenced by the geomorphology, the groundwater-level and its Na-salt content in this landscape. On dunes open sand grasslands with scattered patches of polar or oak forests are typical due to the thin, nutrient-poor soils and deeper groundwater-level, whereas on flat sand sheets and residual ridges closed sand steppes are typical with formerly more distributed closed poplar and oak forests. Uniquely *Molinia* fens, rich fens, tall sedge-beds, mesotrophic wet meadows and Pannonian, more continental salt vegetation (salt meadows, *Puccinellia* and annual salt pioneer swards, salt marshes) appear in the blow-out depressions next to each other. The precipitation is not enough for fens, sedge-beds and wet meadows, so they appear in the groundwater up-welling zones, while further from these zones salt meadows, even further *Puccinellia* and annual salt pioneer swards are dominant in the deepest parts as soil solutions become concentrated due to evapotranspiration and salt-accumulation. In the Dorozsma-Majsa Sand-ridge this pattern shows a local and landscape level northwest-southeast gradient covering soil-type, pH, salt and organic material gradients as well. The observed groundwater-decrease due to precipitation-decrease, drainage and groundwater take-out resulting the out-leaching of salt habitats as well threatens the communities of the depressions. Sand steppe grasslands can occupy their places, but landscape-use decreased their extension significantly making this shift more difficult. So conservation of the full zonation is important for the better adaptation to climate change. Decreasing groundwater-level, desalinization, increasing differences and uncertainties in precipitation, accumulation of organic materials and nutrients due to the lack of cow-grazing, mowing result the decrease of plant diversity, spread of reeds making wet communities more characterless.

T3-21-09

Facilitation by leguminous shrubs increases along a precipitation gradient

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Combining nutrient dynamics (plant nutrient uptake and soil fertility) can help uncover mechanisms of shrub-grass interactions and assess the validity of the stress-gradient hypothesis, which predicts that facilitation between plants increases in stressful environments. However, how facilitation via shrub-mediated nutrient increases varies with precipitation is poorly resolved. We first synthesized a global dataset from 66 studies and evaluated how shrubs affected soil organic carbon (C) and nitrogen (N) in grasslands along a precipitation gradient. We then made new measurements in a single grassland type encroached by leguminous shrubs from the same genus (*Caragana*) to constrain the variations caused by combining different grassland types and shrubs traits in the meta-analysis. Specifically, we investigated how shrubs mediated N dynamics and how shrub-grass interactions varied along a precipitation gradient (147-342 mm) in a temperate steppe of China. At the global scale, nurse plant effects on soil N pool increased with precipitation only for leguminous but not for non-leguminous shrubs. Results from the field study showed that facilitation became relatively weak as water stress increased, indicating a collapse of facilitation. Our results highlight that plant-plant interactions would be altered by precipitation and shrub functional traits (leguminous or not) should be included as mechanisms in conceptual frameworks of plant facilitation.

T3-21-10

How can botanical compostion be managed by grazing in desert steppe

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The desert steppe of Inner Mongolia is traditionally used for year-long sheep grazing. However, overgrazing reduced vegetation production, thereby changing the botanical composition. Grazing is one of important ways to adjust the botanical composition for the development of successful grazing management strategies. A grazing experiment was conducted from 2004 to 2015 in which four stocking rate ranging from 0 sheep equivalent ha^{-1} mo^{-1}, 0.15 sheep equivalent ha^{-1} mo^{-1}, 0.30 sheep equivalent ha^{-1} mo^{-1} to 0.45 sheep equivalent ha^{-1} mo^{-1}. Each stocking rate treatment has 3 replications. Variables measured included dry matter, species number, tiller numbers of dominant species in different treatments. The results showed that the peak dry matter in September has an significant impact on the total dry matter of next June, which means that grass initialed in the grassland will be influenced the dry matter reservation on the previous year. With the reservation dry matter in September increased, total tillers on next May increasing sharply. Plant functional groups (PFGs) changes are good indicators for the evaluation of grassland conditions. Shrubs and perennial grasses were the components most affected by grazing treatments. The higher grazing pressure coincided with the lower the shrub content, and lower species diversity. When we made the management strategies, grazing is a great tool to control the shrub growth in desert steppe. Manage the ration of perennial grasses and shrub fit 2:1 will increase the stability of desert steppe ecosystem.

T3-21-11

The effects of herbivore assemblages on vegetation and soils on steppe grasslands

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Most previous research has indicated that grazing intensity can exert various impacts on steppe vegetation and soils, but little information is available on how different herbivore assemblages influence potential grassland multifunctionality in grazing contexts, despite the fact that mixed grazing by different species is adopted in many grasslands systems. For this study, we investigated the effects of herbivore assemblages on plant and soil characteristics such as plant diversity and productivity, and soil heterogeneity, nitrogen cycling and carbon flux with a set of grazing manipulation (no grazing, cattle grazing, sheep grazing and mixed grazing) experiments in the Eurasian steppe grasslands. We obtain the following results: (1) Grazer effect on plant diversity strongly depended on herbivore species and combination. Single-species grazing by cattle and mixed grazing of cattle and sheep significantly increased plant diversity, while single-species grazing by sheep significantly reduced plant diversity. Such increasing effects of cattle grazing can be attributed to the fact that cattle had more inhibition on the competitive dominant grasses than sheep. Mixed grazing had a consistently positive effect on plant diversity and it is most likely that the additive effect of mixed grazing enhanced plant diversity through the reduction of competition from grasses. (2) Herbivore assemblages had significantly varying effects on plant biomass. Single-species grazing by cattle decreased plant biomass, while grazing by sheep alone and mixed grazing of cattle and sheep didn't affect plant biomass. Cattle, a large-bodied and less selective herbivore that commonly coped with the low plant nutrient content but requires a higher abundance of energy-rich plants, tended to negatively affect plant biomass accumulations by consuming dominant grasses. (3) Cattle grazing increased the spatial heterogeneity of available N in the soil of *Leymus chinensis*-dominated steppe. However, the effects of sheep grazing and mixed grazing strongly depended on grassland plant diversity, with an increased spatial heterogeneity of available soil N in the high diversity sites. In such grassland systems with high plant diversity, herbivore grazing and plant diversity would jointly improve soil spatial heterogeneity, thus feeding back to maintain higher plant diversity. (4) Herbivore grazing significantly improved soil N availability regardless of herbivore assemblages. Single-species sheep grazing and mixed grazing of cattle and sheep had a greater effect on soil N availability than single-species cattle grazing. It demonstrated that herbivore grazing had a predominantly positive effect on N cycling rate under moderate grazing intensity. (4) Grazing by cattle alone significantly increased net ecosystem CO_2 exchange, but exchange was reduced under sheep grazing. Yet NEE was significantly higher under mixed grazing of sheep than under single-species cattle or sheep grazing. We conclude that, given that herbivore assemblages have varying effects based on different grassland functions, herbivore assemblages should be considered an important affecting factor in future grassland management besides grazing intensity.

T3-21-12

Differential responses of canopy nutrients to experimental drought along a natural aridity

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Droughts and periods of water limitation are projected to increase in both magnitude and frequency due to climate change. It is, however, poorly understood how canopy nutrient (N and P) concentrations (community weighted by species abundance) will respond to drought and how these responses may vary along a gradient of precipitation and shifting plant community composition. Nutrients in grassland plant canopies can be used to assess and predict the likely responses of fundamental ecosystem processes to environmental changes. At three sites along a natural aridity gradient in the grasslands of northern China, short-term drought manipulation caused irregular effects on canopy nutrient concentrations, and these were driven by combined effects of species turnover and intraspecific variation at each site. In contrast, along the aridity gradient, canopy nutrient concentration was inversely related to precipitation (the arid site had the highest canopy nutrient concentration) and this pattern was driven mostly by species turnover (i.e., an increase in abundance of species with higher nutrient concentrations). Short-and long-term reductions in precipitation directly affected plant nutrient status rather than indirectly via alterations in soil nutrient availability. We conclude that nutrient-rich canopies were more resistant to drought at drier sites, because of the opposing effects of species turnover and intraspecific variability and the higher drought tolerance of nutrient-rich species. Differing responses of canopy nutrient concentration to drought conditions along natural gradients vs. manipulative experiments in the same grassland biome highlights the complexity in predicting how these grasslands will respond to climate change.

T3-22: Atmospheric nitrogen deposition and its impact on plant growth and sustainability of ecosystems

T3-22-01

Nitrogen deposition in China and its environmental impacts

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Atmospheric nitrogen (N) deposition is not only an important component in the human-accelerated global N cycle but also an indicator of atmospheric N pollution. Excess N deposition has aroused environmental concerns about negative impacts on ecosystem health and services such as loss of biodiversity, forest soil acidification, and increased greenhouse gas emission. Rates of N deposition, especially oxidized N deposition, have leveled off or decreased in Europe and US since the 1980s or early 1990s with the implementation of stricter legislation to limit atmospheric pollution. In contrast, emissions of both NH_3 and NO_x in China have been increasing continuously since the 1980s mainly due to growing agricultural and industrial activities. These increased reactive N emissions to the atmosphere have aroused widespread concern on air pollution in China. However, scientific gaps to understand regional and temporal variability in atmospheric N emissions and deposition still exist. Meanwhile, the ecological and environmental impacts of N pollution and deposition are of great concern in China. In this paper, the authors summarize recent progress on N deposition studies in order to identify: 1) the spatio-temporal variability of N deposition fluxes; 2) the major impacts of N pollution and deposition on air quality and ecosystem health; 3) some future research recommendations and regulatory strategies for mitigation of atmospheric N pollution in China. Recently a number of atmospheric N deposition monitoring networks have been established since the early 2000s. There are increasingly more research on quantification of spatial and temporal variations of atmospheric N deposition and its impact on terrestrial ecosystems, using different atmospheric deposition modelling approaches. The major findings in this paper include three parts: (1) anthropogenic reactive N (e.g., NH_3 and NO_x) emissions contribute largely (up to 40%) to secondary inorganic aerosol formation and haze pollution; (2) dry N deposition is proven to be the same importance to wet N deposition, suggesting the necessity to re-examine the previously identified N critical loads based on wet deposition alone; (3) the enhanced N deposition over China have negatively affected air quality, eutrophication, soil acidification and decline of plant species richness especially in grassland ecosystems. Future research challenges on atmospheric N emission and deposition are discussed as well. China needs to (1) reduce the uncertainties of national N emission inventory; (2) establish national networks for atmospheric N concentration and deposition monitoring; and (3) evaluate ecological and environmental impacts of N pollution and deposition in typical ecosystems. Cross-site N addition experiments along with various forests, grasslands, deserts and aquatic ecosystems are required in order to provide systematic information on the impact of elevated N deposition on both terrestrial and aquatic ecosystems against the background of climate change. In particular, the response of carbon sequestration and/or greenhouse gas balance to elevated N deposition should be taken into account in future studies. Last but not least, N deposition modeling tools should be improved based on localized parameters and further used in future N regulation.

T3-22-02

Recovery from long-term nitrogen deposition

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Rothamsted Research

The negative effect of increasing atmospheric nitrogen (N) pollution on grassland biodiversity is incontrovertible. Nitrogen deposition from the atmosphere to cropland, grassland and woodland has been measured at Rothamsted Research, UK, for many years. Surprisingly large amounts of N, both wet and dry, oxidised and reduced, were deposited to local ecosystems in the 1990s. However, the recent introduction of cleaner technologies in the UK has led to reductions in the emissions of nitrogen oxides and some reduction in ammonia, with concomitant decreases in N deposition. The effects of these decreases on grassland biodiversity has been investigated in the 160-year old Park Grass Experiment to see the extent to which grassland biodiversity can be expected to ‘bounce back’, i.e. recover, in response to these improvements in air quality. The hypothesis was that long-term chronic N addition may lead to an alternative low biodiversity state. However, data from the Park Grass Experiment show a positive response of biodiversity to reducing N addition from either atmospheric pollution or fertilizers. The proportion of legumes, species richness and diversity increased across the experiment between 1991 and 2012 as both wet and dry N deposition declined. Plots that have not received inorganic N fertilizer since 1989 recovered much of the diversity that had been lost, especially if lime (calcium carbonate) was applied to overcome acidification. There was no evidence that chronic N addition has resulted in an alternative low biodiversity state on the Park Grass plots, except where there had been extreme acidification. Recovery was probably also facilitated by the twice-yearly mowing and removal of grass, removing much of the N deposited. The fact that natural and semi-natural grassland is not cut and removed might explain why a comparable response of plant communities to reduced N inputs has yet to be observed in the wider landscape.

T3-22-03

Atmospheric reactive nitrogen deposition and effects in the U.S. Rocky Mountain region

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Increases in reactive nitrogen deposition are a growing concern in the U.S. Rocky Mountain west. Alpine ecosystems in Rocky Mountain National Park (RMNP), Grand Teton National Park (GTNP) and elsewhere evolved under low nitrogen conditions. Increases in inputs of oxidized and reduced nitrogen in recent decades have created new stresses on these ecosystems. Current inputs of reactive nitrogen in this region now substantially exceed the established critical load: the deposition flux at which irreversible changes occur to the ecosystem. For the past decade we have been actively examining the sources, transport and deposition of reactive nitrogen in the Rocky Mountain region. Both RMNP and GTNP are located at high elevation, but can experience periodic, polluted conditions associated with transport of emissions from a variety of regional sources, including urban areas, power plants, agricultural regions, wildfires, and areas of active oil and gas development. While wet deposition of nitrate and ammonium, on which the critical loads for nitrogen deposition are based, are important, our measurements reveal that dry deposition of gaseous ammonia and wet deposition of organic nitrogen are both major contributors to regional reactive nitrogen deposition budgets. Neither form of reactive nitrogen has historically been measured in U.S. air quality or deposition monitoring networks. The presentation will review observed effects of excess reactive nitrogen deposition on regional alpine ecosystems, discuss the establishment of critical loads, summarize findings regarding major sources and deposition pathways, and highlight recent efforts to formulate policies and strategies to reduce reactive N deposition in the future below ecosystem critical loads.

T3-22-04

Modeling atmospheric nitrogen deposition: Sources, processes, and surface ozone responses

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I will present our recent work on better understanding the sources, processes, and consequences of nitrogen deposition using an atmospheric chemistry model (GEOS-Chem). (1) We will show that satellite observations of atmospheric ammonia concentrations from the Tropospheric Emission Spectrometer (TES aboard EOS Aura) can provide top-down constraints to improve our estimate of ammonia emissions over China. Assimilating TES ammonia observations into the model indicates a strong seasonal variability in the Chinese ammonia emissions with 1.9 Tg N emitted in July compared with 1.2 Tg N in April for the year 2008. This is consistent with surface measurements of ammonium wet deposition flux, as well as an improved bottom-up emission estimate (12.6 Tg N annually) with more practical fertilizer application rates and temperature-dependent livestock ammonia emission factors. (2) We simulate atmospheric inorganic nitrogen deposition over China for 2008-2012, and extensively evaluate model results with available measurements. Total inorganic nitrogen deposition fluxes are simulated to be generally less than 10 kg N ha^{-1} a^{-1} in western China (less than 2 kg N ha^{-1} a^{-1} over Tibet), 15-50 kg N ha^{-1} a^{-1} in eastern China, and 16.4 kg N ha^{-1} a^{-1} averaged over China. Annual total deposition to China is 16.4 Tg N, with 62% (10.2 Tg N) from reduced nitrogen (NH_x), 39% via dry deposition process, and 86% attributed to domestic anthropogenic sources. We estimate that about 15% of the land over China is experiencing critical load exceedances. (3) We will also present an exploratory study to understand how surface ozone pollution would respond to anthropogenic nitrogen deposition using the GEOS-Chem atmospheric chemistry model and the CLM land model. Enhanced nitrogen deposition since industrialization altered the land vegetation coverage and soil mineral nitrogen content, leading to increased biogenic VOC emissions and soil NO_x emissions but also higher ozone uptake via dry deposition. Our results show that anthropogenic nitrogen deposition would overall change the summer mean surface ozone pollution by -2.4 to 2.7 ppbv in the northern hemisphere, with significant regional differences. These values are relatively small but comparable to the previously estimated surface ozone changes driven by changes in land cover and climate.

T3-22-05

Impacts of nitrogen deposition on soil biogeochemical cycles: Perspectives based on enzymology

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Over the past decades, atmospheric nitrogen (N) deposition has substantially increased worldwide due to increased fossil fuel combustion and widespread use of chemical fertilizer N. Increased atmospheric N deposition has been found to alter processes and functions of terrestrial ecosystems including the biogeochemical cycling of carbon (C), N, phosphorus (P) and sulfur (S). Soil extracellular enzymes control the rate-limiting steps in soil organic matter decomposition and soil nutrient return, and hence play key roles in determining the biogeochemical cycles of the above elements. Accordingly, dynamics of soil extracellular enzymes activities (EEAs) provide useful information of the alteration of soil biogeochemical cycles under global change. In order to get a general view of the responses of soil EEAs relating to C, N, P and S acquisition to N deposition, a meta-analysis of published studies was carried out in the current study. N addition significantly increased cellulase activity by 13.0%, but repressed ligninase activity by 11.2%. N addition had no significant effect on protease activity, but significantly elevated the activities of N-acetylglucosaminidase and urease by 5.5% and 11.6%, respectively. N addition enhanced phosphatase activity by 42.3%, but decreased arylsulfatase activity by 34.4%. The ratios of C:N:P acquisition enzyme activities converged on 1:1:1, and was not affected by N addition. Substrate C quality, estimated using an enzyme-based lignocelluloses index revealed elevated soil C quality under N addition. The stoichiometric relationships between nutrient acquisition enzyme activities also revealed that the terrestrial ecosystems changed from N limited to P limited status under N addition. Our results suggest that dynamics of EEAs provide valuable information of soil biogeochemical cycle responses to N deposition.

T3-22-06

Effects of nitrogen deposition on the carbon and nitrogen cycle of secondary forests in Northeast of China

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With continues increasing in nitrogen (N) deposition for several decades in China, the deposited N has induced large impacts on the plant growth, soil property and elements biogeochemistry cycles on forests ecosystems. Northeastern China was one of the largest areas of plantations and secondary forests. However, we still know little about the exact pattern of N deposition and its integrated environmental effects to the forest ecosystems in this region. Our study was conducted at Qingyuan Forest CERN station, Chinese Academy of Sciences. The station is located in a mountainous region in the eastern Liaoning Province, Northeast China. The objectives of our study were 1) to examine the exact status of N deposition (fluxes of different N forms and patterns of isotope composition) in typical secondary forests, 2) to determine fates of deposited N in two typical secondary forests, using ^{15}N labeling method, and 3) to assess possible effects of increasing N deposition in future on trees growth and litter decomposition. During 2014 to 2015, total dissolved inorganic N (DIN) deposition in precipitation were 18.1 and 20.8 kg N ha^{-1} yr^{-1}, of which NH_4^+ accounted for about 67%. Total DIN leaching via stream water were 6.0 and 9.3 kg N ha^{-1} yr^{-1}, which were higher than the threshold of N saturation (5 kg N ha^{-1} yr^{-1}) reported for European forests. The ^{15}N labeling experiment for 1 year in the two secondary forests shows that 9.7-23.2% of ^{15}N tracer was incorporated into plant tissues. 26.7-49.2% was retained in soil. 35.9-52.6% was not recovered in the ecosystems. Retentions of deposited N at ecosystems scale were also lower than that in many temperate forests in Europe and America. These findings suggested that the forests in the study area may have N saturated. However, the effect of N addition (50 kg N ha^{-1} yr^{-1}) on the basal area of tress was not significant, but the production of litterfall was enhanced significantly in two forests. As TIN concentration in stream water declined obviously in autumn, we concluded that the forests may be seasonally N-limited in the area. We also observe that litter decomposition was inhibited after the N addition in first two years.

T3-23: Tropospheric ozone as part of global change - effects on plant growth and terrestrial ecosystems

T3-23-01

Interaction of ozone exposure of trees with belowground pro-

cesses

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Ozone exposure of trees does not only interact with aboveground processes, particularly in the leaves, but also indirectly affects belowground processes both, at the tree and the ecosystem level, by disturbing shoot-root interactions. This disturbance is mainly due to ozone-mediated impairment of phloem loading and phloem transport of sucrose. Since sucrose transport is the driver of phloem transport, the shoot-to-root allocation of numerous other intermediates and signalling compounds is affected. Therefore, the consequences of ozone exposure of the shoot for belowground processes can be studied by girdling experiments. The results of such studies show that disturbed anti-oxidant allocation to the roots impairs the homeostasis of reactive oxygen species (ROS) in the roots and, hence, ROS signalling in root growth and development. In addition to this effect at the whole plant level, reduced sucrose transport to the roots causes a decline in rhizo-deposition that in turn affects ecosystem-level processes, such as ectomycorrhizal biodiversity as well as soil microbial abundance in the rhizosphere. As a consequence, e.g., competition for nitrogen is changed in favor of the tree.

T3-23-02

Growth, physiological and biochemical responses of woody species to ground-level ozone in China, with an emphasis on plant functional types comparison

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The carbon-sink strength of northern hemisphere temperate and boreal forests is decreased by rising ozone concentration [O_3], but lacking of the evidence from China, especially unique subtropical evergreen broadleaved forests. This meta-analysis quantitatively evaluated the effects of elevated [O_3] on growth, biomass, functional leaf traits and plant growth form of woody plants representative of Chinese forests. The database from China consisted of 46 peer-reviewed studies published between 2006 and 2016, taking into account plant functional type, climatic zone, O_3 concentration, experimental duration, and additional treatments such as elevated carbon dioxide concentration, drought and nitrogen addition. The results suggested that 116 ppb [O_3] reduced total biomass of woody plants by 14% compared with control (mean [O_3] of 21 ppb). Significant decreases in root (13%) and shoot biomass (8%) but the root-to-shoot ratios were not significantly affected by O_3. At elevated [O_3], woody plants had significant reductions in height, diameter, leaf mass per area, chlorophyll content, chlorophyll fluorescence parameters, enzymatic antioxidant (ascorbate peroxidase) and soluble sugar which underlie significant damage in photosynthesis. Further, subtropical species were significantly less sensitive than temperate ones while deciduous broadleaf species were significantly more sensitive than evergreen broadleaf and needle-leaf species. Few observations of the interaction of [O_3] with additional treatments limited the understanding of the combined effects of O_3, climate change and/or nitrogen on forests. These findings suggest that carbon-sink strength of Chinese forests has been reduced by rising [O_3]. Given that subtropical broadleaved forests across China are much more resistant to O_3, the suppression strength of the northern hemisphere carbon sink as increases in [O_3] by model results could be likely over-estimated.

T3-23-03

Enhanced tropospheric ozone - hemispheric view in relation to free-air exposure experiments with crop and woody plants in E-Asia

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An introduction in the rationale of the session "*Tropospheric Ozone as Part of Global Change - Effects on Plant Growth and Terrestrial Ecosystems*" and sequence of presentations is envisaged by this contribution, which will pursue two perspectives: highlighting (i) enhanced anthropogenic ozone (O_3) regimes as a component of global change and air pollutant inhibiting plant growth, and (ii) recent advancements in free-air O_3 exposure experiments as particularly conducted in E-Asia on crop and woody plants. Regarding (i), formation of enhanced tropospheric O_3 will be demonstrated as fostered by high insolation upon precursor release from combustion processes and, under climate warming, in relation to atmospheric CO_2 accumulation. O_3 precursors do not only arise from industrial activities and traffic transportation, but also from land-use changes, often in relation to forest burning, which is a world-wide phenomenon. Hence, O_3 becomes an intrinsic component of global change, the more so, as once being formed, is spread at hemispheric scales and contributes to climate warming. "Hot spots" of O_3 formation under (sub)tropical and Mediterranean climate drive increases in chronic background concentrations worldwide. Interrelationships between O_3 action and such by other drivers of global change (CO_2, warming, drought/flooding, N deposition, BVOCs, N_2O, CH_4) and biotic stressors (competitors, herbivores, pathogens) are addressed, including atmosphere-vegetation feedbacks in O_3 formation versus decomposition. Advanced experimentation and modelling claim enhanced tropospheric O_3 regimes to globally lower carbon sink strength and storage capacities of forest ecosystems (with feedback on climate warming) and reducing yield and quality of agricultural crops (with risks for food security of mankind). However, validation of modeling outcome on carbon and water relations at high spatio-temporal scales is scarce and empirically demanding. On such grounds, perspective (ii) of the presentation will survey three recent free-air O_3 exposure experiments in China and Japan with agricultural crops and forest trees, regarding cause-effect relationships in stress response. 1) In Jiangdu, China, wheat/rice systems were under focus, started in 2007. Effects upon enhancement of the ambient O_3 regime by up to 50% were reported on growth, grain yield and soil microbial communities. Derived O_3 dose-response relationships demonstrated 23% enhancement of ozone has already reduce wheat yield by 20% in four cultivars, reproduced over three growth seasons. Food security was concluded to be at risk in China in the face of further rising O_3 impact. 2) In Sapporo, Japan, monarch birch has been exposed to 60 ppb O_3 during daylight hours since 2011, showing seasonally changing O_3 sensitivity of photosynthesis. The effect was related to inconsistent stomatal O_3 response rather than altered photosynthetic capacity. 3) In Yanqing, China, an O_3 free-air system is currently built for poplar plantations to analyze combined elevated O_3/ fertilization effects on long-term carbon sequestration, water use and N cycling. Clarification is strived for regarding O_3 impact on forest ecosystem functionality and services. Ecosystem-level research approaches are to be strengthened for reconciling O_3 effects on climate, food security, plant health, which altogether are cross-linked through land-use concepts, energy-use and the uncertain role of biodiversity in mitigating O_3 stress.

T3-23-04

Difference in ozone susceptibility of photosynthesis between upper and lower canopy leaves of Siebold's beech

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Ozone (O_3) in the troposphere is a phytotoxic gaseous air pollutant, and a growing threat for carbon sink strength of forest worldwide. To determine the effect of O_3 on the carbon budget of the whole-canopy, it is necessary to study the distinct responses of individual leaves to O_3 in relation to a gradient of light conditions within the canopy. In this presentation, we will report the results obtained from two O_3 fumigation experiments with Siebold's beech (*Fagus crenata*) and discuss the difference in ozone susceptibility of photosynthesis between upper and lower canopy leaves. We established free-air O_3 fumigation system in 2011. Ozone at 60 nmol mol^{-1} was fumigated to ten-year-old Siebold's beech saplings (as of 2011) during the daytime for the leafy periods. We determined the leaf photosynthetic traits in various canopy positions of Siebold's beech saplings under elevated O_3 during the second growing season. The susceptibilities of photosynthesis and respiration to O_3 were greater in upper canopy leaves than in lower canopy leaves, and the O_3-induced reduction of photosynthetic activity was because of biochemical limitation in the chloroplasts rather than stomatal closure. We then calculated the canopy-level photosynthetic carbon gain (PCG) and respiratory carbon loss (RCL) using a canopy photosynthesis model. The canopy net carbon gain (NCG) was reduced by O_3 by 12.4% during one growing season. The contributions of changes in PCG and RCL under elevated O_3 to NCG were different between late summer and autumn. To clarify the mechanism of difference in O_3 susceptibility of photosynthesis between upper and lower canopy leaves of Siebold's beech, we analyzed the relationships between photosynthetic parameters such as maximum rate of carboxylation (V_{cmax}) and accumulative stomatal O_3 uptake (AF_{st}). Siebold's beech seedlings were grown in greenhouse type O_3 fumigation chamber for two growing seasons. We set three levels of gas treatments (charcoal-filtered air, O_3 at 1.0-time and 1.5-times the ambient concentration) in combination with three levels of soil nutrient treatments (non-fertilized, low- and high-fertilized) as sub-factor. The degree of O_3-induced reduction in V_{cmax} in upper canopy leaves was higher than that in lower canopy leaves in August of second growing season, similarly with above-mentioned free-air O_3 fumigation experiment. The AF_{st} of upper canopy leaves was higher than that of lower canopy leaves. On the other hand, the reduction rate of V_{cmax} per unit AF_{st} in upper canopy leaves was low as compared to that in lower canopy leaves, indicating higher detoxification capacity for O_3 in upper canopy leaves. When we assumed a threshold value of stomatal O_3 uptake rate above which adverse effects of O_3 occurs is proportional to gross photosynthetic rate, reduction rate of V_{cmax} per unit AF_{st} over a threshold became similar between upper and lower canopy leaves. This indicates photosynthate is associated with detoxification capacity for O_3. In conclusion, the susceptibility of photosynthesis to O_3 in upper canopy leaves of Siebold's beech is higher than that in lower canopy leaves, and this is not due to lower detoxification capacity in upper canopy leaves, but to higher stomatal O_3 uptake.

T3-23-05

Ozone impact on crop yields and quality – risk for food security?

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Tropospheric ozone is a threat to food production as it has negative impact on the yield and quality of important staple crops. Ozone reduces plant productivity by entering leaves through the stomata, causing oxidative stress and decreasing photosynthesis, plant growth and biomass accumulation and allocation. Even short ozone episodes can cause visible injury symptoms to foliage on leafy vegetables (e.g., lettuce, spinach, salad onions), thereby lowering their market value. However, there is a wide intra- as well as interspecific variation in sensitivity to ozone. Important food crops such as wheat, soybean and rice are highly sensitive to ozone, with potato, maize or barley being less sensitive. Data from ozone exposure experiments have been used to develop flux-based ozone dose-response relationships for crops which formed the basis to estimate regional or global yield losses. For example, in Europe, estimated loss of wheat grain yield from ozone was €3.2 billion in 2000. Even with current legislation to reduce ozone pollution in Europe, economic losses of wheat are predicted to be about €2 billion in 2020. A first ozone flux-based global assessment of economic losses due to ozone effects on wheat yield (9.4% loss) are estimated at $24.3 billion. Highest losses are estimated in areas with medium to high ozone fluxes in combination with medium to high area of wheat cultivation (e.g., Western Europe, SE Asia, China)

T3-23-06

Tree and forest responses to interacting elevated atmospheric CO_2 and tropospheric O_3: A synthesis of experimental evidence

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The goods and services provided by forest ecosystems will be needed in greater amounts than ever before in the coming decades, yet are being threatened by climate change and air pollution. We surveyed the peer-reviewed literature on elevated atmospheric CO_2 (eCO_2) and elevated tropospheric O_3 (eO_3) experiments with the goal of determining the general significance of the interaction of these antagonistic air pollutants on forest physiology, biomass production and soil carbon cycling. The review was restricted to eCO_2xeO_3 experiments because the literature on eCO_2 and eO_3 has been extensively reviewed and we specifically wanted to highlight the extent to which this understudied, but important interaction has been investigated. Data were sorted by duration of exposure and vegetation age class (young: 0-5 y, intermediate: 6-20 y, and mature: > 20 y). We were able to locate 58 studies published between 1990 and 2012 reporting primary data on forest responses to eCO_2xeO_3. The total number of observations was greatest from FACE experiments (176), followed by open-top chambers (104), growth chambers (88) and then greenhouses (29), indicating the importance and efficiency of large scale, long-term experiments. Across experiments, average exposure levels were 347 and 603 ppm for ambient and elevated CO_2, respectively; and 35 and 70 ppb, for ambient and elevated O_3, respectively. In young systems, the duration of exposure averaged 3.3 y, with some studies as short as 0.2 y and the longest over 5.0 y. In intermediate age systems, average duration of exposure was 7.7 y, but the single longest running study, The Aspen FACE Project, lasted 12.0 y. We found no eCO_2xeO_3 studies in mature forest ecosystems, representing a corresponding gap in our knowledge. There were commonalities in how eCO_2 and eO_3, alone and in combination, affected physiology, biomass production and litter decomposition, and the net effect of the eCO_2xeO_3 interaction was fairly consistent. In general, eCO_2 enhanced and eO_3 decreased photosynthesis, instantaneous water use efficiency, leaf area index, above- and belowground growth, and tissue concentrations of carbon-based defense compounds and lignin. The interaction treatment (eCO_2xeO_3) was generally intermediate between the control and elevated levels of both factors, although often it was not statistically significant. Contrary to expectation, responses of intermediate age trees were greater than those of young trees. Changes in litter chemistry did not appear to affect decomposition rates or formation of soil organic C under either pollutant, although longer duration experiments are needed to assess the cumulative impact of altered (NPP-driven) soil carbon inputs. Remaining gaps in scientific understanding are identified, key remaining questions proposed, and suggestions made for integrating eCO_2xeO_3 interaction into future experimental designs and integrated research infrastructures.

T3-24: Linking aboveground and belowground interactions under global changes

T3-24-02

Short-term interactive effects of soil warming and precipitation exclusion on fine root production of Chinese fir seedlings in subtropical China

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Global warming and shifted precipitation patterns are expected in subtropical China. However, our understanding on how global warming interacted with precipitation changes affecting belowground processes of subtropical forests are lack, which limits our ability to predict the productivity and carbon dynamics of subtropical forests in response to global changes. By establishing a mesocosm experiment in Chenda State-owned Forest Farm in Sanming, Fujian Province, the short-term interactive effects of soil warming and precipitation reduction on fine root production of Chinese fir (*Cunninghamia lanceolata*) seedlings were examined. The design was factorial soil warming (ambient, +5℃) and precipitation exclusion (ambient, -50%), with five replicate plots (2 m × 2 m) for each combination, i.e., control (CK), warming only (W), precipitation exclusion only (P), warming plus precipitation exclusion (WP). Each plot was planted with four 1-year-old Chinese fir seedlings. Fine roots dynamics were monitored by minirhizotron from March 2014 to March 2016. After two years treatment, the WP significantly decreased both seedling height and basal diameter growth, while there was no significant change between other treatments and the CK. Soil warming alone significantly increased fine root production, but there was no significant change in the other treatments. There was significant interaction effect of soil warming and precipitation exclusion on total fine root production. Compared with CK, the W treatment significantly increased fine root production in spring 2014 and winter 2015, the P treatment significantly increased fine root production in autumn 2014, while the WP treatment significantly decreased fine root production in summer 2014. Repeated-measures ANOVA showed there was significant interaction of soil warming, precipitation exclusion and season. Moreover, the interaction of warming and precipitation exclusion was found significant for fine roots at 20-40 cm soil layer. Compared with CK, fine root production increased significantly at 20-40 cm depth in the W and P treatment, but no significant change in the WP treatment. Together, these results showed that soil warming and precipitation exclusion had significant interaction effects on fine root production of Chinese fir seedlings, which varied with season and soil depth.

T3-24-03

Long-term experimental warming have small effect on above and below ground species richness and effective number of species across Alpine Tundra communities, but change correlations among groups and tropic levels

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Alpine and arctic tundra are predicted to be highly sensitive to global warming. There is a growing number of studies on the response of species composition and ecosystem function of plant communities. However, soil fauna, fungi, and bacteria, are less studied although they are important in arctic terrestrial ecosystems through their role in symbiotic relationships, decomposition, and nutrient cycling. In 2013 we collected species richness data from above ground vascular plants (deciduous shrubs, evergreen shrubs, forbs, and graminoids), bryophytes, lichens, and belowground soil fauna (collembola and mites), fungi and bacteria from our long-term warming experiments (using open top chambers, OTCs) at Latnjajaure, Northern Sweden. Three contrasting Alpine Tundra plant communities (wet meadow, rich meadow and dry heath) represent a natural nutrient and moisture gradient. The warmed plots had been experiencing two decades of experimental warming with OTCs since 1993 (wet meadow), and 1995 (rich meadow and poor heath). Here we present the effect of the long-term warming and site on 1) above and belowground species richness and effective number of species, and 2) the correlations in species richness, and effective number of species, between vascular plants, bryophytes, lichens, bacteria, fungi, collembola and mites. We found that warming and the interaction of warming and site had no significant effect on species richness or effective number of species for any group. Site had strong effect on species richness and effective number of species of all groups except fungi. The correlations in species richness and effective number of species was found to vary between below and aboveground groups. Notably, fungi had no significant correlation with any species group. Importantly, warming was found to both weaken and strengthen the correlation of species richness and effective number of species between below and aboveground groups. This suggest that while below and aboveground diversity may be resistant to direct effects of climate change, climate change may indirectly affect the linkages between below and aboveground groups, and tropic levels. Furthermore, the effect on species richness correlation may differ among groups.

T3-24-04

Bamboo invasion above and below a treeline ecotone: Seasonal soil moisture dry-down rates and high stress tolerance promote invasion success of *Sasa kurilensis*

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How species invasions impact ecosystem structure and function at important ecotones or boundaries is unknown, but may provide insight into the impacts of climate change and the mechanisms underlying community change. The dwarf bamboo, *Sasa kurilensis*, may be a good system to understand these issues, as the species impacts ecosystem features as it encroaches beyond treeline into alpine systems. We used remote sensing imagery to quantify *S. kurilensis* expansion patterns across its range, measured growth and stress tolerances of *S. kurilensis* above and below treeline, and evaluated components of growth to reveal how shifts in light and water limitations influence the ontogeny of height, branching, and leaf production. We show that *S. kurilensis* more than doubled its abundance across its range, but more than tripled its abundance near and above treeline. Soil dry-down rates were a key driver of invasion above and below treeline, where growth rates decreased with more rapid rates of soil moisture dry-down. We found *S. kurilensis* responds to competition and climate stress by increasing allocation to belowground structures at high elevations. Further, it invests more carbon in fewer—yet taller and heavier—aboveground structures in low-light, low elevation environments. It appears this species' success is driven by considerable morphological and physiological flexibility, coupled with changes in water balance associated with snowmelt that in each habitat results in sites increasingly hospitable to bamboo. Overall, this study links resource allocation strategies and physiological responses to climate change and provides a mechanistic explanation of invasion success.

T3-24-05

Nitrogen competition drives plant-soil microbe relationships along a snow depth gradient in temperate steppe

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The effects of rainfall changes on soil microbial communities have been extensively studied, but the effects of snowfall changes on soil microbes and plant-microbe interactions are poorly known. Using snow fences at 8 sites in temperate steppe ecosystems, we investigated how snow depth affects interactions among soil microbes and plants. Both soil microbial biomass (measured as phospholipid fatty acids) and plant biomass increased with increasing snow depth, but the interaction between soil microbes and plants shifted from positive to negative. Soil inorganic nitrogen (N), total N, and microbial biomass N pools were not affected by snow depth, but plant N pools increased with deeper snow. Increased snow depth altered soil microbial community structure and increased the fungal/bacterial ratio. Plant community composition also shifted, with greater grass and lower forb abundance as snow depth increased. Structural equation modelling showed that the direct effects of plant biomass and indirect effects of N (mediated through plants) on soil microbial biomass switched from positive to negative with increasing snow depth, suggesting an increase in competition for N between plants and microbes. Our work implies that changes in snow depth will drive shifts in plant and microbial community composition that are mediated by N availability. Together, our results demonstrate the potential for climate change to alter nutrient competition mechanisms underlying community structure and ecosystem processes.

T3-24-06

Intercropping enhances productivity and sustainability of agroecosystems

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Intercropping advantages are mainly derived from interspecific interactions, including above- and below-ground complementarity, competition and facilitation. There were lots of research on interspecific interactions on above-ground parts, but relatively limited on below-ground interactions between intercropped species. The aim of the study was to examine role of interspecific root-root interactions in overyielding and efficient nutrient utilization of intercropping. Both field and greenhouse experiments were conducted to determine yield and nutrient acquisition advantages by adding sparingly soluble and organic P into soils, interspecific root-root interactions by root barrier, N complementarity by natural or enriched isotope abundance methods. Soil fertility parameters were determined in long-term field experiments. There are significant productivity advantages of intercropping in various legumes/cereals and cereals/cereals intercropping. One-thirds to 100 percentage of intercropping advantages are contributed to root-root interactions. There are two main mechanisms underlying the interspecific root-root interactions, facilitation and complementarity. The interspecific facilitation is that some crop species mobilize unavailable forms of one or more limiting soil nutrients (such as phosphorus, iron and zinc), and improve phosphorus, iron or zinc nutrition for themselves and neighboring non-mobilizing species by releasing acid phosphatases, protons and/or carboxylates, and phytosiderophores into the rhizosphere, respectively. When intercropped with legumes, cereals usually have more root competitive to soil mineral nitrogen, and lead to reduction in soil mineral nitrogen, which facilitates to nodulation and biological N_2 fixation of associated legumes. The root-root interactions maintains soil fertility on a relative fertile soil and enhances soil fertility on a poor soil under continuous overyielding. Our results highlight that interspecific root-root interactions below-ground play an important role not only in overyielding and nutrient acquisition, but also maintaining or enhancing sustainability of agriculture. The mechanisms underlying the root-root interactions include interspecific facilitation on sparingly-soluble soil nutrients and complementary utilization of soil and atmosphere nitrogen in legumes-based intercropping.

T3-25: Forest diversity and degradation in Asia/Pacific

T3-25-01

The genus *Aspidistra* (Asparagaceae) in Vietnam: Diversity, intraspecific variation and distribution patterns

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Aspidistra is one of the most fast-growing genera in the World flora. It currently comprises more than 150 species, about one hundred of which were discovered since 2000. During the last several years, each three weeks a new species of *Aspidistra* was described, mainly from southern China and Vietnam. One can notice the tendency that most of the newly obtained flowering specimens of *Aspidistra* are provisionally regarded to represent undescribed taxa. Indeed, the morphology of flowers in this genus is incredibly diverse. However, quite a lot of its species show only minute differences from their morphologically similar congeners. The comparison becomes especially problematic for the plants being remote geographically from each other, because the species descriptions are commonly based on a single population, and degree of intraspecific variations remains scarcely known for the whole genus. We have performed first analysis of geographical distribution of a number of Vietnamese species of *Aspidistra*, basing on our extensive original collections. Our data show that along with the narrow endemics, there are species with rather large distribution areas, including the recently described *A. bogneri* Tillich, *A. opaca* Tillich, *A. oviflora* Aver. & Tillich, *A. paucitepala* Vislobokov, Nuraliev & D.D.Sokoloff, *A. phanluongii* Vislobokov. Furthermore, *A. connata* Tillich occurs both in northern limestone areas of Vietnam and in the southern mountains. For several species, we have found significant gradual variation of morphological traits, e.g., the contrasting perianth coloration in two varieties of *A. xuansonensis* Vislobokov. On the basis of our data on intraspecific variation, we propose *A. parviflora* N.S.Ly & Tillich as a new synonym of *A. paucitepala*. The other side of the species richness of *Aspidistra* combined with wide distribution range of some of its species consists in a hidden diversity within a taken protected area. As only few plants usually produce flowers simultaneously within a population, all the sterile individuals are commonly assigned to the same species as the blooming material. Meanwhile, species of *Aspidistra* sometimes grow sympatrically. We have registered up to four species inhabiting the same forest. In some cases they form mixed populations, while in others they occur separately, possibly being adapted to different ecological conditions (including examples with elevation-based distribution). Besides, the sympatric species of *Aspidistra* can demonstrate overlapping or strictly opposite flowering phenology. An alternative way of distinguishing species of *Aspidistra* and establishing the species boundaries (and also higher-level taxa) is the DNA barcoding. Our results show that plastid *psb*A-*trn*H and nuclear 5S-NTS regions can be used as such barcodes. Our molecular database will be important for further ecological investigations of *Aspidistra* as it will allow identifying sterile specimens for the full inventory of species number and distribution within a model area. The knowledge of species diversity of *Aspidistra* in the local floras is crucial for forest management and conservation. Efficient species identification and assessing their rarity and ecological niches should greatly improve the interaction between conservation biology and forest industry. This study is supported by the Russian Science Foundation (project 14-14-00250) and Russian Foundation for Basic Research (15-04-00419A).

T3-25-02

Altitudinal gradient analyses of the Balakot Forests of District Swat in the Hindukush range of mountains

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In the Swat District, the Balakot Forests is the part of Hindukush range of mountain that is one of the remote area where vegetation of different areas have not been studied correlated to environmental variables. The recent research were carried out to study the different effects of environmental variables specially altitude on plant species association and variation among plant communities of Balakot Forests. Also this exploration identifies pressures from different environmental factors and recommendation for corrective measures. For valuation of environmental changeability quantitative ecological methods were carried out during summer 2014-15. The technique line transect method were used. Size of the transect was 50 m which were further divided into sub transects 10 m. The stands were planned along 15 altitude on different aspects (South, North, East and West). Total 27 transects were studied in all stands in different station. Phytosociological attribute were observed, i.e. frequency, density and cover for trees, shrubs and herbs in each stands of vegetation communities. A total of 120 plant species were collected in Balakot Forests. After getting initial result, Canonical Correspondence Analysis (CCA) was used to analyze the environmental pitch of vegetation's. The environmental data and species abundance were used in CANOCO software version 4.5. The presence absence data of 120 plant species were elaborated with Cluster and Two Way Cluster Analysis techniques by using PC-ORD version 5 to show different species composition that resulted nine stands. Altitudes, aspect, soil texture show that these are the strongest variables and show significant effect on vegetation and distribution in different communities. It is suggested to protect and use wisely whole of the flora and particularly the rare species.

T3-25-03

An overview of the forest vegetation of Irano-Turanian phytogeographical region

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The vegetation distribution pattern is of great importance in conservation and biogeographical investigations. The threatened vegetation is one of the most important indicators for decision-making process in the conservation of priority habitats. Irano-Turanian floristic region comprises a vast territory of Southwest and the Middle Asia. It is connected to the Mediterranean, Euro-Siberian (boreal) and Saharo-Sindian regions. The region is chgaracterised by a high rate of plant speciation and endemism, particularly in some large genera like; *Astragalus, Cousinia, Acantholimon, Acanthophyllum* and *Allium*. The heterogeneous topography together with a relatively stable continental climate have lead to its long-term uninterrupted isolation as well as high rates of allopatric speciation. The climate of the region is highly differentiated from adjacent regions, especially in its degree of continentality and seasonality. Vegetation of this area shows an important genetic, biological and ecological diversity. Unique topography, habitat heterogeneity and long vegetation history have resulted in the formation of diverse community types. These include scattered montane forests, widespread *Juniperus* woodlands, wild *Pistacia vera* woodlands, open shrublands and scrub, diverse mountain steppe communities, cliff vegetation, loess and gypsophilous vegetation, semi-desert steppes and halophytic formations, as well as aquatic, ruderal and weed communities. Several forest vegetation types are found in natural and seminatural environments of the area, due to its biogeographical position and diverse topography and habitats. Although a great variation is observed in different sub-phytogeographic regions and altitudes, in general forest vegetation is dominated by the forest tree species like; *Pinus brutia* var. *eldarica, P. nigra* ssp. *pallasiana*, *P. sylvestris, Quercus aegilops, Q.pubescens, Q. brantii, Q. cerris, Q. infectoria, Q. libani, Q. longipes, Q. macranthera, Juniperus excelsa, J. oxycedrus, Betula pendula, Pistacia atlantica* and *P. khinjuk.* In this study we will try to evaluate the impact of current and potential changes in the near future, the scope of sustainability, and ecological analysis of the forests of this region vis-a-vis the prospects and challenges.

T3-25-04

Protection and economic evaluation of forests: Case study from East Mediterranean, Central and Southwest Asia

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Currently nearly 30 percent of the global land area is forested, more than 1/3 of all forests are considered primary, but around 60,000 km^2 of this is lost or modified annually. In this presentation attempt has been made to evaluate the forest diversity and its conservation priorities in the East Mediterranean, Central /Southwest Asia. The estimates of forest resources in terms of total forest area covered and percentage of land area in the East Mediterranean is 10,854,000 ha (25.7%); Central Asia 39,220,000 ha (51.7%) and Southwest Asia 14,689,000 ha (18%). Important forest types occuring in these regions include predominantly broadleaved forests dominated by non-fruit bearing broadleaf species; Shrublands in arid zones; Riverside forest and Mangroves, and predominantly coniferous forests dominated by spruce, pine or fir species; Juniper forest and Cedar forest. There is an old tradition in the West and Central Asia to cultivate *Populus* as well as other trees like *Morus* and *Platanus* along irrigation channels, for furniture and other purposes. This tradition needs to be kept and maintained, or even enlarged, as part of the economy. These trees give shade in summer and can be used for timber, in the case of mulberries not only for fruit production but even again for silkworm culture and silk production. Overgrazing, industrial activities and housing hand in hand with an expansion in agriculture, particularly into woodlands, coupled with use of different technologies is one of the most devastating causes for deforestation. All these factors lead to a decrease in forest area. A widespread conversion of natural forests into monocultural plantations of commercial value reduces habitat diversity, especially in forest lands where micro-habitat differences are much needed for wildlife. There is a great need to maintain ecosphere for its sustainable use. In spite of our need to manipulate nature we have to understand it in order to be on the proper junction within the ecosphere. A healthy ecosphere needs healthy trees and forests because these have many direct and indirect values to the people in the form of maintaining and improving the soil conditions and the hydrological systems; help in moderating climate by soaking up and storing CO_2; while assuring self renewal processes of the land base and its sustainability. A great stress is being laid on to recognize the factors that threaten our forest ecosystems. We need to take immediate measures to prevent loss of habitats and species diversity, prepare inventory of renewable natural resources on forest lands, develop necessary scientific, technical, legal and administrative measures to improve quality and quantity of forest biodiversity, manage and utilize forest biological resources based on sound ecological and sustainable management principles.

T3-25-05

Forest diversity, ecosystem and landuse changes in Malaysia

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Malaysia is endowed with one of the richest forest diversity in the world which is represented by various forest ecosystems and habitats, species and the genomes contained in the plants, animals and microbes. Forest ecosystem diversity is represented by the rich lowland and hill dipterocarp forests, the mangrove forests and the montane forests at Mt. Kinabalu. To-day a total of 1.39 million ha (about 7.6%) of the forest of all types have been set aside for biodiversity protection and conservation. Forest species diversity is represented by about 4,000 species of trees whilst the diversity of animals is represented by more than 6,000 species, excluding that of the insects, the largest and most diverse single group which is unadequately known. As the country is also pursuing industrialisation by the year 2020, conflicts occur between biodiversity conservation efforts and socio-economic development. Measures for sustainable agriculture and forestry are in place but those in biodiversity prospecting, eco-tourism and impacts of urbanisation on biodiversity are yet to be addressed. In the absence of concrete data of biodiversity loss in the country it is difficult to ascertain the lists of endangered or otherwise threatened forest species as the various ecosystems are prone to changes through landuse patterns. Many factors have contributed to forest diversity loss; among them is the rapid socio-economic development of the country that transformed vast forested lands by logging activities, land openings for agriculture and resettlement and subsequently creating new built-up areas such as urban and industrial areas which are relatively poor in biodiversity. These changing landuse patterns are affecting not only biodiversity but the environment per se. Other factors such as over-harvesting and forest plantations have also contributed in small part. These activities had led to significant forest loss, degradation and forest fragmentation. What is needed are frameworks and strategies for biodiversity conservation, some socio-economic strategies for sustainable use and benefit sharing of biodiversity and also some legal measures for sustainable use and protection of forest biodiversity and assessment of landuse patterns.

T3-25-06

Do protected areas effective in preventing land use conversions? A case study in Xishuangbanna, Southern China.

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Forested areas worldwide are declining in a rapid rate as a result of sustaining increasing needs of growing human population. As many methods and strategies are implemented to prevent this decline it is crucial to evaluate whether these efforts are successful in the conservation agenda. Xishuangbanna is 0.2% of total land of China, harbouring > 20% of China's biodiversity, is now threatened with monoculture rubber plantations expansion and other land use conversions replacing lowland rainforest and shifting cultivation practices. This study was designed to evaluate the effectiveness Protected Areas (PAs) which are specifically designed for species and forest conservation. We measured the success of PAs in preventing expansion of rubber and other non-conservation land uses within their boundaries. We used matching methods to minimize the bias of PAs location and other factors influencing PAs establishment and to compare land use conversion rates within PAs zones and with outside PAs. By 2010, Xishuangbanna had designated an area of 3,455.52 km^2 (~ 18%) as PAs while rubber (22%) showed an expansion rate of 153.4 $km^2\ y^{-1}$ and approximately 16% of PAs were deforested by 2010 with an annual rate of 6.8 $km^2 yr^{-1}$ and if this rate continues assuming that the rate is constant, within next 40 years another 10% of PAs land will be deforested but because drivers of deforestation continuously change and accelerate rather than decelerate Xishuangbanna's PAs might lose more forest than we predicted in this analysis. Therefore it should be a timely concern to review or to strengthen current land sparing policies, rules and regulations.

T3-26: Small RNAs and Plant Response to Climate Change and Environmental Stress

T3-26-01

Abiotic stress miRNome in cereals

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There are two dilemmas in the world: the first one is a/biotic stress and the second one is malnutrition. Crop species such as wheat and barley, suffer from the effect of many different abiotic stresses such as drought, heat, salinity or nutrient deficiencies and toxicities which cause large yield losses resulting in economic and environmental damage. The destructive effects of a/biotic stresses have increased recently and are predicted to further deteriorate simultaneously with climate change. In order to cope with the changing environmental conditions, generation of more stress-tolerant plant varieties which are also high yielded is vitally important. New generation technologies have discovered an essential tool for improving crop tolerance to the abiotic stresses. miRNAs are small regulators of gene expression that act on many different molecular processes such as development, environmental adaptation and stress tolerance. miRNAs can act at both the transcriptional and post-transcriptional level, although post-transcriptional regulation is the most common in plants where miRNAs can inhibit the translation of their mRNA targets via complementary binding and cleavage respectively. To date, several miRNA families such as miR156, miR159 and miR398 have been detected as responsive to severe environmental conditions by taking charge in the regulation of stress associated molecular mechanisms individually and/or together with their various miRNA partners. Handling of such miRNAs together with their targets may pay the way for improved crop performance under several abiotic stresses. Exploration of further miRNA families together with their functions under stress conditions will improve our knowledge and provide opportunities to enhance plant performance to help us meet with world food demand. We will discuss an outline to set out the workflow to study a/biotic stress miRNome in wheat and barley.

T3-26-02

The sudy of the response and the molecular mechanisms of *Astragalus Membranaceus* Bge. var. *mongolicus* (Bge.) Hsiao to drought stress

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The shortage of the water resource is a common phenomenon in Northwestern China, which seriously affects the development of the local economy and production. *Astragalus membranaceus* Bge. var. *mongolicus* (Bge.) Hsiao (*A. mongolicus*) is primarily is cultivated on a large scale in northwestern China. Understanding the plant responses to drought has important effects on ecological environment recovery and local economic development. Here, we studied the effects of drought stress on the growth, physiological and biochemical responses and secondary metabolites accumulation of two-year-old *A. mongolicus*, and combined transcriptomics (Illumina Hiseq 2000) and metabolomics (^{1}H-NMR) to investigate how the roots of *A.mongolicus* responded to 14 days of progressive drought stress. The results of the present study are as follows: 1) The dried soil reduced the relative water content (RWC) of the leaves and biomass during the 14 days of progressive drought stress. Mild and moderate drought stress enhanced some antioxidative enzyme activity, the content of proline and soluble sugar content, but antioxidative enzyme activity was limited by severe stress. A certain degree of drought stress can enhance accumulation of astragaloside IV, calycosin-7-*O*-*β*-D-glycoside, ononin, calycosin and formononetin. 2) Metabolomics demonstrated that the metabolic processes were significantly affected by drought stress. PCA analysis demonstrated that the sucrose, proline, and malate metabolites contributed greatly to the separation. Strikingly, proline was increased by almost 60-fold under severe stress compared to the control. Some backbone pathways, including glycolysis, tricarboxylic acid (TCA) cycle, glutamate-mediated proline biosynthesis, starch and sucrose metabolism, were significantly affected by drought. 3) Transcriptomics demonstrated that the differential expression of a large fraction of the transcriptome after drought stress. Compared with control, there are 18,292, 15,085, 20,986 differentially expressed genes (DEGs) in mild stress, moderate stress and severe stress. The expression of *P5CS, PDH, MDH*, zinc finger proteins genes and cytochrome P450s genes were different during drought stress, which are more likely to determine tolerance to drought. 4) We obtained the key enzyme genes in the biosynthesis pathway of calycosin-7-*O*-*β*-D-glycoside, ononin, calycosin and formononetin by transcriptional sequencing technique, analyzed the expression of key enzyme genes PAL, C4H, 4CL, PKR and CHI in the drought stress, and combined with the changes in the content of four flavonoids to explore the effects of drought stress on flavonoids accumulation of *A.mongolicus* and the molecular mechanisms of metabolic regulation. Our study presented a comprehensive overview of the changes in the *A. mongolicus* under progressive drought stress and identified important pathways through which this herb adapts to and tolerates drought stress. We found a certain degree of drought stress not affect the biomass and can enhance the accumulation of secondary metabolites.

T3-26-03

MicroRNA-mediated biotechnology for improving plant tolerance to abiotic stress

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MicroRNAs (miRNAs) are an extensive class of endogenous, small RNA molecules that sit at the heart of regulating gene expression in multiple developmental and signalling pathways. Recent studies have shown that abiotic stresses induce aberrant expression of many miRNAs, thus suggesting that miRNAs may be a new target for genetically improving plant tolerance to certain stresses. These studies have also shown that miRNAs respond to environmental stresses in a miRNA-, stress-, tissue-, and genotype-dependent manner. During abiotic stress, miRNAs function by regulating target genes within the miRNA-target gene network and by controlling signalling pathways and root development. Generally speaking, stress-induced miRNAs lead to down-regulation of negative regulators of stress tolerance whereas stress-inhibited miRNAs allow the accumulation and function of positive regulators. Currently, the majority of miRNA-based studies have focused on the identification of miRNAs that are responsive to different stress conditions and analysing their expression profile changes during these treatments. This has predominately been accomplished using deep sequencing technologies and other expression analyses, such as quantitative real-time PCR. In the future, more function and expression studies will be necessary in order to elucidate the common miRNA-mediated regulatory mechanisms that underlie tolerance to different abiotic stresses. The use of artificial miRNAs, as well as overexpression and knockout/down of both miRNAs and their targets, will be the best techniques for determining the specific roles of individual miRNAs in response to environmental stresses.

T3-26-04

Genome-wide reshaping of DNA methylation and its association with gene expression in cadmium-exposed rice (*Oryza*

***sativa*)**

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Cadmium (Cd) is one of the naturally occurring toxic heavy metals in the Earth's crust and negatively affects plant growth and development. Exposure to trace metal cadmium (Cd) affects transcriptional responses. Whether Cd-modified DNA methylation marks are associated with transcription and functional consequences in plants remains unknown. We present the genome-wide single-base-resolution maps of methylated cytosines in Cd-exposed rice, along with global transcriptional change in mRNA. Widespread differences were identified in the composition and patterning of CG and non-CG methylation marks between Cd-exposed and control rice genomes. There are 2,320 non-redundant differentially-methylated regions detected in the genome. RNA-sequencing reveals 2,092 DNA methylation-modified genes differentially expressed under Cd exposure. Rice exposure to Cd led to transcriptional changes in DNA methyltransferases, histone methyltransferases and DNA demethylases. By profiling global DNA methylation and gene transcription, we found more genes hypermethylated than those hypomethylated in CG, CHH and CHG (where H is A, C or T) contexts in the regions of upstream, genebody and downstream under Cd stress. Many of the genes were involved in stress response, metal transport, and transcription factors. Most of the DNA methylation-modified genes were transcriptionally altered under Cd stress. A subset of loss of function mutants defective in DNA methylation/demethylation and histone modification activities were applied to identifying transcript abundance of selected genes. Compared to wide-type, mutation of MET1 and DRM2 resulted in general lower transcript levels of the genes under Cd stress. Transcripts of OsIRO2, OsPR1b and Os09g02214 in drm2 were significantly reduced. A commonly used DNA methylation inhibitor 5-azacytidine was employed to investigate whether DNA demethylation affected physiological consequences. 5-azacytidine provision decreased general DNA methylation levels of selected genes, but promoted growth of rice seedlings and Cd accumulation in rice plant. Fifteen loci generating microRNAs such as OsmiR164a and OsmiR812c were found to be differentially methylated under Cd stress. Thousands of transposable elements that were transcriptionally regulated under Cd stress were also found to be differentially methylated. These results indicate that variation of genome-wide DNA methylation may alter gene expression and biological functions in cadmium-exposed rice plants.

T3-26-05

Small RNA deep sequencing reveals the important role of microRNAs in the halophyte *Halostachys caspica*

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MicroRNAs (miRNAs), an extensive class of small regulatory RNAs, play versatile roles in plant growth and development as well as stress responses. However, the regulatory mechanism is unclear on miRNA-mediated response to abiotic stress in plants. *Halostachys caspica* is a halophytic plant species and a great model for investigating plant response to salinity stress. However, no research has been performed on miRNAs in *H. caspica*. In this study, we employed deep sequencing to identify both conserved and novel miRNAs from salinity-exposed *H. caspica* and its untreated control. Among the 13–19 million sequences generated from both treatments, a total of 170 conserved miRNAs, belonging to 151 miRNA families, were identified; among these miRNAs, 31 were significantly up-regulated and 48 were significantly down-regulated by salinity stress. We also identified 102 novel miRNAs from *H. caspica*; among them, 12 miRNAs were significantly up-regulated and 13 were significantly down-regulated by salinity. qRT-PCR expression analysis validated the deep sequencing results and also demonstrated that miRNAs and their targeted genes were responsive to high salt stress and existed a negative expression correlation between miRNAs and their targets. miRNA-target prediction, GO and KEGG analysis showed that miRNAs were involved in salt stress-related biological pathway, including calcium signalling pathway, MAPK signalling pathway, plant hormone signal transduction and flavonoid biosynthesis, *etc*. This suggests that miRNAs play an important role in plant salt stress tolerance in *H. caspica*. This result could be used to improve salt tolerance in crops and woods.

T3-26-06

Ecophysiological responses of *Camellia japonica* (*Naidong*) to different water and light deposition

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Camellia japonica (*Naidong*) is the research object in this paper, which is from Qingdao, the city of Shandong province. The paper set two light regimes L1, L2 (65%, 15% of full sunlight), different water supply regimes W1, W2, W3 [75%, 50%, 25% of field capacity (FC)]. The responses of ecophysiological of *Camellia japonica* (*Naidong*) during different water, light and their interactions were studied. The results showed that height (H), basal diameter (BD) and crown area (CA) of *Naidong* were increased with the treatment time under L1 and L2, the morphological index was highter with the L1 treatment as compared to the L2, and the relative growth rate of each index increased rapidly. But with the extending of stress time, the relative growth rate increased slowly. With the decreasing of water regimes, the Photosynthetic Rate (A), Ranspiration Rate (E), Stomatal Conductance (Gs) and Water Use Efficiency (WUE) decreased, Intercellular CO_2 Concentration (Ci) increased gradually, and Vapour Pressure Deficit (VPD) increased firstly and then decreased. The value of Maximal Fluorescence in the dark (F_m) and Maximal Quantum Yield (F_v/F_m) under the L1 conditions were higher than L2 conditions, and the Initial Fluorescence in the dark (F_0) showed the opposite trend. With the decreasing of moisture gradient, the F_0 increased gradually under L1, while showed the opposite trend under L2. Significant differences were found in photosynthetic pigment contents of leaves among various light and water treatments. Under high irradiation, chlorophyll a, chlorophyll b and total chlorophyll were higher than low irradiation, and the Chla/Chlb value showed the opposite trend. The contents of Superoxide Dismutase (SOD), Peroxidase (POD), Catalase (CAT), Malondialdehyde (MDA) and Proline (Pro) were significantly difference at every level of light and water treatment. And the contents of POD, SOD increased firstly and decreased later with the water gradient decreasing. The content of CAT decreased with the decreasing of water content under L2 conditions, and the contents of MDA and Pro increased gradually with the water gradient decreasing. Drought or shade has some restrictive effect to the growth of *Naidong*, certain moisture and light were favorable for plant growing. *Naidong* could tolerate a certain degree of drought resistance and adapt the larger amplitude of light intensity. At the same time, it can change the enzyme activity and osmotic adjustment substances to against damage.

THEME IV: DEVELOPMENT & PHYSIOLOGY

T4-01: Stomatal guard cells: Model systems for signaling and development

T4-01-01

Convergence of signal transduction events in guard cells at reactive oxygen species and calcium channels during stomatal closure by abiotic or biotic stress signals

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Rapid regulation of stomatal aperture is essential for terrestrial plants not only to optimize water use/CO_2 uptake, under abiotic stress conditions, but also restricting pathogen entry during biotic stress. Stomatal guard cells evolved efficient ways to sense abiotic and biotic stress signals and respond rapidly to induce stomatal closure. Stomata close when the guard cells lose turgor and become flaccid. When triggered by abiotic/biotic stress signals, osmotically active components, such as potassium ions, anions, malate and sucrose, move out of guard cells causing an efflux of water and decrease in turgor. Among several effectors, the mechanism of ABA (a well-known phytohormone) action on stomata has been studied in detail. ABA induced stomatal closure is mediated by many signalling components like cytoplasmic pH, reactive oxygen species (ROS), nitric oxide (NO), cytosolic free calcium, G-proteins, protein kinases, protein phosphatases, phospholipases, phospholipids and sphingolipids. These signalling components appear to operate during stomatal closure by also methyl jasmonate and elicitors such as chitosan and flagellin. During signal transduction pathway leading to stomatal closure by plant hormones or microbial elicitors, three signalling components stand out as the major converging points. These are ROS, cytosolic calcium and ion channels. Once formed, the ROS and free calcium of guard cells regulate both downstream and upstream events. A major influence of ROS is to increase the levels of NO in guard cells. The rise in ROS and NO would cause an increase in free calcium and modulate ion channels, through a network of events, in such a way that the guard cells lose K^+/Cl^-/anions. The efflux of these ions decreases the turgor of guard cells and then stomatal closure. We propose that ROS, NO and cytosolic calcium act as points of divergence. The interrelationships and interactions of ROS, NO, cytosolic pH, and calcium are quite complex and need further detailed examination. The levels of ROS and NO in guard cells play an important role in not only stomatal closure but also in integrating stimuli from abiotic or biotic stress. The cytosolic pH, G-proteins and phospho-/sphingolipids are also important components during stomatal closure but they may act in parallel. Further work is required to elucidate the perception of signals, such as methyl jasmonate or elicitors and how they activate NADPH oxidase leading to ROS production. Several of the unresolved questions and the ease of using epidermis make the stomatal guard cells an ideal system for studying signal transduction mechanism in plant cells.

T4-01-02

Regulation of aquaporins and plant hydraulics under water and salt stress

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Aquaporins of the Plasma membrane Intrinsic Protein (PIP) subfamily mediate multiple controls of plant tissue water transport. New insights into the mechanisms at work in their regulation in stomata and roots under water, salt and oxidative stress will be presented. Fluorescently tagged *At*PIP2;1 aquaporin expressed in *Arabidopsis* roots was instrumental in studying its subcellular trafficking in response to stimuli (Luu *et al.,* 2012; Hosy *et al.,* 2015; Wudick *et al.,* Mol. Plant, 8: 1103-1114). For instance, salt (150 mM NaCl) triggered AtPIP2;1 internalization in a time frame (15 min) that paralleled the fast inhibition of root hydraulic conductivity (Lp_r). These effects are mediated through a salt-induced accumulation of Reactive Oxygen Species (ROS). The mechanisms and routes of ROS-dependent trafficking of PIPs in root cortical cells and evidence for an enhanced cycling of PIPs between the plasma membrane and endosomes in response to salt stress and ROS will be presented. The dynamic properties of AtPIP2;1 in the plasma membrane in normal, salt and oxidative stress conditions were also addressed by means of single particle tracking and fluorescence correlation spectroscopy. Both NaCl and hydrogen peroxide (H_2O_2) treatment induced an increase in lateral motion and a reduction in the density of a fluorescently tagged AtPIP2;1. In normal conditions, membrane rafts play an important role in the partitioning of AtPIP2;1 and clathrin-mediated endocytosis is predominant. In salt stress conditions, AtPIP2;1 and endocytosis is cooperated by a membrane raft-associated salt-induced pathway and a clathrin-dependent pathway. Based on natural variation of Lp_r in *Arabidopsis* and linkage mapping, we also have identified several genes regulating Lp_r (e.g. Shahzad *et al.,* 2016). One of these encodes a protein kinase that acts as a positive regulator of root hydraulics and, surprisingly, of abscisic acid (ABA) signalling. ABA-induced stomatal movements are also crucial for control of plant water status. A role for AtPIP2;1 in stomatal closure induced by ABA (Grondin *et al.,* 2015) or the Pathogen Associated Molecular Pattern flg22 was recently uncovered. Both ABA and flg22 induced an increase in osmotic water permeability of guard cell protoplasts. HyPer, a genetic probe for intracellular H_2O_2, revealed that ABA and flg22 also induce an accumulation of H_2O_2 in guard cells. These two responses were lacking in *pip2;1* knock-out plants indicating that AtPIP2;1 plays a dual hydraulic and signaling role in guard cells. *In vitro* assays indicate that the

OST1 (SnRK2.6) protein kinase likely mediates AtPIP2;1 phosphorylation during guard cell responses to ABA. Based on expression in *pip2;1* of *At*PIP2;1 phosphorylation mutants, we propose a mechanism whereby ABA and flg22 enhance phosphorylation of AtPIP2;1 at a new site (Ser121), to activate its water and H_2O_2 transport activities and induce guard cell closure

T4-01-03

Predawn xylem water potential and leaf hydraulic conductance influence the stomata behavior of mangrove plants

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Mangroves grow in the circumstances with high salinity and high light, their xylem is constantly under high tension, which could be a risk to induce hydraulic dysfunction. It is important for mangrove plants to control transpirational water loss through rigorously stomatal control. However, it has been hardly understood the stomatal regulation behavior of mangrove plants. Mangrove roots largely exclude salt from sea water. In addition, the leaves of some mangrove species secrete salts. In another study, we found the predawn water potential of mangrove plants are related to salt management mechanisms, with salt secretion species having high water potential than other species with only salt exclusion mechanism. Other researchers have reported the stomatal behavior of terrestrial plants is related to predawn water potential and leaf hydraulic conductance. In this paper, we tested whether these findings also work on the mangrove plants. By examining stomatal conductance (g_s) response to transient change in leaf-to-air vapor pressure deficit (VPD) and photosynthetic photon flex density (PPFD) in intact sun leaves of nine mangrove plants in a mangrove forest, we found linear g_s response to transient increase of PPFD, while three modes of g_s to increasing VPD, i.e. (1) decrease of g_s until to a very low value; (2) initially increase then decrease to a very low value; (3) continuously increase until to a stable value. These different g_s response modes might be related to leaf anatomical structure and water use strategies. The g_s sensitivity to PPFD, as indicated by linear regression slope of the regression between g_s and PPFD, was correlated with predawn water potential. We also found that there were significant linear correlations between the predawn water potential or midday water potential of the leaves and maximum g_s in a clear day, and between the diurnal maximum g_s and maximum leaf hydraulic conductivity across species. Our studies demonstrate the stomatal behavior of mangrove plants are regulated by xylem water potential, which is related to salt management mechanisms, and leaf maximum hydraulic conductance. We are now examining the stomatal conductance, foliar ABA concentration and transcriptome expression during increasing water stress in mangrove plants.

T4-01-04

Diverse stomatal signaling and the signal integration mechanism

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A pair of guard cells forms stoma in the epidermis of leaves, stems and other organs. The sophisticated cells sense a variety of abiotic and biotic stresses and convert the input signals to turgor pressure regulation through modulation of ion channel activities. The changes in turgor pressure are followed by changes in cell volume and adjustments of stomatal apertures. The stomatal responses allow plants to sustain their growth under the stressful conditions. Guard cells achieve the signal integration to optimize stomatal aperture. In other words, plants encode the input signals to calcium signaling and reactive oxygen species signaling and decode these signaling to stomatal responses. We summarize the details of guard cell signaling regulated by plant hormones. Furthermore, we discuss the low-molecular compounds such as glutathione and reactive carbonyl species involved in the guard cell signaling.

T4-01-05

Stomatal responses to changes in environmental variables

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Stomata are pores found on the surfaces of leaves that control the uptake of CO_2 and the loss of water vapour. The former is important for photosynthesis and dry matter accumulation while the latter is the driving mechanism for the transpiration stream that brings water and mineral nutrients from the soil to the upper parts of the plant. Restricting water loss also provides the plant with an ability to conserve existing water during times of reduced soil water availability. Plants are able to control loss of water and CO_2 by controlling the aperture of the stomatal pore and by controlling the number of stomata that develop on the surface of leaves. Both stomatal aperture and stomatal development are under the control of environmental signals such as light, atmospheric relative humidity, atmospheric CO_2 and endogenous hormonal signals such as abscisic acid. During the course of this lecture responses to changes in atmospheric CO2 and light will be considered with a focus on signalling and sources of ATP used to support stomatal movements.

T4-01-06

L-Met activates *Arabidopsis* GLR Ca^{2+} channels upstream of ROS production and regulates stomatal movement

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2. DGIST

Plant glutamate receptor homologs (GLRs) have long been proposed to function as ligand-gated Ca^{2+} channels, but no in planta evidence has been provided. Here we present genetic evidence that *Arabidopsis* GLR3.1 and GLR3.5 form Ca^{2+} channels activated by L-methionine (L-Met) at physiological concentrations and regulate stomatal apertures and plant growth. *glr3.1/3.5* mutations resulted in a lower cytosolic Ca^{2+} level, defective Ca^{2+}-induced stomatal closure, and Ca^{2+}-deficient growth disorder, all of which involved L-Met. Patch-clamp analyses of guard cells showed that GLR3.1/3.5 Ca^{2+} channels are activated specifically by L-Met, with the activation abolished in *glr3.1/3.5*. Moreover, GLR3.1/3.5 Ca^{2+} channels are distinct from previously characterized ROS-activated Ca^{2+} channels and act upstream of ROS, providing Ca^{2+}

transients necessary for the activation of NADPH oxidases. Our data indicate that GLR3.1/3.5 constitute L-Met-activated Ca^{2+} channels responsible for maintaining basal $[Ca^{2+}]_{cyt}$, play a pivotal role in plant growth, and act upstream of ROS thereby regulating stomatal aperture.

T4-02: Recent cell biological insights in plant sexual reproduction

T4-02-01

The roles of MON1/CCZ1-mediated Rab7 activation in vacuolar transport, vacuole biogenesis, and pollen development in plants

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The plant secretory pathway contains multiple membrane-bound organelles that mediate vacuolar protein trafficking from the endoplasmic reticulum (ER) to vacuole via Golgi apparatus, trans-Golgi network (TGN) and prevacuolar compartment (PVC) or multivesicular body (MVB) (Cui *et al.,* 2016a). We have recently showed that 1) the *Arabidopsis* RABG3f, a member of the plant Rab7 small GTPase family, localizes to PVC/MVB and tonoplast; 2) the proper activation of Rab7 is essential for both PVC-to-vacuole trafficking and vacuole biogenesis; 3) the *Arabidopsis* MON1 (MONENSIN SENSITIVITY1) and CCZ1 (CALCIUM CAFFEINE ZINC SENSITIVITY1) dimeric complex functions as the Rab7 guanine nucleotide exchange factor; and 4) MON1/CCZ1-mediated Rab7 activation is essential for tapetal programmed cell death (PCD) and pollen development. Thus, Rab7 activation by the MON1-CCZ1 complex is critical for vacuolar trafficking, vacuole biogenesis, tapetal PCD and pollen development in plants. Supported by grants from the Research Grants Council of Hong Kong (CUHK465112,466613,14130716, CUHK2/CRF/11G, C4011-14R, C4012-16E, and AoE/M-05/12).

T4-02-02

Membrane lipids and proteins steering pollen tube tip cell polarity machine – exocyst focus.

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Directionality and speed of growth are crucial parameters of pollen tubes reproduction success resulting in a selection at the level of male gametophytes in Angiosperms. During the pollen grain germination process pollen tube tip growth machinery is assembled aided mechanically by preexisting germination pore or pores formed within the exine during the young pollen grain development within the anthers. But polar growth of transiently stopped or depolarized pollen tubes might be re-established spontaneously from within. Circumstantial evidence implicates small GTPases of RHO family (RHO of plants - ROPs) as important regulators in this process in accordance with the generally accepted model of eukaryotic cell polarization. Small GTPases function as a molecular switches which regulate downstream processes via interacting effectors which orchestrate cytoskeletal and endomembrane dynamics in an intimate cross-talk with specific membrane lipids and their regulators. Exocyst is CATCHR type octameric vesicle tethering complex and was originally described as an effector of exocytosis RAB GTPase in yeast by Peter Novick′s lab, but soon not only in yeast, but also in animals RHO/RAC GTPases turned-out to impose regulatory function upon this complex too. While in most organisms exocyst eight different subunits are encoded by single genes, land plants evolved multiple paralogous subunits esp. in the case of EXO70 subunit - from original three basic classes resulting e.g. in 23 *Arabidopsis* EXO70 isoforms. There is an intriguing question - what is the functional significance of this multiplication. Here, using pollen tubes of tobacco and Arabidopsis as a model, we will present evidence, that in single plant cells several EXO70 subunits are not only co-expressed but show specific, non-overlapping localizations and functions. We have also shown that as in other eukryotic models, also plant exocyst subunits are able to directly bind membrane lipids and these exocyst-lipid interactions are vital for vectorial vesicle delivery supporting rapid tip polarized cell expansion in pollen tubes. Acknowledgement – This work was and is supported by the Czech Sci. Foundation/GACR projects 13-19073S (M.P.) and GA15-14886S and GF16-34887L (V.Z) and Czech Min. of Education/MSMT proj. NPUI LO1417 (V.Z.).

T4-02-03

Male meiotic cytokinesis and pollen ploidy consistency is affected by cold shock and exogenous GA applications through independent signaling

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The genomes of higher plants show multiple incidences of whole genome duplications, which have been suggested to emerge from sexual reproduction involving unreduced gametes. Diploid pollen are for instance formed after a spell of cold temperatures in *Arabidopsis*, underlining the importance of environmental cues affecting ploidy consistency. Recently, we have shown that GA treatment of flowers induces defects in cytokinesis in a subpopulation of male meiocytes, leading to meiotic restitution and the production of diploid pollen grains. Plants depleted of RGA and GAI, two members of the DELLA family that function as suppressors of GA signaling show similar aberrations in meiocyte cytokinesis. As cold- and GA treatment induces similar aberrations in the spatial organization of the phragmoplast-like radial microtubule arrays in dividing meiocytes, we hypothesized that GA mediates the cold response. However, the GA biosynthesis blocker paclobutrazol does not prevent cold-induction of meiotic restitution and GA insensitive mutants are still responsive to cold. Because GAs a transcriptional fusion of *RGA-GFP* is predominantly expressed in the tissue surrounding the meiocytes under GA limiting conditions we hypothesize that meiocyte cytokinesis is controlled by non-cell autonomous GA signaling. This signaling may find its origin from the tapetum as mutants impaired in early tapetum development display cytokinesis defects similar to that observed upon cold and GA treatment.

T4-02-04

Coordination of actin assembly and turnover by ADF and CAP1 regulates the pollen tube growth of *Arabidopsis thaliana*

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Pollen tube transports the sperm cells from pistil into ovule to finish double fertilization. The rapid growth of pollen tubes (>200 μm/ h in *Arabidopsis*) relies largely on the myosin based vesicle transport mediate by actin filaments. To maintain a dedicate and highly dynamic actin filament structures, various actin binding proteins (ABPs) work in concert in actin assembly, branching, bundling, depolymerizing *etc.* In the hundreds of ABPs, the highly-expressed Actin depolymerizing factor (ADF) and Cyclase associated protein 1 (CAP1) have been reported to play vital roles in actin filament dynamics in various models species. We generated the *adf10* mutant by TALEN technology and carefully examined the changes of actin structures and its effects in the pollen tubes of *adf7*, *adf10* and *cap1* mutants. We found that: 1) Actin filament structures are disordered in pollen tubes of *adf7*, *adf10* and *cap1* mutants. Actin dynamics as well as the velicity of vesicle transport is also downregulated. 2) ADF10 plays a key role in shaping the actin fringe structure to facilitate vesicle transport both to the tip and the base of pollen tube. Disordered actin organization will lead to irregular vesicle movements. 3) Elucidation of the biochemical activity of ADFs and CAP1 *in vitro*. Both ADF7 and ADF10 coworks with CAP1 to promote actin filament turnover, in accordance with *in vivo* results in 1). 4) CAP1 promotes the nucleotide exchange of actin monomers to promote actin assembly, proved by the actin phenotypes in the tip of pollen tube and by CAP1 point mutants, both *in vitro* and *in vivo*. Thus, we provide solid evidence for the working mechanism of how ADFs and CAP1 promote actin turnover, regulate formation of specific actin structures and vesicle transport for the rapid growth of pollen tubes.

T4-02-05

DEEP-mediated modification of tRNAs is essential for endosperm-to-embryo communication in *Arabidopsis*

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Modification of transfer RNAs (tRNAs) is essential for protein synthesis in both prokaryotes and eukaryotes. However, how modified tRNAs affect the development in multi-cellular organisms remains elusive. Here, we report that atRNA modifying enzyme, Defective in Embryo and Endosperm Phenotypes (DEEP), plays an essential role in early embryo and endosperm development in *Arabidopsis*. *DEEP* was expressed both in embryo and endosperm. In the *deep-/-*mutant, embryo and endosperm development were arrested at very early stage. DEEP protein was localized in cytoplasm, mitochondrial and chloroplast and the localization of DEEP in cytoplasm is sufficient for its function in *Arabidopsis* seed development. Endosperm-specific expression of *DEEP* in the *deep*-/- mutant could completely rescue the developmental defects both in embryo and endosperm, while embryo-specific expression of DEEP in the *deep*-/- mutant could only largely rescue the defects in embryo but not those in the endosperm. Our data demonstrate that DEEP-mediated tRNA modification is essential for endosperm-to-embryo communication and thus for embryo/endosperm development in *Arabidopsis*.

T4-02-06

Kinase partner protein KPP controls pollen tube growth

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Pollen receptor kinases from tomato, LePRKs, have been implicated in signaling during pollen germination and tube growth. Kinase partner protein, KPP, is a guanine exchange factor (GEF) for RopGTPases and a downstream signaling component of LePRKs signaling. We found that *KPP* RNAi pollen tubes grew slower both *in vitro* and *in vivo*, and elongated with severe F-actin aggregation close to the germination pores. Overexpression full length of *KPP* promotes pollen tube growth both in tobacco and in tomato and over-expression of truncated *KPP* lacking C-terminal fused with fluorescent proteins triggered branching tips. In addition, we showed that KPP can form dimers during pollen tube growth, that the C-terminal region is necessary for KPP to form homodimers. KPP preferentially activates a pollen-expressed ROP, SlROP4, and the C-terminal region is also necessary for KPP to activate SlROP4. We used affinity column chromatography to identify proteins associated with KPP and thereby identified COPI subunits which co-localized with the ARP2/3 complex. Co-expression assays showed that KPP and SlROP4 recruited coatomer proteins and ARP2/3 to the apical plasma membrane during pollen tube growth, and GST pulldown assays showed that COPI subunits and the ARP2/3 complex associated with KPP and SlROP4. Over-expression of the SHD domain of SlSCAR or dominant negative SlARP2 (G151D) the phenotypes caused by over-expression KPPΔC or KPPΔN. These data suggest that KPP transduces signals from the LePRKs at plasma membrane, activates ROP, recruits COPI and ARP2/3 complex to the apical plasma membrane, and activates ARP2/3 complex to affect actin dynamics during pollen tube growth.

T4-03: Breaking the mold: a role for non-model species in plant development

T4-03-01

Non-model systems for understanding mechanisms of leaf morphogenesis

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Previously it was nearly impossible to study mechanisms of leaf morphogenesis other than in the model species, *Arabidopsis thaliana.* Even application of our knowledge on the *Arabidopsis* system to non-model species was also challenging. But thanks to the recent development of new technologies including Next-generation sequencing, the gap between the model and non-model is rapidly filled. As typically shown by a historical finding of the contribution of class I *KNOX* genes in compound leaf formation in tomato (Hareven *et al.,* 1996), comparative studies among different systems must be done to reveal the ‘real’ roles of particular genes on organogenesis. There is awide range of diversity in leaf shape and organogenesis in the world (Tsukaya 2014), and some species with unique morphologies would provide us with important clues to understand mechanisms of leaf organogenesis. Here I will introduce you to some of our present studies on leaf morphogenesis in non-model species. For example, some species of the genus *Callitriche* (Plantaginaceae) are amphibious and exhibit dimorphism in leaf shape according to external environmental cues (mainly water level). We are trying to know what genetic mechanisms are involved in the switching between the dimorphism from RNAseq analyses; how such switching systems were acquired from comparative studies between amphibious and terrestrial species of the above genus. We are also studying indeterminacy of leaves in a one-leaf plant, *Monophyllaea glabra* (Gesneriaceae) by analyzing expression of several key genes for activities of leaf primordia and shoot apical meristem. Comparison between our present data and past reports on another one-leaf plant genus, *Streptocarpus,* suggested that the genetic background for the indeterminacy of leaves differs between these two genera. On the other hand, we are also interested in mechanisms of domatia formation in leaves of an ant plant, *Callicarpa saccata* (Lamiacae). The domatia are used by symbiotic ants as nests anddomatia formation in this genus is known only in this species. Anatomy indicates that the domatia formation is caused by ectopic activation of cell proliferation at the leaf meristem. We are currently trying to understand the genetic factors involved in it. We are also analyzing flattening mechanisms in unifacial leaves by using *Juncus prismatocarpus* (Juncaceae). Our previous work revealed that the *DL* gene is one of the key factors that make non-polarized terete leaves into a flat shape (Yamaguchi *et al.,* 2010), but our detailed analysis also showed that *DL* action is not cell-or tissue- autonomous. Through our trials, I wish to indicate the fruitful potential of Evo-devo studies on leaf morphogenesis in this era.

T4-03-02

Virus-Induced Gene Silencing, the second best tool for gene function analysis in non-model plants

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Plants are amazingly diverse in their metabolism, vegetative and reproductive morphology which allows them to adapt to ecological niches. Understanding the molecular mechanism orchestrating this biochemical and developmental diversity is one of the major research foci in plant biology. The current model plants examined on the molecular level are, like *Arabidopsis thaliana* (thale cress) or *Oryza sativa* (rice) from highly derived plant lineages, which impedes a direct transfer of knowledge to other species in most cases. With genome sequences available for many species and RNAseq of specific tissues and organs becoming affordable for even smaller labs, the availability of sequencing data is not rate limiting anymore. New model species can be now selected based on e.g. interesting phylogenetic position, important morphological or metabolic traits, and with these new model species at hand, one can study e. g. ecologically relevant traits (such as the origin of zygomorphy) in closely related taxa. However, the limiting step now is the amenability of the new model species to genetic manipulation. Protocols for stable transformation of many species are, if available at all, very labor and time consuming. A feasible alternative is Virus-Induced-Gene Silencing (VIGS), a method that utilizes the plant’s reaction towards double stranded RNAs to inhibit viral gene transcription. Many plant viral vector systems have been adopted to serve as VIGS vectors and VIGS-based functional gene analysis in a large number of plant species can be achieved by now. Unfortunately, the VIGS method is transient and lacks standardization. I will provide a brief overview over this technique and its applications and outline the pitfalls. Further, I will present ideas on how this very useful method can me employed in a meaningful way to provide reliable data on gene functions and genetic interactions in new model organisms.

T4-03-03

Exploring floral senescence without a model – The rich rewards of diversity

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Floral senescence does not follow the same pattern in all flowering species. Flowers can be broadly divided between those in which petals abscise while still turgid and those in which petals wilt and may or may not abscise. Furthermore, flower senescence can also be divided into a further two groups. In one group of species ethylene is produced by the flowers as the senescence progresses, and acts as a major regulator of this process. In another group ethylene is not produced, or only at very low levels and does not act as a major senescence regulator. Broadly most species that show rapid abscission without wilting are ethylene sensitive whereas those that wilt are generally ethylene insensitive, although the demarcations are not absolute. Many monocotyledonous flowers including lilies, as well as most Asteraceae are ethylene insensitive, while ethylene sensitive species include orchids, carnations petunia and Brassicaceae including *Arabidopsis*. Given these major differences it has been difficult to find a suitable model to study floral senescence. However new tools such as transcriptomic analyses are offering new approaches to investigate common and divergent regulatory mechanisms. In the obvious model, *Arabidopsis*, senescence proceeds rapidly and within 48h petals are abscised with only moderate wilting. Some progress has been made in identifying genes regulating ethylene-regulated senescence in *Arabidopsis* flowers, and some of these genes may also regulate senescence progression in other ethylene sensitive species. Abscission signals also appear to be shared across different floral senescence groups. A comparison of two lily genotypes that have contrasting senescence programmes identified genes related to abscission previously identified in *Arabidopsis*. However the regulation of senescence in ethylene-insensitive species remains problematic. Recent transcriptomic studies in lily and other species reveal families of transcription factors that may be involved. A further complexity in floral senescence is offered by composite flowers. A transcriptomic analysis of Dahlias is comparing senescence between florets and the whole inflorescence.

T4-03-04

Lace Plant: A novel model to study programmed cell death in plants

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The lace plant, *Aponogeton madagascariensis* (Aponogetonaceae) and a few genera in the Araceae develop a complex leaf shape with distinct holes, or perforations, formed through the process of programmed cell death (PCD). Although *Monstera deliciosa* and *Monstera obliqua* (Araceae) form holes via PCD during leaf morphogenesis, they are much less tractable than the lace plant for use as a model to study PCD. We demonstrated the outstanding potential of the lace plant as a system in which to study developmentally-regulated PCD. In the lace plant, perforations form at highly predictable locations and specific developmental stages that are accessible for observation and experimental manipulations. PCD begins at the center of each areole in window stage leaves, develops outwards, and stops approximately 4-5 cell layers from the veins. These peripheral cells do not undergo PCD and are therefore referred to as NPCD (non-PCD) cells. The presence of visible changes within an areole provides a convenient gradient of cell death for analysis. The lace plant is an extremely useful system to study developmental PCD for several reasons: the accessibility and predictability of perforations; the existence of an established protocol for propagation of lace plant via sterile cultures; the tractability of the PCD process with live cell imaging *in vivo*; the ability to separate PCD and NPCD cells using laser capture micro-dissection; the ability to grow detached leaves in Petri Plates; the feasibility of performing pharmacological experiments; the possibility to regenerate lace plant via callus; the potential for genetic transformation, and the existence of optimized standard protocols for plant molecular biology research. The challenges in transforming the non-model lace plant species to a potential model species in the study of programmed cell death will be discussed.

T4-03-05

Juncus prismatocarpus (Juncaceae) as an emerging model to study unifacial leaf development

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In recent years we have learned much about leaf development in bifacial leaves, particularly from model plants. However, although evolved repeatedly in the monocot, we know very little about leaf development in unifacial leaves, which lack adaxial side in their leaf blade. One particular reason is that in the past an efficient method to analyze the direction of cell division and thus leaf morphogenesis in non-model plants does not exist. To address this problem, we have first developed a method to efficiently and rapidly quantify the direction of cell division (Yin and Tsukaya, 2016). This method is applicable to all plant species and therefore it is a perfect tool to study leaf morphogenesis in non-model plants. Next we have applied this method to *Juncus prismatocarpus* (Juncaceae), a unifacial plant, to analyze its cell division direction in details. We have found that cell divisions contribute to the thickening growth is most active in the P2 stage. We have also found that cell divisions contribute to the thickening growth is present not only in the putative “adaxial meristem” domain but also in other domains. These data does not support the classical “adaxial meristem” hypothesis (Kaplan, 1975) which asserts that such thickening growth is restricted to the “adaxial meristem”. To better understand the underlying mechanism of unifacial leaf mechanism, we have also compared cell division pattern to various key genes expression patterns. Previously we have shown that in *J. prismatocarpus*, *DROOPING LEAF* (*DL*) is responsible for the leaf blade development towards the shoot apical meristem (SAM) in the P2 stage; *PRESSED FLOWERb* (*PRSb*) is responsible for the leaf blade secondary margin development in the P3 stage; and *PRSa* is responsible for the leaf sheath development (Yamaguchi *et al.,* 2010). The comparison of cell division pattern and genes expression patterns indicated that *DL* acts in a non-cell-autonomous manner. In addition, the frequency of cell division in the presumed *PRSa* expression area is higher than that in the presumed *PRSb* area. Our newly developed method is an invaluable tool to study leaf morphogenesis in non-model plants. Using this method and other techniques, we are establishing *J. prismatocarpus* as an emerging model-plant to study unifacial leaf development. We will discuss our latest progress on this topic.

T4-03-06

Enhancing the lace plant model system through genetic transformation

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The lace plant (*Aponogeton madagascariensis*) is an aquatic monocot with a unique perforated leaf morphology. The distinct lattice-like pattern of the leaves is created by programmed cell death (PCD), which occurs at a specific point in leaf development known as the window stage. The high degree of predictability of pattern formation, nearly transparent leaves that are ideal for live cell imaging, and the existence of established sterile cultures are the primary features that have led to the emergence of the lace plant as a model for studying developmentally regulated PCD.

Protocol development has been a key focus in the enhancement of the lace plant model system over recent years. This study is the first to report on genetic transformation of the lace plant, also a first within the taxonomic family. Two *Agrobacterium tumefaciens* strains, GV2260 and AGL1 were used in combination with two different plasmids (pJP and pJLU13) containing reporter genes for rapid determination of transformation efficiency. The PJP and pJLU13 plasmids confer constitutive green fluorescent protein (GFP) expression driven by *CaMV 35S* and a rice *ubiquitin* (*rubi3*) promoter, respectively. The plasmids and bacteria were tested on various explants including: inflorescences, leaves, leaf sheaths, embryos, seedlings, calli, and isolated shoot apical meristems (SAMs). Results show that stable transformation has been most successful using callus derived from lace plant corm tissues and isolated SAMs. GFP detection through fluorescence microscopy and PCR analysis indicates that the lace plant is amenable to *A. tumefaciens*-mediated transformation. Current work aims to improve the efficiency of the transformation process and achieve the regeneration of whole plant transformants from SAMs and calli.

T4-04: Comparative genomics perspectives on crassulacean acid metabolism (CAM)

T4-04-01

Origin and mechanism of crassulacean acid metabolism in orchids

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Crassulacean acid metabolism (CAM) is a CO_2 fixation pathway that maximizes water-use efficiency (WUE), compared with the C_3/C_4 CO_2 pathway, which permits CAM plants to adapt to arid environments. The CAM pathway provides excellent opportunities to genetically design plants, especially bioenergy crops, with a high WUE and better photosynthetic performance than C_3/C_4 in arid environments. The information available on the origin and evolution of CAM is scant, however. Here, we analyzed transcriptomes from 13 orchid species and two existing orchid genomes, covering CAM and C_3 plants, with an emphasis on comparing 13 gene families involved in the complete carbon fixation pathway. The dosage of the core photosynthesis-related genes plays no substantial role in the evolution of CAM in orchids; however, CAM may have evolved primarily by changes at the transcription level of key carbon fixation pathway genes. We proposed that in both dark and light, CO_2 is primarily fixed and then released through two metabolic pathways via known genes, such as *PPC1*, *PPDK* and *PPCK*. This study reports a comprehensive comparison of carbon fixation pathway genes across different photosynthetic plants, and reveals the importance of the level of expression of key genes in the origin and evolution of CAM.

T4-04-02

Molecular signatures of convergent evolution in crassulacean acid metabolism (CAM) plants

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Flowering plants of diverse lineages have independently acquired crassulacean acid metabolism (CAM) as photosynthetic adaptations to dry environments. CAM plants have much higher water-use efficiency (WUE) than C_3 or C_4 plants due to an inverted day/night pattern of stomatal closure/opening in CAM species relative to C_3 and C_4 species. Convergence of gene function may play an important role in the evolution of isomorphic phenotypes in phylogenetically independent lineages. We hypothesized that convergent genomic evolution underpins the multiple and independent emergences of CAM from C_3 photosynthesis. Our genome-wide comparative analyses revealed the genomic signatures of convergence shared between eudicot (represented by *Kalanchoë*) and monocot (represented by pineapple and orchid) CAM species. Specifically, approximately 60 genes were found to have undergone convergent evolution in the divergent eudicot and monocot lineages, providing strong evidence that molecular convergence underpins CAM evolution. Engineering of CAM pathway into C_3 crops using synthetic biology approach would have great potential for genetic improvement of WUE and drought tolerance for sustainable production of food and bioenergy in arid, semi-arid and marginal lands. The results obtained from this research provide new insights into the molecular basis of CAM evolution, and will facilitate CAM-into-C_3 engineering to enhance drought tolerance in crops.

T4-04-03

Elucidation of evolutionary transitions between C_3 photosynthesis and CAM in Yuccas, Agaves and related species

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The subfamily Agavoideae in the Asparagaceae has three independent origins of Crassulacean acid metabolism (CAM), a photosynthetic modification that allows plants to thrive in water-stressed environments. While multiple origins of CAM have been documented across the flowering plant phylogeny, the mechanism by which C_3 plants transition to CAM is not well understood. We used comparative transcriptomics to describe gene expression changes associated with two independent shifts from C_3 to CAM within the Agavoideae: one within the genus *Yucca* and one on the ancestral lineage leading to *Agave*. *Yucca* has two main subgenera which diverged roughly 5 Mya from a C_3 ancestor – CAM evolved in one of these lineages encompassing approximately half of extant *Yucca* species. CAM evolved independently in the ancestor of *Agave sensu lato* after divergence from the C_3 *Beschorneria* lineages. However, low-level CAM activity was found in *Polianthes*, which is deeply nested within the *Agave sensu lato* clade, implying a possible reversion from CAM to C_3 photosynthesis. Genes with time-structured expression profiles, as well as canonical CAM genes, were compared within lineages and between C_3 species and independently derived CAM lineages. *Beschorneria* and C_3 *Yucca* exhibit time-structured expression of CAM genes and some of their regulators, perhaps indicating a genomic predisposition in the Agavoideae to evolve CAM. At the same time, analysis of gene interaction networks inferred from expression correlations suggest transitions to CAM in *Agave sensu lato* and *Yucca* involve a common theme of expanded regulatory functions for clock-related transcription factors. These findings highlight the importance of phylogenetically-informed comparative analysis of closely related CAM and C_3 species.

T4-04-04

Engineering crassulacean acid metabolism (CAM) and tissue succulence to improve water-use efficiency of bioenergy feedstocks

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Global warming trends are leading to increased terrestrial soil drying, reduced terrestrial net primary production and carbon sinks, global food security and future biofuel production, and the global expansion of drylands that already cover 42% of the earth's surface. In order to offset these negative effects, an increased reliance upon crassulacean acid metabolism (CAM) crops or the introduction of CAM, a water-wise form of photosynthesis, into C_3 food and bioenergy crops might serve as a useful strategy to improve the water-use efficiency (WUE) of sustainable biomass production systems in the future. CAM features inverse stomatal behavior, in which stomata are open at night for CO_2 uptake when evapotranspiration rates are reduced compared with the daytime and closed during all or part of the day, thereby maximizing WUE. CAM also exploits a temporal separation of nocturnal CO_2 uptake and fixation by phosphoenolpyruvate carboxylase (PEPC), which leads to the formation of C_4 organic acids that are stored in the vacuole. The subsequent decarboxylation of these organic acids during the day releases CO_2 and concentrates it around ribulose-1-5-bisphosphate carboxylase/oxygenase (RUBISCO), suppressing photorespiration, while resulting in carbohydrate production via the C_3 Calvin–Benson cycle. Detailed comparative, functional, and integrative 'omics analyses of several CAM model or crop species have recently defined the basic genetic requirements for CAM. Such information has led to the development of new synthetic biosystem design approaches to modify and transfer photosynthetic modes into different species (http://cambiodesign.org). Facile gene stacking strategies for the assembly of a large number of transcription units (TUs) with appropriate circadian and drought-inducible expression patterns are necessary for the genetic reconstitution of facultative CAM into host C_3 species. We have created a plant-specific position/adaptor/carrier vector system originally designed for engineering mammalian cells that enables the rapid, reliable, and scalable creation of complex gene circuits using the Gibson isothermal assembly process. Design and construction of CAM-specific carboxylation and decarboxylation gene circuits containing 9 and 15 genes has been completed and are in the process of being introduced into Arabidopsis and Poplar. The gene circuits were designed to include mesophyll-specific, drought-inducible, and appropriately timed circadian expression patterns of the transgenes in order to engage the CAM pathway only during water-deficit stress conditions. Lastly, tissue succulence has been successfully engineering in the C_3 photosynthesis model species A. thaliana in order to increased mesophyll cell size for increased malate storage capacity and reduced intercellular air space to limit internal CO_2 diffusion out of the leaf during the day to favor refixation by ribulose 1,5-bisphosphate carboxylase oxygenase (RUBISCO) and thereby increases the capacity to perform CAM. Added benefits of engineered tissue succulence included increased biomass production, increased WUE, and tolerance to water-deficit (drought) and salinity stress. The combination of engineered CAM and tissue succulence is expected to increase the WUE of bioenergy feedstocks and potentially expand their production into more marginal, abandoned, or semi-arid regions. This material is based upon work supported by the U.S. Department of Energy, Office of Science, Genomic Science Program under Award Number DE-SC0008834.

T4-04-05

Evolution and regulation of crassulacean acid metabolism in the genus *Kalanchoë*

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Forward engineering of crassulacean acid metabolism (CAM) into non-CAM crop species in order to enhance their water use efficiency requires a comprehensive knowledge of the minimal 'parts-list' for CAM. Here we describe progress with functional genomics research that aims to define and characterize the complete CAM genetic blueprint from model species in the genus *Kalanchoë* (1). A draft assembly of the 246 Mbp *K. fedtschenkoi* genome has recently been further improved through the addition of 100X coverage of PacBio long reads. Quantitative RNA-seq analysis of light/dark time course samples from C_3 and CAM leaves identified candidate CAM-associated genes. Many of these CAM-induced genes also undergo robust oscillations in transcript abundance over the light/dark cycle, consistent with a role in the circadian optimization of the pathway. The RNA-seq data has guided the reconstruction of a comprehensive model of CAM wherein candidate CAM-recruited genes are allocated to each step in the pathway. This in turn has allowed targeted RNAi gene silencing and over-expression approaches to be applied to each candidate CAM gene through the generation of stable transgenic lines of *K. fedtschenkoi* and *K. laxiflora*. Detailed analysis of CAM-associated phenotypes in the transgenic lines is revealing which genes are essential for efficient CAM, and which genes are dispensable (2). Data will be presented for RNAi lines of *K. laxiflora* lacking key CAM genes. Several lines fail to fix atmospheric CO_2 in the dark period, and the phenotypic consequences of this will be described. In addition, there is a wide diversity in the level of the CAM phenotype in the genus *Kalanchoë*, from C_3 with inducible, weak CAM in the most basal/ancestral species in section Kitchingia, through to strong constitutive CAM in the most derived species in section *Kalanchoë*. Section Bryophyllum, which includes *K. fedtschenkoi* and *K. laxiflora*, resides in the centre of the genus and these species possess intermediate levels of CAM. The genus thus represents an excellent system for comparative analysis of CAM gene evolution. Data will be presented on the CAM physiology of a range of species sampled from across the Liverpool *Kalanchoë* diversity set that we have built up for the genus. Furthermore, preliminary insights into *CAM* gene evolution across this diverse genus will be presented based on analysis of a new *Kalanchoë* genome that we have generated for a basal species. *This material is based upon work supported by the U.S. Department of Energy, Office of Science, Genomic Science Program under Award Number DE-SC0008834.*

T4-04-06

Comparative genomics of CAM photosynthesis

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The altered carbon assimilation pathway of crassulacean acid metabolism (CAM) results in up to six times greater water-use efficiency than C_3 photosynthesis, making it an excellent pathway for engineering crop plants with improved drought tolerance. Central to the success of this approach is a deep understanding of the underlying genetic mechanisms controlling CAM photosynthesis. We surveyed detailed temporal and spatial transcriptomic and metabolomic changes in the obligate CAM plant pineapple (*Ananas comosus*) and facultative CAM plant *Sedum album*. Pineapple has a normal number of CAM pathway genes compared to grasses and other monocots, but CAM isoforms are enriched with cis-regulatory elements associated with regulation by circadian clock genes. This suggests CAM in pineapple evolved through the regulatory neofunctionalization of preexisting genes and not through the acquisition of neofunctionalized genes via whole-genome or tandem gene duplication. *Sedum album* shows strong CAM induction under progressive drought stress with strong co-expression of drought and CAM pathways genes. Sedum uses the same orthologs of CAM pathway genes as the model CAM plant Kalenchoe supporting parallel evolution. Together these resources provide targets for functional validation and increase our understanding of this complex trait.

T4-05: Revealing the secrets of angiosperm resurrection plants

T4-05-01

Towards understanding mechanisms associated with desiccation tolerance in Angiosperms

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The seeds of 90% of Angiosperms are desiccation tolerant (DT), whereas only 0.01% of such species (termed resurrection plants) display vegetative DT. It has been proposed that while vegetative DT first arose with the transition from aquatic to terrestrial life forms, it was lost as plants acquired mechanisms for water deficit (drought) resistance and evolved DT seeds. We propose that resurrection plants subsequently reactivated aspects of mechanisms associated with seed DT in vegetative tissues, through mechanisms not yet completely understood. Using a systems biology approach in which molecular (including all the omics), biochemical, biophysical and physiological studies were utilized, this presentation will provide evidence for similarities and differences in mechanisms associated with DT in seeds and resurrection plants.

T4-05-02

Arabinose-rich polymers as an evolutionary strategy to plasticize resurrection plant cell walls against desiccation: Understanding the woody angiosperm *Myrothamnus flabellifolia*

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A variety of desiccation-tolerant resurrection plants from Southern Africa were surveyed using high-throughput cell wall profiling tools (Moore *et al.,* 2009). Hydrated and desiccated leaf (frond) material, were analysed using (Moore *et al.,* 2013). Arabinan-rich pectin and an abundance of arabinogalactan proteins were found associated with the resurrection fern *Mohria caffrorum* and the woody angiosperm *Myrothamnus flabellifolia.* In contrast, *Craterostigma plantagineum* up-regulated wall proteins and osmoprotectants (Moore *et al.,* 2013). The hemicellulose-rich walls of the grass-like *Xerophyta* spp. and the resurrection grass *Eragrostis nindensis* possess highly arabinosylated xylans and arabinogalactan proteins for protection (Moore *et al.,* 2013). Of these species, only the resurrection bush *Myrothamnus flabellifolia* (Moore *et al.,* 2007) is a true woody perennial, displaying the remarkable capacity to be able survive extensive periods (i.e. years) in the desiccated (quiescent) state. Recent research has highlighted a number of anatomical (xylem modification), physiological (photosynthetic adjustment), chemical (antioxidant load), biochemical (cell wall flexibility and membrane stabilizers) and molecular (sugar transport) properties which facilitate survival of this plant under adverse environmental conditions (Moore *et al.,* 2007). This presentation will review the current hypotheses and data that support a general evolutionary mechanism of 'plasticising' the cell walls of resurrection plants (Moore *et al.,* 2007) to desiccation and implicate arabinose-rich polymers (pectin-arabinans, arabinogalactan-proteins and arabino-xylans) (Moore *et al.,* 2013) as the major contributors in ensuring flexibility is maintained and rehydration is facilitated in these remarkable plants.

T4-05-03

Adaptation mechanisms of the resurrection plant *Boea hygrometrica* to dry environment

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Vegetative tissues of *Boea hygrometrica*, a member of the Gesneriaceae family, can tolerate severe water loss to desiccated state and fully recover upon rehydration, a capacity termed as desiccation tolerance (DT). Studies have been conducted to identify the physiological, cellular, and molecular mechanisms underlying DT in the last two decades. Both specific responses to water stress, such as cell wall folding and pigment-protein complex stabilizing and common response such as the accumulation of osmoprotectants and antioxidants have been revealed in this plant during dehydration. The desiccation-triggered "dormancy"-like state could benefit the plant to adapt to multiple environmental stresses, such as cold and alkaline. This explains the fact that *B.hygrometrica* is the only species in Gesneriaceae family that distributes widely from the tropics to northern temperate regions in East Asia and grows vigorously in thin, calcareous and alkaline soil on limestone rocks, where dehydration occurs frequently, rapidly, and profoundly. Besides, *B. hygrometrica* is one of the few resurrection angiosperms have been reported to survive rapid dehydration. Such ability is primed by a period of acclimation of slow soil-drying in *B. hygrometrica*. Although phenotypically indistinguishable, principal component analysis revealed that the gene expression profiles in hydrated, acclimated plants resemble those of desiccated plants more closely than those of hydrated, non-acclimated plants. Anti-oxidation, protein quality control, transposons and epigenetic modifications may contribute to the priming and acquisition of DT in *B. hygrometrica*. These studies implied the existence of drought stress-induced transcriptional memory in *B. hygrometrica* that facilitates plant adaptation to the extreme habitat.

T4-05-04

Surviving metabolic arrest: What can we learn from resurrection plants?

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Resurrection plants can tolerate severe water loss, and mostly adjust their water content with the relative humidity in the environment. The plants grow in ecological niches with seasonal rainfall, they can remain in the desiccated state, comparable to dormancy in dry seeds. When rainfall occurs, the plants take advantage of the conditions, resurrect, grow and produce seeds before other species growing from seeds can do so. The objective of our research is to understand why resurrection plants survive desiccation. Our studies are focused on the desiccation tolerant resurrection plant *Craterostigma plantagineum* and the desiccation tolerant grass *Oropetium thomaeum*. Desiccation tolerance is a complex trait which requires the synthesis of proteins and sugars. To identify essential components of desiccation tolerance close relatives of *C. plantagineum* are being studied on the molecular and cellular level. Some of these species are desiccation tolerant and others are not. This comparative approach demonstrates that expression levels of genes encoding protective molecules are differentially regulated in desiccation tolerant and non-tolerant plants. This differential expression is linked to promoter architecture and the presence of specific regulatory *cis*-elements. The comparative studies are complemented by large scale analyses of gene expression patterns that have been performed to characterize the transcriptomes at different physiological conditions. These studies showed that increased expression of genes encoding protective components, species-specific genes as well as non-protein-coding RNAs are essential components of desiccation tolerance. Desiccation tolerance in resurrection plants and seeds have many metabolic pathways and molecular components in common. However, some components are specific to resurrection plants.

T4-05-05

Identification and characterisation of microRNAs in the resurrection grass *Tripogon loliiformis*

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MicroRNAs (miRNAs), a class of endogenous 20-22 nt non-coding RNAs that play critical regulatory roles in plant growth, development and in response to environmental stress have been characterised in many drought sensitive and tolerant plant species. Resurrection plants, a group desiccation tolerant plants can withstand extreme water deficit with the ability to recover on rehydration due to their ability to effectively regulate stress response. It is postulated that post-transcriptional regulatory mechanisms could be involved in desiccation tolerance. *Tripogon loliiformis*, a native Australian resurrection grass that recovers from desiccated state in 72 hrs was used to investigate this phenomenon. High-throughput sequencing of *T. loliiformis* small RNAs libraries under water deficit conditions identified 668 conserved and 119 novel miRNAs. Differential expression showed enhanced miRNA accumulation in the shoots compared to the roots. Interestingly, miRNAs were recorded even in desiccated state suggesting the existence of enhanced regulatory activities as the plant switched towards desiccation with possible repackaging and storage of miRNAs. Predicted targets included transcription factors, kinases, chaperones, carbohydrate metabolism and programmed cell death (PCD) associated genes. The targets were enriched in biological process associated with stress responses, regulation of development, gene expression, PCD and post-transcriptional gene silencing. Identification and characterisation of *T. loliiformis* miRNAs not only points to their evolutionary conservation but also their indispensable regulatory role during desiccation stress. Understanding of the underlying mechanisms is paramount in devising strategies for the development of genetically improved crop plants with enhanced drought tolerance and water use efficiency.

T4-05-06

Mining the resurrection genome of *Xerophyta viscosa* for a footprint of desiccation tolerance

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Desiccation tolerance (DT) is common in seeds and various other organisms but only a few Angiosperm species possess vegetative DT. These resurrection species may serve as ideal models for the ultimate design of crops with enhanced drought tolerance. Combining genome sequence information with genome-wide gene expression data may provide insight into the 'footprint' of vegetative DT. We produced a high-quality whole-genome sequence for the grass-like resurrection plant *Xerophyta viscosa* and assessed transcriptome changes during its de- and rehydration. We found 126 putative LEA motif-containing proteins divided into eight families, of which 90 were differentially expressed during de- and rehydration of adult plants. The number of LEAs is significantly higher than identified in the genomes of the homoiochlorophyllous resurrection species *Boeia hygrometrica* (67 LEA genes) and *Oropetium thomaeum* (94 LEA genes) as well as in a phylogenetic cross-section of 25 plant species. These differences might relate to the poikilochlorophyllous nature of *X. viscosa* as well as to the habitat in which it occurs. Data revealed induction of transcripts typically associated with DT in seeds, and involvement of orthologues of ABI3 and ABI5, both key regulators of seed maturation. The bZIP transcription factor ABI5 is strongly associated with the acquisition of seed bank longevity in orthodox seeds, as well as with several LEAs. Here we show that ABI5 may be a main regulator of expression of the LEA_4 family, which, together with the LEA-6 family, is significantly expanded in the genome of *X. viscosa*. Thus, this may be an important factor in the extreme longevity of X. *viscosa* in the dry state. Furthermore, we identified two structural orthologs of ABI3, a major regulator of seed maturation and DT along with the majority of the ABI3 regulon expressed in leaves. Again, this exemplifies the strong 'seed character' of vegetative DT in this species. Dehydration resulted in the down regulation of genes located in genomic 'clusters of desiccation associated genes' (CoDAGs), reflecting the cessation of growth that allows for the expression of DT. They may represent genes related to mechanisms that suppress growth and development or energy metabolism in vegetative tissues and may trigger or inhibit the induction of the mechanisms that lead to vegetative DT. The genes in these CoDAGs may also supress the expression of DT in vegetative tissues, allowing the induction of DT only at critical levels of water loss. Their down regulation may thus be part of the reactivated mechanism that evolved to deliver DT in vegetative tissues. We provide strong (molecular) support for the hypothesis that vegetative desiccation tolerance arose by redirection of genetic information from desiccation tolerant seeds.

T4-06: Calcium transport and signaling

T4-06-01

Plant osmosensor OSCA1 calcium channel family

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The increases in cytosolic free Ca^{2+} ($[Ca^{2+}]$) induced by osmotic stress have been speculated to act in the perception of the stress. However, the molecular components remain poorly understood. Using Ca^{2+} imaging-based forward genetic screens we have isolated *Arabidopsis* mutants defective in osmotic stress-induced $[Ca^{2+}]$ increases (osca). The *osca1* mutant displays impaired osmotic Ca^{2+} signaling and attenuated responses to osmotic stress at the cellular and whole plant levels. OSCA1 is a plasma membrane Ca^{2+} channel gated by osmotic stress. OSCA1 belongs to a conserved yet previously uncharacterized gene family in eukaryotes. These genes represent the long-sought components of stimulus responsive $[Ca^{2+}]$ increases, and could provide potential molecular targets for engineering drought resistant crops.

T4-06-02

Oxygen sensing in plant roots linked with ion channels

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Over 17 million km^2 of land is affected by flooding, resulting in substantial yield losses and jeopardising food security across the globe. The crucial role of Ca^{2+} and K^+ in response to abiotic and biotic stimuli has been widely recognized in plants but still poorly understood within specific cell types in different root zones under hypoxia stress. As dependence and reliance of mammalian system on O_2 is much stronger than in their plant counterparts, acute responses to hypoxia must operate within a timeframe of seconds. O_2-regulated ion channels fit this description very well. To further understand the mechanisms by which plants sense low-oxygen stress, we first summarise and identify several known candidates for oxygen sensing in the mammalian literature. We then identify key oxygen sensing domains (PAS; GCS; GAF; PHD) in mammalian systems and use the sequences of those oxygen sensing domains to identify the potential plant counterparts in *Arabidopsis*. Several plasma- and tonoplast based ion channels (such as TPC; AKT; KCO) and oxygen domain-containing proteins with predicted oxygen sensing ability were identified in plants. Whole-plant physiological and tissue-specific Ca^{2+} and K^+ changes were studied using knock-out *Arabidopsis* mutants of Ca^{2+}-ATPase (ACA), Ca^{2+}/proton exchanger (CAX), depolarization-activated guard cell outward-rectifying potassium (GORK) channels and respiratory burst oxidase homologue D (RBOHD) in waterlogging treatment. In the wild-type (WT) plants, the expressions of *ACA8*, *CAX4*, *CAX11*, *GORK* and *RBOHD* were down-regulated by up to 3-fold by hypoxia treatment. Ca^{2+} accumulation in root tissues was much higher in stelar cells in the mature zone of *aca8, aca11, cax4* and *cax11* mutants. In addition, we also show that CAX11 plays a key role in maintaining cytosolic Ca^{2+} homeostasis and/or signalling in root cells under hypoxic conditions. Phenotyping experiments found that *gork1-1* mutant has better K^+ retention ability in both the elongation and mature zone compared with the WT and *rbohD* under hypoxia. Besides, hypoxia induced Ca^{2+} increase in each cell type was much less pronounced in *rbohD* than in the WT. In most tissues except the elongation zone in *rbohD*, the H_2O_2 concentration had decreased after 1 h of hypoxia, but then increased significantly after 24 h of hypoxia in each zone and tissue, further suggesting that RBOHD may shape hypoxia-specific Ca^{2+} signatures via the modulation of apoplastic H_2O_2 production. In summary, Ca^{2+} efflux systems especially CAX11, RBOHD and GORK channel play critical roles in plant adaptive to hypoxia stress by shaping the stress-specific Ca^{2+} and H_2O_2 'signatures' and K^+ homeostasis.

T4-06-03

Nuclear calcium signalling in symbioses and beyond

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Nuclear calcium signalling is known to be essential in legumes to promote associations with nitrogen fixing bacteria and phosphate delivering arbuscular mycorrhizal fungi. Legumes are among the world's most important agricultural food crops that are beneficial to billions of farmers and consumers worldwide and provide an essential aspect of natural soil enrichment of organic nitrogen compounds. The mechanisms of plant nuclear calcium signalling were poorly understood. Using a wide range of approaches, we have discovered a number of ion channels located at the nuclear envelope that are responsible for symbiotic nuclear calcium release. Among them, we defined the first plant nuclear-associated calcium channels encoded by cyclic nucleotide gated channels (CNGC15s). The CNCG15s sit at the nuclear envelope in a complex with a potassium permeable channel (DMI1), also required for the generation of the symbiotic nuclear calcium signals. Interestingly, CNGC15s and DMI1 are conserved across all land plants, including non-symbiotic species, strongly suggesting that they have other functions during plant development. The function of this nuclear calcium channel complex in and outside root legume symbioses will be discussed.

T4-06-04

Coordination of the sorting and activity of glutamate receptor-Like channels (GLRs) by cornichon-like proteins

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Two decades after the discovery of Glutamate receptor-like channels (GLRs) in the genome of *Arabidopsis*, their molecular properties, localization, physiological relevance and regulation are still under debate. Of relevance, the evolution and apparent explosion of their number and diversity in angiosperms lacks an appropriate rational framework. While this diversification is suggestive of functional specialization, the complexity inherent to redundancy and compensation mechanisms associated with Ca^{2+} signaling have been hindering any generalizing functional model for their physiological functions in plants. Here, we report on a number of approaches to address this enigma, using *Arabidopsis* pollen grains and tubes, and root protoplasts as model cell systems, and a combination of genetic, electro-physiological and imaging methods. Single or multiple mutations in GLRs (up to quadruple mutants) resulted in reduced Ca^{2+} fluxes across the plasma membrane in both pollen and root protoplasts. To our surprise, this was not reflected by the subcellular localization of the channels, as we localized two representative pollen-expressed GLRs at the membrane of the sperm and vegetative vacuole, respectively. With the aim of detecting GLR-interacting proteins mediating the differential targeting of the channels to their target membrane, we identified a family of chaperone-like proteins homologous to the *Drosophila* CORNICHON family. Interestingly, *cornichon* pollen and root protoplasts revealed similarly low Ca^{2+} fluxes as *glr* mutants. Moreover, expression in *cornichon* plants resulted in the mislocalization of GLRs. To understand the specificity of this targeting we localized several integral membrane proteins in *cornichon* single or higher order mutant backgrounds. We observed that most, but not all, had their localization changed in the mutants, while soluble or membrane-attached proteins were unaffected. Remarkably, the heterologous co-expression of GLRs and cornichons lead to increased Ca^{2+} fluxes, suggesting a regulatory effect of the cornichons. We therefore posit that, beyond their implication in the sorting of various protein families, cornichon-like proteins are necessary to regulate the trafficking and activity of GLRs.

T4-06-05

Circadian regulation of calcium signals in *Arabidopsis thaliana*

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The circadian clock is a 24 h time keeper that is present in all multicellular organisms. The structure of these circadian clocks differ between the Kingdoms but broadly they have similar features resulting in persistent rhythms of cellular activity in constant conditions with a period close to 24 h, regulation of a swathe of gene expression, physiology and development and entrainment by light and dark cycles so that these circadian oscillations of activity occur in concert with the light and temperature cycles of the rotating Earth. In *Arabidopsis* the circadian oscillator comprises a translational transcriptional feedback loop but in addition there is circadian regulation of cytosolic calcium signals that peak towards the end of the photoperiod. We have been dissecting the physiological and genetic basis of the regulation of these calcium signals. We have new data suggesting that the circadian regulation of calcium occurs through a coincidence between light and the circadian clock. Additionally we have performed a mutant screen identifying plants with altered responses to nicotinamide, an antagonist of circadian calcium signalling. We have identified genes that are required for the correct regulation of calcium signals. A physiological role for calcium signalling the dynamic adjustment of circadian period is suggested by our phenotypic analysis of nicotinamide over sensitive mutants. Using a new approach for the identification of systems changes based on dynamical modelling of biological networks in different experimental conditions, we have identified the physiological and genetic network that constitutes a calcium signalling based feedback loop in the circadian clock.

T4-06-06

Interaction of calcium and ROS signaling in *Arabidopsis* roots in response to environmental cues

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Calcium (Ca^{2+}) and reactive oxygen species (ROS) are important second messengers that function in many different biological processes in plants. Spatially and temporally defined changes in their cytoplasmic concentrations represent stimulus-specific signals. However, the modes of interaction and integration of these two important second messengers are largely unknown. Respiratory burst oxidase homologs (RBOHs) are membrane localized plant NADPH oxidases that produce ROS and form a family of 10 proteins in *Arabidopsis*. Cellular calcium signals are detected and transmitted by calcium-binding proteins that function as calcium sensors. In higher plants, calcineurin B-like (CBL) proteins and their CBL-interacting protein kinases (CIPKs) represent important relays in calcium signaling in many different response reactions. In *Arabidopsis*, 10 CBL-type calcium sensor proteins form a dynamic interaction network with 26 CIPKs. Here, I will report in detail how distinct Ca^{2+}-activated CBL/CIPK complexes regulate ROS production of specific RBOH proteins in *Arabidopsis*. In this way I will detail mechanisms that integrate Ca^{2+} and ROS signaling, how activation of ROS signals occur and discuss a model how Ca^{2+} and ROS could function together in plant long distance signaling.

T4-07: Plant responses to flooding

T4-07-01

Ethylene priming: A new mechanism that confers flooding tolerance

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The severity and frequency of floods is predicted to increase with changing global weather patterns. These floods adversely affect crop growth and yields. Research aimed at increasing plant resistance to abiotic stresses such as flooding is therefore a high global priority. The volatile plant hormone ethylene accumulates in submerged plant tissues very quickly (within 1 h) and acts as a reliable proxy for submergence and probably therefore this hormone operates upstream of many flood adaptive processes. In *Arabidopsis*, ethylene can 'prepare' (prime) plants for flooding tolerance to later occurring oxygen deficiency (hypoxia). This novel mechanism of flooding tolerance is regulated via increased transcription and stabilisation of group VII Methionine-Cysteine (MC) Ethylene Response Factor (ERF) transcription factors. Upon hypoxia these MC-ERFs regulate the transcription of adaptive core-hypoxia genes. We hypothesize that ethylene-regulated increase of non-symbiotic hemoglobin causes stabilization of MC-ERFs via its capacity to scavenge NO.

T4-07-02

Regulation of root water transport under flooding

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Soil water uptake by roots is central for plant growth and survival. Flooding results in root hypoxia, which as many other environmental constraints such as drought, nutrient deprivation or oxidative stress, exerts deep effects on root transport functions and alters root water permeability (root hydraulic conductivity; Lp_r). We previously showed that the early inhibition of Lp_r by hypoxia is mediated through proton-dependent gating of aquaporin water channels (Tournaire-Roux *et al.,* 2003). More recently, quantitative genetics approaches based on the natural variation of Lp_r in the model plant *Arabidopsis* were used to identify Hydraulic Conductivity of Root 1 (HCR1), a Raf-like MAPKKK (Shahzad *et al.,* 2016). This protein kinase accumulates as a function of nutrient and oxygen (O_2) availability and regulates Lp_r accordingly. In brief, HCR1 reduces water entry into the roots when there is a lack of O_2, but only when the soil is also rich in potassium (K^+), a mineral salt essential for plant growth. *HCR1* acts on abundance of RAP2.12, a key transcription factor triggering the core anaerobic response. We propose that *HCR1* delineates a combinatorial signaling pathway integrating two soil signals, K^+ and O_2 availability, for resilience of plants to multiple flooding scenarios including root waterlogging or complete submergence. The adaptive significance of this mechanism is supported by substantial variation of *HCR1* in regulating Lp_r at the *Arabidopsis* species level.

T4-07-03

The mitochondrial retrograde signaling regulates flooding tolerance in rice

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Rice is the only cereal crop capable of anaerobic germination and seedling growth, which represents a unique adaptive trait to oxygen (O_2) deficiency under flooding. Our previous study showed that sugar and O_2 deficiency signaling processes converge into a common CIPK15-SnRK1A-dependent regulatory pathway to activate alpha-amylase expression, resulting in hydrolysis of seed starch to sugars to be used for anaerobic energy metabolism in rice seedlings. The underlying mechanism of how rice senses and initiates this anaerobic tolerance response is unclear. Recently, we observed that a coordinated signaling cascade regulates the expression of genes necessary for sugar production, fermentation, and chloroplast biogenesis to sustain anaerobic seedling growth. Studies with mutants of components in the mitochondrial electron transport chain (mtETC) indicates that mtETC monitors O_2 and sugar availability through the two parallel cytochrome c oxidase (COX) and alternative oxidase (AOX) pathways to initiate the retrograde signaling process. Ca^{2+} serves as a second messenger in the signaling pathway, and a Ca^{2+} sensor, CBL, interacts with and translocates CIPK15 from cytoplasm to plasma membrane in a

Ca^{2+}-dependent manner. N-myristoylation & N-acylation of CBL is necessary for the interaction of CBL and CIPK15 in the plasma membrane. Our studies reveal that the mitochondrial retrograde regulatory network and the downstream CBL-CIPK15 interaction regulating alpha-amylase expression confer the flood-adaptive trait in rice seedlings.

T4-07-04

Jasmonate regulates plant responses to post-submergence reoxygenation through transcriptional activation of antioxidant synthesis

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Submergence induces hypoxia in plants; exposure to oxygen following submergence, termed reoxygenation, produces a burst of reactive oxygen species. The mechanisms of hypoxia sensing and signaling in plants have been well studied, but how plants respond to reoxygenation remains unclear. Here, we show that reoxygenation in *Arabidopsis thaliana* involves rapid accumulation of jasmonates (JAs) and increased transcript levels of JA biosynthesis genes. Application of exogenous methyl jasmonate improved tolerance to reoxygenation in wild-type *Arabidopsis*; also, mutants deficient in JA biosynthesis and signaling were very sensitive to reoxygenation. Moreover, overexpression of the transcription factor gene *MYC2* enhanced tolerance to post-hypoxic stress and *myc2* knockout mutants showed increased sensitivity to reoxygenation, indicating that MYC2 functions as a key regulator in the JA-mediated reoxygenation response. MYC2 transcriptionally activates members of the *VITAMIN C DEFECTIVE* (*VTC*) and *GLUTATHIONE SYNTHETASE* (*GSH*) gene families, which encode rate-limiting enzymes in the ascorbate and glutathione synthesis pathways. Overexpression of *VTC1* and *GSH1* in the *myc2-2* mutant suppressed the post-hypoxic hypersensitive phenotype. The JA-inducible accumulation of antioxidants may alleviate oxidative damage caused by reoxygenation, improving plant survival after submergence. Taken together, our findings demonstrate that JA signaling interacts with the antioxidant pathway to regulate reoxygenation responses in *Arabidopsis*.

T4-07-05

The carbon and phosphorus trade market between arbuscular mycorrhizal fungi and rice under different flooding regimes

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Arbuscular mycorrhizal fungi (AMF) is arguably the most important and widespread symbiotic microorganisms in nature. They form symbiosis with the majority of terrestrial plants, in which the fungal partner benefits the host plant by facilitating plant access to mineral nutrients (especially phosphorus (P)), in exchange for the plant-assimilated carbon (C). This C-P nutrient exchange between AMF and host plant can greatly influence plant productivity and community structure, and plays a significant role in the global C and P cycling. In recent years, evidences are accumulating that AMF are prevalent in paddy wetlands. AMF are aerobic microorganism, while the soil environment in the paddy field is usually hypoxic. Therefore, the nutrient exchange between them in wetland environment may be different from that in terrestrial ecosystems. Until now, the nutrient exchange in AM symbiosis from wetland environment has not yet been explored. The present study aims to clarify (a) whether the C-P trade, which were commonly present in AM symbiosis in terrestrial ecosystems, also occurs between AMF and its wetland hosts under wetland environments? and (b) the response of the C-P trade to flooding regimes? A pot experiment, using rice (cv. Guinongzhan) and *Rhizophagus irregularis* as the plant and fungal materials, respectively, was conducted. Rice plants were cultivated under three different flooding conditions, and the plant growth parameters and gene expression files relating to P absorption were analyzed. The results showed that under all flooding conditions AMF structure were commonly present in the rice roots with AMF inoculation. Considering the obligate nature of AMF, it suggested that there was C input from rice to AMF under these flooding regimes. The AMF-specific P transporter gene *OsPT11* were detected in rice roots with AMF inoculation, indicating the P delivery from AMF to rice plants. The C-P nutrient exchange was inhibited by increasing flooding intensity, as indicated by decreased AMF colonization rates and down regulation of *OsPT11* expression level. The AMF colonization should also decrease the direct P absorption pathway by rice roots, since the *OsPT2* and *OsPT6* expressions in rice roots were much lower in AMF colonized plants than those without AMF inoculation. The present study provide the first evidence, as far as we know, showing the presence of the C-P trade between AMF and wetland plants in wetland environments. The inhibitary effects of flooding on the nutrient exchange between AMF and rice plants are indicative to the application of AMF in rice cultivation system, but further research were needed to explore, in more details, the mechanisms involved in this inhibitory effects of flooding on the C-P exchange in AM symbiosis at wetlands.

T4-07-06

Elucidating the molecular mechanisms behind adventitious root formation in *Solanum dulcamara*

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Adventitious root (AR) formation is an adaptive trait to partial flooding in many plant species. Phytohormones are extensively involved in this process. Ethylene and ABA were shown to play contrasting roles in AR formation. Ethylene induces AR emergence by activating the activities of cell cycle genes, and promoting programmed death of overlying epidermal cells, while ABA inhibits AR formation in monocot species deepwater rice which mechanisms were unclear yet. In our research, *Solanum dulcamara*, a dicot species, was used. We investigated the interaction between ethylene and ABA in regulating AR formation, and further elucidated the role of ABI5, a bZIP transcriptional factor in ABA-inhibited AR formation. We found that, ethylene treatment, on one hand, highly induced cell wall degrading genes expression which resulted in the loosening of overlying tissues; on the other hand, decreased ABA biosynthesis and thus led to a reduced ABA level in primordia. Locally applying ABA inhibited flooding-induced AR emergence, while removal of ABA signal by fluridone application released primordia from dormancy. These data together confirmed the positive role of ethylene and inhibitory role of ABA in AR emergence. Moreover, One *SdABI5* candidate was figured out by screening in the *Solanum dulcamara* transcriptome database and by blasting with that of *Solanum lycopersicum*. *SdABI5* was highly expressed in primordia and seeds, and was induced by ABA treatment, whereas inhibited by flooding and ethylene treatment. RNA interference (RNAi)-mediated repression of *SdABI5* resulted in an earlier activation of dormant primordia during flooding. To further explore the role of SdABI5, a microarray expression analysis will conducted on *S.dulcamara abi5* mutants.

T4-08: Development and genetics of dioecy and sex chromosomes in plants (two sessions)

T4-08-01

Evolution of flexible sex determination system in polyploid persimmon

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Although the field of epigenetics has strong roots in plant biology, evidence for its adaptive role is limited. This is surprising because plants are sessile and can be long lived, especially in trees. Epigenetic regulation can add a flexible layer to genetic variation, potentially enabling long term, but reversible cis-regulatory changes of an allele while maintaining DNA sequence. Here, we present a case in which alternative epigenetic states lead to reversible sex determination in hexaploid persimmon *Diospyros kaki*, in an evolution from dioecious diploid *Diospyros* species. Previously, we elucidated the molecular mechanism of sex determination in diploid persimmons and demonstrated the action of a Y-encoded sex determinant pseudo-gene called *OGI*, via the production of small-RNAs against the gene encoding the female-determining factor *MeGI* and resulting in separate male and female individuals (dioecy). On the other hand, in hexaploid Oriental persimmon (*D. kaki*), this regulatory mechanism leads to the formation of both male and female flowers (monoeocy), in genetically male individuals. In these Y-chromosome carrying hexaploid persimmon trees, the *MeGI* and its promoter region exhibits methylation and repression in male flower buds but not in developing female flower buds or any other tissue. Inhibition of DNA methylation by treatment of demethylating agents resulted in the reversion of male developing buds to form semi-feminized flowers, while it did not affect female flowers. In hexaploid persimmon, *OGI* expression is generally undetectable, possibly due to the SINE-like transposon insertion of a transposon in the 5’ region of its promoter. This transposon insertion is highly methylated as is reported SINE families in other plant species, and is conserved in all Y-carrying *D. kaki* cultivars, suggesting either natural selection or a strong bottleneck for the silenced *OGI* gene during the establishment of this species. Our findings are consistent with the idea that the evolution of this more flexible system is an adaptation to polyploidization and associated enhanced sex bias. They might also provide an example of an adaptive scenario involving epigenetic plasticity.

T4-08-02

Pattern and process of unisexual flower development

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Unisexual flowers are all defined by a single attribute: they have only a single set of functional reproductive structures; the androecium or gynoecium. Yet, the developmental patterns and process that underlie the absence of function in the androecium or gynoecium of unisexual flowers are diverse. I will summarize the common processes involved in flower sex determination, the stages of development affected, and examine the distribution of stages and process across the angiosperms.

T4-08-03

Hill-robertson interference causes reduced genetic diversity on a young plant y-chromosome following recent recombination suppression

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X and Y chromosomes differ in effective population size (*Ne*) rates of recombination, and exposure to natural selection, all of which can affect patterns of genetic diversity. On Y chromosomes with suppressed recombination, selection is expected to eliminate neutral variation and reduce the *Ne* of Y compared to X chromosomes or autosomes. However, non-selective factors including female biased sex ratios and high variance in male reproductive success can also reduce Y-linked *Ne*, making it difficult to infer the causes of low Y-diversity from natural populations. Here, we investigate the factors affecting levels of polymorphism during sex chromosome evolution in *Rumex hastatulus* (Polygonaceae), a dioecious plant with young sex chromosomes. Strikingly, we find that neutral diversity for genes on the Y is on average 2.1% of the value for their homologues on the X, corresponding to a chromosome-wide reduction of 93% compared to the neutral expectation. We demonstrate that the magnitude of this diversity loss is inconsistent with a reduced male *Ne* caused by neutral processes including female-biased sex ratios and high variance in male reproductive success. Instead, using forward simulations and estimates of the distribution of fitness effects of deleterious mutations, we show that diversity loss on the Y can be explained by purifying selection acting over a large number (~ 800 kb) of genetically-linked sites. Our results are in agreement with theory on “interference selection”, and provide empirical evidence that this effect can substantially reduce neutral diversity on a non-recombining chromosome. Given the recent origin of *R. hastatulus* sex chromosomes (15MYA), our results imply that Y-chromosome degeneration in the early stages may be largely driven by such interference effects rather than by positive selection for gene silencing followed by neutral genetic drift.

T4-08-04

Towards understanding the gene network controlling sex determination in cucurbits

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How the gender of a flower or plant is determined is an important issue in plant developmental biology. Understanding this process also has practical applications in agriculture and plant breeding, as the gender of a flower or plant often limits how it is bred and cultivated. Sex determination is a process that leads to the physical separation of male and female gamete producing structures in separate flowers on the same plant (monoecious species) or on separate individuals (dioecious species). Several species in the *Cucurbitaceae*, including melon, have bisexual floral primordia, but often have flowers limited to a single sex. Sex determination occurs by the selective arrest of either the male stamen or female carpel during development. In melon, sex determination is governed by three genes *andromonoecious* (M), *androecious* gene (A) and *gynoecious* (G) and the interplay of alleles of these three genes results in a range of sexual types. In addition, cucurbit sex expression patterns can be modified by hormones, such as ethylene, and by environmental factors. We have previously shown that the *gynoecious* (G) gene encodes for a zinc finger transcription factor, *CmWIP1*, the *andromonoecious* (M) gene encodes for an ethylene biosynthesis enzyme, *CmACS*-7 and the *androecious* gene (A) encodes *CmACS11*. Now that the identity of the androecy, monoecy and gynoecy sex genes are revealed and genetic pathway controlling sex determination in *Cucurbitaceae* discovered, among the next challenges are to decipher how the sex determination signals are perceived and how the information is translated to cause organ-specific abortion at the flower and the plant level. Our progress in this direction will be discussed.

T4-08-05

New insights about the sex chromosomes in *Coccinia*, a dioecious genus ‘between’ *Cucumis* and *Citrullus*

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The angiosperm species with the relatively largest Y-chromosome is *Coccinia grandis* (Cucurbitaceae), with a male/female difference of 10% of the total genome (male genome size: 0.925 Gb/2C). The Y chromosome arose ca. 3 my ago and is entirely heterochromatic. The chromosomal distribution of repetitive and organellar DNA (detected with probes from high-throughput sequencing) shows that the size of the Y is largely due to an accumulation of certain Ty1/copia and Ty3/gypsy repeats, satellites, and organellar DNA. The centromere of the Y differs from those of all other chromosomes including the X in lacking a highly abundant centromeric repeat (CgCent) and in extended histone marks, raising questions about spindle attachment. PacBio sequencing of *C. grandis* is ongoing. The genus *Coccinia* also includes 24 other dioecious species, and we also tested the repetitive DNA accumulation patterns and performed immunostaining with (peri-) centomere-specific antibodies in three species close to *C. grandis*. Male nuclei of two of the relatives indeed accumulate distinct repeats (in each case on one chromosome pair), and CgCent also labels their centromeres and pericentromeric regions.

T4-08-06

The secrets of sex determination in strawberries

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Sex chromosome evolution is thought to be a universal feature of separate-sexed organisms but our understanding of the process is heavily influenced by animal systems, the majority of which have ancient sex chromosomes. We will present research on species of wild strawberries (*Fragaria*, Rosaceae) demonstrating that plants not only provide valuable insight into the earliest stages of sex chromosome evolution from autosomes but also offer key parallels to dynamics seen at later stages in animal systems. Using comparative approach across multiple species in this young genus we have discovered at least four transitions from hermaphroditism to separate sexes, and multiple genetic avenues to loss of male function. Within the nascent ZW sex determining system in an octoploid and decaploid clade, we revealed novel recombination heterogeneity between the sexes, as well as at least four different genomic locations of a homologous sex determining region. Within this dynamic system, the translocated sex determining region shows surprising fidelity to a single homoeologous set of chromosomes. If this pattern is found in other genera, it could help explain the prevalence of separate sexes in polyploid plants.

T4-08-07

Using common garden and transcriptomics to understand the genomic architecture of sexual dimorphism

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Sexual dimorphism evolves because selection favors different phenotypes in males and females. However, the trajectory of phenotypic evolution in species with separate sexes is determined by not just nature of selection on the two sexes, but also the correlation between the phenotypic effects of allelic substitutions in males and females. To examine these two phenomena, we studied population differentiation in males and females of the moss *Ceratodon purpureus*, and transcriptomic differences between males and females in early growth rate, a sexually dimorphic trait. We hypothesized that traits in which the difference between males and females evolved among populations may be subject to the strongest selection. In contrast, we found unambiguous evidence for adaptation in development time, a modestly dimorphic trait, but no signature of selection on the most sexually dimorphic traits (leaf size, early development growth). Instead, dimorphism in these traits appears to evolve as a consequence of sexually dimorphic correlated responses to selection on development time. These findings suggest that the genetic architecture of trait variation in males and females is quite different. To begin to understand the genetic basis of sexual dimorphism in early development, we surveyed gene expression and metabolite profile in ten male-female sibling pairs (i.e., two haploid plants derived from the same diploid meiosis) isolated from seven populations spanning the geographic distribution of the species. We identified a core set of ~1000 genes (both autosomal and sex-linked) that were consistently differentially expressed between females and males, of about 15,000 expressed in this tissue, and 25 differentially represented metabolites. These data highlight the importance of pleiotropy and genetic background in governing the phenotypic response to selection. We are currently testing these findings in other tissues to directly link sexually antagonistic selection to its molecular targets.

T4-08-08

Genome-wide association studies reveal different sex determining systems in two poplar species

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All species of the genus *Populus* are dioecious plants, suggesting a common origin of this trait in their ancestral lineage. Although chromosome XIX was revealed as sex chromosome in all studied poplars, the location of sex determination region differs among species and both male and female heterogametic systems (XX/XY and ZZ/ZW) could be present, indicating that sex determination is highly dynamic in poplar. In the present study, we performed whole genome resequencing of 60 *P. alba* and 60 *P. euphratica* individuals of known sex and used a genome-wide association approach (GWAS) to determine the genomic regions associated with sex in these two species. We identified 114 SNPs located in scaffold 1,514 significantly associated with sex in *P. alba*. Gene syntenic analysis revealed that this sex-linked region were mapped to chromosome XIX in *P. trichocarpa* reference genome. Otherwise, we also identified one unbalanced large duplication in female individuals at this region. The segregation pattern of sex associated SNPs revealed this to be a ZW system in which the female was the heterogametic gender. In contrast, 102 SNPs located in 4 different scaffolds were identified to be associated with sex in *P. euphratica* and we found that the sex of this species was determined by an XY system. Unlike *P. alba*, these regions showed homologous relationships with chromosome I and XIV in *P. trichocarpa*. Results of this study demonstrated a high turnover rate of sex determination mechanisms in poplar and provided valuable information for better understanding the evolutionary processes of sex chromosome in dioecious plants.

T4-08-09

The identification and evolution of sex determination genes in *Asparagus*

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An elegant model for the conversion of an autosomal pair in a hermaphroditic species to a sex chromosome in a dioecious species was formalized by Deborah and Brian Charlesworth in 1978. Briefly, the conversion from autosome to sex chromosome could require just two loci linked perfectly in non-recombination on a young Y chromosome: one locus must dominantly suppress female (pistil) organogenesis, while another locus must promote the formation of male (anther) organogenesis, but this model has never had strong genic support in any dioecious species. Garden asparagus (*Asparagus officinalis*) is an ideal system to test this question, given that the X and Y sex chromosomes are young and cytologically homomorphic. Leveraging a doubled haploid YY individual and a doubled haploid mapping population, we generated a chromosome-level genome assembly using Illumina, PacBio, and Bionano optical maps and identified a 1Mb region of non-recombination on the Y chromosome that is largely missing from the X. Several independent male-to-hermaphrodite mutants (gamma irradiation, spontaneous SNP) implicate a single gene *SOFF* (Suppressor of Female Function) on the Y chromosome as being responsible for female suppression. While only 12 gene annotations are in this non-recombining region, there exists *Tapetum Development and Function 1*, a gene with an *Arabidopsis* knockout phenotype very similar to Asparagus females. With an additional PacBio and optical map genome assembly for a sibling XX female, we have compared the structure of the X and Y chromosomes and identified ~130kb of X-specific sequence, including several X-specific genes. We are currently performing *in situ* hybridizations to test for the developmental timing and localization of gene expression of our candidate sex determination genes. This is the strongest evidence to date for the Charlesworth's classic "two gene" model.

T4-08-10

Efficient inference of the genetic basis of dioecy in Nepenthes pitcher plants

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Dioecy has evolved multiple times independently in plants, which may facilitate comparative studies of its molecular basis and identification of ecological factors promoting the evolution of separate sexes. However, in the vast majority of plant groups, nothing is known about the genetic basis of dioecy. We present a method to efficiently characterize the genetic sex determination system in plants with homomorphic sex chromosomes without requiring breeding efforts. The method can easily be extended to develop sex-specific markers for application in ecology, conservation or agriculture. We have applied the new method to *Nepenthes* pitcher plants, a genus encompassing approximately 150 dioecious species. We genotyped wild populations of flowering *Nepenthes* from Southeast Asia and the Seychelles using ddRAD-sequencing and developed a novel empirical statistical procedure to robustly distinguish sex-limitation of genomic contigs from stochastic missingness, a major problem inherent to most methods that have to date been used to study the genetic basis of dioecy. We found consistent results in our separate analysis of three *Nepenthes* taxa. Comparison with results from *Silene latifolia*, a model species with heteromorphic sex chromosomes, indicates that the putative sex-specific region in *Nepenthes* is much smaller, as expected for homomorphic sex chromosomes. Characterization of expressed genes located in the sex-specific region reveals that sex chromosomes are shared across the *Nepenthes* radiation and may thus have a single origin.

T4-08-11

Genetic transformation and CRISPR/Cas9-mediated targeted mutagenesis in a dioecious plant model *Silene latifolia*

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Silene latifolia is a dioecious plant model with heteromorphic sex chromosomes. It has been successfully used as a model in studying numerous biological questions including sex chromosome evolution, sex determination, sexual dimorphism, epigenetic processes in flower development, sexually transmitted diseases, invasive species biology *etc.* With the progress of massive parallel sequencing during the past decade, a number of candidate genes for important biological traits increased dramatically. However, a functional evaluation of these genes is difficult because of the lack of methods for gene delivery to *S. latifolia*. We have developed an efficient and reproducible protocol for genetic transformation and *in vitro* regeneration of S. *latifolia*. We demonstrated a successful T-DNA integration in *S. latifolia* genome using GUS assay and standard molecular analyses. Subsequently, we assessed the transgene segregation in T_1 generation and confirmed Mendelian manner of T-DNA transmission that suggests a single T-DNA integration event per T_0 plant. Furthermore, we employed the CRISPR/Cas9 nucleases to disrupt a coding sequence of a sex-linked, MADS-box gene *APETALA3* (*SlAP3*) that is involved in floral organ development. We recovered mutants in both X- and Y-linked alleles of the *SlAP3* gene. To our knowledge, this is the first case of genome editing on plant sex chromosomes.

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T4-08-12

Different autosomes evolved into sex chromosomes in the sister genera of *Salix* and *Populus*

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Willows and poplars are dioecious plants that originate from a common ancestor. Sex chromosome in poplar genome was consistently reported to be associated with chromosome XIX. In this study, chromosome XV was identified as sex chromosome in willow, and willow's sex determination was found to occur through a ZW system in which the female was the heterogametic gender. High collinearity was revealed between sex chromosomes and the corresponding autosomes in these two lineages. By contrast, no syntenic segments were found to share between poplar's and willow's sex chromosomes. We proposed that evolution of autosomes into sex chromosomes in *Salicaceae* occurred after the divergence of *Salix* and *Populus*. Syntenic analysis also revealed severe chromosome rearrangements between willow's Z and W chromosomes. Since sex chromosomes in *Salicaceae* are still at their early evolutionary stage, our findings provided valuable information for better understanding the genetics and evolution of sex chromosome in dioecious plants.

T4-09: Foliar vasculature: transport and communication hub

T4-09-01

Observing Real-Time Air Bubble Formation in the Leaves, Petioles and Stems of a Woody Plant

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The ease with which water flows through different tissues of the leaf to arrive at sites of evaporation near the stomata (defined as leaf hydraulic conductance; Kleaf) is a fundamental determinant of photosynthetic capacity (Brodribb et al. 2007). The magnitude of Kleaf and its interaction with external signals are constrained by the nature of the hydraulic pathway through the leaf xylem and mesophyll tissues (Sack and Holbrook 2006). Xylem embolism during dehydration has been strongly associated with declining Kleaf, and ultimately to tissue death. Understanding the spread of xylem embolism, and its timing relative to critical plant responses such as stomatal closure and tissue damage is fundamentally important. New non-invasive methods allow us for the first time to observe embolism formation in real-time and map where and when they occur in the plant with high resolution. We used the woody tree Magnolia grandiflora to examine embolism formation in the stem, leaf and petiole using a new optical technique developed by our lab group. The timing of embolism formation in these organs was compared with losses in plant hydraulic conductance in the laboratory and field.

Brodribb TJ, Feild TS, Jordan GJ (2007) Leaf maximum photosynthetic rate and venation are linked by hydraulics. Plant Physiology 144, 1890-1898.

Sack L, Holbrook NM (2006) Leaf hydraulics. Annual Review of Plant Biology 57, 361-381.

T4-09-02

TWI1, a transcription factor binding protein, regulates cell-to-cell movement of OSH15 to control bundle sheath and sclerenchyma development in *Oryza japonica* leaves

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Cell pattern formation in plant leaves has attracted much attention from plant biologists and breeders. However, the relevant molecular mechanism remains unclear. Here, we describe the isolation and functional characterization of *TWIST-LEAF1 (TWI1)*, a critical gene involved in development of the vascular bundle sheath and sclerenchyma in the rice leaf. Mutant *twi1* plants have twist leaves due to the compromised development and disordered patterning of bundle sheath and sclerenchyma cells. Expression of *TWI1* can functionally rescue these mutant phenotypes. *TWI1* encodes a transcription factor binding protein which interacts with OSH15, a class I KNOTTED1-like homeobox (KNOX) transcription factor. The cell-to-cell trafficking of OSH15 was shown to be restricted by its interaction with TWI1. During leaf development, widespread distribution of OSH15 impedes cell lignification, an essential step in sclerenchyma formation. Knock-out or knock-down *OSH15* in *twi1* could rescue the twisted leaf phenotype. These studies identified a key factor controlling cell pattern formation in rice leaves.

T4-09-03

Water supply and demand remain coordinated during breakdown of the global scaling relationship between leaf size and major vein density

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Vein networks that disobey the global scaling of major vein density with leaf size shed light on functional constraints of vein network formation in dicotyledons. Based on vein traits of 55 species of pantropical Ochnaceae, stomata and climatic niche data, and a dated molecular phylogeny, we unveiled major structural shifts in vein networks through deep time, relationships between leaf size, vein and stomatal traits. Our study shows that the breakdown of the vein-leaf size scaling is associated with vein networks that exhibit densely spaced 2° veins and reduced minor veins, and that this character combination evolved multiple times independently in Ochnaceae. In spite of the major shifts in vein architecture in this venation type, the coordination of vein and stomatal densities remains similar to that in species with normally spaced 2° veins and abundant minor veins.

T4-09-04

Daily dynamics of xylem sap properties and plant hydraulic traits in hybrid aspen

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Plants have to cope with very different environmental conditions, which vary circadially enormously in most habitats, shaping daily patterns in physiological processes including long-distance water transport and xylem sap properties. Some of them are poorly documented in natural conditions; especially there is a lack of night-time measurements. The daily dynamics of physico-chemical properties of xylem sap and their covariation with tree hydraulic traits was investigated in hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx) in field conditions. K^+ concentration ($[K^+]$), electrical conductivity (S_{sap}), osmolality (Osm) and pH of the xylem sap, as well as branch hydraulic traits including whole-branch (K_{wb}), leaf blade (K_{lb}) and petiole hydraulic conductances (K_P) and bare branch hydraulic conductance (K_B), were measured in the field over 24-h cycles. The study aimed also to clarify which environmental drivers influencing the daily dynamics of these parameters. All studied xylem sap properties and hydraulic characteristics including K_{wb}, K_{lb} and K_P presented clear daily dynamics. Air temperature (T_A) and photosynthetic photon flux density (PPFD), but also water vapour pressure deficit (VPD) and relative humidity (RH), had significant impacts on K_{wb} K_{lb}, K_P, $[K^+]$, and s_{sap}. In the same time Osm varied only with light intensity (PPFD), while K_B was influenced by atmospheric evaporative demand expressed as T_A, VPD or RH. Xylem sap pH depended inversely on soil water potential (Ψ_S), and during daylight, also on VPD. Although soil water content was close to saturation during the study period, Ψ_S influenced also $[K^+]$ and S_{sap}. The enhancement of branch hydraulic conductance in the morning was associated with the upregulation of potassium ion concentration in xylem sap. The present study presents evidence of coupling between circadian patterns of xylem sap properties and plant hydraulic conductance providing adequate water supply to foliage under environmental conditions characterised by diurnal variation. A combination of different atmospheric variables generating AED rather than irradiance predominantly controls the circadian patterns of tree's hydraulic conductance and xylem sap ionic content in field conditions. The ionic content of xylem sap also affects plant hydraulic traits, but atmospheric variables mask this effect: the direct impact of environmental conditions on branch hydraulic efficiency outweighs that of xylem sap properties.

T4-09-05

Global analysis of the evolution of leaf venation networks in angiosperms

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Understanding the mechanistic implications of functional trait variation and how this variation evolved through time is key to understanding species adaptation to their environments and how they will respond to global environmental changes. Leaf vein traits are proving especially important to understanding plant performance, environmental adaptation including drought tolerance and even the paleohistory of ecosystems. We quantified leaf morphological and venation characteristics for >5,000 angiosperm species distributed worldwide based on the cleared leaf collections of D. Axelrod and J. Wolfe, to describe the evolution of vein densities and vein architecture types across biomes including their relationship with life form, climate and evolutionary diversification.

T4-09-06

The forgotten side of leaf veins: Phloem hydraulic architecture of Poplar and *Ginkgo*

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Over the last decade, research in leaf vasculature has unveiled fundamental form-function properties that govern leaf hydraulics, consequently affecting traits such as drought tolerance and maximum rates for gas exchange among others. Most research dealing with leaf hydraulics has however focused on water transport properties and the xylem, whereas the form-function relationships that affect sugar flow through the phloem continue to be heavily understudied. Part of the discrepancy between the phloem and the xylem research is due to the inherent difficulty in directly measuring flow through a living tissue such as the phloem, as well as the availability of analytical methods required to quantify pressure and sugar translocation *in vivo*. Biophysical inferences based on leaf phloem structure can provide basic principles for understanding phloem flow at the leaf level, including upper boundaries for sugar translocation rates, and xylem–phloem interconnectivity.

We describe and compare the leaf phloem hydraulic architecture of two topologically distinct model species: a species with dichotomous, open leaf venation (*Ginkgo biloba*), and a species with hierarchical, reticulate venation (*Populus x canescens*). Using a combination of electron and light microscopy, we compare the conductive areas of phloem and xylem, as well as sieve and tracheary element size across all vein classes observed in fully mature samples of both leaf types. We have found that the conductive area of phloem scales isometrically with that of xylem in both Ginkgo and poplar. Phloem conductance varies within each of these leaf types, as sieve element or sieve tube diameter and volume are largest in the major veins of poplar and the largest, basalmost veins of Ginkgo. Poplar leaf venation, as in most eudicots, is composed of a system of major and minor veins. The conductive area of minor veins is notably lower than that of the major veins, matching the functional distinction (sugar loading vs. translocation) of minor and major veins. At the whole leaf level, the number and length of minor veins drastically exceed the number of major veins, such that the total conductive area of minor veins to which sugar is loaded drastically exceeds that of major veins and the petiole. Contrarily, the phloem conductive area at the petiole level in Ginkgo leaves exceeds the total phloem conductive area of the collecting veins near the margin of the leaf. In this sense, the total phloem conductive area of Ginkgo tapers from the leaf base towards its margin, despite the characteristic fan-shape of the leaf blade. The application of first principles to our data shows different implications to phloem transport in light of Münch's pressure hypothesis. Using the phloem structural parameters previously presented, we will present a spatially-explicit model for phloem transport at the leaf level to estimate translocation and infer the xylem–phloem interconnectivity required for sugar export. The distinction between major and minor veins in angiosperms may provide faster phloem export rates when only considering structural differences between the studied species.

T4-10: Stem cell and root meristem regulation

T4-10-01

Ribosome biogenesis, cell cycle, and cell size – how an adenylate kinase and its natural genetic variants regulate the root meristem and root growth in *Arabidopsis*

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Root growth is dependent on cellular proliferation and expansion, both of which are determined in the apical meristems of roots. It follows that the tremendous variation in root growth between different species, and different strains of one species, originates from the root apical meristem. While many genes involved in stem cell maintenance, cellular proliferation, and differentiation are known, it is not clear whether genetic variation in these genes is responsible for the differences in root meristem activities across and within species. Indeed, many of the important regulators show little genetic variation between *Arabidopsis thaliana* accessions, and are therefore unlikely to be major determinants of natural variation in root growth. We used genome wide association mapping (GWAS) to identify novel regulators of cell proliferation and expansion, where natural genetic variation is responsible for variation in root growth rates between different *Arabidopsis* accessions. One of these regulators is a gene encoding for an adenylate kinase (AK) that regulates cell proliferation and cell size in the meristem. In contrast to many known meristem regulators, loss of this AK does not lead to correlated changes in meristem and mature cell size, but to an opposite effect - meristem size is reduced while mature cell size is increased. At the molecular level, this AK is involved in small ribosomal subunit maturation and ribosome biogenesis. Overall, we have uncovered an unknown regulatory mechanism in the root apical meristem that links ribosome biogenesis, cell cycle, and cell size control. Importantly, genetic variation in a key gene in this process is involved in determining root growth in natural accessions of *Arabidopsis*.

T4-10-02

Glycogen synthase kinases refine the specificity and output of auxin signaling in plant growth and development

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Peptide-initiated signaling networks play central roles in various aspects of plant growth and development. In particular, ligand TDIF (tracheary element differentiation inhibitor factor) and its receptor TDR (TDIF receptor) modulate lateral root development via a glycogen synthase kinase BIN2 (brassinosteriod insensitive 2) which directly controls the activity of ARF7/19. We also found that BIL1 (BIN2-like 1) acts as a negative regulator of the cambium activity. The loss-of-function *bil1* mutant shows increased thickness of stems and hypocotyl, but not *bin2* loss-of-function mutant, suggesting the regulatory specificity in GSK3-mediated vascular development. TDR directly interacts with BIL1 *in vivo*, and this interaction is sustained after TDIF treatment. In addition, BIL1 increases transcriptional activity of ARF5 which negatively regulates vascular cambial activity. TDIF/TDR suppresses BIL1-mediated ARF5 activation, suggesting that TDIF/TDR could maintain vascular cambial activity by partially inhibiting BIL1-ARF5 signaling module. Taken together, we propose that TDIF/TDR-GSK3 signaling cascade has a critical role to refine ARF activity in diverse plant developmental processes.

T4-10-03

Hemigraphis alternata as a novel model plant for understanding root morphogenesis

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Developmental processes that underlie plant anatomy and architecture uses root as an excellent model. Its modular structure, lack of cell movement and relative accessibility to microscopic visualization facilitate research in a number of areas of plant biology. It is often difficult to visualize and analyze, the so-called 'hidden half' of plants and requires innovative approaches to understand its developmental processes and responses to external cues. The development of model plants to study these interactions will allow a better knowledge of those mechanisms. Computational plant models or 'virtual plants' are increasingly seen as a useful tool now a days, for comprehending complex relationships between gene function, plant physiology, plant development, and the resulting plant form. However, the mechanisms governing the interaction between various cell layers are yet not well understood, including those genes responsible for lateral rooting. In the present work, Hemigraphis alternata was used as a model to understand the developmental pathways of root regeneration. Hemigraphis alternata, commonly known as red ivy is a creeping, herbaceous, perennial groundcover growing to about 6 inches in height and can spread indefinitely on the ground with its creeping growth habit, rooting at the stem nodes along the way. It is native to India and Indonesia. In vitro culture systems for rooting were standardized using different plant growth regulators and were chosen for further studies. Nodal, internodal regions and leaf petioles were used as explants and inoculated on MS medium supplemented with auxins such as NAA, IAA, IBA and 2,4-D. Rooting observed from all the culture systems showing variations in root morphology, number and branching pattern. Roots developed from culture systems were compared with both acclimatized and ex vitro plant. Development of root from each system was studied at morphological, biochemical and genetic level. The present study also focused on the developmental aspects involved in establishing root anatomy and architecture.

T4-10-04

Auxin mediates Al stress signaling to control root growth through the regulation of local cytokinin biosynthesis in *Arabidopsis*

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Aluminum stress, which inhibits root growth, is one of the widespread environmental cues in acid soils. Our recent studies showed that Al stress induced a local auxin accumulation which is mediated through TAA1 and YUCs in root transition zone (TTZ). The high accumulated auxin in root TZ causes root-growth inhibition. Since it is well known that auxin acts synergistically with cytokinin to control the shoot stem-cell niche, while they act antagonistically to maintain root meristem. However, the role of cytokinin has not yet been addressed. Therefore, we investigated the role of CK in Al-induced inhibition of root growth, and the protential interaction between auxin and CK in this process. Our study showed that CK enhanced root-growth inhibition under stress through mediating Al-induced auxin signaling. Al stress induced a local up-regulation of CK response in the root-apex TZ that is dependent on *IPT*s, which encode adenosine phosphate isopentenyltransferases and regulate CK biosynthesis. *IPTs* are specifically up-regulated in the root-apex TZ in response to Al stress in an auxin-dependent manner, and AUXIN RESPONSE FACTORS, ARF7 and ARF19 play an important role in this process. In summary, different from the current general view in the root meristem, auxin acts with CK in a synergistic way to regulate aluminum-induced root growth inhibition in *Arabidopsis*.

T4-10-05

The SMC5/6 complex couples cell cycles checkpoint and DNA repair to maintain genome stability and stem cell identify

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Stem cells are hypersensitive to DNA damage. In response to DNA damage, cells activate checkpoints to arrest cell cycle progression and provide sufficient time to repair DNA. Previous studies have identified different sets of checkpoint and repair proteins. How cells coordinate these two processes is not well-understood. The evolutionarily conserved SMC5/6 complex plays multiple essential roles in DNA repair. Here we show that the SMC5/6 complex also regulates G1/S checkpoints by repressing E2F transcription factors. Mutation in SNI1, a subunit of the SMC5/6 complex, results in defective G1/S transition and eventually causes disorganized root meristem and short-root phenotypes, mimicking E2F overexpression. Consistently, mutations in E2Fs suppresses the *sni1* mutant and overexpression of SNI1 suppresses E2F overexpression plants. Remarkably, SNI1 directly binds the activation domain of E2Fs and represses their transcriptional activity. Our study demonstrates that the SMC5/6 complex is the key link to couple checkpoint and DNA repair to maintain genome stability and stem cell identity.

T4-10-06

Temperature-compensated cell production rate and elongation zone length in the root of *Arabidopsis thaliana*

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To understand how root growth responds to temperature, we used kinematic analysis to quantify division and expansion parameters in the root of *Arabidopsis thaliana*. Plants were grown at temperatures from 15 to 30°C, given continuously from germination. Over these temperatures, root length varies more than threefold in the wild type but by only two-fold in a double mutant for *phytochrome-interacting factor 4* & *5*. For kinematics, the spatial profile of velocity was obtained with new software, Stripflow. We find that 30°C truncates the elongation zone and curtails cell production, responses that probably reflect the elicitation of a common pathway for handling severe stresses. Curiously, rates of cell division at all temperatures are closely correlated with rates of radial expansion. Between 15 to 25°C, root growth rate, maximal elemental elongation rate, and final cell length scale positively with temperature whereas the length of the meristem scales negatively. Non-linear temperature scaling characterizes meristem cell number, time to transit through either meristem or elongation zone, and average cell division rate. Surprisingly, the length of the elongation zone and the total rate of cell production are temperature invariant, constancies that have implications for our understanding of how the underlying cellular processes are integrated.

T4-11: Plant kinase and sugar signaling network

T4-11-01

Transcription factor ANAC060 represses *ABI5* to atteuate the sugar-abscisic acid pathway in *Arabidopsis*

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Arabidopsis seedling establishment is inhibited on media containing high levels (6%) of sugars. A sugar-ABA pathway has been illustrated to render this sugar sensitivity. Previously, the nucleus presented ANAC060 transcription factor was involved in the negative feed back loop in this sugar-abscisic acid pathway. In this study, microarray analysis was undertaken in wild type and *anac060* mutant under sugar (glucose or frucotse) treatment and sorbital control to identify the downstream genes of ANAC060. In wild type, expression levels of 2,122 and 2,509 genes were altered under glucose and fructose treatment respectively. In *anac060* mutant, up to 5,955 and 8,716 genes were regulated by glucose and fructose, respectively. Mapman analysis indicated that the genes involved in light reaction, light respiration, culvin cycle and tetrapyrrol synthesis were supressed by sugar treatment in wild type, and much stronger supression in these genes were found in *anac060* mutant. These results confirmed the attenuater role of *ANAC060* in sugar-ABA pathway on transcriptome level. Further more, ChIP-Seq analysis was undertaken using the line in which the functional ANAC060-GFP fusion protion was over expressed in *anac060* background to identified the targets of ANAC060. A total of 5,631 ANAC060 binding sites were identified. The percentages of the ANAC060 binding sites in premoter, exon, intron TES and intergenic sequence were 66%, 15%, 3%, 6% and 10% respectively. Most of the ANAC060 binding sites in promoter region were in the regions of 3 kb upstream of transcript start sites. The G-Box motif (CACGTG) was identified as ANAC060 binding motif. GO analysis indicated that the ANAC060 binded genes were enriched in chitin response, salt stress, membane target protein, plant hypersensitization response, calcium ion response, abscisic acid (ABA) activated signaling pathway, wouding response, ABA response, cold response, drought response, defense response, endoplasmic reticulum protein folding, programing cell death, jasmonic acid signaling pathway, salicylic acid signaling pathway and insect resistance response. These results indicated that ANC060 was involved in response of biostess and abiostess. Interestingly, *ABA INSENTIVE 5* (*ABI5*) which has been unrevealed to play an important role in ABA signaling and sugar-ABA pathway was identified as a target of ANAC060 by ChIP-Seq. The binding of ANAC060 to *ABI5* promoter was confirmed by ChIP-qPCR and in vitro EMAS assay. The expression of *ABI5* was induced by sugar treatment in wild type. Much stronger glucose upregulation of *ABI5* occurred in the *anac060* mutant. The *anac060 abi5* double mutant showed the sugar insensitive phenotype. These results indicated that ANAC060 atteuates the sugar-ABA pathway partially through repressing of ABI5.

T4-11-02

PRRs act as circadian hubs to regulate TOR pathway in *Arabidopsis*

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Circadian clocks are not only the intricate time-keeping mechanisms, also regulating many aspects of plant growth and development, such as cell elongation of hypocotyl and stress response by synchronizing environmental cues. To date, it still largely unknown about regulation of cell proliferation by circadian clock in higher plants. The conserved eukaryotic TOR signaling can promote root cell proliferation and growth by gauging endogenous nutrient and energy status. Here we found Pseudo Response Regulators (PRRs), essential components of circadian core oscillator, redundantly modulates cell division activities in root meristems mediated via TOR signaling. PRRs appear to be positive regulators of TOR signaling, as their null mutants display the reduced sensitivities to reactivation TOR-signaling. We further demonstrated *Arabidopsis Tandem Zinc Finger 1* (*TZF1*), encoding a processing body and stress granule localized RNA-binding protein, is a direct downstream target of PRR proteins in mediating reactivation TOR signaling. PRRs differentially bind to *TZF1* promoter and repress its expression, in accodance with genetic analysis in which TZF1 acts as a negative regulator of TOR signaling. Reciprocally, blocking TOR signaling by either pharmacological approach or genetic knockdown of *TOR* by artificial microRNA significantly lengthens circadian period. Collectively, our studies support a notion that the PRR-TZF1-TOR interacting network plays a crucial role in root meristem activities and root architecture and the reciprocal intersection between circadian clock and TOR signaling is evolutionary conserved in plants.

T4-11-03

Mitogen-activated protein kinases and their downstream WRKY transcription factors in *Arabidopsis* pollen development

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Mitogen-activated protein kinase (MAPK, or MPK) cascades are highly conserved signaling modules downstream of receptors/ sensors that transduce extracellular stimuli into cellular responses in eukaryotes. *Arabidopsis* MPK3 and MPK6, two closely related MAPKs, are involved in signaling multiple developmental processes, including gametogenesis, embryogenesis, morphogenesis, senescence, abscission, fertilization, and seed formation. We are interested in not only the biological functions of these two MAPKs, but also how the signal specificity is maintained. It is possible that different upstream components such as the receptors and their ligands are involved in spatiotemporal activation of MPK3/MPK6, and/or different downstream targets such as the MAPK substrates are expressed in different cell types, which confers the signaling specificity of MAPKs. MAPK cascades are known to function as molecular switches in animal system. Plant MAPK cascades are likely to be the same and can carry out distinct functions depending on their upstream ligand–receptor interactions and downstream targets, both of which can be spatiotemporal specific during plant growth and development. Previously, we demonstrated that MPK3/MPK6 are critical to *Arabidopsis* oval development. In *mpk6 mpk3/+* plants, cell division of the integuments is hindered during oval development, resulting in a female sterile phenotype as a sporophytic effect. More recently, we found that MPK3/MPK6 also play essential roles in pollen development by regulating WRKY34 and WRKY2, two redundant WRKY transcription factors, in a spatiotemporal-specific manner. WRKY34 can be phosphorylated by MPK3 and MPK6 in both *in vitro* and *in vivo* assays. Phos-tag gel mobility shift assay revealed a spatiotemporal-dependent phosphorylation of WRKY34 by MPK3/MPK6 between bicellular and tricellullar stages during pollen development. *WRKY34* and *WRKY2* are highly expressed during pollen development, and mutation of both genes results in reduced pollen viability, pollen germination, and transmission efficiency. At the meeting, we will report that WRKY2 and WRKY34, and their upstream MPK3/MPK6 cascade, regulate carbon metabolism and storage lipid accumulation during pollen maturation, therefore, influence pollen fitness and plant reproduction.

T4-11-04

Mechanistic studies of light-enhanced translation in de-etiolating *Arabidopsis* seedlings

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Photomorphogenesis, or de-etiolation, is a developmental process transforming young plant seedlings to vegetative phase with photosynthetic activities. In addition to transcriptomic adjustments, translation for thousands of genes was enhanced in de-etiolating *Arabidopsis* seedlings. However, the molecular mechanism and signal transduction underlying the translational enhancement remains unclear. Here we show the far-red (FR) light photoreceptor phytochrome A (phyA) and the E3 ubiquitin ligase, CONSTITUTIVE PHOTOMORPHOGENESIS 1 (COP1), positively and negatively regulate translation in photomorphogenic *Arabidopsis*, respectively. The survey of known translational regulators revealed that light could induce the activity of TOR (target of rapamycin) and followed by the phosphorylation of a key ribosomal protein small subunit 6, RPS6. Our results indicated that the phosphorylation of RPS6 depends on light signals but independent of photosynthesis activity or glucose. phyA mediates the phosphorylation of RPS6 under FR, whereas COP1 represses the phosphorylation of RPS6 and TOR activity in darkness. In addition to FR, blue light could also activate TOR-RPS6 pathway, a process requiring functional photoreceptors phyA, cry1 and cry2. Consistent with a delayed cotyledon opening phenotype observed in de-etiolating *tor* mutant, the RPS6 phosphorylation and the *de novo* protein synthesis were markedly reduced in *tor* mutant. The survey of light-regulated and TOR-dependent association of transcripts with polysome fraction revealed a higher representation of ribosome biogenesis genes. We uncovered a new signaling pathway involving phyA, cry1, cry2, COP1, TOR and RPS6 in regulating translation in de-etiolating *Arabidopsis* seedlings.

T4-11-05

Tandem CCCH zinc finger proteins in plant growth and stress response, mRNA decay, and beyond

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Tandem CCCH zinc finger (TZF) proteins are evolutionarily conserved regulators of gene expression at the post-transcriptional level. TZFs target AU-rich RNA elements at 3′ un-translated region and recruit catabolic machineries to trigger mRNA degradation. The plant TZF families are over-represented by a class of proteins with a unique TZF domain preceded by an arginine-rich motif (RR-TZF). RR-TZF proteins are mainly involved in hormone-, sugar- and environmental cues-mediated plant growth and stress responses. Numerous reports have suggested that RR-TZF proteins control seed germination, plant biomass, flowering time, and biotic and abiotic stress responses via regulation of gene expression. Despite growing genetic evidence, the underlying molecular mechanisms are elusive. We have found that AtTZF1 binds specific RNA elements and trigger corresponding mRNA degradation. We have also found that RR-TZF proteins are dynamically localized in Processing bodies (P-bodies, PBs) and stress granules (SGs), two cytoplasmic messenger-ribonucleoprotein foci mediating mRNA decay, in response to various internal and external cues. Finally, we demonstrate that RR-TZF proteins preferentially and specifically interact with stress response mediators, particularly protein kinases involved in an array of stress signal transduction.

T4-11-06

Parent-of-origin effect seed mutants regulate endosperm development in maize

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Genomic imprinting in plants is an epigenetic phenomenon by which a subset of genes is expressed in a parent-of-origin-dependent manner. Imprinted gene expression primarily occurs in the endosperm and is thought to influence seed size and embryo development. Although many maize imprinted genes have been identified through transcriptome analysis, imprinted genes with developmental functions in the maize seed have not been identified. We screened rough endosperm mutants from the UniformMu transposon-tagging population for parent-of-origin effects using reciprocal crosses between heterozygous mutant plants and inbred lines. Six maternal rough endosperm (*mre*) and three paternal rough endosperm (*pre*) mutants were identified. When inherited from the female parent, *mre* seeds show a rough, etched, or pitted endosperm surface as well as a reduced seed size and weight. The *pre* mutants show the converse inheritance pattern with pre pollen conferring a seed phenotype after fertilizing wild-type ovules. Characterization of the *mre* and *pre* isolates shows a range of endosperm defects. *mre1* mutants have a general delay in endosperm development with smaller starchy endosperm cells, delayed basal endosperm transfer cell layer (BETL) development, and delayed accumulation of starch granules. The *mre2* mutant shows multiple starchy cell differentiation defects, while *mre3* mutants have an endosperm phenotype consistent with reduced sink strength. In addition to the defects in BETL and starch endosperm development, *pre1* mutant is deficiency in embryo development in consistent with the significant reduction in oil content. Our results showed that imprinting is important for endosperm development as well as the seed size diversity. Molecular mapping experiments have identified four loci: *mre1* is on chromosome 4, *mre2* is on chromosome 6, and both *pre1* and *mre3* are on chromosome 10. Additional mutant alleles obtained from the UniformMu reverse genetics resources appear to identify *mre1*, *mre3*, and *pre1* as imprinted genes in maize. RNAseq analysis from F1 crosses of the *mre1*, *mre2* and *mre3* is in progress and will identify imprinted genes that are differentially expressed in the maternal-effect mutants.

T4-12: Intracellular communication in higher plants (two sessions)

T4-12-01

Choreography of plastidial retrograde signaling network in interorganellar communication

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Interorganellar communication is an evolutionary necessity for maintenance of cellular homoeostasis in response to prevailing environment that, in part, is exquisitely controlled via retrograde-signaling pathways. We have identified a novel stress-specific plastidial retrograde signalling metabolite, methylerythritol cyclodiphosphate (MEcPP), previously known solely as an intermediate in the isoprenoid biosynthetic pathway. The additional function of MEcPP as a stress sensor and a coordinator of transcriptional and post transcriptional regulation of key stress-responsive nuclear genes, has unraveled the central role of this metabolite in cellular functions in response to a wide range of environmental and developmental cues. To identify the underlying molecular mechanism of the MEcPP-mediated stress responses, we have performed a multi-omics approach. These studies have led to the identification of a transcriptional hub activated by MEcPP, and have further established a previously unrecognized link between this plastidial retrograde signal and the transcriptional reprogramming of endoplasmic reticulum genes critical for readjustment of protein-folding capacity in stressed cells. Lastly, we have gained an insight into the molecular mechanism by which MEcPP regulates plant growth and development in response to stress. In brief we have advanced our understanding of how MEcPP reprograms a repertoire of intricate networks crucial for coordinating the physiological and metabolic processes required for stress-induced developmental responses and stress adaptation. These studies offer an insight into functional module concept of biological organization and regulation by MEcPP, the plastidial retrograde signaling metabolite and a coordinator of interorganellar communication.

T4-12-02

Roles of GOLDEN2-LIKE 1 transcription factor in the regulation of plastid-to-nucleus retrograde signaling

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Arabidopsis thaliana GOLDEN2-LIKE (AtGLK) transcription factors promote chloroplast development by regulating the expression of photosynthesis-related genes. AtGLK1 is also known to participate in plastid-to-nucleus retrograde signaling. The expression level of *AtGLK1* is tightly correlated with the expression of photosynthesis-related nuclear genes in *Arabidopsis* possessing damaged chloroplasts. Furthermore, the activity of plastid-localized GENOMES UNCOUPLED 1 (GUN1) appears to down-regulate the expression of *AtGLK1* when chloroplasts are dysfunctional. These data suggest that plastid signals repress the expression of photosynthesis-related nuclear genes through the repression of *AtGLK1* expression. In addition to transcriptional regulation of *AtGLK1*, our recent study have demonstrated that ubiquitin-proteasome dependent regulation of AtGLK1 participates in plastid-to-nucleus retrograde signaling. Norflurazon- or lincomycin-treated *gun1-101* mutant expressing normal levels of *AtGLK1* mRNA failed to accumulate AtGLK1 protein. This suggests that plastid signals directly regulate the accumulation of AtGLK1 protein in a GUN1-independent manner. Treatment of the *glk1glk2* mutant expressing functional GFP-AtGLK1 with a proteasome inhibitor, MG-132, induced the accumulation of polyubiquitinated GFP-AtGLK1. Furthermore, the level of endogenous AtGLK1 in plants with damaged chloroplasts was partially restored when those plants were treated with MG-132. Taken together, these data suggest that plastids have evolved multiple mechanisms to regulate the level of AtGLK1 in response to plastid signals.

T4-12-03

GUN1, a jack-of-all-trades in chloroplast protein homeostasis and signaling

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The *GUN1* (*GENOMES UNCOUPLED 1*) gene has been reported to encode a chloroplast-localized pentatricopeptide-repeat protein, which acts to integrate multiple indicators of plastid developmental stage and altered plastid function, as part of chloroplast-to-nucleus retrograde communication. However, the molecular mechanisms underlying signal integration by GUN1 have remained elusive, up until the recent identification of a set of GUN1-interacting proteins, by co-immunoprecipitation and mass-spectrometric analyses, as well as protein-protein interaction assays. Here, we review the molecular functions of the different GUN1 partners and propose a major role for GUN1 as coordinator of chloroplast translation, protein import and protein degradation. This regulatory role is implemented through proteins that, in most cases, are part of multimeric protein complexes and whose precise functions vary depending on their association states. Within this framework, GUN1 may act as a platform to promote specific functions by bringing the interacting enzymes into close proximity with their substrates, or may inhibit processes by sequestering particular pools of specific interactors. Furthermore, the interactions of GUN1 with enzymes of the tetrapyrrole biosynthesis pathway support the involvement of tetrapyrroles as signaling molecules in retrograde communication.

T4-12-04

Identification of novel auxiliary factors involved in the post-translational regulation of tetrapyrrole biosynthesis in higher plants

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Tetrapyrrole biosynthesis (TBS) is a multiple enzymatic pathway, is exclusively located in plastids, and provides vital cofactors such as chlorophyll (Chl) and heme for a wide range of essential cellular processes. While the biochemical properties of tetrapyrroles and almost all genes encoding enzymes in the TBS pathway have been intensively characterized in higher plants, the post-translational regulation and spatiotemporal organization of the tetrapyrrole metabolism remain to be clarified. To ensure adequate synthesis of tetrapyrrole end-products at any time of developmental and environmental conditions, a wide range of auxiliary factors such as chaperones, scaffold proteins, and proteases have been characterized in tetrapyrrole metabolism. In this study, we aimed to identify a group of novel regulators involved in the post-translational control of TBS pathway in higher plants. To this purpose, the bioinformatics analysis, such as gene coexpression analysis, reverse genetic approach, and multiple biochemical approaches were applied for the identification of the potential candidates. Among them, two types of proteins, chloroplast signal recognition particle 43-kDa protein (cpSRP43) and CAAX-type Endopeptidase-like Protein1 (CPL1), participate in the control of different enzymatic steps of TBS pathway and likely in plastid-to-nucleus retrograde signaling. Firstly, cpSRP43 is well known as an essential component of the post-translational cpSRP pathway, by which the light-harvesting chlorophyll-binding proteins (LHCPs) are transferred from the stroma to thylakoid membranes. Here, we provide evidence supporting the function of cpSRP43 as a chaperone in the regulation of the rate-limiting step of tetrapyrrole metabolism, 5-aminolevolinic acid (ALA) synthesis. Co-fractionation analysis and protein-protein interaction assays suggest that cpSRP43 directly interacts with the first enzyme of ALA synthesis, glutamyl-tRNA reductase (GluTR), and its regulator GluTR-binding protein (GBP). The cpSRP43 deficiency led to reduced GluTR content without altered transcript levels of *HEMA1*, the GluTR encoding gene. Furthermore, cpSRP43, as well as GBP interact with the N-terminal domain of GluTR, which is also the target site at GluTR for the Clp protease. Our data provide a first link between the cpSRP43 chaperone function in the TBS. Secondly, we demonstrated that the previously uncharacterized chloroplastic transmembrane protein CPL1 is required for the activation of the chlorophyll-synthesizing branch of TBS. The *cpl1* null mutants display a significantly reduced ALA synthesis rate, and dramatically reduced accumulation of Chl and its precursors, but not of heme. Moreover, the CPL1 mutation leads to a reduced GluTR and an increased GUN4 (genome uncoupled 4) level. The direct regulation of GluTR and GUN4 was supported by their CPL interaction. In summary, our data suggest that CPL1 serves as a scaffold protein in the thylakoid membrane, and functions in the activation of both ALA synthesis and magnesium chelatase in the Chl branch of TBS.

T4-12-05

Tetrapyrrole-dependent retrograde signalling pathways that function during chloroplast development

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The development of chloroplasts during de-etiolation is a critical time for a plant seedling as it races towards a photoautotrophic existence in the light. Retrograde signalling between the developing chloroplasts and the nucleus is important during this time to ensure an appropriate supply of chloroplast proteins. We have been investigating two tetrapyrrole-dependent pathways that have been proposed to function during chloroplast biogenesis: a positive, promotive pathway related to heme and mediated by ferrochelatase 1 (FC1) activity and an inhibitory pathway that is dependent on Mg-porphyrin synthesis and mediated by singlet oxygen. To examine the role of a heme-related signal we have constructed a series of transgenic *Arabidopsis* lines in which we alter heme pools through compartment-specific expression of FCs and heme oxygenase (HO1). The results demonstrate that overexpression of *FC1* in the chloroplast results in rescue of nuclear gene expression, but that overexpression in the mitochondrion does not. Analysis of multiple lines with altered FC1 expression showed a strong correlation between changes in a regulatory heme pool and nuclear gene expression supporting a role for FC1-dependent heme synthesis in retrograde signalling. Pharmacological experiments using inhibitors of the tetrapyrroles biosynthesis pathway are also consistent with this interpretation. The positive retrograde signal increases expression of tetrapyrrole genes including *HEMA1* encoding the rate-limiting step for tetrapyrrole synthesis and the Mg-chelatase genes *CHLH* and *GUN4*. What therefore prevents such a signal causing an over accumulation of chlorophyll precursors such as protochlorophyllide? Accumulation of excess protochlorophyllide after transfer from far-red (FR) to white light, after feeding of the tetrapyrrole precursor aminolevulinic acid, or in the *flu* mutant of *Arabidopsis* results in the production of singlet oxygen and rapid and strong inhibition of nuclear photosynthetic genes, and especially tetrapyrrole biosynthesis genes. This inhibitory signal would therefore serve to function as a safety valve to prevent overproduction of photo-toxic tetrapyrroles. We are now identifying mutants in the inhibitory pathway. We have already shown that the singlet oxygen signalling mutants *executer1* and *executer2* show partial rescue of photosynthetic gene expression. In addition, we have undertaken a genetic screen for mutants that show elevated expression of *HEMA1* after a FR pre-treatment. Progress on characterization of these *safe after far-red light* (*saf*) mutants will be reported.

T4-12-06

Intracellular communication of abiotic stress, memory and recovery

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Abiotic stress such as excess-light and drought cause significant crop losses by reducing photosynthetic efficiency and preventing yield potentials to be realized, conversely, slow recovery from, or constitutive acclimation to, stress can impair growth and yield. Multiple components of excess-light and drought stress induction pathways have been identified. However, we have very little understanding of the reverse processes that are required to signal recovery and for resetting the transcriptome. Systems biology approaches provide valuable tools for assessing these competing interests for energy allocation. Here, several examples of the application of systems biology to understanding stress signaling, recovery and memory will be presented with a focus on gene expression profiling. We are employing large scale biology approaches to investigate the processes and mechanisms enabling both stress tolerance and recovery from stress, including transcriptome, degradome and methylome profiling together with predicting retrograde stress-signal interactomes.

T4-12-07

Mitochondrial biogenesis and signalling

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It is widely accepted that in addition to the extensive biochemical metabolic functions of mitochondria, they play an essential role as a cellular signalling hub. Mitochondria act as a sensor to environmental and cellular signals to trigger signal transduction pathways to modify nuclear gene expression. Much of the investigation in this area has focused on stress-induced components of mitochondria, e.g. alternative oxidase (AOX), and the identification of the signalling pathways and components that are involved in induction of AOX under a variety of different conditions. However, in addition to responding to adverse conditions it is hypothesized that mitochondrial signalling is required during normal growth and development. We hypothesize that mitochondrial function and mitochondrial biogenesis must be linked through normal development, to ensure that any change in mitochondrial signalling can be executed. To investigate this we have investigated the changes that occur in mitochondrial function when biogenesis is altered, and vice versa, changes that occur in biogenesis when mitochondrial function is altered. Using knock-out mutants of mitochondrial complex I as an example of altering mitochondrial function, i.e. respiration, and knock-out mutants of the TIM17:23 complex as altering biogenesis, we have uncovered a previously unknown link between protein complex involved in protein import (mitochondrial biogenesis) and respiration (complex I – respiration). Further studies indicate that mitochondrial complex I is a signalling hub in plants and interacts with a variety of signalling protein in the mitochondrial inter-membrane space. Alterations of these proteins not only impacts mitochondrial function, but chloroplast and cellular protein abundance. Results will be presented to show that a variety of proteins interact with complex I and that upon perturbation signalling cascades are initiated to alter total cellular function. The alteration of cellular function occurs at both a transcriptional and post-transcriptional level. Overall these results show that altering mitochondrial function directly impacts the protein complement and function throughout the cell.

T4-12-08

Glycosylphosphatidylinositol-anchored proteins (GPI-APs) as chaperones and co-receptors for FERONIA receptor kinase signaling in *Arabidopsis*

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The *Arabidopsis* receptor kinase FERONIA (FER) has been characterized as a critical multifunctional regulator in many aspects of plant growth and development, including hormone signaling, reproduction, and defense. FER perceives the peptide regulator Rapid Alkalinization Factor 1 (RALF1) at the plasma membrane and activate the downstream signaling. LORELEI (LRE), a female reproductive tissue-specific glycosylphosphatidylinositol (GPI)-anchored protein (AP), has been reported to have similar function as FER in mediating pollen tube reception in the ovule. In this study, we show that mutants in vegetative tissue-expressed LRE-like GPI-anchored protein1 (LLG1), *llg1*, resemble *fer* defects in vegetative tissues. Interestingly, FER relies on LLG1 for efficient localization to the cell membrane. Further investigation showed that LLG1/LRE interacts with FER in the endoplasmic reticulum and on the cell surface where LLG1/LRE and FER associate with the RHO GTPase signaling apparatus to mediate multiple downstream processes. Loss of LLG1 function induces cytoplasmic retention of FER, consistent with transport of FER from the endoplasmic reticulum to the plasma membrane in a complex with LLG1. Moreover, we determined that LLG1 binds to the extracellular juxtamembrane region of FER, lacking of which caused retention of FER in the endoplasmic reticulum. Our results support LLG1/LRE acting as a chaperone and co-receptor for FER. These findings elucidate a novel mechanism that underlies FER function and new insights on how GPI-APs might enable the signaling capacity of a cell surface receptor.

T4-12-09

Interaction between brassinosteriod and glucose signaling in regulation of seed germination in *Arabidopsis thaliana*

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Seed germination is a critical biological process that is regulated by multiple phytohormones, as well as metabolic and environmental signals. Brassinosteriods (BRs) and glucose (Glc) are known to regulate developmental process including lateral root growth, shoot gravitropism, and hypocotyl elongation. However, little is known about the involvement of BRs in seed germination. Here, we demonstrate that BR induces seed germination through promoting testa and endosperm rupture. The positive effect of BR is through cell elongation at the hypocotyl-radicle transition region of hypocotyl during endosperm rupture, rather than cell division. Glc appreciably inhibits testa rupture and strongly inhibits endosperm rupture. Moreover, we found that BRs partially counteract the Glc-induced inhibition of cell elongation during endosperm rupture. The role of BR in Glc-induced inhibition of cell elongation is apparently independent of the hexokinase function. We also identified that two key transcription factors (TFs) in BR signaling, BR ENHANCED EXPRESSION2 (BEE2) and HOMOLOG OF BEE2 INTERACTING WITH IBH1 (HBI1), are involved in the regulation of endosperm rupture by direct binding to E-box elements of the *GA-Stimulated Arabidopsis 6* (*AtGASA6*) promoter. Our findings reveal that BRs utilize different signaling cascades to partially reverse the Glc-induced inhibition of seed germination, highlighting the complexity of BR response in regulation of seed germination.

T4-12-10

A novel chloroplast translocase, Sec2, is involved in protein targeting to the chloroplast inner envelope membrane and chloroplast biogenesis

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Chloroplasts are essential organelles of plant and green algae. In plant cells, a chloroplast contains about 3,000 proteins and most of them are synthesized in the cytosol and post-translationally imported into chloroplasts. Correctly target these proteins to different sub-compartments within chloroplasts is essential for chloroplast biogenesis and the plant development. After import into the chloroplast, many proteins are further targeted to the thylakoid membrane and the thylakoid lumen by three thylakoid-localized translocases, the Sec1, Tat and SRP/Alb3. Others are targeted to the inner chloroplast envelope membrane through at least two pathways, the "stop-transfer" pathway and the post-import integration pathway. However, the mechanisms of these two pathways are still unknown. Recently, we identified a second Sec translocase system (Sec2) consisting of three protein components: SCY2 (forming the protein-conducting channel), SECE2 (SCY2 binding protein), and SECA2 (ATP-dependent motor protein). SCY2 and SECE2 are integral membrane proteins and localized mainly in the inner envelope membrane where they form a stable complex, while SECA2 is a soluble protein and localized in the stroma. Null mutants of any component in Sec2 in *Arabidopsis* exhibit severe embryo lethal phenotype. To investigate the function of Sec2 system in plants, we used inducible RNAi to knockdown *SCY2* in *Arabidopsis*. Seedlings cultured with inducer were chlorotic with aberrant chloroplasts and undeveloped thylakoids, indicating an essential role for SCY2 in chloroplast biogenesis beyond embryo development. In *SCY2* knockdown seedlings, several thylakoid membrane proteins including SCY1, ALB3 and TATC, were substantially reduced, as well as some inner envelope membrane proteins TIC40, TIC110 and FTSH12, which are through the post-import integration pathway for their targeting. Our data suggested that those down-regulated membrane proteins might be Sec2 substrates. Furthermore, *in vitro* reconstitution of protein integration into chloroplast membranes showed that both SCY1 and ALB3 target directly to the thylakoid membrane and are likely independent of Sec2. FTSH12 was integrated into the envelope membrane in a coupled import/integration reaction that was impaired by the SECA inhibitor sodium azide. The stromal intermediate of TIC40 integrated into the envelope in a reaction that was largely inhibited when antibodies against epitope-tagged SCY2 or SECE2 were applied. These data demonstrate that the Sec2 translocase likely integrates a subset of inner envelope membrane proteins through the post-import integration pathway. More detailed functional studies on Sec2 system are in progress. Our work shed new light on the current understanding of membrane protein trafficking within chloroplasts, which play essential roles in chloroplast biogenesis and plant development.

T4-12-11

ER-localized SNAREs are involved in transport of seed storage proteins

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Plant seeds accumulate large amount of storage proteins as N resorce for plant inicial growth. Storage proteins are synthesized as large precursors on the ER. The precursors are delivered to the vacuole by vesicle transport and processed to smaller mature forms. In our previous study, we found that ER-localized tethers, MAG2, MIP1, MIP2 and MIP3, form a tethering complex-MAG2 compplex, function on ER-Golgi vesicle transport in *Arabidopsis*. When they are defective, transport of newly synthesized proteins from the ER are affected. To further clarify mechanism underlying MAG2-relating protein transport pathway, we investigated function of predicted ER-localized SNAREs, including Qa-SNARE, Qb-SNARE and Qc-SNAREs. Our results indicated that these ER-localized SNAREs interact with each other to form a SNARE complex. This SNARE complex interacts with MAG2 complex.

When these SNAREs are defective, efficiency of transport of seed storage protein precursors from the ER is decreased. On the other hand, plant growth and development is also affected. Our findings indicate that these ER-Localized SNAREs collaborate with MAG2 complex to regulate ER-Golgi vesicle transport.

T4-12-12

AP-3-dependent vacuolar trafficking of *Arabidopsis* PAT10 involves a subpopulation of COPII and the vacuolar tethering complex HOPS

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Plant vacuoles are versatile organelles critical for plant growth and responses to environment. Vacuolar proteins are transported from the endoplasmic reticulum (ER) via multiple routes in plants. Two classic routes bear great similarity to other phyla with major regulators known, such as COPII and Rab5 GTPases. By contrast, vacuolar trafficking mediated by adaptor protein-3 (AP-3) or that independent of Golgi has few recognized cargos and none of the regulators. In search of novel regulators for vacuolar trafficking routes and by using a fluorescence-based forward genetic screen, we demonstrated that the multi-span transmembrane protein, *Arabidopsis* PROTEINS-ACYL TRANSFERASE 10 (PAT10), is an AP-3-mediated vacuolar cargo. We show that the tonoplast targeting of PAT10 is mediated by AP-3 complex but independent of Rab5-mediated post-Golgi trafficking route. We also report that AP-3-mediated vacuolar trafficking involves a subpopulation of COPII and requires the vacuolar tethering complex HOPS. In addition, we have identified two novel mutant alleles of AP-3δ, whose point mutations interfered with the formation of the AP-3 complex as well as its membrane targeting. Results presented here shed new lights on the vacuolar trafficking route mediated by AP-3 in plant cells.

T4-13: Programmed cell death and senescence

T4-13-01

ORE1, an aging regulator feedbacks into the circadian clock and exerts age-dependent doppler effect on the circadian system to sustain rhythmicity in old plants

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Aging and the following death are the inevitable fates of most organisms. In plants, aging is associated with age-dependent senescence and death of various organs such as leaf organ. The process of age-dependent senescence of plant leaf organs is critical for plants' fitness and productivity, which is representatively observed in leaf senescence. The physiological effect of aging encompasses the circadian clock system. In animals, aging is associated with disruption of many circadian rhythm, such as change of circadian period, delayed recovery from jet-lag, and reduced amplitude of clock genes. Conversely, the circadian system affects the aging processes. Disruption of circadian rhythms hastens aging process in several animal systems. In plants, the interaction of circadian clock and aging has been rarely explored despite of the importance of the two processes in plant physiology. However, it was recently noticed that plants appear to robustly maintain circadian rhythmicity even upon aging. Here, we show that ORE1, a pro-aging factor is regulated by circadian clock and in turn positively feedbacks into the circadian system, leading to robust circadian rhythmicity in aged plants. We found, in *Arabidopsis*, that most of core circadian components affect aging. Consistently, *ORE1* expression was transcriptionally activated by PRR9, a clock component and posttranscriptionally repressed by clock-controlled miR164. Reciprocally, ORE1 activated transcription of two clock genes, TOC1 and GI, and mediated age-dependent "jet-lag" response and circadian period shortening. ORE1 also stabilizes TOC1 through direct protein-protein interaction. ORE1 controls the age-dependent period shortening, or a temporal Doppler effect through TOC1. We propose that in plants, a cyclic positive feedback loop (PRR9→ORE1→TOC1) between circadian clock components (PRR9, TOC1) and an aging regulator (ORE1) modifies circadian rhythmicity and controls aging.

T4-13-02

Roles of *miR319* and TCP transcription factors in plant growth and senescence

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Leaf development includes the differentiation of leaves from the flank of a shoot meristem and the growth and senescence of leaves. The underlying mechanism of leaf differentiation, growth and senescence consists of many pairs of microRNAs (miRNAs) and their target genes. It has been reported that several miRNAs including *miR164* and *miR319* affect broad ranges of leaf differentiation, growth and senescence, while others affect specific aspects of leaves. Despite the fact that *miR164* and *miR319* respectively target NAC and TCP transcription factor genes, these miRNAs function in common regulatory programs, namely, the specification of the boundary between leaves and the shoot meristem, formation of leaf serrations and onset of leaf senescence. This implies that *miR164*, *miR319* and their target genes comprise a regulatory network for the regulation of leaf differentiation, growth and senescence. The role of the *miR319*-TCP pair in leaf development is obvious, because the downregulation of *TCP* genes in *Arabidopsis thaliana* induce jagged and wavy leaves as well as delayed senescence whereas ectopic expression of the *miR319*-resistant *TCP* genes inhibit the formation of serration in the leaf margin and stimulate precocious senescence. This versatility of the *miR319*-TCP pair in leaf development largely rely on the TCP's ability of regulation of various developmental regulators and senescence-stimulating hormone, jasmonate. Particularly, TCP transcription factors directly regulate *miR164A*, one of three *miR164*-precursor genes, and therefore TCP transcription factors act as master regulators of miR164 and the NAC-related CUP-SHAPED COTYLEDON genes. To obtain a detailed view on the role of the *miR319*-TCP pair, two of three precursor genes for *miR319* were mutated in *A. thaliana*. This *miR319* double mutant inhibited the formation of serration in the leaf margin, which is similar to the plants expressing the *miR319*-resistant *TCP* genes, and thus the mutant gained the functions of *TCP* genes. In this presentation, I will talk about our recent advance in the genetic link between the *miR319*-TCP pair and the regulatory mechanism of leaf differentiation, growth and senescence.

T4-13-03

Hormone crosstalk during rose petal senescence

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Rose (Rosa hybrid) is one of the most important ornamental plants in the global flower trade. Roses have enriched flower shapes and colors, and attractive fragrance. Trade share of cut rose accounts for 32% of total cut flower trade worldwide. With the globalization of cut flower trade, production of cut rose shows a new trend of development. Long-distance transportation often causes more than 30% value loss of cut rose. Our research aims to understand the causes of quality deterioration and molecular mechanism in terms of influence of flower opening and senescence in cut rose. We firstly established small RNA and RNA transcriptome databases during rose flower senescence, and obtained the ethylene-responsive miRNA and mRNA profiling in rose petals. We then identified an ethylene-responsive *miRNA164*/NAC100 module and found a gene network of cell expansion in response to ethylene in rose petals according to integral analysis of expression profiles of miRNAs and their predicted targets. Intriguingly, our work also found that subfamily I of homeodomain-leucine zipper transcription factor contributes to rose petal senescence. Among them, RhHB1 mediates the antagonistic effect of GAs on ABA and ethylene during rose petal senescence. However, RhHB6 functions as a brake of ethylene promoted senescence through increasing the CTK content. Our results suggest that homeodomain-leucine zipper I transcription factors involve in regulation of petal senescence through hormone crosstalk in rose flower.

T4-13-04

Hormonal regulation of leaf senescence in *Arabidopsis*

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Leaf senescence or ageing is an intricate and highly orchestrated developmental process that is subject to regulations by numerous environmental and endogenous signals, including plant hormones. Ethylene is known to be an endogenous modulator of senescence, including fruit ripening and flower and leaf senescence. However, the underlying molecular mechanism of ethylene-induced leaf senescence starts to be elucidated until recently. We have reported that EIN3, a key transcription factor in ethylene signaling, is a functional senescence-associated gene (SAG). Loss of EIN3 and its homolog EIL1 function leads to a delay in both age-dependent and ethylene-, JA- or dark-induced leaf senescence. We further define a novel signaling pathway involving EIN2-EIN3-miR164-NAC2 in the regulation of leaf senescence, and provides a mechanistic insight into how ethylene promotes the progression of leaf senescence in *Arabidopsis*. It has long been known that leaf senescence is a progressive natural degenerative process, which can be delayed or accelerated, but not prevented. To understand the progressive feature of leaf senescence at the molecular level, we have screened and collected *Arabidopsis* plants with altered senescence phenotype, from which a positive regulator of leaf senescence, WRKY75, was identified. We found that *WRKY75* transcription was induced by age, ethylene, jasmonic acid (JA), abscisic acid (ABA), salicylic acid (SA), and H_2O_2 treatment. Further studies showed that WRKY75 acted to enhance SA biosynthesis by inducing *SID2* transcription, and enhance tolerance to oxidative stress due to directly repressing *CAT2* transcription. Based on these results, together with the previously observation that SA and H_2O_2 induce each other, WRKY75, SA and H_2O_2 form a tripartite positive feedback loop, which is defined as an aging accelerator that speeds up gradually and irreversibly to accelerate leaf senescence. Further implication of this feedback amplification loop in integrating multiple hormonal signals will be discussed.

T4-13-05

Proteomes and ubiquitylomes analysis reveals the involvement of ubiquitination in protein degradation in petunias

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South China Agricultural University

Petal senescence is a complex programmed process. It has been previously demonstrated that treatment with ethylene can extensively alter transcriptome and proteome profiles in plants. However, little is known regarding the impact of ethylene on post-translational modification (PTM) or the association between PTM and the proteome. Protein degradation is one of the hallmarks of senescence, and ubiquitination, a major PTM in eukaryotes, plays important roles in protein degradation. Here, we first obtained reference petunia transcriptome data via RNA sequencing. Next, we quantitatively investigated the petunia proteome, ubiquitylome, and the association between them in petunia corollas following ethylene treatment. In total, 51,799 unigenes, 3,606 proteins, and 2,270 ubiquitination sites were quantified 16 hours after ethylene treatment. Treatment with ethylene resulted in 14,448 down-regulated and 6,303 up-regulated unigenes (absolute log2-fold change > 1 and FDR < 0.001), 284 down-regulated and 233 up-regulated proteins, and 320 up-regulated and 127 down-regulated ubiquitination sites using a 1.5-fold threshold ($P < 0.05$), indicating that global ubiquitination levels increase during ethylene-mediated corolla senescence in petunia. Several putative ubiquitin ligases were up-regulated at the protein and transcription levels. Our results showed that the global proteome and ubiquitylome were negatively correlated, that ubiquitination could be involved in the degradation of proteins during ethylene-mediated corolla senescence in petunias, and that ethylene regulates hormone signaling transduction pathways at both the protein and ubiquitination levels in petunia corollas.

T4-13-06

Molecular mechanisms of DNA damage- induced leaf senescence in *Arabidopsis*

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Environmental and endogenous sources constantly trigger DNA damage, which has long been suspected to be the cause of aging. However, knowledge about the underlying molecular mechanisms of DNA damage-induced leaf senescence is still lacking. Here, we show that the levels of DNA double strand breaks (DSBs), one of the most cytotoxic forms of DNA damage, increase as leaf aging; by contrast, DNA damage repair efficiency decreases. DSBs triggered by chemicals or inducible expression of endonuclease promote leaf senescence in an age dependent manner. Transcript levels of *ATAXIA TELANGIECTASIA MUTATED* (*ATM*) that signals the existence of DSBs and *SUPPRESSOR OF GAMMA RESPONSE 1* (*SOG1*), a plant-specific NAC transcription factor that plays a central role in DNA damage response (DDR), decline as leaf aging. Loss of functions of ATM and SOG1 accelerate DNA-damaged induced leaf senescence process. In addition, mutations in *ATAXIA TELANGIECTASIA MUTATED AND RAD3-RELATED* (*ATR*), *Ku70* and *Ku80* cause earlier senescence. To make clear how DNA damage affects leaf senescence process, we make a comparison between the transcript profiles affected by DNA damage chemical and senescence-associated genes. Our results suggest that DNA damage induces leaf senescence in part by regulating expressions of senescence-associated genes.

T4-14: Organ Size Control

T4-14-01

Molecular basis of flower-size evolution associated with the transition from outbreeding to selfing

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Understanding the molecular basis of morphological change remains a central challenge in evolutionary-developmental biology. The transition from outbreeding to selfing is often associated with a dramatic reduction in reproductive structures, such as a reduced flower size in plants. We are using the pair of closely related species *Capsella grandiflora* and *C. rubella* to study the molecular basis of evolutionary changes in flower size. The outbreeding ancestor *C. grandiflora* forms large attractive flowers, while the derived selfing species *C. rubella* only shows small, inconspicuous flowers. Using a quantitative-genetic approach, we have identified three genes whose variation between the two species contributes to flower-size reduction in *C. rubella*. One of these encodes the F-box protein STERILE APETALA (SAP), the other a putative transcriptional regulator and the third a cytochrome P450 enzyme involved in brassinosteroid biosynthesis. For the latter, the primary transcript of the *C. rubella* allele is spliced more efficiently than that of *C. grandiflora*, resulting in higher brassinosteroid levels. These restrict organ growth by limiting cell proliferation. More efficient splicing of the *C. rubella* allele results from two *de novo* mutations in the selfing lineage. Splicing variation has recently been suggested as a major contributor to complex traits in humans. Thus, our results highlight the potentially wide-spread importance of differential splicing and higher-than-optimal hormone levels in generating phenotypic variation, and identify the molecular changes underlying flower-size reduction in selfing compared to outbreeding species.

T4-14-02

Leaf size control mechanisms – Systems revealed from studies of compensation

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Compensation is a queer phenomenon that is seen in mutants/transgenics having severe defects in cell proliferation in leaf primordia: abnormally enhanced cell expansion triggered by decreased cell proliferation activity. Thanks to this phenomenon, the overall size decrease in leaf area is at least partially recovered than expected from the pure decrease in the number of leaf cells. While few cases can be simply explained by switching from mitosis to endoreduplication, the majority of the compensation-exhibiting mutants do not show enhanced endoreduplication in leaf cells. Moreover, the impact of endoreduplication on cell volume in palisade tissue is much weaker than observed in epidermis. For example, *KRP2* over-expressor in *Arabidopsis thaliana* shows typical Class III compensation and also displays a decrease in endoreduplication. The Class III compensation is unique since increased cell volume occurs even during the mitotic phase. Our genetic studies revealed that the enhanced cell expansion in Class III compensation depends on the activity of vacuolar proton-pump V-ATPase, provided that a mutation in *DET3* cancelled increased cell volume in the *KRP2* over-expressor. Interestingly, the requirement of the V-ATPase activity is not seen in the other classes of compensation, indicating the different nature of the compensation among differed classes. On the other hand, suppressor screening of *fugu5*, that shows Class II compensation characterized by an extended period for post-mitotic cell expansion, suggested that the monofunctional peroxisomal enoyl-CoA hydratase 2 (ECH2) is required for the compensation of *fugu5* mutant. This may be related to biosynthesis of biologically active auxin. Importantly, the peroxisomal ECH2 is not involved in the other classes of compensation. Finally in the *an3* mutant exhibiting typical Class I compensation, that is defined by an enhanced post-mitotic cell expansion rate, we found that compensation signal (s) can be transmitted among cells from analyses of chimeric leaves of *an3*-defective and *AN3*-over-expressing cells. Further detailed analysis demonstrated that the non-cell autonomous induction of the compensated cell enlargement is context-sensitive. Knowledge on how the context affects cell-cell communication in compensation will supply us with clues to identify the factors involved in the Class I compensation.

T4-14-03

Molecular networks governing biomass productivity and

seed yield

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Plant and plant organ growth are regulated by an exceedingly complex interplay of many genes and their interaction with the ever changing environment. The long-term goal of our research is to obtain a holistic understanding of plant organ growth. Numerous genes of which the modified expression enhances plant organ growth have now been identified, and a detailed study of these genes provided novel insights in the molecular machines driving growth. Furthermore, evidence obtained both in the model plant *Arabidopsis* and in maize, demonstrated that the combination of multiple growth enhancing genes can have very profound effects on organ sizes. Field experiments with transgenic maize also show that genes enhancing leaf growth have profound effects on seed yield. Tremendous progress has also been made in understanding how environmental cues, such as mild drought stress, negatively affect plant growth. In unpredictable environments, growth reduction enables plants to redistribute and save resources, ensuring reproduction, even when the stress becomes extreme. However, when the episode of stress does not threaten plant survival, and from the agricultural point of view, growth reduction can be seen as counter-productive, leading to unnecessary yield loss. Limiting growth reduction may thus provide a strategy to boost plant productivity under stress. I will discuss how our insights open up new perspectives for the identification of optimal growth regulatory networks that can be selected by advanced breeding, or for which more robust variants (e.g. reduced susceptibility to drought) can be obtained through genetic engineering. The ability to improve growth of maize and, in analogy other cereals, could have a major impact in providing food security.

T4-14-04

Ubiquitin-mediated control of seed size and weight

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Seed size is one of the most important yield traits in plants. In angiosperms, a mature seed consists of the embryo, the endosperm and the seed coat, which develop from the zygote, the fertilized central cell and the maternal integument. Thus, the characteristic size of plant seeds is coordinately determined by maternal and zygotic tissues. However, the genetic and molecular mechanisms that set the final size of seeds are largely unknown. We are focusing on understanding how plants determine their final seed size in *Arabidopsis* and rice. We have recently identified several seed size regulators that are involved in ubiquitin-related activities. Here, we will discuss the role of the ubiquitin pathway in seed control.

T4-14-05

New pathways in stem cell regulation and impact on cereal crop yields

David Jackson

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Shoot growth depends upon meristems, pools of stem cells that are maintained in a number of ways, including a negative feedback loop between the *CLAVATA* pathway and *WUSCHEL*. CLAVATA signaling involves a secreted peptide, CLAVATA3 (CLV3), and its perception by cell surface leucine-rich repeat (LRR) receptors, including the CLV1 receptor kinase, and an LRR receptor-like protein, CLV2. We are interested in finding novel players in the CLV-WUS pathway, as well as new regulators that could affect meristem size in parallel pathways. Maize is an excellent model system for these studies, because there are many clavata- type mutants, and their isolation is becoming routine. For example, we isolated the *COMPACT PLANT2* (*CT2*) gene, and found it encodes the predicted a subunit (Gα) of a heterotrimeric GTP binding protein. *ct2* mutants have CLAVATA-like meristem proliferation phenotypes, and genetic, biochemical and functional assays indicate that CT2/Gα signaling transmits a stem cell restrictive signal from a maize CLAVATA LRR receptor, suggesting a new function for Gα signaling in plants. Recent studies have questioned the idea that plant heterotrimeric G proteins interact with canonical GPCRs, and our findings suggest that single pass LRR receptors act as GPCRs in plants, challenging the dogma that GPCRs are exclusively 7TM proteins. We also characterized a new CLV- related receptor, *FASCIATED EAR3 (FEA3),* that appears to function in a different pathway to control meristem size, in parallel to CLV-WUS. We found that FEA3 responds to a CLV3-related ligand that is expressed in differentiating cells in leaf primordia, so it could provide a feedback from these differentiating primordia to the meristem. This pathway is therefore complementary to the known CLV-WUS feedback, and computational modeling suggests the 2 pathways interact. I will discuss the functions of these new pathways, as well as their potential use in improvement of maize yields, for example through enhancing seed number.

T4-14-06

Towards a better understanding of the grain size regulation in crops: cases from the perspective of QTL analyses

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Enhancing crop production is a matter of cardinal significance in view of the increasing global population. Grain size and weight is a key determinant of crop yield and is determined by multiple naturally occurring quantitative trait loci (QTLs); however, our current knowledge of the underlying molecular mechanisms is still fragmentary, although a dozen such QTLs have been identified. I will describe our work that uncovered two quite different mechanisms, a RING-type E3 ubiquitin ligase and a putative protein phosphatase, underlying major QTLs for, respectively, grain width and grain length (Song *et al.,* 2007; Qi *et al.,* 2012), and a third example of QTL for grain length being due to a chromatin modifier (Song *et al.,* 2015). I will also describe recent work in my lab that shed light on how previously unknown genetic networks and/or signaling pathways operate to fine tune grain size in crops.

T4-15: Plant Regeneration

T4-15-01

Epigenetic control of wound-induced cell reprogramming

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During normal development, a vast rearrangement of the epigenetic landscape is correlated with the progression from undifferentiated cells into differentiated cells. This epigenetic reprogramming serves as a barrier, preventing cells to regress from a differentiated state back into an undifferentiated state. Interestingly, many plant species can overcome this barrier after injury to heal wounds and regenerate new organs. Molecular mechanisms controlling this wound-induced organ regeneration remain largely unknown. We have previously identified *WOUND INDUCED DEDIFFERENTIATION1-4* (*WIND1-4*) as central transcriptional regulators of wound-induced cellular reprogramming in *Arabidopsis*. More recently we demonstrated that WIND1 promotes shoot regeneration by directly up-regulating another transcription factor *ENHANCER OF SHOOT REGENERATION1* (*ESR1*). We have also shown that genes in the WIND-mediated pathway need to be epigenetically repressed by POLYCOMB REPRESSIVE COMPLEX 2 (PRC2) to prevent unscheduled cellular reprogramming in intact plants. Currently, we are investigating the epigenetic and transcriptional rearrangements needed to activate the WIND-mediated pathway.

T4-15-02

Type-B *ARABIDOPSIS* RESPONSE REGULATORs is critical to the specification of shoot stem cell niche by dual regulation of *WUSCHEL*

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Plants are known for their capacity to regenerate the whole body under appropriated cultural conditions. During this process, apical meristems are *de novo* formed from a mass of proliferating cells named callus. Exogenous cytokinin and auxin determine cell fate for the establishment of stem cell niche, which is the vital step of shoot regeneration. However, the mechanisms underlying remains elusive. In the present study, we show that type-B ARABIDOPSIS RESPONSE REGULATORs (ARRs), critical components of cytokinin signaling, activate the transcription of *WUSCHEL* (*WUS*), which encodes a key regulator for maintaining the stem cells. In parallel, type-B ARRs inhibit auxin accumulation by repressing the expression of *YUCCA*s, which encode a key enzyme for auxin biosynthesis, indirectly promoting *WUS* induction. Both pathways are essential for *de novo* regeneration of shoot meristems. In addition, the dual regulation of type-B ARRs on *WUS* transcription is required for the maintenance of shoot apical meristem *in planta*. Thus, our results reveal a long-standing missing link between cytokinin signaling and WUS, and the findings provide critical information for understanding cell fate specification.

T4-15-03

Brachypodium tissue culture as a model system to reveal the functions of the components of the cell wall

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Brachypodium Beauv. is believed to be one of the oldest genera within the Poaceae. This genus is predominantly distributed in Europe and Asia with disjunctions occurring in Central America and Southern Africa. *Brachypodium distachyon* (Brachypodium) is a model system for functional genomics in grasses. Although there are some studies of *in vitro* Brachypodium cultures including somatic embryogenesis, detailed knowledge of the composition of the components of the main cell wall in the embryogenic callus in this species is lacking. Therefore, we used histological, scanning electron microscopy as well as the immunocytochemical approach against the arabinogalactan proteins (AGP), extensins and hemicelluloses to understand the localisation and possible functions of these cell wall components during the embryogenic mass that appears in Brachypodium callus. We found that the distribution of pectins, AGPs and hemicelluloses can be used as molecular markers of the embryogenic cells. Furthermore, we showed that AGPs and pectins are components of the extracellular matrix. The presented data extends our knowledge about the chemical composition of the embryogenic cells in the Brachypodium callus.

T4-15-04

Auxin and cytokinin interplay controls *de novo* shoot regeneration *in vitro*

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Shandong University

Exogenous auxin and cytokinin are required for shoot regeneration under appropriate culture conditions. Some of the gene products participating in *in vitro* shoot formation act within an auxin and/or a cytokinin signal transduction pathway. However, the molecular mechanisms underlying the auxin and cytokinin interplay during *in vitro* shoot formation remain unresolved. Here, we show that the *ARR1-IAA17* and *ARF10-ARR15* regulatory cascades mediate phytohormonal interaction and control shoot regeneration in *Arabidopsis thaliana*. On the one hand, higher numbers of SAMs and shoots are generated in the *arr1* mutant than in the wild type, but lower numbers when *ARR1* was over-expressed. *ARR1* is expressed in nascent shoot apical meristems (SAM). ARR1 promotes expression of *IAA17* by binding to its promoter, thereby lessening the explants' responsiveness to auxin. The loss-of-function of *IAA17* promotes shoot formation, and over-expression of the *VP16-IAA17mImII* construct rescues the shoot regeneration defect shown by explants harboring a p*35S::ARR1* transgene. On the other hand, the shoot regeneration is more prolific in a *miR160*-resistant form of *ARF10* (*mARF10*)*,* but fewer in the *arf10* mutant than that in wild type. ARF10 repressed the expression of *Arabidopsis Response Regulator15* (*ARR15*) via its direct binding to the gene's promoter. The loss-of-function of *ARR15* enhanced shoot regeneration and over-expression of *ARR15* rescues the shoot regeneration defect of *mARF10* plants. Our findings define the ARR1 and ARF10 as key regulators of shoot regeneration and shed light on the molecular mechanisms underlying the auxin and cytokinin interplay during *in vitro* shoot formation.

T4-15-05

Identification and characterization of meiotic recombination suppressors in *Arabidopsis thaliana*

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In plants sexual reproduction, meiotic recombination plays a central role in creating genetic variability, making it an important factor for plant breeding in the background of climate change. However, the molecular mechanisms controlling the frequency and patterning of meiotic recombination events are not well understood. Until now, seven meiotic recombination suppressors have been reported that control the minority type class II crossovers. To identify additional regulators, we initiated a forward genetics screen in *Arabidopsis thaliana*. An extensive EMS mutant screen was performed by using the segregation of fluorescent transgene markers in the male reproduction system as a read-out for male meiotic recombination frequency. Among ~12,000 M2 individual plants obtained from self-fertilization of mutagenized M1 generation, a range of mutants with significant higher (281 lines) and lower (72 lines) recombination frequencies have been isolated, including 22 lines showing more than three-fold increase in male meiotic recombination frequency compared to wild type. Most of the mutations are recessive, except for two, which are dominant. Causative mutations are currently being mapped by whole-genome sequencing. Using highly heterozygous Col-0/Ler hybrids that contain the dominant mutation, we found that meiotic recombination frequency increased up to three-fold in both male and female sporogenesis, and this equally spread across the genome. Our study and research could provide insight into the molecular constraints on meiotic recombination processes, but also could help plant breeders to make fuller use of the genetic variability that is available within crop species.

T4-16: Mechanism of hybrid vigor

T4-16-01

Analysis of transcriptional and epigenetic regulation in hybrid vigor of allopolyploid *Brassica napus* uncovers key roles for small RNAs

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Heterosis is a fundamental biological phenomenon characterized by the superior performance of a hybrid compared to its parents. The underlying molecular basis for heterosis, particularly for allopolyploids, remains elusive. In this study, we analyzed the transcriptomes of *Brassica napus* parental lines and their F_1 hybrids at three stages of early flower development. Phenotypically, the F_1 hybrids show remarkable heterosis in silique number and grain yield. Transcriptome analysis revealed that various phytohormone (auxin and salicylic acid) response genes are significantly altered in the F_1 hybrids relative to the parental lines. We also found evidence for previously undescribed decreased expression divergence of the homoeologous gene pairs in the allopolyploid F_1 hybrids and suggest that high-parental expression level dominance plays an important role in heterosis. Small RNA and methylation studies aimed at examining the epigenetic regulation of the gene expression changes in the F_1 hybrids showed that the majority of the small interfering RNA (siRNA) clusters had a higher expression level in the F_1 hybrids than parents, and that there was an increase of the genome-wide DNA methylation in the F_1 hybrid. Transposable elements associated with siRNA clusters had a higher level of methylation and a lower expression level in the F_1 hybrid, implying that the non-additively expressed siRNA clusters resulted in lower activity of the transposable elements through DNA methylation in the hybrid. Our data provide new insights into the role that gene expression pattern changes and epigenetic regulation mechanisms contribute to heterosis during early flower development in allopolyploid *B. napus*.

T4-16-03

Up-regulating the abscisic acid inactivation gene *ZmABA8ox1b* contributes to seed germination heterosis by promoting cell expansion

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Heterosis has been widely used in agriculture, but the underlying molecular principles are still an area for further investigation. Maize F_1 hybrid plants are taller, display increased biomass, grow more rapidly, and have greater yields than their inbred parents. Notably, one of the key aspects of maize heterosis is accelerated seed germination. Here, we reported that the seed germination rate of hybrid B73/Mo17 was significantly faster than that of its parental lines. The T_{50} of parental inbred lines B73 and Mo17 was greatly increased by exogenous ABA treatment compared with the hybrid B73/Mo17, indicating that ABA inactivation plays an important role in seed germination heterosis. Furthermore, we found that the expression level of *ZmABA8ox1b* rapidly increased and displayed an above high parent or high parent expression pattern at the early stage of seed germination. In *Arabidopsis*, the overexpression transgenic lines of *ZmABA8ox1b* showed lower ABA content in dry seeds and faster seed germination rate compared with the wild type. Notably, microscopic observation revealed that cell expansion played a major role in the ABA-mediated maize seed germination heterosis, which could be attributed to the altered expression of cell wall-related genes. Taken together, we propose a simple model for ABA-mediated seed germination heterosis. Briefly, rapid ABA inactivation occurs in hybrids during seed germination due to the up-regulation of *ZmABA8ox1b*. Consequently, the expression levels of genes involved in cell wall loosening and expansion are then altered, which accelerates cell expansion in hybrids and contributes to the observed seed germination heterosis.

T4-16-04

The chromatin remodeler DDM1 regulates hybrid vigor of *Arabidopsis*

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Hybrid vigor, or heterosis, refers to the improved performance of hybrid offspring relative to their parents. In *Arabidopsis*, F1 hybrids, derived from various accession combinations, show obvious growth hybrid vigor in early development stage. Although epigenetic modifications, including small RNA and DNA methylation was altered in F1 hybrid progenies after hybridization between parents, and then RNA-directed DNA methylation (RdDM) is proposed to influence heterosis, we identified that RdDM is not involved in regulation of biomass hybrid vigor of Col and C24 accessions using genetic mutants. However, we uncovered that the chromatin remodeler DECREASED DNA METHYLATION 1 (DDM1) promotes early seedling growth heterosis in Col/C24 hybrids by regulating salicylic acid metabolism, suggesting that stress-induced hormesis may underlie specific hybrid vigor traits. We will investigate the epigenetic mechanisms of DDM1 promoting hybrid vigor associated with salicylic acid metabolism.

T4-16-05

Circadian and epigenetic perspectives on heterosis

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We investigate mechanisms and evolution for advantages and disadvantages of being polyploids and hybrids and their impact on plant breeding and crop improvement. Most crop plants including wheat, cotton, and canola are polyploids, and many other crops such as maize are ancient polyploids and/or are grown as hybrids. Moreover, polyploidy provides a means to permanently fix the heterozygosity and heterosis in interspecific hybrids. Although polyploidy and heterosis have been widely applied in animal and plant production, the molecular bases remain largely elusive. Using *Arabidopsis*, cotton, and corn as experimental systems, we have deployed integrated approaches of genomics, epigenetics, molecular biology, and systems biology to investigate genetic and epigenetic mechanisms for gene expression changes and phenotypic variation in allopolyploids and hybrids. We found a direct link of epigenetic regulation of circadian clock regulators with the increased levels of chlorophyll biosynthesis and starch metabolism in *Arabidopsis* hybrids and allotetraploids. This link of altered circadian rhythms to growth vigor is provocative but logical because circadian rhythms regulate many metabolic and physiological processes in plants and animals. Moreover, the circadian regulation mediates the adaptation of strains to local environments (stress conditions). Under normal conditions, the basal level of stress response is compromised in the hybrids to promote growth vigor, while under stress conditions, the stress response genes are induced at certain times of the day to combat stress as well as to maintain growth vigor. Recent studies also reveal a role for diurnal repression of exogenous ethylene levels and endogenous ethylene signaling in heterosis. In maize hybrids, the clock-protein binding targets are shifted to morning-expressed genes, promoting early activation of photosynthetic and metabolic activities and leading to biomass heterosis. These mechanistic insights into polyploidy and heterosis will ultimately help us improve the production of hybrid and polyploid crops and plants to meet the growing demands for food, feed, fiber, and biomaterials.

T4-16-06

Molecular mechanisms of heterosis for biomass and bacterial defense in plants

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The rapid development of genomics provides excellent opportunities to explore the molecular mechanism of heterosis in plants. We have made efforts to investigate the genome-wide differences in gene expression and epigenetic modifications between hybrid and their parents, and to identify genetic or epigenetic components potentially contributing to heterosis for biomass and disease resistance in plants. Our studies revealed that insertions/deletions (INDELs), many of which result in the formation/disruption of putative *cis*-regulatory elements is one plausible molecular mechanism underlying heterotic gene action and thus heterosis in rice. Epigenetic components, the regulators of chromatin states and genome activity, were found to correlate with transcriptomic variation in hybrids, and thus also have the potential to impact biomass heterosis in rice, maize and *Arabidopsis*. We identified *Arabidopsis* hybrids showing increased resistance to biotrophic bacterial pathogen, and subsequent transcriptomic and genetic analyses demonstrated that enhanced activation of the salicylic acid biosynthesis pathway play a central role in heterosis for bacterial defense in *Arabidopsis*. Furthermore, combining the identification of candidate genes for heterosis by genome-wide association analysis with the comparative transcriptomic analysis indicate the potential contribution of stimulus-responsive and cell cycle regulation pathways to biomass heterosis in *Arabidopsis*.

T4-17: Hormone biosynthesis, metabolism and transportation

T4-17-01

Cytokinin importer PURINE PERMEASE 14 confines the cytokinin responses

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Multicellular organisms operate via the coordination of specialized cellular identities and functions dictated by signalling systems. However, the mechanisms that define the precise spatiotemporal cell domains and subcellular locales responding to specific signals are not well understood. In plants, cytokinins direct essential cell-to-cell signalling systems to initiate and maintain differential cell functions during gametophyte development, embryogenesis and postembryonic shoot and root development. Guided by the live green fluorescent protein (GFP) imaging of the synthetic reporter, *TCSn::GFP* (Two Component signalling Sensor), we illuminate precise cell populations activating conserved phosphorelay signalling circuitry despite much broader intracellular cytokinin signalling competence and potential. Here, we report a surprising finding that *Arabidopsis* PURINE PERMEASE 14 (PUP14) plays a pivotal role in confining the cytokinin signalling response throughout development by ligand sequestration. The expression patterns of PUP14 are inversely correlated with the cytokinin signalling readout, indicating an antagonistic relation. Indeed, the conditional *PUP14* knock-down by an artificial microRNA (*amiRPUP14*) causes ectopic cytokinin signalling accompanied by aberrant morphogenesis in embryos, roots and the shoot apical meristem. PUP14 protein localises to the plasma membrane and imports bioactive cytokinins as shown by analysing transport in mesophyll protoplasts, seedlings and microsomes. These activities of PUP14 reduce the apoplastic cytokinin pools and extracellular cytokinin perception by cytokinin sensors. The relevance of apoplastic cytokinins in initiating intracellular signalling is corroborated by our findings that signalling output is attenuated by secreted cytokinin-degrading enzyme CYTOKININ OXIDASE 2 (CKX2), but not by intracellular CKX variants. These results uncover the existence of a previously unknown and dedicated transport system that patterns the cytokinin signalling landscape of vascular plants.

T4-17-02

Chemical regulation of plant hormone functions and their cross talk

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Plant hormones are biosynthesized and perceived by their receptors and then elicit their activity to regulate plant life cycles. Various steps in the life cycles of plants can be modulated by each of hormones, sometimes in a synergistic fashion, suggesting physiological redundancy and/or crosstalk between the different pathways. As small molecules can be used to unravel these plant hormone functions, many chemicals that can dissect plant hormone functions have been developed and accelerate our understanding of plant hormone signaling. In this context, we developed several chemical regulators for plant hormone functions, such as biosynthesis inhibitors, catabolism inhibitors, receptor inhibitors and mimics. Here, we will talk about the development and/or characterization of known or new chemical regulators mainly for GA and SL from the viewpoint of the reduction of crop damage by root parasitic weeds. 1) AC94377 and D67I is a GID1 agonists that bind GA receptors to activate the GA signa. 2) Debranones are SL agonists. 3) KUT15 is an ethylene mimic that partially activates ethylene signal.These chemicals can control damage by root parasitic weed such as *Striga*. AC94377 can reduce SL production in rice. TIS108 inhibits biosynthesis of SLs without affecting branching in rice. KUT15 can induce suicidal germination of *Striga* and can reduce the seedbank of *Striga*.

T4-17-03

NRT1.1 mediated nitrate suppression of asymmetric root growth relies on PIN2- and AUX1-dependent auxin transport

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Asymmetric root growth (ARG) on tilted plates, in extreme situation root coiling on horizontally-placed plates, is proposed to be a combination of gravitropism, mechanical sensing, and circumnutation, a word designated by Charles Darwin to describe the helical movement that all plant organs make around the growth direction. ARG is a developmentally controlled process in which microtubule-regulating proteins and cell surface proteins such as MLOs participate. Environmental cues, such as nutrient deficiency, also influence ARG.However, it is unclear what nutrients play key roles in regulating ARG that leads to root coiling, what endogenous components are involved in responding to nutrient deficiency for ARG, and how is nutrient deficiency translated into endogenous responses. We report here that nitrate deficiency resulted in a strong ARG in *Arabidopsis*. Nitrate deficiency caused root coiling on horizontal plates, which is inhibited by auxin transport inhibitor, and by mutations at *PIN-FORMED2* (*PIN2*) and *AUXIN RESISTANT 1* (*AUX1*). We further show that suppression of ARG by nitrate is mediated by the nitrate transporter/sensor NRT1.1. Nitrate deficiency and functional loss of *NRT1.1* both resulted in altered membrane dynamics of PIN2 and enhanced asymmetric auxin signaling, leading to root coiling. This study reveals a signaling pathway in root growth by responding to exogenous nitrate and relaying it into intracellular activities through altered auxin transport.

T4-17-04

Long-distance phytohormonal signalling of drought stress

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Plants respond to drying soil by altering their synthesis and metabolism of phytohormones, thereby changing tissue hormone concentrations and xylem sap composition. While most attention has focused on increased ABA (abscisic acid) concentrations as a putative long-distance signal of drying soil, the advent of multi-analyte phytohormone analyses (Albacete *et al.*, 2009) allows the role of other phytohormones as sensors of soil drying to be investigated. When tomato (*Solanum lycopersicum)* plants were grown in a peat-based substrate and automatically irrigated when soil water content decreased below a threshold value, deficit irrigation increased foliar ABA and ethylene concentrations by *circa* 50% compared to well-watered plants. Although the ethylene precursor ACC (1-aminocyclopropane carboxylic acid) was not detected in root xylem sap that was collected at flow rates matching *in vivo* transpiration, xylem ABA concentration approximately doubled while xylem JA (jasmonic acid) concentrations increased

5-fold. When water was withheld from tomato (*Solanum lycopersicum)* plants grown in a mineral substrate at two different bulk densities (1.4 *versus* 1.74 g cm^{-3}), soil-drying-induced changes in root xylem sap composition were accentuated in plants grown at low bulk density, due to greater soil drying. At the same soil water content, high bulk density increased xylem ABA concentrations. While soil drying increased xylem ABA concentration compared to well-watered plants, xylem JA and cytokinin (trans-zeatin, tZ and isopentenyl adenine, iP) concentrations did not change. When water was withheld from pea (*Pisum sativum)* plants grown in a mineral substrate, there were multiple changes in phytohormone concentration of expanding stem internodal tissue. Soil drying progressively increased stem ABA, ACC, iP and tZ levels, reaching concentrations that were 76-fold, 2.4-fold, 3.4-fold and 2-fold higher compared to well-watered plants respectively, at the time that stem elongation ceased. Moreover, soil drying progressively decreased stem GA1 (gibberellic acid 1) and IAA (indole-3-acetic acid) concentrations by 97% and 80% respectively. Whether these changes in xylem sap and tissue phytohormone concentrations are due to local or long- distance processes will be investigated by self- and reciprocal-grafting of mutants that are deficient in or overexpress selected phytohormone groups.

T4-18: Flavonoid biosynthesis and regulation

T4-18-01

Phenylalanine biosynthetic network: What's left to know?

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In plants, up to 30% of photosynthetically fixed carbon is directed towards formation of aromatic amino acids (AAAs) and their downstream products, making this biosynthetic network one of the most metabolically intensive. Within the AAA network, the highest carbon flux is directed to phenylalanine (Phe), a common precursor of >8000 phenolic compounds, that have profound impacts on plant growth, development, reproduction and defense and constitute approximately 30-45% of plant organic matter. Phe biosynthesis in plants occurs via two alternative routes with either arogenate or phenylpyruvate as a key intermediate. Using a functional genomics approach and petunia flowers, which emit high levels of Phe-derived volatiles as a model system, we have identified genes encoding proteins involved in the arogenate and phenylpyruvate Phe biosynthetic pathways as well as in Phe export from plastids. We have shown that, while plants predominantly synthesize Phe in plastids via the arogenate pathway, the microbial-like phenylpyruvate pathway also contributes to Phe formation. Moreover, flux through the phenylpyruvate route increases when the entry point to the arogenate pathway is limiting. Interestingly, the phenylpyruvate pathway utilizes a cytosolic aminotransferase that strongly favors Tyr as the amino donor. Taken together, our results demonstrate that Phe biosynthesis is not limited to plastids and that there is an interconnection between aromatic amino acid catabolism and biosynthesis *in planta*. This presentation will also discuss the plasticity and complex regulation of Phe and Tyr biosynthetic pathways *in planta*. Another aspect of plant metabolism to be discussed is its compartmentalization and metabolite transport within the cell and how this intracellular transport contributes to the interconnections between and within metabolic networks.

T4-18-02

Flavonoid biosynthesis in pollen: Functional identification of glycosyltransferases responsible for pollen-specific flavonols

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Flavonoids, including flavonols, flavones, anthocyanins and proanthocyanidins, are the most intensely studied plant secondary metabolites and over 9,000 known compounds are widely distributed throughout plant kingdom. Flavonoids play important roles as UV protectants, pigments, attractants of pollinators, phytoallexins, signaling molecules and regulators of fertility and auxin transport. The huge chemical diversity of flavonoids and the intricate distribution patterns in plant tissues and species make it difficult to correlate specific flavonoid structures with their physiological functions. As one of a few exceptions, a relationship between pollen-specific flavonols and pollen fertility is well established in petunia (*Petunia hybrida*) and maize (*Zea mays*) . Pollens of flavonoid-deficient mutants of petunia are unable to germinate, resulting in male sterility. Interestingly, *Arabidopsis* flavonoid-deficient mutants are fertile although petunia and *Arabidopsis* accumulated relatively similar flavonol 3-*O*-diglycosides in pollen. To complete the flavonoid biosynthetic map in pollen of *Arabidopsis* and *petunia*, we identified genes encoding flavonoid glycosyltransferases, *UGT79B6* and *UGT79B31*, responsible for terminal modification of pollen-specific flavonoids in *Arabidopsis* and *petunia*, respectively. *In vitro* characterization of the recombinant proteins and functional complementation of *Arabidopsis* mutants which lacked flavonoid diglycosides, indicated that *UGT79B6* and *UGT79B31* encode flavonoid 3-*O*-glycoside: 2"-*O*-glucosyltransferase. We also discuss the localization of UGT79B6 and UGT79B31 and roles of pollen-specific flavonols.

T4-18-03

Flavone biosynthesis in rice: different biosynthetic routes for *C*-linked and *O*-linked conjugates

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Flavones are flavonoids found extensively in land plants with important physiological functions like UV protection, interactions with other organisms, co-pigmentation in flowers, *etc*. They are increasingly popular as dietary constituents or supplements due to their health-beneficial properties. In grasses, flavones are predominantly accumulated as C- or O-linked conjugates in vegetative tissues. The enzymology of flavone biosynthesis in monocot remained largely elusive until recent years. Our recent work established the cytochrome P450 enzymes CYP93G1 and CYP93G2 as key branch point enzymes in rice channeling flavanones to the formation of flavone O-linked conjugates and C-glycosides, respectively. CYP93G1 functions as a flavone synthase II, which generates flavone aglycones for different O-linked modifications.

On the other hand, CYP93G2 is a flavanone 2-hydroxylase, which produces 2-hydroxyflavanones for immediate C-glycosylation, followed by the formation of the flavone nucleus. We further filled the remaining gap in the tricin biosynthesis pathway with CYP75B4. In tricin, the 3’, 5’-dimethoxyflavone nucleus is formed before O-linked conjugations. CYP75B4 functions as a unique flavonoid 5’-hydroxylase that converts chrysoeriol to selgin, the immediate precursor of tricin. CYP93G1, CYP93G2, and CYP75B4 homologous sequences are highly conserved in Poaceae, suggesting that they are specifically recruited for the biosynthesis of different flavone-derived metabolites that are prevalent in many grass species today. They also represent good targets for engineering flavones in edible tissues.

T4-18-04

Regulation of flavonoid biosynthesis by UV-B photoreceptor pathway

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Flavonoid biosynthesis is dependent on light. In dark, flavonoid biosynthesis is off. Light induces flavonoid biosynthesis through activation of several transcription factors including HY5, MYB11, MYB12 and MYB111 *etc*. These transcription factors directly bind to promoter regions of genes in flavonoid biosynthesis pathway including *CHS*. Among different light spectra, Ultraviolet-B (UV-B; 280-315nm) is most effective in flavonoid biosynthesis induction. Interestingly, accumulation of flavonoids in plant epidermal tissues can absorb UV-B and protect inner tissues from UV-B damage. Thus, flavonoids can serve as “suncream” for plants.

With the characterization of UV-B photoreceptor UV RESISTANCE LOCUS 8 (UVR8) and its downstream signaling components including CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) and HY5, we can now draw a clearer picture from UV-B perception to flavonoid biosynthesis induction. I will present the recent progress from early UV-B signaling biochemical events to downstream flavonoid biosynthesis.

T4-18-05

Arogenate dehydratase isoforms differentially regulate anthocyanin biosynthesis in *Arabidopsis thaliana*

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Anthocyanins, a group of L-phenylalanine (Phe)-derived flavonoids, have been demonstrated to play important roles in plant stress resistance and interactions between plants and insects. Although the anthocyanin biosynthetic pathway and its regulatory mechanisms have been extensively studied, it remains unclear whether the level of Phe supply affects anthocyanin biosynthesis. Here, we investigated the roles of arogenate dehydratases (ADTs), the key enzymes that catalyze the conversion of arogenate into Phe, in sucrose-induced anthocyanin biosynthesis in *Arabidopsis*. Genetic analysis showed that all six ADT isoforms function redundantly in anthocyanin biosynthesis but have differential contributions. ADT2 contributes the most to anthocyanin accumulation, followed by ADT1 and ADT3, and ADT4-ADT6. We found that anthocyanin content is positively correlated with the levels of Phe and sucrose-induced ADT transcripts in seedlings. Consistently, addition of Phe to the medium could dramatically increase anthocyanin content in the wild-type plants and rescue the phenotype of the *adt1 adt3* double mutant regarding the anthocyanin accumulation. Moreover, transgenic plants overexpressing *ADT4*, which appears to be less sensitive to Phe than overexpression of *ADT2*, hyperaccumulate Phe and produce elevated level of anthocyanins. Taken together, our results suggest that the level of Phe is an important regulatory factor for sustaining anthocyanin biosynthesis.

T4-18-06

Key aspects of flavonoid pathway regulation are conserved between angiosperms and liverworts

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Flavonoids are ubiquitous in extant land plants. The pathway is hypothesized to have evolved for protection against UVB radiation during land colonization around 500 million years ago. In angiosperms, an R2R3MYB-bHLH-WDR (MBW) transcriptional complex controls flavonoid production. Our research on dicot (petunia, antirrhinum) and monocot (onion, cymbidium orchid) species has contributed to the current model for the action of the MBW complex, which proposes that the R2R3MYBs are the key components for environmental and developmental regulatory responses in both leaves and flowers. We have also found that R2R3MYB and R3MYB transcriptional repressors are important for modulating the strength of pathway activation and defining spatial activation domains. However, it is an open question whether the MBW complex for flavonoid regulation is a universal system in land plants, as data are lacking from outside of the angiosperms. As an early diverging lineage of land plants, the liverworts (e.g. *Marchantia* sp.) can help to inform us on systems that have an early evolutionary origin. We are using the model liverwort *Marchantia polymorpha* for our studies. Marchantia produces flavones, a key UV-induced flavonoid of many angiosperms, and a red pigment (ricciodinin) thought to be related to anthocyanins. We used RNAseq analysis of UVB-treated plants and Blast interrogation of transcriptome and genome resources to identify biosynthetic and regulatory gene candidates for the marchantia flavonoid pathway. These were then characterized using transgenic over-expression and CRISPR mutagenesis, combined with tests of the environmental signals that might control flavonoid production in marchantia. The studies revealed that an R2R3MYB gene was a key regulator of flavonoid production. Knockout lines for the MYB had greatly reduced flavonoid levels and lost all red pigmentation, while over-expression conferred production of large amounts of flavonoids, activation of the biosynthetic genes, and constitutive red pigmentation. This suggests that R2R3MYB regulated flavonoid production is a conserved character across land plants and arose early during land colonization.

T4-19: Terpenoid biosynthesis and regulation

T4-19-01

Genome mining for sesterterpenoid discovery in Brassicaceae *plants*

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Terpenoids represent the largest and most diverse class of plant specialized metabolites, with diverse physiological functions during plant development. In the biosynthesis of terpenoids, short-chain isoprenyl diphosphate synthases (SC-IDS) account for the majority biosynthesis of linear isoprenyl pyrophosphates, which are the direct precursors for terpenoids. *Arabidopsis thaliana* possess a group of SC-IDSs (GGPPS-like proteins, which were supposed to mainly produce geranylgeranyl diphosphate) whose functions are not clearly known. In this study, we found the neo-functionalization of Arabidopsis GGPPS-like gene family: GGPPS6,7, 9 and 10 have GFPPS activities (the predominant product is geranylfarnesyl diphosphate (C25)) and GGPPS8 produces even longer chain isoprenyl diphosphate (>C25). By solving the crystal structures of GGPPS7 (C25), GGPPS8 (>C25) and GGPPS11 (C20), we disclose the product chain-length determination mechanism and describe it as a “three floor” model. Using this model, we discovered the novel GFPPS in various Brassicaceae species. Genome mining further reveal that these Brassicaceae *GFPPS* genes are usually linked to terpene synthase gene and *P450* genes. The *GFPPS-TPS-P450* gene clusters for sesterterpenoids biosynthesis were functional identified and the potential biological functions of these sesterterpenoids in Brassicaceae plants are also discussed.

T4-19-02

Molecular diversity of terpene synthases in the liverwort *Marchantia polymorpha*

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Marchantia polymorpha is a primitive terrestrial land plant, which like most liverworts accumulates a structurally diverse array of terpenes believed to serve in deterring disease and herbivory. Previous studies have suggested that the mevalonate and methylerythritol phosphate pathways, present in evolutionarily advanced plants, are also operative in liverworts. However, the genes and enzymes responsible for the chemical diversity of terpenes have yet to be described. In the current study, we resorted to a HMMER search tool to identify 17 putative terpene synthase genes from *M. polymorpha* transcriptomes. Functional characterization identified 4 diterpene synthase genes phylogenetically related to those found in higher plants and 9 rather unusual monoterpene and sesquiterpene synthase-like genes. The presence of separate mono-functional diterpene synthases for ent-CPP and *ent*-kaurene biosynthesis are similar to orthologs found in vascular plants, pushing back the date of the underlying gene duplication and neofunctionalization of the ancestral diterpene synthase gene family >400 million years ago. In contrast, the mono- and sesqui-terpene synthases represent a new class of enzymes, not related to plant terpene synthases and only distantly so to microbial-type terpene synthases. The absence of a Mg^{2+} binding, aspartate-rich, DDXXD motif place these enzymes in a new, non-canonical family of terpene synthases.

T4-19-03

Triterpenoid saponins in a wild crucifers - biosynthesis, evolution and structure activity relationships in plant-insect interactions

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It is well known that plants produce specialized metabolites for defense, yet we know little of how they actually evolved. Certain classes of chemical defense compounds have evolved repeatedly in plants, facilitated by recruitment of genes from a few old gene families. This was probably mediated by gene duplications, either locally or by whole-genome duplications, followed by selection on their enzyme substrate and product specificity. The initial assumption that divergent evolution is the major player is being challenged, and it appears that convergent evolution is surprisingly common in specialised metabolite pathways (Pichersky and Lewinsohn 2011). Triterpenoid saponins comprise a highly diverse group of specialised metabolites, derived from 2,3-oxidosqualene, the precursor for sterols in most eukaryotes. They are widespread in different plants families suggesting that their biosynthesis has evolved convergently. We have developed the wild crucifer *Barbarea vulgaris* as a model system to study the biosynthesis of triterpenoid saponins, the genome localization of the genes involved in the pathway, and reconstituted the pathway in tobacco leaves. Structure-activity relationships studies using insects, such as the crucifer pest Diamond back moth, begin to unravel which chemical features of a saponins influences toxicity to specific insects.

T4-19-04

Functional analysis of a promiscuous oxidosqualene cyclases in rice

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Triterpene synthases, also known as 2,3-oxidosqualene cyclases (OSCs), synthesize diverse triterpene skeletons for the biosynthesis of both essential sterols and also a large number of functionally divergent triterpenoids. These complex tetracyclic and pentacyclic triterpenes are generated by OSCs that can use one of two original carbocations, the prosteryl and dammarenyl cations which adopt chair-boat-chair (C-B-C) and chair-chair-chair (C-C-C) conformations, respectively. Here we report a promiscuous OSC (named OsOS) in rice that produces a pentacyclic triterpene orysatinol having an unprecedented conformation derived from an unknown orysatinyl cation adopting a novel C-C-C conformation different from that of the dammarenyl cation. Phylogenetic and structure-based functional analyses revealed I732, L365, L124 of OsOS are three key amino acids driving the formation of orysatinyl cation, which upon mutagenesis, led to complete conformational switch from orysatinyl C-C-C to prosteryl C-B-C cation which yielded end product parkeol. Our results not only elucidate the detailed process by which OsPS is likely to have functionally evolved from OsOS, but also provide fundamental insights and mechanistic clues about generation of triterpene diversity in nature.

T4-19-05

Applications of plant terpenoids in the synthesis of colloidal silver nanoparticles

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Green chemistry is the design of chemical products and processes that reduce or eliminate the generation of hazardous substances. Since the last few years, natural products especially plant secondary metabolites have been extensively explored for their potency to synthesize silver nanoparticles (AgNPs). The plant-based AgNPs are safer, energy efficient, eco-friendly, and less toxic than chemically synthesized counterparts. The secondary metabolites, ubiquitously found in plants especially the terpenoid-rich essential oils, have a significant role in AgNPs synthesis. Terpenoids belong to the largest family of natural products and are found in all kinds of organisms. Their involvement in the synthesis of plant-based AgNPs has got much attention in the recent years. The current article is not meant to provide an exhaustive overview of green synthesis of nanoparticles, but to present the pertinent role of plant terpenoids in the biosynthesis of AgNPs, as capping and reducing agents for development of uniform size and shape AgNPs. An emphasis on the important role of FTIR in the identification and elucidation of major functional groups in terpenoids for AgNPs synthesis has also been reviewed in this manuscript. It was found that no such article is available that has discussed the role of plant terpenoids in the green synthesis of AgNPs.

T4-20: Plant response to drought and salt (two sessions: session 1, drought stress; session2, salt stress)

T4-20-01

Regulation of SOS pathway by 14-3-3 proteins

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The Salt Overly Sensitive (SOS) pathway regulates intracellular sodium ion (Na$^+$) homeostasis and salt tolerance in plants. In this pathway, a salt stress-induced transient increase in cytosolic calcium activates two calcium sensors, SOS3 and SOS3-Like Calcium Binding Protein 8, which interact with and activate the SOS2 protein kinase. The SOS3/SCaBP8-SOS2 complex then activates SOS1, a plasma membrane Na$^+$/H$^+$ antiporter which transports sodium from the cytosol. So far, the mechanisms that control inhibition of the SOS pathway when plants are grown in the absence of salt stress have remained elusive. We demonstrate that the λ and κ 14-3-3 proteins interact with SOS2 and repress its kinase activity. 14-3-3 λ interacts with the SOS2 junction domain, which has previously been shown to be important for its kinase activity. A phosphorylation site (Ser^{294}) was identified within this domain by mass spectrometry. Mutation of Ser^{294} to Alanine (A) or Aspartate (D) did not affect SOS2 kinase activity in the absence of 14-3-3s. However, the S/A mutation decreased the 14-3-3-dependent inhibition of activity, while the S/D mutation increased this inhibitory effect. SOS2^{S294A} rescued the salt-sensitive phenotype of the *sos2-2* mutant to wild-type levels while SOS2^{S294D} did not. Moreover, we find that anther protein kinase phosphorylates the SOS2 Ser^{294} site in normal condition. Upon salt stress, this kinase activity is repressed by the 14-3-3 proteins, which in turn releases the SOS2 activity for activating SOS1. These results identify 14-3-3 proteins as important regulators of salt tolerance and uncover their role in a mechanism that confers basal repression of the SOS pathway in the absence of salt stress.

T4-20-02

Nuclear-localized SOS3 stabilizes GIGANTEA to ensure flowering under salt stress

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The timing of flowering under stress environments is critical for plants to secure reproductive success, and salt stress delays flowering time in *Arabidopsis*. In normal growth conditions, the circadian and photoperiodic flowering time regulator GIGANTEA (GI) makes a complex with and inhibits the salt-tolerance kinase SOS2 (CIPK24). Salt stress causes the degradation of GI protein by the proteasome and the release of SOS2, which is then free to interact with the Ca^{2+}-dependent protein SOS3 (CBL4) to activate the Na$^+$ efflux protein SOS1 and mount successful adaptation to the saline environment. Degradation of GI delays but does not abrogate flowering in a saline environment. Here we show that SOS3, a regulator of SOS2, also makes a complex with GI in the nucleus to ensure the expression of the flowering activator *CONSTANS* (*CO*) under salt stress. Palmitoylation-dependent nuclear import of SOS3 stabilizes GI in the nucleus to proceed with flowering, while degradation of cytosolic GI releases the protein kinase SOS2 to achieve salt tolerance. Thus, SOS3 is a Ca^{2+}- and palmitoylation-dependent molecular switch required to ensure flowering is a saline environment by selectively stabilizing GI in the nucleus.

T4-20-03

Coordinated regulation of potassium uptake and storage in *Arabidopsis*

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Potassium (K) is an essential nutrient for all living organisms, but in plants K reaches organismal contents of up to 10% of dry weight. The ability of plants for K uptake and storage is critical for stress tolerance and a key issue in agricultural practices. While cytosolic K is kept at homeostatic concentrations close to 100 mM, surplus K is stored in cell vacuoles in large quantities. An array of K transporters and regulators (protein kinases and phosphatases, interacting proteins, diffusive chemical messengers) have been identified as the basic elements underpinning K fluxes at the plasma membrane and tonoplast, but the regulatory networks involved in controlling K homeostasis in individual cells and whole organisms remain largely unknown. Current evidence indicates that active K uptake (i.e., against the electrochemical K gradient) involves K-H symporters at the plasma membrane (HAK, High-Affinity K transporters) and K/H antiporters (NHX) at the tonoplast. These transporters, together with K-selective channels that fine-tune the membrane electrical potential by K uptake and release, share common regulators that are effectors of K nutrition. We have shown that the Ca^{2+} sensors Calcineurin B-Like CBL1, CBL8, CBL9, and CBL10, together with CBL-Interacting Protein Kinase 23 (CIPK23) activated HAK5 in vivo. CIPK23 is also known to regulate the root specific K-inward channel AKT1. Measurement of K^+ (Rb^+) uptake and growth rate in low-K^+ medium with Arabidopsis mutants bearing mutations *hak5*, *akt1* and *cipk23* in various combinations confirmed the key regulatory role of CIPK23 in K acquisition in planta. Cytosolic K is trafficked further to the vacuolar lumen and the tonoplast-localized K/H exchangers of the NHX family are pivotal to the vacuolar accumulation of K. Hypomorphic and loss-of-function double mutants *nhx1 nhx2* of Arabidopsis had impaired K acquisition, failed to osmoregulate and were unable to create sufficient turgor for cellular expansion. Moreover, they had defective stomatal opening and closure responses. Disruption of K^+ accumulation in guard cells correlated with more acidic vacuoles and the disappearance of the highly dynamic remodeling of vacuolar structure associated with stomatal movements. Notably, vacuolar NHX proteins are also regulated by CIPKs, which are emerging as central regulators of K transport processes occurring at various cells membranes and plant organs.

T4-20-04

High-throughput screening tools for identification of traits contributing to salinity tolerance

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Non-invasive capturing and interpreting of plant structural and functional phenotypes in controlled or dynamically changing environment is long-standing and necessary requirement for genetic and physiological research by crop breeders, agricultural industry, and academia. To sustain global food security the major challenge global agriculture and plant biology field has been facing is the identification of new high-yielding genotypes of agricultural crops that are adapted to our future climate. Soil salinity is one of the main stress factors that are severely affecting the agriculture land in global scale and results in significant reduction of plant growth and yield. It was shown that plants suffer a rapid growth reduction upon the first exposure of their roots to salt stress, which is occurring prior to the accumulation of ions to toxic concentrations in the shoots. During this early phase, symptoms of growth reduction include slower leaf emergence and a small growth size. The phenotypic traits associated with this type of tolerance can be quantified in the days immediately after imposition of stress using non-destructive image-based phenotyping. To enhance our understanding of the early responses to salinity, we designed an experimental protocol based on using high-throughput and non-invasive imaging technologies developed at Photon Systems Instruments (PSI, Czech Republic). The methodology presented is based on automated integrative analysis of photosynthetic performance, growth analysis and color index analysis at the onset and early phase of salinity stress response of *Arabidopsis thaliana* ecotypes grown in soil. Here we show that stress imposition significantly and rapidly affected photosystem II operating efficiency, subsequently impacted growth dynamics and greening index of Arabidopsis plants at different stages of stress response. Our work provides quantitative insights into early phase of salinity response and provides robust protocol for high-throughput image-based analysis of phenotypic traits associated with this early phase of salinity response. We show that the integrative concept of PlantScreen^{TM} high-throughput phenotyping platform provides a powerful tool for acquisition and selection of morphological, physiological and biochemical parameters, which can be used for identification of various components underlying early plant responses to various environmental conditions.

T4-20-05

Regulation of alternative polyadenylation in *Arabidopsis thaliana* response to salt stress

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Alternative polyadenylation (APA) is an important post-transcriptional regulatory process during eukaryotic gene expression. It has been reported to be involved in abiotic stress such as oxidative stress. However, the roles of alternative polyadenylation in plant response to salt stress remains elusive. In this study, genome-wide studies of polyadenylation sites have been carried out in *Arabidopsis thaliana* seedlings treated with 200mM NaCl using the poly (A) tag sequencing (PAT-seq) approach. We found that a large number of genes were alternative polyadenylated under salt stress and the ratio of genes having more than two poly (A) sites increased. Genomic distributions of poly (A) sites seen only in salt stress condition, unstressed condition and common were quite different, suggesting the genes 3'-end formation pattern being changed under salt stress. We claimed that most of the differential expression (DE) APA genes were also differently expressed genes, which means that APA may cooperated with gene expression regulating in response to salt stress. Besides that, the group of differential expression (DE) APA genes were enriched in salt stress response and plant hormone signal transduction pathway, revealing the effect of APA on salt and hormone response genes. Morever, a dozen of transcription factors and salt response genes were exclusively possessed by APA under salt stress, unveiling a complex loop between APA and transcriptional regulation. Taken together, our study provided new insights into the potential roles of APA in plant response to salt stress.

T4-20-06

Possible roles of RING E3 ubiquitin ligases in drought and proteotoxic stress responses in *Arabidopsis*

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Ubiquitination is implicated in diverse cellular processes in higher plants. Arabidopsis contains at least 477 RING-motif containing E3 ubiquitin (Ub) ligases. *AtAIRP1* and *AtAIRP2* encode cytosolic C3H2C3- and C3HC4-type RING E3 ligases, respectively, whose expressions were induced by ABA and dehydration stress. Both *AtAIRP1*- and *AtAIRP2*-overexpressing transgenic plants exhibited hypersensitive phenotypes to ABA in terms of stomatal movement. They were highly tolerant to severe drought stress. In contrast, *atairp1* and *atairp2* loss-of-function mutants showed opposite phenotypes in response to ABA and drought stress. Constitutive expression of *AtAIRP1* and *AtAIRP2* in *atairp2* and *atairp1* mutant plants, respectively, reciprocally rescued the loss-of-function ABA-insensitive and drought-hypersensitive phenotypes of the mutant plants. These results suggest that AtAIRP1 plays combinatory roles with AtAIRP2 in ABA-mediated drought stress responses in Arabidopsis. We identified that over-expression of *MPRR1* (Misfolded Protein Responsive RING 1) resulted in the markedly increased tolerance to AZC and arsenate treatments, both of which induced cytosolic proteotoxic stress, in Arabidopsis. Possible role of MPRR1 E3 ligase in response to proteotoxic damage will be discussed.

T4-20-07

Reactive oxygen species in the regulation of stomatal movements

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Water availability is the most limiting factor for agricultural production and insufficient water supply can cause major reductions in crop yields. Guard cells that form stomatal pores regulate plant water balance since plants with more open stomata allow faster water evaporation. Assimilation of CO_2 requires stomatal opening, but also opens the gates for excessive water loss and consequently, several environmental factors, such as light intensity and quality, soil and air water content, and CO_2 regulate stomatal aperture. Thus, guard cells sense and respond to diverse stimuli optimizing photosynthetic carbon dioxide uptake with minimal water loss. Stomatal closure is controlled by a complex network of signaling compounds, including the drought phytohormone abscisic acid (ABA), rise in cytoplasmic calcium (Ca^{2+}) and reactive oxygen species (ROS). ROS are used by the plant as secondary messengers in several abiotic stresses, such as drought, and salt. In responses to these stresses regulation of stomatal aperture requires coordinated activity of ROS-generating enzymes, signaling proteins and downstream executors such as ion pumps, transporters and plasma membrane ion channels that control guard cell turgor pressure. Accumulation of reactive oxygen species (ROS) in the apoplast and chloroplasts is among the earliest hallmarks of stomatal closure. Subsequent increase in cytoplasmic Ca^{2+} concentration governs the activity of multiple kinases that regulate the activity of ROS-producing enzymes and ion channels. In parallel, ROS directly regulate the activity of multiple proteins via oxidative posttranslational modifications to fine-tune guard cell signaling. I will describe recent advances in the role of ROS in stomatal closure as a response to water limitation and discuss the importance of ROS in regulation of signal amplification and specificity in guard cells. Detailed knowledge of how a specific components are employed in constitutive and inducible complexes in response to a specific stimuli, such as drought, at a given time and location will be required for full understanding of the mechanisms underlying stomatal responses and can be used to identify components that regulate plant water use efficiency in the shifting global climate.

T4-20-08

Regulation of clade A type PP2Cs in ABA signaling

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The phytohormone abscisic acid (ABA) is quickly accumulated under drought stress, which plays crucial roles in plant development and responses to abiotic and biotic stresses. The accumulated ABA is perceived by PYR1/PYL/RCAR ABA receptors that then interact with and inhibit the clade A protein phosphatases 2Cs (PP2Cs), and release their inhibition on the downstream protein kinases such as CDPKs, SnRKs and GHR1. Thus the core negative regulation PP2Cs play crucial roles in transducing ABA signaling. However, besides ABA receptors, how other factors regulate PP2Cs is not well known. Recently, We found that one of PP2c members, ABI1 (ABA INSENSITIVE1) can be degraded by PUB12/13 E3 ligases only after it interacts with PYR1/PYL/RCAR ABA receptors either in presence of ABA or no ABA. Furthermore, we found that EAR1 (Enhancer of ABA co-Receptor 1), an uncharacterized protein, can interact with N-termini of 6 members of the clade A type PP2Cs and enhance the PP2Cs activities. Removing N-termini of PP2Cs releases their autoinhibition, but EAR1 does not add any enhancement on their PP2C activities. These studies highlights the novel paradigms for regulation of the PP2C activities in *Arabidopsis*.

T4-20-09

The extremophyte *Schrenkiella parvula* as an emerging model to study salt stress adaptations

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Schrenkilla parvula (*Syn Thellungiella parvula)*, a close relative of *Arabidopsis thaliana* and *Brassica* crops, thrives in highly saline soils. It is also drought and cold tolerant as adaptations that mark its lifestyle in the dry and extreme weathers of salt flats in Central Anatolia, Turkey. *S. parvula* compared to *A. thaliana*, presents a unique system to identify naturally selected physiological traits, developmental responses, and genome reorganization behind adaptations to salt stress. Despite the stark differences in adaptations to extreme salt stresses, *S. parvula* and *A. thaliana* show extensive genome-wide macrosynteny. However, explicit genome structural variations, including tandem gene duplications, gene transpositions, and transposable element insertions interrupt the co-linearity observed throughout the genomes and distinguish *S. parvula* from *A. thaliana* and other closely related non-halophytic species. Comparative physiological observations on *S. parvula* support the comparative transcriptomic studies between *S. parvula* and *A. thaliana* that highlight the stress-prepared responses of *S. parvula* even under stress neutral conditions. These studies have led to the identification of key physiological and developmental traits in *S. parvula*, and the gene families involved in ion transport and other metabolic pathways associated with salt stress responses. Genomic structural variants appeared to have led the two species into two distinct lifestyle trajectories by their enrichment in significantly differently expressed homologous genes in root and shoot transcriptomes under neutral and varying salt stress conditions. Furthermore, we observed tissue-specific isoform expression contributing to species-specific salt stress responses. The extremophyte genome of *S. parvula* acts as a repository of genetic changes that have enabled its successful niche adaptation to a hyper-saline environment. Our genomic and transcriptomic dissection offers a framework to identify adjustments of genome architecture and expression that control a set of genes regulating fundamental physiological responses in salt stress adaptations.

T4-20-10

Origin and evolution of the plant succulent syndrome in arid regions of the world

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Of all the major climatic groups, arid climates are the most extensively distributed over the land surface of the earth. Ecosystems in arid environments have low precipitation, highly variable and random, occurring in infrequent discrete events, making water availability the dominant factor controlling biological processes. It is estimated that arid biomes appeared recently, being in most cases no older than the Miocene/Pliocene, and the rapid origin of these environments created the selective pressure under which several plants evolved a diversity of strategies and specialized structures to withstand periods of severe drought. Some drought-adapted lineages went through the most dramatic evolutionary modifications in the plant kingdom by developing extremely modified organs and tissues to store water, i.e. succulence. Some of the succulent lineages are currently the most species rich and structurally diverse plant families on earth. Current studies project increases in the frequency and intensity of drought related to global climate change. Thus, understanding the details of the diversification mechanisms of succulent plants in arid environments could help us develop better predictions about how fast can plants adapt to increasingly harsh conditions, and how they may respond to an even warmer environment in the near future. Moreover, understanding the evolution of the anatomical, physiological and morphological adaptations of these plants, and especially the genetic bases of these adaptations, may provide information that could lead to the development of genetic tools to improve food crops and generate bioenergy for the future. Recent estimates indicate that there are ~12,500 species of succulents, distributed among 690 genera and 83 families. The fact that lineages with the succulent syndrome independently evolved convergent solutions to the problem of water scarcity could indicate that there is a parallel adaptive response to a global trend. It has been proposed that succulent families originated contemporaneously, in response to a global aridification trend and a global scenario of a decline in atmospheric CO_2 concentrations. Here I discuss the most recent knowledge about the morphological and anatomical adaptations of succulents to water scarcity, as well as current knowledge about the genetic and molecular bases of these adaptations. I present estimated dates of origin of major succulent lineages, and discuss the hypothesis of a synchronic origin in response to a global aridification trend. I present data on the diversification patterns of major succulent lineages in different arid regions of the world, and discuss the possible drivers of their diversification. Finally, I discuss the possible outcome of succulent plants under a global warming scenario and the possibility of using information about the origin and evolution of the succulent syndrome to design strategies to understand and improve the future outcome of food crops.

T4-20-11

Revealing the causal relationship between salinity and oxidative stress tolerance in wheat and barley

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Drought and salinity are two major abiotic stresses limiting crop production worldwide. Both stresses result in an increased production and accumulation of reactive oxygen species (ROS), causing oxidative stress. The latter disturbs the metabolic balance of the cells and causes damage to major cell structures. ROS also activate several different types of ion channels affecting intracellular ionic homeostasis and thus plant's ability to adapt to adverse environmental conditions. At the same time, more moderate quantities of ROS are essential for a range of signalling pathways mediating a diverse range of processes such as plant growth, development, gravitropism, or hormonal action. Thus, the causal link between ROS production and stress tolerance is not as straightforward as one may expect. In this work, we used non-invasive microelectrode ion flux estimation (the MIFE) technique to establish a causal relationship between salinity and oxidative stress tolerance in two cereal crops: barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*). By screening 80 different genotypes we show that exogenous application of H_2O_2 to 3d hydroponically-grown roots results in massive, dose-dependent K^+ efflux and Ca^{2+} influx in both elongation and mature zones of root epidermis. A strong positive correlation was found between H_2O_2-induced K^+ efflux and overall salinity tolerance in both species in mature root epidermis. The same positive trend was also found in Ca^{2+} influx. Pharmacological experiments suggested that non-selective cation channels (NSCCs) mediated such Ca^{2+} influx and played a major role in controlling K^+ efflux in both species. The above trends were not applicable to cells in the elongation root zone, indicating high tissue specificity of ion flux responses to ROS. Consistent with H_2O_2 data for mature zone, application of a hydroxyl radical ($\cdot OH$)-generating Cu^{2+}/ascorbate (Cu/A) mixture did not reveal any clear correlation between K^+ and Ca^{2+} flux responses and an overall salinity tolerance. Implication of these findings for plant breeding for salinity stress tolerance are discussed.

T4-20-12

Genome-wide association study of drought tolerance and gene cloning in maize seedlings

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Maize production is frequently threatened by drought stress on a global-scale. Identification of the genetic components underlying the drought tolerance in maize is of great importance. Here, we report a genome-wide association study (GWAS) of maize drought tolerance at the seedling stage that identified 83 genetic variations which were resolved to 42 candidate genes. Firstly, a Miniature Inverted-repeat Transposable Element (MITE) inserted in the promoter of a *NAC* gene (*ZmNAC111*) was found to be significantly associated with the natural variation in maize drought tolerance. We discovered that the 82-bp MITE represses *ZmNAC111* expression via RNA-directed DNA methylation and H3K9 dimethylation. Increasing *ZmNAC111* expression in transgenic maize conferred enhanced drought tolerance by improving water use efficiency and upregulation of drought-responsive genes under water stress. Secondly, the peak signal of GWAS uncovered that the natural variation in *ZmVPP1*, encoding a vacuolar-type H^+-pyrophosphatase, most significantly contributes to maize drought tolerance. Further analysis found that a 366-bp insertion in the promoter, containing three MYB *cis*-elements, confers drought-inducible expression of *ZmVPP1* in drought-tolerant genotypes. Transgenic maize with enhanced *ZmVPP1* expression exhibits improved drought tolerance, probably due to enhanced photosynthetic efficiency and root development. Taken together, this research provides important genetic insights into the natural variation of maize drought tolerance at seedling stage. The identified loci/genes can serve as direct targets for both genetic engineering and selection for the trait improvement of maize.

T4-21: Plant responses to ambient and stress temperature

T4-21-01

Regulation of protein kinases in plant response to cold stress

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Cold stress is an environmental factor that affects plant growth, development and distribution. The well-studied cold signaling pathway is the ICE1-CBF transcriptional cascade in *Arabidopsis*; however, the specific protein kinases involved in this pathway remain elusive. We report that SnRK2.6/OST1 (OPEN STOMATA1), a well-known Ser/Thr protein kinase in ABA signaling, acts upstream of *CBF*s to positively regulate freezing tolerance. The *ost1* mutants show freezing hypersensitivity, whereas overexpression of *OST1* enhances freezing tolerance. Cold can activate OST1 kinase activity, and activated OST1 phosphorylates ICE1, which in turn suppresses HOS1-mediated ICE1 degradation under cold conditions, thereby promoting *CBF* expression and plant freezing tolerance. In addition, we isolated another protein kinase CRPK1 (Cold-responsive protein kinase 1) which negatively regulates plant freezing tolerance. The cold activated plasma membrane protein CRPK1 phosphorylated its interacting protein KIP1 (CRPK1 interacting protein 1), which shuttled from the cytosol to the nucleus to promote CBF protein degradation under cold stress, thus fine-tuning CBF signaling during cold response. Our results thus uncover the important roles of different kinases in regulating CBF-dependent cold signaling in *Arabidopsis*.

T4-21-02

Involvement of a subfamily of SAUR genes in growth architecture thermosensitivity in *Arabidopsis*

Jian Hua

Cornell University

We are interested in how diversity in thermosensitivity of growth architecture is evolved. Responses in development and growth architecture to a moderate temperature change differ greatly among accessions of *Arabidopsis thaliana*. Although molecular genetics on the reference accession Col-0 have revealed molecular identities of some regulatory components in temperature responses, it is not known how diversity in thermo-responses is generated. We used natural accessions of *Arabidopsis thaliana* to reveal the major molecular loci conferring the genetic diversity in growth architecture thermosensitivity. Genome Wide Association Study (GWAS) has revealed a major quantitative trait locus (QTL) for variation in thermosensitivity of growth architecture. The major QTL contains a sub-family of auxin response *SAUR* (*Small Auxin Up Response*) genes which likely are central in temperature responses in rosette leaf architecture. The function and diversity of these *SAUR* genes in temperature responses of growth architecture will be discussed.

T4-21-03

HOS15, a WD40 protein, mediated chromatin remodeling in plant freezing tolerance

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Histone modification in chromatin such as histone acetylation and deacetylation plays key role in gene regulation in eukaryotic cells. Previously, we reported that HOS15, a WD40 repeat protein, is involved in histone modification and cold tolerance in *Arabidopsis* plant (Zhu *et al.,* 2008). However, it remains unknown the molecular function of HOS15, and how HOS15 regulates cold responses in chromatin level. Here, we show that HOS15 functions as a substrate receptor for CULLIN4 (CUL4)-based ubiquitin E3 ligase and regulates C-repeat binding factors (CBFs)-dependent cold responsive genes (*CORs*) expression in chromatin level by degrading histone deacetylase 2C (HD2C) through ubiquitin/proteasome pathway. HOS15 forms complex with CUL4-DDB1 *in vitro* and *in vivo*. A yeast two-hybrid screening identified a HD2C as a substrate of HOS15. Accordingly, HD2C interacts with HOS15 in the nucleus and interacts with histone H3. Cold induced HD2C degradation and increased histone H3 acetylation is dependent on HOS15. Loss of HD2C in plant leads to higher accumulation of *CORs*, such as *RD29a*, *COR15a* and *COR47*, and increased freezing tolerance. Chromatin Immunoprecipitation (ChIP) assays show that a HOS15/HD2C complex is associates with CBFs-dependent *CORs* chromatin in normal condition. However, in response to cold, CBFs binds to *CORs* chromatin in a HOS15–dependent manner and removal of HD2C by HOS15 is essential for CBFs-binding to the *CORs* chromatin and *CORs* expression. Altogether, our data establishes a function for HOS15 as a CUL4-based ubiquitin E3 ligase receptor and uncover a mechanism for cold acclimation and tolerance in plant based on the HOS15/HD2C/CBFs-mediated epigenetic regulation of *CORs*.

T4-21-04

Temperature perception and response in plants

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Ambient temperature affects several aspects of plant growth and development and the underlying molecular mechanisms are just beginning to be elucidated. Recent studies implicate phytochromes as potential thermosensors in plants. In addition a role for histone modifications have been implicated in thermal response. By using a combination of mutant analysis and natural variation, we uncover the genetic architecture of natural variation in thermal responses of *Arabidopsis*. I will present our recent findings on a novel gene that appears to be essential for themo-morphogenesis in *Arabidopsis*. In addition, I will describe our novel finding that implicates a role for alternative splicing coupled with nonsense-mediated mRNA decay in thermal responses of *Arabidopsis thaliana*.

T4-21-05

Chromatin regulation of heat stress memory

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In nature, plants often encounter chronic or recurring stressful conditions. An increasing number of observations suggest that plants can remember a past exposure to stress in order to be better prepared for a future stress incident. My lab studies heat stress memory in plants as a model case for environmental stress memory. Seedlings acquire thermotolerance through a heat treatment at sublethal temperatures (priming heat stress (HS)) that enables them to survive an otherwise lethal HS. This thermotolerance is actively maintained for several days as indicated by the existence of mutants which are able to establish thermotolerance, but fail to maintain it. We have been exploring the molecular basis of HS memory in the model plant *Arabidopsis thaliana*. We have found that HS induces sustained histone methylation at HS memory-related loci that outlasts the transcriptional activity of these loci and hence marks them as recently transcriptionally active. At certain loci, this is correlated with hyper-induction of gene expression upon a recurring HS, - a signature readout of transcriptional memory. In a forward genetics approach, we have found that regulated nucleosome occupancy is also required for sustained activation of memory gene expression. Sustained low nucleosome occupancy is mediated by the highly conserved FORGETTER1 protein through direct interaction with chromatin remodeling proteins. I will present latest our finding on the role of chromatin structure in stress memory. In summary, the physiologically defined phenomenon of HS memory has a molecular equivalent in the transcriptional memory and changes in chromatin structure at the level of histone modifications and nucleosome occupancy are associated with this. In the long term, our research will provide new approaches to improve stress tolerance in crops.

T4-21-06

Genetic control of thermomorphogenesis in rice

Yongbiao Xue

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Global warming is having strong negative impacts on crop productivity. Being sessile, plants have evolved numerous intrinsic strategies to acclimate to ambient temperature increases often referred to as thermomorphogenesis. However, we know little about genetic control of thermomorphogeneis in rice (*Oryza sativa*), a staple crop for more than half of the world population. To dissect its genetic basis, we did a large scale screen for genetic mutations affecting rice thermomorphgenesis and several independent genetic loci were subsequently identified. Genetic and molecular analyses of these loci revealed insights into the molecular control of rice thermomorphogenesis. In this talk, I will present our recent progresses and highlight the challenges and opportunities to breed new heat-tolerant rice cultivars.

T4-22: Plant response to phosphorus and nitrogen (two sessions)

T4-22-01

STOP1-ALMT1 a new signalling pathway inhibiting root growth in low-phosphate

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When grown on a low-phosphate (–Pi) medium, the Arabidopsis primary root growth is inhibited as a consequence of reduced cell elongation and proliferation. We used a classical genetics approach to identify genes involved in these responses. By QTL analysis we identified the *LPR1* gene that inhibits the primary root growth in –Pi; LPR1 is a feroxidase targeted to the apoplast. From a collection of new EMS mutants that display a long root in –Pi, we identified many alleles of two other genes: *STOP1* and *ALMT1*. STOP1 is a transcription factor that directly regulates *ALMT1,* encoding a malate-exuding transporter. The –Pi stress enhances the expression of *ALMT1*, independently of PHR1 and PHL1. This makes the STOP1-ALMT1 module a new pathway of the –Pi signalling. We show that STOP1, ALMT1 and LPR1 rapidly inhibit root cell elongation in the transition zone, whereas LPR1 inhibits of cell proliferation in the meristem. The inhibition of cell elongation and cell proliferation is correlated with deposition of Fe and callose in the cell walls of the elongation zone and the meristem, respectively. By using atomic force microscopy (AFM) indentation, we also demonstrate that the rapid inhibition of cell elongation is correlated with stiffening of the cell wall, in an iron and peroxidase-dependent manner. We will present additional results and a model about the role of these genes in the –Pi-induced primary root growth arrest.

T4-22-02

Phosphate uptake by plant and its regulation

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Phosphate (Pi) is a crucial and often limiting nutrient for plant growth. It is also a very insoluble ion heterogeneously distributed in soil triggering numerous morphological and biochemical adaptations. The uptake of this element relies on the presence of nine high affinity transporters (PHT1 family) located in plasma membranes. Multiple and complex steps of transcriptional and post-transcriptional regulations (phosphorylation, degradation) of these proteins were identified illustrating the capacity for plants to tightly control the level of these transporters into the cells. Combining several genetic approaches we reduced 95% of PHT1 family activities offering unique opportunities to investigate the physiological role of these proteins. It highlighted their crucial role for Pi absorption and reveals that at least some (if not all) PHT1 members should exhibit dual affinity properties to improve Plant adaptation to the phosphate concentration present in the environment. Innovative techniques, such as radioisotope live micro-imaging system was used to image the spatial distribution of Pi absorption along the root. It revealed unexpected location of the uptake and provides opportunity to dissect the specific role of different root cell layers.

T4-22-03

The molecular mechanism of ethylene-mediated root hair development induced by phosphate starvation

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Enhanced root hair production, which increases the root surface area for nutrient uptake, is a typical adaptive response of plants to phosphate (Pi) starvation. Although previous studies have shown that ethylene plays an important role in root hair development induced by Pi starvation, the underlying molecular mechanism is not understood. In this work, we characterized an *Arabidopsis* mutant, *hps5*, that displays constitutive ethylene responses and increased sensitivity to Pi starvation due to a mutation in the ethylene receptor ERS1. *hps5* accumulates high levels of EIN3 protein, a key transcription factor involved in the ethylene signaling pathway, under both Pi sufficiency and deficiency. Pi starvation also increases the accumulation of EIN3 protein. Combined molecular, genetic and genomic analyses identified a group of genes that affect root hair development by regulating cell wall modifications. The expression of these genes is induced by Pi starvation and is enhanced in the *EIN3* over-expressing line. In contrast, the induction of these genes by Pi starvation is suppressed in *ein3*and *ein3eil1*mutants. EIN3 protein can directly bind to the promoter of these genes, some of which are also the immediate targets of RSL4, a key transcription factor that regulates root hair development. Based on these results, we propose that under normal growth conditions, the level of ethylene is low in root cells; a group of key transcription factors, including RSL4 and its homologs, trigger the transcription of their target genes to promote root hair development; Pi starvation increases the levels of the protein EIN3, which directly binds to the promoters of the genes targeted by RSL4 and its homologs and further increase their transcription, resulting in the enhanced production of root hairs. This model not only explains how ethylene mediates root hair responses to Pi starvation, but may provide a general mechanism for how ethylene regulates root hair development under both stress and non-stress conditions.

T4-22-04

Identification of root cell-specific regulators of phosphate signalling and their effect on whole plant phosphorus status

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Plant roots mediate the acquisition of most nutrients needed for growth and development. The balancing of these nutrient supplies and inherent demands has been shown to be regulated in different tissues across the root, with the root epidermis controlling acquisition and symplastic transport, the endodermis merging symplastic and apoplastic fluxes and the loading of the xylem ultimately determining nutrient supply to the shoot. Recent research indicates that nutrients such as nitrate, potassium or phosphate have different control points along their transport route. Phosphate is a major limiting nutrient for plant growth and we were interested in identifying components of the phosphate signalling network that are expressed in a root-cell specific manner, making them prime candidates for gatekeeping functions of phosphate flux. We identified these candidates by merging transcriptome data on phosphate-starvation induced (PSI) genes with those on root-cell enriched genes. The resulting list of root-cell enriched PSI genes was then expanded by adding predicted interactors and co-expression in a given root cell type. Candidate *PSI-interacting root-cell enriched* (*PRCE*) genes were then selected based on their predicted interaction with phosphate transporters (*WDD1*, *PRCE1*, *PSF2*, *STP10*) or on their potential to alter transport activities. The latter group comprised protein kinase components (*CBL1*, *CIPK2*, *CIPK14* and *S6K2*) as well as other membrane proteins (*MFS1*, *PRCE2* and *PRCE3*). Differential expression across root cell files was confirmed by promoter-GFP lines. Only two candidates, *CBL1* and *CIPK2* were ubiquitously expressed. Analysis of T-DNA insertion lines of the eleven *PRCE* genes revealed altered biomass and organ phosphate accumulation as well as transcriptome profiles in response to limited phosphate supply. The *s6k2* mutant showed a very similar transcriptome profile to the *phr1-1* mutant, a loss-of-function allele of master regulator PHOSPHATE STARVATION RESPONSE 1, a MYB transcription factor controlling the majority of PSI genes. However, in contrast to the *phr1-1* mutant, the *s6k2* mutant showed higher root biomass and shoot phosphate concentration than P-limited wild-type. Root transcriptome profiles of *cbl1* and *wdd1* mutants showed the opposite response to those of *phr1-1*. Together with their sustained biomass accumulation under limited Pi supply, these two genes therefore encode strong negative regulators of the plant's phosphate-starvation response. Consequences of manipulation of these root-cell specific networks for overall plant performance in response to phosphate fertilisation will be discussed.

T4-22-05

Molecular regulation mechanisms of phosphate transporter in rice

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Phosphorus is an essential macronutrients for plant growth and development. Under current situation of limited phosphate resources, low phosphate fertilizer use efficiency and high demand for green crops, it is important to improve the absorption and utilization efficiency of phosphorus in crops to reduce the amount of chemical fertilizers applied in agricultural production. Phosphate transporters (PTs) are direct executors of phosphorus uptake and transport, which directly involved in plant uptake of phosphate from soils. Phosphate transporters are regulated at transcriptional and posttranscriptional levels which allows plant cells to adapt to changes in cellular phosphorus environment. Rice OsPHF1 is a facilitator protein which facilitates the exit of phosphate transporter (PT) from the endoplasmic reticulum and localized to plasma membrane (PM). Protein kinase CK2 phosphorylates the phosphate transporters under high phosphorus conditions, and the phosphorylated PT does not interact with OsPHF1 thus remains on the endoplasmic reticulum. When plant is moved to low phosphate environment, CK2β subunit would be degraded, it cannot phosphorylated PT anymore. In the same time, the phosphorylated PT would further be dephosphorylated by protein phosphatase. The non-phosphorylated PT exits from the endoplasmic reticulum and localizes to the cytoplasm membrane with the help of OsPHF1, thereby facilitating the transport of phosphate from the extracellular to the intracellular to allow the plant to adapt to a low phosphate environment. The mechanism of the phosphorylation of PT on membrane localization would be discussed. The strategy for improving phosphorus uptake efficiency by using phosphate transporters is put forward.

T4-22-06

Identification of phosphate-stress-induced mRNA-protein complexes involved in phloem-mediated long-distance signaling

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In plants, phosphate (Pi) is an essential macronutrient, and consequently, it is a main constituent of fertilizers required to sustain agricultural output. Given that rock Pi supplies are finite and non-renewable, achieving the goal of food security will require engineering of crops with enhanced Pi uptake and use efficiency. To this end, our group is exploring the role of the plant vascular system, and the phloem in particular in systemic Pi-stress-associated signaling. Using cucumber as a model system, we have shown that, as part of the plant's early Pi stress response, many hundreds of mRNAs move long distance through the phloem to be targeted to specific sinks, including developing tissues of the root and shoot and young expanding leaves. Proteomics studies conducted on control and Pi-stressed cucumber plants identified 319 phloem sap proteins that are Pi-stress responsive, with 244 being phloem-mobile and, here again these proteins were being delivered to specific sink tissues. In addition, we mapped the response of some 105 phloem RNA-binding proteins (RBPs) that were associated with the imposed Pi-stress condition. These RBPs are being used to characterize the phloem-mobile ribonucleoprotein complexes that mediate delivery of mRNAs to specific tissues, under control and Pi-stress conditions. Transcripts bound by these RNP complexes are also being analyzed to ascertain the sequence/secondary structure motifs, within these mobile mRNAs, that are recognized by each specific RBP. The nature of the surveillance system that appears to operate, within the vascular system, to ensure selective delivery of phloem-mobile transcripts to a specific target tissue is also being investigated. These studies will be discussed in the context of developing breeding strategies to engineer crop plants with the capacity for sustained yields when grown on soils with reduced levels of available Pi.

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T4-22-07

Improvement of nitrogen use efficiency in rice: Theory and practice

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Nitrogen fertlizer is one of the driving force to increase crop productivity. It is estimated that more than 120 million tons of nitrogen is used as fertilizer annually and will still increase with growing demand for food production. However, overconsumption of nitrogen fertilizer causes severe problems such as soil acidification, eutrophication and other environmental pollution. Thus, reducing fertilizer usage but maintaining crop yield is a pressing challenge and the ideal way to alleviate this problem is to improve nitrogen use efficiency (NUE) of crops. Nitrate is one of the major nitrogen source for higher plants, plants absorb and transport nitrate mainly via nitrate transporters. Our previous work indicated that *indica* rice has much higher nitrate use efficiency over *japonica* rice, and a natural variation at nitrate transporter NRT1.1B/OsNPF6.5 contributes to this nitrate use divergence between *indica* and *japonica*. NRT1.1B-*indica* exhibits higher nitrate transport activity than NRT1.1B-*japonica*, which corresponds well with *indica* having higher nitrate use efficiency. Evolutionary analysis reveals that *NRT1.1B-indica* is a later derived allele and subjected to artificial selection. The large-scale field tests in three different locations across China showed that the NUE and grain yield increase could be achieved by about 30% in *japonica* variety with *NRT1.1B-indica* introgression under 1/2 nitrogen supply conditions. In addition to NRT1.1B, we also found that some other nitrate transporters also play important roles in nitrate uptake and transport in rice. Our results suggested that nitrate transporters might be the potential targets in improved NUE of rice, and in principle, can be also used for NUE improvement of other crops.

T4-22-08

Regulating membrane transporters to increase rice yields and nutrient use efficiency

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Cellular pH homeostasis is fundamental for life and all cells adapt to maintain this balance. In plants the chemical form of nitrogen supply, nitrate and ammonium, is one of the cellular pH dominators. The activities of plasma membrane transporters of nitrate and ammonium regulate cation/anion balance in cytosol. In addition, NHX-type K^+ (Na^+)/H^+ antiporters play critical roles in establish vesicle and vacuole pH homeostasis. The cycle of K^+ as major accompanying ion for translocation of nitrate and sucrose can act as an important signal for feedback control of nutrient uptake. We report that the rice nitrate transporter *OsNRT2.3* is transcribed into two spliced isoforms with natural variation in their expression ratio. One splice form, OsNRT2.3b is plasma membrane located mainly expressed in the phloem, and which has a regulatory motif on the cytosolic side that acts to switch nitrate transport activity on or off by a pH sensing mechanism. High OsNRT2.3b expression in rice enhances the pH buffering capacity of the plant, increasing N, Fe and P uptake. Interestingly, expression of a vesicle-localized transporter gene *HtNHX2* from *Helianthus tuberosus* could significantly improve rice tolerance to nutrient deficiency by up-regulating some membrane transporters for efficient acquisition of the nutrients. Increased expression of *OsNRT2.3b* or *HtNHX2* could very significantly increase grain yield and nutrient use efficiency. These results indicate that the sensing of cytosolic pH by OsNRT2.3b can function to improve rice NUE and pH balance, providing an explanation for plant adaptation to changes in ammonium-nitrate supply shift between the waterlogged and drained soil environment, while the transporters like HtNHX2 can strengthen vesicle pH and K^+ homeostasis for enhancing K^+-accompanying translocation of nutrients, thus, total uptake and use efficiency.

T4-22-09

A role for bHLHm1 and AMF1 in the AM symbiosis of *Medicago truncatula*

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GmbHLHm1 is a membrane bound basic helix-loop-helix DNA-binding transcription factor. In soybean, it has been localised to the symbiosome membrane, plasma membrane, endoplasmic reticulum and nucleus of nodule cells. *GmbHLHm1* expression is enhanced in nodules relative to roots with the onset of nitrogen fixation and loss of bHLHm1 activity reduces nodule fitness and nodule number. A preliminary study indicated that expression of bHLHm1 increased in roots when Pi and N availability was reduced. In this study, we investigated the activity of bHLHm1 homologs in the model legume *Medicago truncatula* colonised by either rhizobia or the arbuscular mychorrizal (AM) fungal strain, *Rhizophagus irregularis*. We have identified that Medicago homologs of *GmbHLHm1* are expressed in both nodules and AM-colonised roots. Localisation of GFP::MtbHLHm1 indicates membrane localization in arbuscular-containing cortical cells as well as in the nucleus. Loss of *MtbHLHm1* expression through RNAi silencing impairs nodule development and reduces AM colonization and arbuscule development. Plants of AM colonized *bHLHm1* roots displayed a negative growth response. In soybean, bHLHm1 is linked to the activity of the ammonium channel protein GmAMF1. Homologs of *AMF1* in *Medicago* were similarly found localized to arbuscule-containing cells. Loss of *AMF1* via RNAi, disrupted arbuscule development similar to the *bHLHm1* RNAi phenotype. Furthermore, RT-qPCR gene expression analysis indicates that *MtbHLHm1* activity is linked to changes in Pi (PhT) and N (AMT, AMF) transport genes in AM-colonised roots. This research highlights conservation of bHLHm1 activity between soybean and medicago and indicates both bHLHm1 and

AMF1 are intimately involved in the AM fungal symbiosis.

T4-22-10

Nitrogen Limitation Adaptation (NLA) is involved in source-to sink remobilization of nitrate by mediating the degradation of NRT1.7 in *Arabidopsis*

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Recent studies on nitrate transporters (NRT) have greatly increased our knowledge of the mechanisms regulating N homeostasis in plants. However, an understanding of how these NRTs are regulated is still lacking. n*la* mutant is hypersensitive to N limitation. In *nla* mutant, ^{15}N-nitrate spotted on old leaves preferentially accumulated in youngest leaves. Analysis of leaf cross sections indicated *NLA* expression was expressed in the sieve element and companion cell system. The results of BiFC, Split-ubiquitin Yeast Two-hybrid and CoIP assays demonstrated that NLA interacts with NRT1.7 in in the plasma membrane. The following findings suggested that NLA directs the ubiquitination of NRT1.7: down regulation of NRT1.7 protein abundance in *35S::NLA/35S::Myc-NRT1.7* double transgenic plants compared to *35S::Myc-NRT1.7* transgenic plants; and up regulation of NRT1.7 protein abundance in *nla* mutant compared to wild-type plants; and direct degradation of truncated NRT1.7 recombinant protein by NLA. Furthermore, analysis of NLA and NRT1.7 protein abundance in *mirna827* knock-out plants showed that N deficiency-guided translational repression of NLA depends on miRNA827. Our findings reveal that plants regulate source-to-sink remobilization of nitrate by the ubiquitin-mediated posttranslational regulatory pathway.

T4-22-11

Root microbiome and plant nutrient uptake

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Plants harbor abundant and diverse bacterial microbiota. Culture-independent 16S rRNA gene profiling revealed distinctive bacterial communities associated with plant roots. The availability of comprehensive pure cultures of root- and leaf-derived bacteria is critical to deeply understand functions of root-associated bacteria to a variety of plant physiological processes. We established *Arabidopsis* root-derived microbiota culture collections representing the majority of bacterial species that are reproducibly detected by previous culture-independent community sequencing. Using defined bacterial communities and a gnotobiotic *Arabidopsis* plant system we illustrated that root bacteria facilitate plants to uptake nutrients from natural soil. The comprehensive bacteria culture collections and high quality genomes together with the remarkable reproducibility of the gnotobiotic reconstitution system enable future studies on the cooperation between root bacterial community and plant nutrient uptake ability.

T4-22-12

Constructing a transcriptional regulatory network for nitrogen metabolism in *Arabidopsis*

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Humans and animals depend on plants for fitness and survival, which is particularly true in obtaining nitrogen (N) directly from plants. N is the nutrient required in the greatest quantity by plant growth, nevertheless, being the most limiting element for plant productivity due to its poor availability. Nitrate is the predominant form of nitrogen in most agricultural soils. In addition to being an essential nutrient, nitrate is also a key signal for plant growth, development and stress responses. Therefore, deciphering nitrate signaling and regulatory network will provide direct guidance for improving nitrogen use efficiency (NUE) and achieving low-input sustainable agriculture. Much effort has been made to identify nitrate regulatory Transcription factors (TF) and DNA *cis*-elements, but progress has been slowed by the lack of a rapid and large-scale method to study the nitrate regulatory network. In our lab, we have developed a tripartite nitrate-responsive promoter (NRP) containing enhancer fragments from two nitrate-regulated genes, a 109 bp fragment from nitrate reductase (*NIA1*) and a 130 bp fragment from nitrite reductase (*NiR*), and a 35S mini promoter. This construct was used in a forward genetic screen to identify many nitrate regulatory mutants. We also developed a rapid transient expression system in *Nicotiana benthamiana* for analyzing nitrate responses and were able to identify multiple DNA *cis*-regulatory elements in the 109 bp fragment from *NIA1*. In addition, the 109 bp fragment was utilized as bait in a yeast-one hybrid system to screen 2000 clones encoding known and predicted *Arabidopsis* TFs. Among those TFs, we have identified and characterized *teosinte branched1/cycloidea/proliferating cell factor1-20* (*TCP20*), a broad regulator of plant growth and development that plays a key role in nitrate systemic signaling underlying nutrient foraging by roots and discovered TCP20-NLPs (NIN-like proteins) protein interactions and their specific roles in orchestrating the regulation of nitrate assimilation with that of the cell cycle and root meristem growth. The discovery is largely thanks to a DNA *cis*-element- and TFs-based screening system that we have been developing. These regulators (TFs and *cis*-elements) provide important components and information to construct nitrate regulatory network in plants.

T4-23: Germline development and fertilization mechanisms

T4-23-01

FERONIA and related receptor kinases in the regulation of pollen tube growth and pollen-pistil interaction

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FERONIA receptor kinase was first discovered as a critical regulator of female fertility; loss of its function results in a dramatic ovule phenotype of pollen tube pile up inside the female gametophyte and severely suppressed female fertility. It was demonstrated more than a decade ago that FERONIA plays a dual function: one in mediating female gametophyte-induced rupture of a penetrating pollen tube, resulting in sperm release and enabling fertilization; the other in preventing multiple pollen tube entrance to the female gametophyte, ensuring against polyspermy. Loss of FERONIA results in multiple pollen tubes entering an ovule and penetrating the female gametophyte; they continue growth without discharging sperm, thus precluding fertilization. Our earlier work demonstrated that the two functions of FERONIA in regulating pollen tube-ovule interaction are not obligatorily coupled and FERONIA induces a high oxidative environment in the filiform apparatus/micropyle region to induce pollen tube rupture in a Ca^{2+}-dependent process. Here we discuss FERONIA-regulated properties that underlie its role in preventing multiple pollen tube entrance into the female gametophyte. FERONIA is expressed almost ubiquitously except in pollen. We will also discuss the functional role of a FERONIA-related receptor kinase that are specifically expressed in pollen and how it impacts the pollen tube growth process in the pistil.

T4-23-02

How do plant female gametes prevent and allow polyspermy in double fertilization?

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Fertilization in flowering plants is very unique and called as double fertilization. A set of two sperm cells enclosed by an endocytic membrane is conveyed by the pollen tube, which is a tip growing cell precisely attracted to the egg-containing tissue. One of two sperm cells fuses with the egg cell to form an embryo and the other fuses with the central cell on the side of the egg cell to form a nutritive tissue endosperm. Precise one-to-one fusion in the two female gametes is critical for normal seed development. Polyspermy of flowering is likely to be prevented by at least two distinct mechanisms: one is called as blocking polytubey to prevent attraction of supernumerous pollen tubes, and the other is gametic polyspermy block. We have been working on plant reproduction by live-cell imaging analysis (e.g., Science 2001, Nature 2009, 2016, Curr. Biol. 2011, Nat. Commun. 2014). We found double fertilization was intimately related to cessation of additional pollen tube attraction (Curr. Biol. 2012, Dev. Cell, 2013). To our surprise, the fertilized central cell fused with an attractive synergid cell to stop pollen tube guidance (Cell 2015). Secretion of LURE attract peptide by the synergid cell rapidly decreased and the synergid cell nucleus degenerated rapidly due to sudden cell cycle entry. On the other hand, in the step of double fertilization, gametic polyspermy block was also shown by using laser ablation and mutant pollen tubes conveying supernumerous sperm cells. In this symposium, I will talk about both blocks for polytubey and gametic polyspermy.

T4-23-03

Functional analysis of CRPs during reproduction and defense response in *Zea mays*

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Double fertilization is a unique sexual reproduction process restricted to angiosperms (flowering plants). Two sperm cells have to be transported to the female gametophyte via a pollen tube and fuse with egg cell and central cell to generate embryo and endosperm, respectively. To accomplish double fertilization, extensive male-female communication and accurate guidance is needed at many steps along the pollen tube journey. Previous studies in different plant species revealed that various highly specific receptor-ligand binding activities are critical to accomplish cell-cell communication between the pollen tube and various tissues of the female flower organ during fertilization. Small secreted peptides, a major class of extracellular signaling ligands in plants, can be classified into two major groups, CRPs (cysteine-rich peptides) and non-CRPs. Functional analysis of these two peptide classes showed that most non-CRPs are involved in signaling pathways of vegetative growth and environment response, while CRPs are more presented in different stages of reproductive growth. Originally CRP subgroups such as defensins were thought to be involved in pathogen defense. RNA-seq data of our group indicates that more than 400 CRPs showed specific expression pattern during fertilization and fungal invasion. To further elucidate the biological functions of candidate CRPs during reproduction and defense responses in maize, RNAi transgenic plants were generated with Agrobacterium-mediated stable transformation and are currently used for phenotypic analyses. For example, we identified three CRP genes encoding rapid alkalinization factors (*RALFs*), which are highly and exclusively expressed in germinated maize pollen tubes. Down-regulation of pollen-specific *RALFs* affects pollen tube growth *in vitro* indicating that *RALFs* could be involved in stabilizing pollen tube growth during fertilization. A CRP subgroup restricted to C4 grasses appears as pollen tube interactor of the egg apparatus-secreted guidance peptide EA1. We will report and discuss these and other functional studies of CRPs in maize to provide a deeper insight into the understanding of the different roles of CRPs during fertilization and stress response, their evolution and their role in speciation (reproductive isolation) in maize and plants in general.

T4-23-04

Generating clonal progeny in maize

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Apomixis is asexual reproduction through seed. The production of seeds though apomixis, which generates plants that are genetically identical to the mother plant, has considerable agricultural potential to maintain desired complex genotypes, e.g. those of F1 hybrids, over many generations. Gametophytic apomixis deviates from sexual development in three major steps: (1) meiosis is circumvented or aborted, leading to the formation of unreduced, unrecombined embryo sacs (apomeiosis); (2) embryogenesis initiates without fertilization of the unreduced egg cell (parthenogenesis); and (3) developmental adaptations enable the formation of functional endosperm. The aim of our research is to identify mutations that mimic the major components of apomixis, and to combine them to engineer apomictically-reproducing maize plants. In a genetic screen we identified the *n*on-*r*eduction in *f*emale 4 (*nrf4*) mutant, which mimics the first step of apomixis: apomeiosis. Homozygous *nrf4* plants produce up to 95% unreduced embryo sacs. Using SAIFF-by sequencing technology, the mutation was mapped to *GRMZM2G148133* on the long arm of chromosome 7, and the identity of *Nrf4* was confirmed by two additional mutant alleles. To identify whether *nrf4* leads to first or second division restitution (FDR vs SDR), we analyzed maintenance of heterozygosity in the progeny of *nrf4* mutant plants in comparison to mother plants using a SNP array that enabled the analysis of 10-20 SNPs on each chromosome. The effect of the *nrf4* mutation turned out to be more complex than expected and leads to both FDR and SDR. Nonetheless, depending on the genetic background of the mother plant, up to 11% of the unreduced female gametes were genetically identical to the mother. Indeed, pollination of *nrf4* plants by a tetraploid haploid inducer resulted in some clonal individuals. To our knowledge, this is first evidence that production of clonal individuals through seed is possible in maize.

T4-23-05

Chromatin remodeling complex SWR1 coordinates with cytochrome P450 KLU to regulate plant megasporocyte formation

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Germline cell specification is essential for sexual reproduction. In the ovules of most flowering plants, only a single hypodermal cell enlarges and differentiates into a Megaspore Mother Cell (MMC), the founder cell of the female germline lineage. The molecular mechanisms restricting the specification of the MMC to a single cell remains elusive. We showed that the transcription factor *AKD28,* exclusively expressed in hypodermal somatic cells surrounding the MMC, is required to repress those cells to differentiate into MMC in *Arabidopsis*. We also showed that the SWR1 chromatin remodeling complex mediates the incorporation of the histone variant H2A.Z into *AKD28* nucleosome and modulates its structure, and that cytochrome P450 *KLU,* expressed in the inner integument primordia, controls H2A.Z deposition at *AKD28* in a non-cell autonomous manner. Our findings show how somatic cells cooperatively use chromatin remodeling to restrict germline cell specification to a single cell in the ovule primordia.

T4-23-06

AP1G mediates vacuolar acidification during synergid-controlled pollen tube reception

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Double fertilization in angiosperms requires the delivery of immotile sperm through pollen tubes, which enter embryo sacs to initiate synergid degeneration and to discharge. This fascinating process, called pollen tube reception, involves extensive communications between pollen tubes and synergids, within which few intracellular regulators involved have been revealed. Here we report that vacuolar acidification in synergids, mediated by AP1G possibly through the activity of V-ATPases, is critical for pollen tube reception. Functional loss of *AP1G*, encoding the γ subunit of adaptor protein 1, impaired synergid degeneration and pollen tube discharge, resulting in partial female sterility. Although vacuolar trafficking was compromised in the *ap1g* synergids, genetic and pharmacological interferences suggested that it was not the determinant for defective pollen tube reception. We further showed that vacuolar acidification in the *ap1g* synergids was compromised. Consistently, functional loss of V-ATPases also resulted in the failure of synergid degeneration and pollen tube reception despite that the targeting of V-ATPases was not affected by AP1G. We propose that vacuolar acidification-mediated synergid degeneration might represent a distinct cell death mechanism specifically adopted by the plant phylum.

T4-25: Photosynthetic efficiency and environmental regulation

T4-25-01

Functional analysis of IsiA-containing complexes in *Synechocystis* sp. PCC 6803

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Iron is an essential element that functions as a cofactor in numerous redox reactions and is a key component of the electron transport system of photosynthesis. Iron stress-induced protein A (IsiA) is a major chlorophyll-binding protein in the thylakoid membrane that is significantly induced under iron deficiency conditions. An IsiA-PS I supercomplex, which contains IsiA, trimeric PS I, Slr1128 and HliA/B was detected by sucrose gradient ultracentrifugation in the model cyanobacteria *Synechocystis* sp. PCC 6803, and when trimeric PS I is absent, the other components would form a novel high light-inducible carotenoid-binding protein complex (HLCC). Monitoring the dynamic changes of IsiA-containing complexes in *Synechocystis* sp. PCC 6803 during exposure to long-term iron deficiency discovered two other types of IsiA-PS I supercomplexes, namely IsiA-PS I high fluorescence supercomplex (IHFS) and IsiA-PS I low fluorescence supercomplex (ILFS). Further analysis revealed a role for IsiA as an energy dissipater in the IHFS and as an energy collector in the ILFS. The trimeric structure of PS I mediated by PsaL was found to be indispensable for the formation of IHFS/ILFS. Dynamic changes in IsiA-containing complexes in cyanobacteria during long-term iron deficiency may represent an adaptation to iron limitation stress for flexible light energy distribution, which balances electron transfer between PS I and PS II, thus minimizing photooxidative damage.

T4-25-02

The mechanism and significant of chloroplast gene expression in higher plants

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The chloroplast of higher plants is a unique organelle evolved from a photosynthetically active cyanobacterium by an endosymbiotic event. Strategies and regulation of chloroplast gene expression are distinct from those of eubacterial as well as from those of eukaryotic cytoplasm and reflect a unique chimerical system assembled by multiple origins during endocytobiosis. Proper expression of chloroplast genes relies on coordinated interplay between *cis*-acting elements at the plastid DNA/RNA level and *trans*-acting factors, most of which are nuclear-encoded and specifically bind to their *cis*-acting targets. The significant of chloroplast gene expression has been expanded greatly. The growing evidence has show that it not only regulates the chloroplast biogenesis but also involved in plant development via retrograding signaling. The chloroplast gene expression has been integrated into plant development program. In this presentation, we will summary the advance in chloroplast gene expression and highlight the roles of nuclear-encoded factors e have identified in recent years. Based on our work, we will also discuss how the chloroplast gene expression influence root and leaf development via interaction with auxin signaling.

T4-25-03

Physiological traits mediate bioclimatic segregation of epiphytic CAM bromeliads in Trinidad

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Trait-mediated divergences in bioclimatic relations could represent an important pathway to the evolution and maintenance of genus-level diversity, but there are relatively few comprehensive case studies of this effect in regional vascular plant florae. To investigate this topic, we mapped the distributions of epiphytic CAM bromeliads of the genus *Aechmea* across the Northern Range of Trinidad, a topographically and climatically heterogeneous continental island in the southern Caribbean. Distinct elevational and latitudinal effects on species presence and abundance were apparent. Bioclimatic and vegetational species distribution modelling was performed in MaxEnt to project the potential ranges of four species across Trinidad, on Tobago and along the Venezuelan Coastal Cordillera. Using the same models, projections of species distributions under an aggressive 2,070 climate scenario revealed particular vulnerability to upslope shifting of bioclimate envelopes in the montane species. To explore the contribution of ecophysiological traits to observed divergences in occupation of environmental niche space, plants of each species were collected and subjected to anatomical and physiological characterisation. Bioclimatic differentiation was associated with contrasting water-use strategies, stomatal sensitivity, temperature relations and drought responses. Measured physiological traits were used to construct a mechanistic species distribution model and probability distribution grid maps were generated for comparison with MaxEnt output. Distributions modelled by correlative and mechanistic approaches showed a high degree of congruence, consistent with a physiological explanation for regional- and landscape-scale patterning in the distributions of *Aechmea* bromeliads. Interspecific variation in quantitative and qualitative traits related to abiotic tolerances is probably an important contributor to the ecological diversity of the Bromeliaceae.

T4-25-04

Effects of close planting on chlorophyll content and photosynthetic function in field grown sorghum

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To study the effects of mutual shading on photosynthesis in field-grown sorghum. Under absence of root competition, we investigated the effects of planting density on light environment, leaf chlorophyll and photosynthetic function. Increased planting density not only modified the light intensity and spectrum, but also induced a marked increase in chlorophyll content; the chlorophyll increased gradually with increased leaf position in upper canopy at the medium and high density, while it decreased in low density; compared with sorghum grown at high density, the lower canopy leaves at medium density had longer duration of stay-green period and photosynthetic function, and the leaves senescence was quicker at low density. In the absence of mineral deficiency, close planting induce a significant increase in chlorophyll content, and the chlorophyll increase gradually with rise of leaf position in upper canopy; close planting not only changes the duration of stay-green period, but also promotes the optimization of photosynthetic function in field-grown sorghum.

T4-25-05

Regulation of stomatal patterning by CO_2 across *Arabidopsis* tissues occurs during spacing divisions

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Stomatal development is regulated by the atmospheric concentration of carbon dioxide ($p[CO_2]$). Mature leaves can convey developmental cues to immature leaves in response to $p[CO_2]$, but the developmental mechanisms are unknown. We constructed a dual-chamber growth system in which rosette and cauline leaves of *Arabidopsis thaliana* were subjected to differing $p[CO_2]$, to characterize changes in stomatal patterning resulting from signals moving from mature to developing leaves. Young rosette tissue was found to adjust stomatal index (SI) in response to both the current environment and the environment experienced by mature rosette tissue, whereas cauline leaves appear to be insensitive to $p[CO_2]$ treatment. We also found that leaf epicuticular wax across the entire plant is dependent upon only the $p[CO_2]$ experienced by young tissue. The effect of $p[CO_2]$ on stomatal development reappears in cotyledons of the next generation, but only after germination. Moreover, these data suggest that $p[CO_2]$ affects regulation of stomatal development specifically through the development of satellite stomata and not the core pathway. It is likely that cauline leaves and cotyledons deploy mechanisms for controlling stomatal development that are related to, but distinct from, that operating in rosette leaves.

T4-25-06

Transport of photosynthate

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In order to meet increasing demands for energy and food as the world population grows, there is an urgent need to produce sustainable fuel and increase crop yield beyond existing yield potentials. A better understanding of the mechanisms of fixed carbon allocation from photosynthetic tissues to non-photosynthetic ones is critical to achieve the goal of redirecting sugar flux to storage tissues. Sugar transporters are required to facilitate the carbon allocation. We have recently characterized the SWEET family of sugar transporters, which are involved in different biological efflux processes: *Arabidopsis* AtSWEET11 and 12 export sucrose into the apoplasm from the phloem parenchyma cells prior to phloem loading. *atsweet11;12* double mutant impairs sucrose export out of the leaf. We found more SWEETs are involved in this efflux step before phloem loading. Arabidopsis sucrose transporter AtSWEET11,12 and 15 are required for seed filling. Triple mutant *atsweet11;12;15* exhibits severely delayed embryo development and reduced seed yield. Overexpression expression of *SWEETs* leads to the unexpected results-stunted growth. How to engineer sugar flux will be discussed. The structure-guided functional study of SWEETs offers a valuable foundation for engineering sugar flux to increase crop yields and biofuel.

T4-26: Hormone and plant development

T4-26-01

Structural variations of long-distance translocated cytokinins control the specificity of the action in shoot growth and development

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Cytokinin (CK) plays an important role in regulation of plant growth and development, and its action is finely controlled by various steps including biosynthesis and metabolism, transport, and signaling. We have demonstrated that IPTs, CYP735As, and LOGs, which are key genes for *de novo* CK biosynthesis, are expressed in various parts during growth and development, and differentially regulate the synthesis of N6- (Δ^2-isopentenyl)adenine (iP) and trans-zeatin (tZ). In addition, ABCG14, a member of ABC transporter family, has been recently identified as a key gene for root-to-shoot translocation of CKs via xylem. Detailed studies on CYP735As mutants show that tZ is important for the normal growth of shoot rather than that of root, suggesting a mechanism that modulates physiological function of CKs by modification of the side-chain structures. This regulation is one of the qualitative controls of CK action involved in shoot growth regulation by root-borne signal. Furthermore, our recent studies suggest importance of translocation of active form CK via xylem (i.e. tZ) for regulation of specific traits in shoot growth and development. These findings suggest that complex action of long-distantly transported cytokinins could be organized by the side chain structure and the dependency of the activation pathway. The dual long-distance cytokinin signaling system would be important for fine-tuning shoot growth manner in response to environmental conditions. We will outline our recent progress in CK study, and discuss the physiological significance of regulation of CK action to optimize growth and development at whole plant level.

T4-26-02

Reprogramming of rice root development under water-deficit stress

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Rice roots grow longer under mild water-deficit stress, yet their elongation rate is reduced when encountering moderate to severe stress or treated with 2 uM or more ABA, generating shorter and thicker roots. The root biomass is increased in both cases compared to unstressed roots, and consequently the root to shoot ratio is enhanced under stress. Transport of ^{14}C-sucrose from leaves to roots is enhanced under stress conditions, suggesting that the sink strength of roots has become stronger. Storage lipids and starch accumulate to higher levels in root tissues under stress. Transcriptomics analysis reveals that genes for the biosynthesis of fatty acids and starch are preferentially induced in roots by ABA/stress with concomitant increase in the activity of enzymes encoded by these genes. In addition, a novel class of highly proline-rich glycoproteins, RePRPs (Repetitive Proline-Rich Proteins) is also preferentially induced by ABA/stress in rice roots. RePRP proteins contain more than 40% prolines and numerous PEPK (pro-glu-pro-lys) motifs, and are heavily O-glycosylated. Unlike other plant proline-rich proteins, RePRPs are not secreted to the cell walls, but localized on the plasma membrane as well as in the cytosol. Although RePRPs are intrinsically disordered proteins due to the high proline content, they are able to specifically bind to the cell wall polysaccharide arabinogalactan (AG), actin and tubulin. The binding of actin or tubulin is independent of the binding of AG. Overexpression of RePRP leads to shorter and thicker roots in transgenic rice and the reduction of actin filaments, mimicking the effect of ABA/stress. Knocking down the expression of RePRP by RNAi prevents roots from becoming shorter and thicker under ABA/stress treatment. It is suggested that the plasma membrane-localized RePRPs regulate cell wall properties through their interactions with cell wall polysaccharides and cytosolic RePRPs reduce actin filaments, causing the formation of shorter yet wider root cells. The synergism between RePRP expression and storage nutrient biosynthesis alters the architecture and function of rice roots so that they are better suited for coping with severe stress conditions.

T4-26-03

Molecular mechanism underlying rice shoot gravitropism and tiller angle

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In higher plants, gravitropism is a key factor that affects plant architecture. The auxin homeostasis and distribution are considered to play an important role in shoot gravitropism, however, the mechanism underlying shoot gravitropism is still largely unknown. Rice shoot gravitropism is also regarded as one of important agronomic traits because it can determine tiller angle and thus affect the grain yields. Our previous studies have identified the rice *LAZY1* (*LA1*) gene, which functions as a negative regulator of polar auxin transport (PAT). Loss-of-function of *LA1* greatly enhances PAT and thus alters the endogenous IAA distribution in shoots, leading to the reduced gravitropism and therefore the tiller-spreading phenotype of rice. To elucidate the mechanism of LA1 involved in the regulation of shoot gravitropism and thus tiller angle, we carried out a genetic screening to identify the suppressors of *la1*, and identified multiple suppressors of *LA1* that are components involved in the strigolactones (SLs) biosynthetic or signaling pathway. We showed that SL biosynthetic or signaling mutants could rescue the spreading phenotype of *la1*. SLs are a group of newly identified plant hormones that are essential for shoot branching/rice tillering. Our study revealed that SLs can inhibit auxin biosynthesis and attenuate rice shoot gravitropism mainly through decreasing the local IAA content. We also verified that the SL-mediated shoot gravitropic mechanism is conserved between dicot and monocot plants. Moreover, we used RNA-Seq technology to identify the genes involved in the regulation of shoot gravitropism and tiller angle, trying to reveal the molecular event and dynamic regulatory network of tiller angle at a whole genome level. We found that *Gravitropic Response Gene 4* (*GTR4*) showed asymmetrically expression in the rice shoot base upon gravistimulation, the similar expression pattern as auxin responsive marker gene *OsIAA20*. *GTR4*-RNAi transgenic plants displayed larger tiller angle and attenuated gravitropic response. Further evidence demonstrated that *GTR4* may function downstream of auxin in the regulation of shoot gravitropism and tiller angle.

T4-26-04

Arabidopsis CLE peptide signals contributes to pollen exine development through precisely regulated transcriptomic and proteomic networks

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The CLAVATA3/ESR-RELATED (CLE) peptide signals are required for cell-cell communication in several plant growth and developmental processes. However, little is known regarding the function and working mechanism of the CLEs in the male reproductive process. Here, through a gene expression survey, we identified 7 Arabidopsis *CLE* genes that are specifically/preferentially expressed in the tapetum cells and microsporocytes in the anther; also dominant-negative (DN) mutant plants of each of these genes exhibited significantly reduced male fertility, with abnormal pollen wall formation. Further study with CLE19 as representative and using *cle19*, DN-*CLE19,* and *CLE19-OX* mutant lines as materials, we demonstrated that CLE19 peptide signal affected the expression of more than 1,000 genes at the RNA level and 595 at the protein level, including the important transcription factor gene*AMS*, 280 AMS downstream genes, and other genes involved in pollen coat and pollen exine formation, lipid metabolism, pollen germination and hormone metabolism. Consistently, mutants of the CLE19 downstream genes *GRP20*, *ACOS5* and *MEE48* all exhibited *CLE19-OX*-like pollen exine structure. In conclusion, these findings demonstrate CLE19 and redundant CLE hormone peptides work as negative regulator of AMS and downstream networks in pollen exine formation, and provide insights into studies of both peptide signaling transduction and anther development fields.

T4-26-05

COG1 regulates brassinosteroid biosynthesis via PIF4 and PIF5 in *Arabidopsis thaliana*

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Brassinosteroids (BRs) are essential phytohormones regulating various developmental and physiological processes during normal growth and development. Within the last two decades, significant progress has been made in elucidating both BR signal transduction and BR biosynthesis pathways. But how BR homeostasis is regulated by internal and external cues remain poorly understood. *cog1-3D* (*cogwheel 1-3D*) was identified as an activation-tagged genetic modifier of *bri1-5*, an intermediate BR receptor mutant. *COG1* encodes a Dof-type transcription factor previously found to act as a negative regulator of the phytochrome signaling pathway. *cog1-3D* single mutants show an elongated hypocotyl phenotype under light conditions. Loss-of-function by ethylmethanoesulfonate (EMS) mutation or inducible expression of a dominant-negative form of *COG1* results in an opposite phenotype. BR profile assay indicated that BR levels are elevated in *cog1-3D* seedlings. Quantitative RT-PCR analyses showed that several key BR biosynthetic genes are significantly up-regulated in *cog1-3D* compared to those of wild-type. Two basic helix-loop-helix transcription factors, *PIF4* and *PIF5*, were found to be transcriptionally up-regulated by *cog1-3D*. Genetic analysis indicated that PIF4 and PIF5 are required for COG1 to promote BR biosynthesis and hypocotyl elongation. Chromatin immunoprecipitation (ChIP) and electrophoretic mobility shift assays (EMSA) demonstrated that COG1 directly binds to the promoter regions of *PIF4* and *PIF5*; and PIF4 and PIF5 directly binds to the promoter regions of key BR biosynthetic genes, such as *DWF4* and *BR6ox2*, to promote their expression. Previous study revealed that both expression of *COG1* and *PIF4* could be induced by far-red and red light. Our results revealed that COG1 and PIF4 and PIF5 are key regulators mediating BR biosynthesis, supporting that light signaling is critical to BR homeostasis.

T4-26-06

Evolutionary history of a gibberellin receptor, GID1

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Gibberellin (GAs) are a large family of tetracyclic diterpenoid phytohormones that induce a wide range of plant growth responses, including stem elongation, seed germination, floral induction and pollen development. Although more than 130 GA derivatives have been identified, only a few GAs, such as GA_4, GA_1 and GA_3, actively function in plant responses. These bioactive GAs essentially have a hydroxyl group at 3-carbon, a carboxyl group at 6-carbon, γ-lactone ring, and no hydroxyl group at 2-carbon. GA receptor can strictly distinguish the structures of bioactive GA from inactive ones. The GA receptor, GID1, has a primary structure of carboxylesterases (CXEs). The crystal structure of the GID1 bound with GA_4 revealed that the GA binding pocket corresponds to the substrate-binding site of CXEs. N-terminal lid of GID1 covers the GA molecule to stabilize at the binding site. The lid also functions for the interaction with DELLA proteins, repressors in GA signaling. Here, we quantified the effect of the amino acids of GID1, which are not shared between *Selaginella moellendorffii* and flowering plant, using physicochemical and phenotypic analyses of transgenic rice plants with various mutant GID1s. We also studied the copy number of *GID1* from comprehensive phylogenetic analysis during plant evolution of GID1 in broad plants species. Based on these observations, we discussed the establishment of GID1 in the process of plant evolution.

T4-27: Light signal and photomorphogenesis

T4-27-01

SPLICING FACTOR FOR PHYTOCHROME SIGNALING promotes photomorphogenesis by regulating pre-mRNA splicing in *Arabidopsis*

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Light signal regulates plant growth and development by controlling a plethora of gene expression changes. Post-transcriptional regulation, especially pre-mRNA processing, is a key step in controlling gene expression; however, the molecular mechanisms linking pre-mRNA processing and light signaling are not well understood. Here we report a splicing factor called SPLICING FACTOR FOR PHYTOCHROME SIGNALING (SFPS) that directly interacts with the photoreceptor Phytochrome B (phyB). *sfps* mutants have long hypocotyls, smaller cotyledon angles under monochromatic light and display early flowering. SFPS colocalizes with U2 snRNP associated factors including, U2AF65B, U2A', and U2AF35A in nuclear speckles, suggesting that SFPS might be involved in the 3' splice site determination. SFPS regulates pre-mRNA splicing of a large number of genes enriched in light signaling, photosynthesis and circadian clock under both dark and light conditions. *In vivo* RNA immunoprecipitation (RIP) assay revealed that SFPS associates with *EARLY FLOWERING 3* (*ELF3*) mRNA, a critical gene linking light signaling and circadian clock. Moreover, *PHYTOCHROME INTERACTING FACTORs (PIFs)*, act downstream of SFPS, as knocking out four prominent PIFs masks *sfps* phenotypes. Taken together, these data strongly suggest that SFPS modulates light-regulated developmental processes by controlling pre-mRNA splicing of many light signaling and circadian clock genes.

T4-27-02

Photobody biogenesis in early phytochrome signaling

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Photobodies are photosensory subnuclear domains containing the red and far-red photoreceptors, the phytochromes. In *Arabidopsis*, biogenesis of phyB-GFP-containing photobodies is triggered by red light and promoted by increasing light intensity. A central mechanism by which phytochromes control photomorphogenetic responses is to trigger the degradation of a group of antagonistically-acting Phytochrome-Interacting transcription Factors (PIFs). During the dark-to-light transition, phyB-GFP is colocalized with PIF3 before PIF3 degradation, it was therefore proposed that photobodies are sites for PIF3 degradation. However, the mechanism linking photobodies and PIF3 degradation is still poorly understood. We show that PIF3 degradation is tightly correlated with photobody biogenesis. Using a forward genetic screen for mutants defective in photobody biogenesis, we identified HEMERA (HMR), a new phytochrome signaling component required for the biogenesis of large photobodies. HMR is localized to the periphery of photobodies and required for the degradation of PIF1 and PIF3, providing genetic evidence supporting the role of photobodies in PIF degradation. We show that HMR interacts directly with all PIFs. The HMR-PIF interaction is mediated mainly by HMR's N-terminal half and PIFs' conserved active-phyB-binding motif. In addition, HMR possesses an acidic nine-amino-acid transcriptional activation domain (9 aa TAD). Unexpectedly, the 9 aa TAD of HMR is required for both PIF3 degradation and the activation of a subset of growth-related PIF target-genes. These results support a novel phytochrome signaling mechanism, in which HMR mediates downstream photomorphogenetic responses by binding directly to PIFs and coupling the degradation of PIF1 and PIF3 with the transactivation of PIF target genes via HMR's 9 aa TAD. We propose that photobodies are gene hubs for the regulation of a distinct set of light responsive PIF target-genes.

T4-27-03

The reds and the blues of the shade avoidance response

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Plants adjust their body plan in response to ambient environmental conditions. Light is among the most relevant environmental signals because light not only drives photosynthesis but also provides critical information about the local growth environment as well as diurnal and seasonal time. Light is perceived by a complex array of photoreceptors, defined by the color of light they absorb. These include the red/far-red-absorbing phytochromes (phyA-E), the blue/UV-A-absorbing cryptochromes (CRY1-2), phototropins (phot1-2), and ZTL-family, and a UV-B-absorbing receptor. Plants have developed various adaptive responses in interpreting and utilize light directionality, quantity, and quality. One such adaptive response is the ability of sun-loving plants to sense and avoid vegetational shading by nearby plants. In vegetational or canopy shading, plants perceive a decrease in the ratio of red to far-red light (R: FR) and there is also a reduction in blue light and the available photosynthetically active radiation (PAR). CRYs control the phenotypic plasticity of plants in response to changes in blue light. Despite their importance, very little is known about the mechanisms by which CRYs affect growth programs. We present data to show that when plants are deprived of blue light under a shaded canopy, CRY1/2 perceive this change and respond by directly contacting two bHLH transcription factors, PIF4 and PIF5. Activities of these factors are controlled by phytochromes, which are the red:far-red light photoreceptors. Intriguingly, transcriptome analysis shows that genes regulated by PIF4 and 5 in response to low blue light are distinct from those regulated by low red/far-red. Results indicate that CRYs signal by modulating PIF activity, and that different plant photoreceptors can mediate distinct phenotypic responses through shared signaling components.

T4-27-04

Coordinated positive and negative regulatory machinery underlies *Arabidopsis* UV-B signaling network

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The ultraviolet (UV)-B light is an indispensible component of the sunlight that reaches the earth surface. UV-B irradiation brings about differential physiological responses in plants, dependent on its wavelength, fluence rate and duration. In higher plants, photomorphogenesis is a pivotal developmental process that allows plant seedlings to experience dark-to-light transition during their emergence from soil. Long-wavelength and low-fluence UV-B specifically induces plant photomorphogenesis, which is characterized by the inhibition of hypocotyl elongation, the promotion of anthocyanin accumulation, and eventually the tolerance against UV-B stress. In *Arabidopsis thaliana*, the environmental UV-B light signal is perceived by the photoreceptor UV RESISTANCE LOCUS 8 (UVR8), a plant-specific chromoprotein. UVR8 initiates UV-B specific signaling by interacting with CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) which is a UV-B inducible protein. However, less is known about the molecular mechanism of the negative regulation in UV-B-induced photomorphogenesis. It thus remains poorly understood how the positive and negative regulatory machinery is coordinated in the UV-B signaling network. In this study, we demonstrate that two sets of E3 ubiquitin ligases promote and repress UV-B-induced photomorphogenesis respectively by differentially targeting specific substrates for proteasome-mediated degradation, so as to constitute positive and negative regulatory circuits for balanced signal transduction. Based on the findings, our research aims at understanding the genetic and molecular basis of plant responses to UV-B light, and over a long term, at establishing the linkage between fundamental plant biology and practical outcomes.

T4-27-05

Plant seedling soil emergence: Regulation of the protein machinery

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The survival of seed plants in natural environments requires the successful emergence from the soil. A key question in seedling emergence is how plants sense changing soil conditions and adjust their growth accordingly, yet this mechanism is largely unknown. Here, we find that CONSTITUTIVE PHOTOMORPHOGENESIS 1 (COP1), a central repressor of light signaling, is a key component required for seedlings to sense the depth of soil overlay. Mutation of COP1 causes severe defects in penetrating soil, due to decreased level of EIN3, a master transcription factor in ethylene pathway that mediates seedling emergence. We show that COP1 directly targets the F-box proteins EBF1 and EBF2 for ubiquitination and degradation, thus stabilizing EIN3. As seedlings grow towards the surface, the depth of soil overlay decreases, resulting in a gradual increase of light fluences. COP1 channels the light signals while ethylene transduces the information on soil mechanical conditions, which cooperatively control EIN3 protein levels to promote seedling emergence from the soil. The COP1-EBF1/2-EIN3 module reveals a mechanism by which plants sense the depth to surface and uncovers a novel regulatory paradigm of an ubiquitin E3 ligase cascade. Upon reaching the surface, light triggers a dramatic developmental transition termed de-etiolation that requires immediate termination of ethylene responses. We further find that light-activation of photoreceptor phyB results in rapid degradation of EIN3, the master transcription factor in ethylene signaling pathway. As a result, light rapidly and efficiently represses ethylene actions. Specifically, phyB directly interacts with EIN3 in a light-dependent manner and also physically associates with F-box protein EBFs. The light-activated association of phyB, EIN3, and EBF1/EBF2 proteins stimulates robust EIN3 degradation by $\mathrm{SCF}^{\mathrm{EBF1/EBF2}}$ E3 ligases. We reveal phyB acting as a light-reversible "molecular glue" that directly manipulates the substrate-E3 ligase interactions to control the stability of EIN3. Our findings illustrate a mechanistic model of how plants transduce the light information to immediately turn off ethylene signaling for de-etiolation initiation.

T4-27-06

Blue light- and low temperature-regulated COR27 and COR28 play roles in the *Arabidopsis* circadian clock

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Light and temperature are two key environmental signals that profoundly affect plant growth and development, but underlying molecular mechanisms of how light and temperature signals affect the circadian clock are largely unknown. Here we report that *COR27* and *COR28* are regulated not only by low temperatures but also by light signals. COR27 and COR28 are negative regulators of freezing tolerance but positive regulators of flowering, possibly representing a trade-off between freezing tolerance and flowering. Furthermore, loss-of-function mutations in COR27 and COR28 result in period lengthening of various circadian output rhythms and affect central clock gene expression. Also, the *cor27 cor28* double mutation affects the pace of the circadian clock. Additionally, COR27 and COR28 are direct targets of CCA1, which represses their transcription via chromatin binding. Finally, we report that COR27 and COR28 bind to the chromatin of *TOC1* and *PRR5* to repress their transcription, suggesting that their effects on rhythms are in part due to their regulation of *TOC1* and *PRR5*. These data demonstrate that blue light and low temperature-regulated COR27 and COR28 regulate the circadian clock as well as freezing tolerance and flowering time.

T4-28: Floral organ development (two sessions)

T4-28-01

VRS2 regulates hormone-mediated inflorescence patterning in barley

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Plant architecture has clear agronomic and economic implications for crops, such as wheat and barley, as it is a critical factor for determining grain yield. Despite this, only limited molecular information is available about how grain-bearing inflorescences, called spikes, are formed and maintain their regular, distichous pattern. Here we elucidate the molecular and hormonal role of Six-rowed spike 2 (Vrs2)— a SHORT INTERNODES (SHI) transcriptional regulator during barley inflorescence and shoot development. We show that Vrs2 is specifically involved in floral organ patterning and phase duration by maintaining hormonal homeostasis and gradients during normal spike developmentand similarly influenced plant stature traits. Furthermore, we establish a link between the SHI-protein family and sucrose metabolism during organ growth and development that may have implications for deeper molecular insights into inflorescence and plant architecture in crops.

T4-28-02

Transcriptome profiling of *Desmos chinesis*: Revealing the molecular basis of dipartite perianth evolution in the early divergent angiosperm family Annonaceae

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During the past 200 million years of speciation and hybridization, angiosperm genome structure has evolved and diversified in size and composition. While many angiosperm taxa experienced genome duplication and different degrees of transposable element proliferation, the homeotic control of floral development has remained conservative. The MIKC-type MADS-domain proteins are transcription factors that dictate organ identity during floral organogenesis. The classic "ABCDE" *MADS*-box gene expression model proposed for the core eudicots is not fully applicable to early-divergent angiosperms, in which a gradual transition is apparent between sepal-like outer tepals and petal-like inner tepals, leading to the proposed "fading-borders" expression model. Flowers in the Annonaceae deviate from other early-divergent angiosperms in possessing a distinctive dipartite perianth. Petals in the Annonaceae ('bracteopetals') have furthermore presumably evolved independently from andropetals in core eudicots. The degree of differentiation of petals from sepals in Annonaceae flowers is generally of functional significance (e.g., enabling the evolution of largely or fully enclosed pollination chambers during anthesis). Little is known of the genome and genetic control of floral development in the Annonaceae. The application of massive parallel sequencing techniques such as RNAseq provides opportunities to profile the transcriptomes of non-model organisms. In addition to a developmental study, we characterized the floral and leaf transcriptome of *Desmos chinensis*, a representative of the Annonaceae that possesses a dipartite perianth and which shares the basic floral Bauplan of the family, in order to understand the genetics of its floral development. Transcriptomes of developing and mature *Desmos chinensis* floral organs and leaves were sequenced using the high-throughput Illumina sequencing platform. After filtering, 16,098 genes and 22,112 transcripts were recovered from *de novo* assembly. We identified possible coding regions and compared them against five public databases. Based on sequence homology against the NCBI non-redundant protein database, transcripts were annotated with 3,991 unique gene ontology (GO) terms and categorized into functional groups. Functional group enrichment analysis was used to identify groups with significant expression level changes during organ development. Transcripts that are commonly differentially expressed among developing and mature floral organs and leaves were identified using the digital gene expression data. We identified 41 putative homeotic MADS-box gene homologs and their respective expression levels in order to determine whether Annonaceae flowers are consistent with the *MADS*-box gene "fading borders" expression model. Other floral developmental regulators of *Desmos chinensis* were also discovered. While many questions remain unresolved at the transcription level, our data emphasises the importance and potential of extending further studies into systems biology, and help understand floral developmental biology as biological networks.

T4-28-03

The *Arabidopsis* CELLULASE 6 and MANNANASE 7 proteins affect cell differentiation in the silique and contribute to silique dehiscence

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Cell wall degradation or modification by cellulases, hemicellulases and pectinases is expected to be crucial for many plant developmental processes. Mutations in pectinase genes *ADPG1* and *ADPG2*, for example, cause silique indehiscence in *Arabidopsis*. At present, no mutations of cellulase or hemicellulase genes have been reported to affect silique development and dehiscence in plants. To investigate the roles of cellulases and hemicellulases in fruit development and dehiscence, we first identified *CEL6,* a cellulase gene, and *MAN7,* a hemicellulase gene, as candidate genes encoding cell wall-degrading enzymes in Arabidopsis siliques. Analysis of the expression of these two genes by qPCR in siliques of the wild type and the silique-indehiscent mutants *ind-7* and *alc-3* revealed that their expression was partially dependent on the IND and ALC transcription factors. GUS staining results from transgenic lines containing the promoter-GUS fusion and protein-GUS fusion of these genes confirmed that *CEL6* and *MAN7* were expressed in the valve and the replum, including the dehiscence zone. Such GUS signals were reduced or hardly detectable in the *ind-7* and *alc-3* siliques, consistent with the aforementioned qPCR results. Phenotypic analysis of *cel6* and *man7* loss-of-function mutants indicated that silique development was delayed and dehiscence impaired in these mutants. Secondary cell wall thickening and cell morphology in the valve margin were also affected by the mutations. Most notably, in sections prepared for transmission electron microscopy, cells in the separation layer in nearly mature siliques of the single mutants and the *cel6-1 man7-3* double mutant remained intact whereas they were broken in the control wild-type siliques. Overexpression of *CEL6* and *MAN7* also seemed to slightly increase the dehiscence rates of nearly mature and mature siliques. Phenotypic analysis of the double, triple, and quadruple mutants involving the *adpg1-1*, *adpg2-1, cel6-1, and man7-3* mutations indicated that the indehiscent phenotypes of the *adpg1-1* single mutant and the *adpg1-1 adpg2-1* double mutant, which were much more severe than those of the *cel6-1* and *man7-3* single mutants and the *cel6-1 man7-3* double mutants, became even more severe by the additions of the *cel6-1* and *man7-3* single mutations or the *cel6-1 man7-3* double mutation. On the other hand, the phenotypes of the *cel6-1* and man7-3 single mutants and the *cel6-1 man7-3* double mutant were epistatic to that of *adpg2-1*. Taken together, our results show that CEL6 and MAN7 act in the cell differentiation process during silique development and contribute to silique dehiscence by promoting cell lysis in the separation layer. Our results also demonstrate that the ability of the silique to dehisce can be manipulated by altering the activities of multiple types of cell wall-degrading enzymes.

T4-28-04

Control of anther development by the EMS1 receptor kinase-linked signaling complex in *Arabidopsis*

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In flowering plants, successful sexual reproduction depends on the specification of distinct reproductive cells that conduct meiosis to form gametes and associated somatic cells that provide nutrition and developmental cues to ensure successful gamete production. Anther, the male reproductive organ in a flower, typically has four lobes (microsporangia). Within each of these lobes in a mature anther, the central reproductive microsporocytes (or pollen mother cells) are surrounded by four concentrically organized somatic cell layers: epidermis, endothecium, middle layer, and tapetum. Microsporocytes give rise to pollen via meiosis, while somatic cells, particularly the tapetum, are required for the normal development and release of pollen. Our previous studies showed that the EMS1 leucine-rich repeat receptor-like kinase (LRR-RLK) plays an essential role in somatic and reproductive cell differentiation during anther development. Recent research in my lab for the first time demonstrates that TPD1 is a novel small secreted cysteine-rich protein ligand for EMS1. The TPD1-EMS1 signaling initially promotes the periclinal division of secondary parietal cells to form a monolayer of tapetal precursor cells and then determines the fate of functional tapetal cells. We also identified the EMS1 co-receptor SERK1/2 and its downstream player CA1. *EMS1* and *SERK1/2* functions in the same genetic pathway. Bimolecular fluorescence complementation (BiFC) and Fluorescence resonance energy transfer (FRET) analyses demonstrate that TPD1 interacts with EMS1 *in vivo*. Trans-phosphorylation between EMS1 and SERK1/2 enhances the EMS1 kinase activity. In addition, we determined phosphorylation sites of EMS1 and their biological significance. Our yeast two-hybrid screening identified the EMS1 downstream player CA1. Besides several lines of evidence supports the biochemical interaction between EMS1 and CA1, our results show that loss-of-function mutants of *CA1* are defective in tapetal cell differentiation, while overexpression of *CA1* results in the formation of additional tapetal cells. A model will be discussed to explain how the TPD1-EMS1/SERK1/2-CA1 signaling pathway controls anther development.

T4-28-05

An ABC transporter, OsABCG26, is required for anther cuticle and pollen exine formation and pollen-pistil interactions in rice

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Wax, cutin and sporopollenin are essential components for the formation of anther cuticle and pollen exine, respectively. Their lipid precursors are synthesized by secretory tapetal cells and transported to anther and microspore surface for deposition. However, the molecular mechanisms involved in the formation of anther cuticle and pollen exine are poorly understood in rice. Here, we characterized a rice mutant *osabcg26* with male sterility. Molecular cloning and sequence analysis revealed a point mutation in the gene encoding ATP binding cassette transporter G26 (OsABCG26). *OsABCG26* was specifically expressed in anther and pistil. Cytological analysis revealed defects in tapetal cells, lipidic Ubisch bodies, pollen exine, and anther cuticle in *osabcg26* mutant. Expression of some key genes involved in lipid metabolism and transport, such as *UDT1*, *WDA1*, *CYP704B2*, *OsABCG15*, *OsC4* and *OsC6*, was significantly altered in *osabcg26* anther, possibly due to disturbance in the homeostasis of anther lipid metabolism and transport. Interestingly, we found most of the wild-type pollen tubes ceased growth in the upper region of *osabcg26* ovary, and only a few pollen tubes could reach the micropyle for fertilization, leading low seed setting in *osabcg26* cross-pollinated with the wild-type pollen. These results indicated that *OsABCG26* plays an important role in anther cuticle and pollen exine formation and pollen-pistil interactions in rice. Further investigations are needed to decipher the mechanism of pollen tube growth defect in *osabcg26* pistil and to pinpoint the role of OsABCG26 in pollen-pistil interactions.

T4-28-06

Variable cell growth channels reproducible organ development though spatiotemporal averaging

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Organ sizes are strikingly precise, despite the stochastic growth and division of individual cells within them. Because their shapes are highly reproducible and relatively insensitive to environmental conditions, *Arabidopsis* sepals, allow robustness in organogenesis to be assessed within a single plant. To reveal how robust organ sizes emerge from the variable growth of cells, we screened for *Arabidopsis* mutants with variably sized sepals and identified mutations in the mitochondrial i-AAA protease FtsH4. Through live imaging, we observed substantially variable cell growth in wild-type sepals, while in *ftsh4* sepals, the variability of neighboring cell growth is reduced. Through computational modeling and quantitative phenotypic analysis, we found that regular organ size results from spatiotemporal averaging of the cellular variability in wild-type sepals, whereas in *ftsh4* mutants the averaging process is disrupted due to the less-variable cell growth. Reactive oxygen species (ROS) accumulated in *ftsh4* mutants, abnormally increasing both cell wall stiffness and organ size variability. In wild-type sepals, ROS accumulate in the maturing cells and limit organ growth. Enzymatically-lowering ROS produced larger sepals. Our results demonstrate that spatiotemporal averaging of cellular variability is required for robustness in organ size, and there is an inverse relationship between the robustness of plant organ size and ROS. We are currently investigating the mechanism of ROS's regulation of the spatiotemporally variability averaging process, and exploring whether mechanical cues contribute to this process.

T4-28-07

Petal elaboration in *Nigella*: From morphology, micro-morphology to molecular basis

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As a special type of floral organ, petals show tremendous diversity in shape, structure, color/coloration, and function, and have increasingly been used as a model for studying plant organogenesis and evolution. Petals of the *Nigella* (Ranunculaceae) species are of particular interest because they are highly elaborate, bilabiate structures with extensive modifications. To understand how *Nigella* petals become elaborated and diversified, we first investigated the tempo, mode, and mechanisms of character evolution during petal elaboration within this genus. We found that the degree of petal complexity increased gradually during evolution, involving both modification of existing characters and *de novo* origination of new features. Through transcriptomic analyses, we then identified regulatory modules and genes contributing to petal elaboration in *N. damascena*. We found that *NdLMI1*, a member of HD-Zip I genes, is responsible for the division of the lower lip as well as the initiation of short trichomes. We also found that *NdPSN01* and *NdYAB5-1* play key roles in the development of pseudonectaries, silence of which led to loss of pseudonectaries. Finally, we explored histological evidences and expression patterns of adaxial-abaxial polarity genes during petal development, and propose that the basic structure of *Nigella* petals may have been related to the repatterning of adaxial-abaxial polarity during morphogenesis.

T4-28-08

The origin of floral organ identity quartets

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The origin of flowers has puzzled plant biologists ever since Darwin referred to their sudden appearance in the fossil record as an abominable mystery. Flowers are considered to be an assembly of protective, attractive and reproductive male and female leaf-like organs. Their origin cannot be understood by a morphological comparison to gymnosperms, their closest relatives, which develop separate male or female cones. Despite these morphological differences, gymnosperms and angiosperms possess a similar genetic toolbox consisting of phylogenetically related MADS-domain proteins. Using ancestral MADS-domain protein reconstruction, we trace the evolution of organ identity quartets along the stem lineage of crown angiosperms. We provide evidence that current floral quartets specifying male organ identity, which consist of four types of subunits, evolved from ancestral complexes of two types of subunits through gene duplication and integration of SEPALLATA proteins just before the origin of flowering plants. Our results suggest that protein interaction changes underlying this compositional shift were the result of a gradual and reversible evolutionary trajectory. Modelling shows that such compositional changes may have facilitated the evolution of the perfect, bisexual flower.

T4-28-09

OsMADS14/15 interact with YABBY6 and specify the perianth organ identity in rice (*Oryza sativa* L)

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The well-known ABC model describes the combinatorial interaction of homeotic genes in specifying floral organ identies. While there is evidence that the B and C functions are highly conserved throughout angiosperm evolution, the status of the A function has been hotly debated because in most plants that have been investigated thus far, with *Arabidopsis* being a remarkable exception, one does not find recessive mutants in which the identity of both types of perianth organs is affected. In our study, we present a comprehensive mutational analysis of all four members (*OsMADS14*, *OsMADS15*, *OsMADS18*, and *OsMADS20*) of the *AP1*/*FUL*-like subfamily of MADS-box genes in rice, and demonstrate that *OsMADS14* and *OsMADS15*, in addition to their function of specifying meristem identity, are also required to specify palea and lodicule identities. Since these two grass-specific organs are very likely homologous to sepals and petals of eudicots, respectively, we conclude that some kind of an floral homeotic A function also exists in grasses. Furthermore, our result of IP-MS showed that OsYABBY6 is an interaction factor of OsMADS14/15. The further experiments, such as Pull-down, BiFC, and Co-IP analyses, confirmed the protein-protein interaction patterns. Our data reveal that, at least to some extent, the mechanism of floret development controlled by multi-gene regulation in rice, and thus expand the understanding of the core theory described by ABC model that are mostly made of MADS-box genes.

T4-28-10

Evolution of the fruit developmental genetic network with particular emphasis on the dehiscence zone bHLH identity genes

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Fruit development in *Arabidopsis thaliana* is controlled by seven key transcription factors from different gene families: FUL and SHP1/2 belong to the MADS-box family, IND, ALC and SPT belong to the bHLH family, RPL belongs to the homeodomain (HOX) family and AP2 belongs to the APETALA2/Ethylene Responsive Element Binding Factor gene family, all of which are large transcription factor families. FRUITFULL (FUL) is responsible for proper cell division during valve development; REPLUMLESS (RPL) is involved in controlling replum identity; both FUL and RPL antagonize SHATTERPROOF genes (SHP1/2), which in turn become restricted to the dehiscence zone. SHP1/2 positively regulate INDEHISCENT, responsible for the formation of the lignified layer, as well as ALCATRAZ and SPATULA, which redundantly control the identity of the non-lignified layer. The entire network is negatively regulated by APETALA2. In order to identify the components of the network conserved across angiosperms, we have studied the evolution of each of these gene lineages involved in fruit development. Our data show that: (1) most MADS box gene duplicates are maintained independently in monocots, basal eudicots and core eudicots; (2) the bHLH SPT and ALC paralogs are exclusive to core-eudicots, while paleoSPT/ALC are found in non-core eudicots; (3) IND orthologs are only present in Brassicaceae while HECATE3 proteins are present in all other flowering plants; (4) AP2 homologs are found in all seed plants, with a specific duplication resulting in the paralogous TOE3 genes in Brassicales; and (5) RPL genes have been mostly retained as single copy genes across angiosperms. In order to better understand the evolution of specific genes targeting the formation of dehiscence zones, we studied expression and function of bHLH, ALC and SPT genes in Solanaceae (core eudicot) and Papaveraceae (basal eudicot). In Solanaceae, ALC genes are predominantly retained as single copy, while SPT genes underwent a specific duplication in *Nicotiana*. Both ALC and SPT genes are expressed in stamens, carpels, fruits and leaves of *Nicotiana obtusifolia*, *Capsicum annuum* and *Solanum lycopersicum*. Functional data from *N. obtusifolia* and *C. annum* show that *SPT* and *ALC* play redundant roles in leaf pigmentation, petal fusion and fruit development. Specifically, they seem to negatively control lignification as downregulated plants show smaller fruits with ectopic lignified patches in the pericarp, independently of whether fruits are dry, dehiscent capsules (i.e. *Nicotiana*) or indehiscent berries (i.e. *Capsicum*). Further evidence for the maintained role of these genes in regulating specific tissues in dehiscence zones comes from conserved expression patterns of *paleoSPT/ALC* preduplication genes in *Bocconia frutescens* (Papaveraceae) compared to their core-eudicot counterparts. Altogether our data shows that by comparison to the preduplication genes, SPT and ALC genes have undergone some degree of sub-functionalization in Brassicaceae while maintaining redundancy in Solanaceae. Finally, we propose that co-option of SPT and ALC genes in leaf pigmentation is likely a new role of these genes exclusively in *Solanaceae*.

T4-28-11

The Origin of peloria in Chinese cymbidium (*Cymbidium sinense*)

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Orchid flowers exhibit zygomorphic corollas with highly specified lips. In *Cymbidium sinense*, plenty of peloric varieties with defects in floral zygomorphy have been bred during centuries of domestication since Song dynasty. However, little is known about the molecular basis regulating orchid floral zygomorphy and producing these peloric varieties. Here, we studied the floral morphogenesis of *C. sinense*, transcriptome-wide examined the spatiotemporal expressional patterns of floral homeotic MADS factors, and detected the expression of interested genes among different types of varieties. The floral zygomorphy was established in early developmental process with retarded ventral organogenesis. We found the interactions among AGL6-clade and AP3-clade MADS genes were crucial to the establishment of its floral zygomorphy. *CsAP3-1* was highly expressed in the two inner whorls of floral organs, which coped with a lip-specific factor, *CsAGL6-1*, regulating the differentiation between dorsal lip and ventral petals. The expression of these two genes were absent in varieties with sepal-like petals and lip. The floral sepal whorl was established by another *AGL6*-clade factor, *CsAGL6-2*, whose expression was significantly down-regulated in varieties with defects in sepals and petals. These findings indicate combinatory actions of MADS factors and changes in these factors may have occurred during artificial selection.

T4-28-12

Mechanisms of floral organ specification by MADS domain proteins

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Flower development is controlled by master-regulatory transcription factors (TFs), which orchestrate gene expression during developmental switches and organ differentiation. However, the molecular mechanisms by which these factors dynamically regulate the expression of their target genes are still poorly understood: how are developmental programmes encoded in plant genomes, and how is this ‘code’ read and executed? We characterized dynamic changes in chromatin structure, gene expression and DNA-binding of floral MADS-domain TFs. Our findings suggest that MADS-domain TFs modulate chromatin accessibility and thereby control target gene expression. MADS-domain TFs control the expression of hundreds of genes in a partly stage- and tissue-specific fashion. In order to study how different MADS-domain TFs achieve their functional specificity, we used an *in vitro* approach to reveal DNA-binding specificities of different heteromeric MADS domain protein complexes that specify distinct organ types in the flower. We are also interested in the evolutionary dynamics of gene-regulatory networks controlling flower development. Using a comparative ChIP-seq approach, we determined the evolutionary variation of DNA binding sites of a developmental key regulatory TF in two closely related species: *Arabidopsis thaliana* and *A. lyrata*. Despite their recent evolutionary divergence of ~10 Mio years, we found a high level of divergence between TF binding site locations. In *A. lyrata*, we found that binding sites can be associated with transposable elements, which likely contributed to the origin of novel binding site locations in this species.

T4-29: Plant branching

T4-29-01

Shoot branching and complex leaf development are controlled by homologous gene modules

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Aerial plant architecture is predominantly determined by the pattern of shoot branching and the morphology of leaves, which are governed by underlying developmental processes, axillary meristem formation and complex leaf development, that appear fundamentally unrelated. In tomato, these processes share essential functions in boundary establishment. Boundaries are established in different regions of the plant body and have diverse functions in development. Firstly, they separate different cell groups, for example the differentiating cells of a leaf primordium from the pluripotent cells of the apical meristem. In compound leaved species, like tomato, boundaries separate young leaflets from each other and from their connecting structure, called rachis. Recent experiments have shown that boundary zones between the meristem and leaf primordia show similar properties as boundaries between leaflets. They are characterized by a low rate of cell divisions and specific patterns of gene expression. We identified potato leaf (*C*), a key regulator of leaf dissection, to be the closest paralog of the shoot branching regulator Blind (*Bl*). Expression studies and complementation analyses indicate that these genes have undergone sub- or neo-functionalization due to promoter differentiation. *Bl* is expressed in the axils of leaves whereas *C* transcripts accumulate in the boundary zones of leaflets. The known leaf complexity regulator Goblet (Gob) is crucial for axillary meristem initiation and leaf dissection, acting in parallel to C and Bl. A combination of loss-of-function alleles of *C* and *Gob* results in simple leaves. Secondly, boundary zones initiate new meristems, which requires a low level of the plant hormone auxin. Disruption of polar auxin transport compromises axillary meristem formation. New meristems do not only initiate in the meristem-to-leaf primordium boundary, but also in the distal leaflet boundary (DLB) of tomato leaves. Initiation of these ectopic meristems is dependent on activities of the well-known branching regulators Goblet (Gob) and Lateral suppressor (Ls), which act in hierarchical order. Finally, we have identified a new regulator, Super determinant (Sde), which plays a critical role in axillary meristem formation. *Sde* encodes a protein with similarity to components of the PRC1 complex. A search for interactors indicates that Sde may be involved in an epigenetic mechanism regulating axillary meristem formation.

T4-29-02

Stem cell specification during shoot branching

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The homeodomain transcription factor WUSCHEL (WUS) directs stem cell specification in the shoot. Extensive studies have shown how *WUS* homeostasis is maintained and how *WUS* promotes shoot meristems, but the mechanisms underlying the establishment of *WUS* expression remain unknown. Seed plants produce meristematic cells that form axillary meristems in the leaf axil; these meristems may develop into branches, and thus affect plant architecture. Floral meristems are also specialized axillary meristems. Here, we show that cytokinin signaling activates *WUS* expression *de novo* in the leaf axil to promote axillary meristem initiation. Furthermore, type-B ARABIDOPSIS RESPONSE REGULATORs (ARRs), transcriptional activators in the cytokinin signaling pathway, directly bind to the *WUS* promoter to activate its expression. Finally, *WUS* expression requires a permissive epigenetic environment to restrict it to highly defined meristematic tissues. Taken together, these findings establish a mechanistic framework for the postembryonic establishment of the shoot stem cell niche.

T4-29-03

De novo organogenesis in *Arabidopsis*: From wounding to cell fate transition

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De novo root organogenesis is a type of plant regeneration for survival after wounding. We established simple methods to study *de novo* root and shoot organogenesis by culturing Arabidopsis explants without added hormones to regenerate adventitious roots and shoots. Wounding is the first event in regeneration. During *de novo* root organogenesis from leaf explants, wound signal spreads rapidly from the wounded site to mesophyll cells and activates auxin biogenesis in mesophyll cells. Auxin is then polar-transported to regeneration-competent cells (i.e. procambium and vascular parenchyma cells) in the vasculature near the wound site. Auxin accumulation in regeneration-competent cells activates *WOX11* and *WOX12*, and this triggers the first step of cell fate transition from regeneration-competent cells to root founder cells. *WOX11* and *WOX12* directly activate *WOX5*, *WOX7* and *LBD16* for the second step of cell fate transition from root founder cells to root primordium. Overall, wound signaling, auxin behavior and cell fate transition are successively involved in *de novo* regeneration of adventitious roots from leaf explants. In addition, *WOX11* and *WOX12* are specifically expressed in founder cells of adventitious roots but not lateral roots, suggesting that the priming and initiation mechanisms for adventitious roots and lateral roots might be different.

T4-29-04

Regulation of rice inflorescence architecture by a cooperative action of *TAWAWA1* and *BOP* genes

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The architecture of rice inflorescence is determined by the pattern of shoot branching, which depends on the timing of a change from indeterminate growth to determinate growth of the meristem. Among meristems produced during inflorescence development, the ones produced early acquire an indeterminate branch meristem (BM) identity and iterates shoot branching, while the later produced ones are specified as a determinate spikelet meristem (SM). *TAWAWA1* (*TAW1*) is a negative regulator of the meristem phase change from the BM to the SM. Therefore, higher activity of TAW1 leads to production of higher order branches, which results in the production of an inflorescence with more spikelets. *TAW1* encoded a nuclear protein containing a conserved ALOG domain. TAW1 binds to specific DNA sequences, interacts with an ortholog of *Arabidopsis BLADE-ON-PETIOLE* (*BOP*) and localizes in the nuclear, suggesting it works as a transcriptional regulator. We analyzed target genes of TAW1-BOP by chromatin immunoprecipitation followed by next-generation sequencing. We will discuss a possible molecular function of TAW1-BOP complex in the control of the meristem phase change.

T4-29-05

Branching out into HD-ZIPIII target genes and interacting proteins

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The production of side shoots, commonly referred to as shoot branching, is a major trait determining plant biomass. Plants can initiate new branches by establishing lateral meristems that form in the axils of leafs. Class III homeodomain leucine zipper (HD-ZIPIII) transcription factors are major determinants of shoot patterning and also involved in controlling branching. The activity of HD-ZIPIIIs is strongly regulated by microRNAs and microProteins at both the post-transcriptional and post-translational level.

In order to deeper characterize HD-ZIPIII function, we have used a combination of genomics (ChIP-Seq and RNA-Seq) and proteomics approaches to identify HD-ZIPIII responsive genes as well as interacting proteins. Further genetic analysis revealed several new factors that act downstream of HD-ZIPIIIs in the control of shoot meristem activity, including shoot branching.

T4-29-06

New evidence of complex shoot and leaf meristems in ferns

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Leaves usually are lateral and determinate organs and these features seem to apply well in most of the cases in seed plants. On the other hand, shoots are characterized by indeterminacy, detected by the expression of Class I *KNOTTED-LIKE HOMEOBOX* (*KNOX*) genes in the shoot apical meristem (SAM). Downregulation of Class I *KNOX* is one of the first signals of the origin of a determinate leaf primordium and plants with defective Class I *KNOX* genes may be unable to maintain the SAM. Ferns are tricky in the sense that most of them produce leaves called fronds that are usually compound with lateral pinnae and a leaf apical meristem (LAM). The LAM and SAM of ferns have a distinctive prominent apical cell, that has been pointed as equivalent to a whole apical meristem by some authors or only the initial cells of a multicellular meristem by other. Organ determinacy seems to

be the key character to understand the evolution and development of fronds. They show intriguing resemblances with indeterminate shoots (e.g. apical meristems presence, lateral organs production, and reduced or absent determinacy) and do not fit on classic morphology concepts of leaves. In order to gather more information concerning fronds and pinnae development, and about LAM and SAM organization, we studied the expression of Class I KNOX in *Mickelia scandens*, a leptosporangiate fern that bears small pinnate fronds in the younger terrestrial form and longer pinnate fronds in the older climbing form. In-situ hybridizations and RTq-PCR experiments were performed in shoot apex and developing leaves and we detected two Class I *KNOX* and one histone genes that were expressed in growth regions of the shoot apex and developing leaves. The expression of these genes shows a significant difference in the smaller developing fronds of the younger form when compared with shoot apices and bigger developing fronds. We also detected a complex meristematic organization, not restricted to the apical cells in the shoot and the leaf apex. Class I *KNOX* genes seems to play a role in the development of pinnae, that are more determinate than shoots and developing leaves; and in the development of the vascular system.
These results challenge the classical view that ferns meristems are simple and very reduced. Instead, they have a complex meristem that allows them to develop one of the most complexes foliar structures in land plants.

T4-30: Molecular and genetic analysis of seed development

T4-30-01

Auxin production following fertilization drives early seed development in *Arabidopsis*

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Seed development in flowering plants is preceded by a double fertilization event, whereby one of the paternal sperm cells fertilizes the female egg cell, giving rise to the embryo, and a second sperm cell fertilizes the central cell, which originates the endosperm. The endosperm is a triploid tissue that supports embryo development and, in species such as *Arabidopsis*, it is consumed as the embryo develops. Surrounding the two fertilization products is the seed coat, which derives from the ovule integuments and is of purely maternal origin. The successful establishment of a seed requires the fine tuning in the development of these three genetically-distinct structures, implicating paternally-derived signals that regulate the developmental processes. Seed development in most plant species is tightly coupled to fertilization, but this requirement can be bypassed in mutants for components of the FERTILIZATION INDEPENDENT SEED Polycomb Repressive Complex 2 (FIS-PRC2), which establish the repressive chromatin mark trimethylation of lysine 27 on histone H3 (H3K27me3). Lack of FIS-PRC2 function results in the formation of autonomous seeds, which develop in the absence of fertilization. We have shown that FIS-PRC2 represses the expression of auxin biosynthesis genes in the female gametophyte, so that the paternal-specific expression of these genes drives both endosperm and seed coat development after fertilization. Our data indicates that auxin production is sufficient to initiate seed development in *Arabidopsis*, but it also has a major role in non-autonomous cell communication between gametophytic and sporophytic tissues. The interplay between auxin and different PRC2 complexes, acting both in the gametophyte and in sporophyte, is therefore a key component for the successful establishment of the distinct seed structures during seed development.

T4-30-02

Are sRNAs involved in the drastic shift of allele-specific expression in hybrid tomato seeds?

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The roles of small RNAs (sRNAs) in plant gametophyte and seed development remain elusive, but recent investigations point to a critical involvement. A transcriptomic study has provided insights into hybrid seed failure in interspecific crosses between wild tomato species. There is a strong postzygotic isolation barrier between *Solanum peruvianum* and *Solanum chilense*, with endosperm-based incompatibilities being responsible for seed failure. We performed laser-capture microdissection coupled with RNAseq of the endosperm and analyzed patterns of gene and allele-specific expression (ASE). We found that ASE is disturbed in hybrid seeds with a genome-wide shift towards increased expression of maternal alleles disrupting normal patterns of ASE. Based on these results, we propose that silencing mechanisms regulating maternal allele transcription are malfunctioning. We investigated whether gene silencing mechanisms involving sRNAs, namely RNA-directed DNA methylation (RdDM), are involved in this process. We hypothesized that there is a lack of sRNA production in hybrid endosperm and thus proper transcriptional regulation/silencing does not take place, leading to the observed increase in maternal ASE, loss of imprinting, and hybrid seed failure. We will present analyses coupling sRNA and methylome sequencing of tomato seeds from intraspecific (viable) and interspecific (failed) crosses. We will present correlates of sRNA expression, methylation, expression and ASE in viable seeds to describe the epigenetic landscape of normally developing tomato endosperm. We will compare and contrast these patterns between viable and inviable seeds and test the hypothesis that sRNAs are down-regulated in hybrid endosperms. We will investigate the proposed link between the malfunctioning of the sRNA machinery and the misexpression of specific candidate genes to test for sRNA involvement in the establishment of reproductive isolation and therefore speciation.

T4-30-03

Temperature modulates seed dispersal in Brassicaceae

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Environmental signals such as temperature affect plant growth and development. Plants respond to diurnal and seasonal changes in temperature to reprogram developmental pathways critical for reproductive fitness and adaptation. Understanding the molecular mechanisms that define environmental modulation of plant growth and reproduction is important in the context of climate change that threatens crop yield. Here we show that elevated temperature accelerates seed dispersal in the model plant *Arabidopsis* and a number of other members of the Brassicaceae family. We found that *THERMAL RESPONSE FACTOR* 1 (*TRF1*), a valve margin identity gene, is upregulated by temperature-induced chromatin dynamics at the *TRF1* locus. Specifically, we show that temperature-induced changes in *TRF1* expression are associated with temperature-dependent H2A.Z nucleosome dynamics. Our findings connect tissue identity with thermal sensing and provide a molecular framework for understanding how temperature influences seed dispersal. These results will be valuable in the production of crops that are able to withstand the predicted rise in temperature at a global scale.

T4-30-04

Pollen tube contents initiate seed enlargement and enhance autonomous endosperm development without fertilization

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In animals, numerous sperm cells swim toward the egg and complete fertilization supported by seminal plasma, a fluid medium required for sperm metabolism, function and carrier to female reproductive tract. The SVS2 protein localized only in the seminal plasma protects sperms from uterine attack and is required for sperm capacitation in mice. In angiosperms, pollen tubes carry two sperm cells toward the egg and central cells to complete double fertilization. However, except for being a sperm cell carrier, nothing is known regarding the function of pollen tube content (PTC), a fluid medium, analogous to seminal plasma in animals. To investigate whether the PTC functions male-female interaction in plants, we performed transcriptome analysis for 3 types of ovules (seed primordia), non-pollinated (NP), pollinated and fertilized (PF) and pollinated but non-fertilized (PN; PTC only). Notably, multiple genes associated with cell expansion, cell division or seed coat development were significantly expressed in PN ovules. Based on the transcriptome analysis, we performed phenotypic analysis for PN ovules and the ovules showed cell expansion, cell division and seed coat development without fertilization. Here, we newly identified the PTC functions that are significant for seed development initiation. We termed this phenomenon as Pollen tube-dependent Ovule Enlargement Morphology (POEM) and placed it between pollen tube guidance and double fertilization. As well as seminal plasma in animals, PTC plays crucial roles in male-female interaction in plant reproduction. The other significant finding is that PTC increases endosperm nuclei without fertilization. PTC can dramatically increase endosperm nuclei number without fertilization in the previously identified mutants, capable of autonomous endosperm formation. This finding could be applied in agriculture, particularly enhancing seed formation without fertilization in important crops, which is called apomixis.

T4-30-05

Genetic and molecular regulation of grain chalkiness in rice

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Improving grain quality is one of the most important goals of plant science research. Grain chalkiness in rice, a chalky texture of endosperm, is a highly undesirable trait of the grain for human food that deteriorates milling, cooking, eating and appearance quality, and thus represents a major problem in many rice-producing areas of the world. However, the genetic and molecular basis of this trait is still poorly understood. Here, we performed genome-wide association studies for all grain chalkiness traits with four-year field replications, and detected 29 stable quantitative trait loci (QTL) and 15 major QTLs sensitive to temperature using the difference values of the two responsive chalky traits between the high (33°C/27°C, 2013 at Wuhan) and low temperature (27°C/21°C, 2014 at Wuhan) natural environments. Many NILs for these gain-chalkiness QTLs were constructed, and a major QTL, *Chalk5*, regulates grain chalkiness and also affects head rice yield and many other quality traits. *Chalk5* encodes a vacuolar H^+-translocating pyrophosphatase (V-PPase) with inorganic pyrophosphate (PPi) hydrolysis and H^+-translocation activity. *Chalk5* functions as a positive regulator of grain chalkiness rate. Elevated expression of *Chalk5* increases chalkiness of the endosperm putatively by disturbing the pH homeostasis of the endomembrane trafficking system in developing seeds, which affects the biogenesis of protein bodies and is coupled with a great increase in small vesicle-like structures, thus forming air spaces or spherical granules among endosperm storage substances and resulting in chalky grain. Introgression of the *Chalk5* allele of non-chalky variety into a chalky variety greatly decreases rice grain chalkiness rate by 50.5% and increases head rice yield by 37.9%, equally increasing the grain yield. Our results indicated that two consensus nucleotide polymorphisms among rice varieties in *Chalk5* promoter partly account for the difference in *Chalk5* mRNA levels causing natural variation of grain chalkiness. The tight linkage of *Chalk5* for grain chalkiness and quality, and *GS5* and *qSW5*/*GW5* for grain size, weight and yield, points to the genetic and molecular evidence for the inconsistency between grain quality and yield during modern breeding. Breaking such linkage by recombination may result in genotypes producing wide grain with low chalk to achieve high yield with good quality in breeding programs of *indica* rice.

T4-30-06

Origin of embryo polarity

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One of the first steps in the development of an embryo from a single celled zygote is the establishment of the main body axis. In model animals the orientation of this axis uses position information provided by surrounding maternal tissue or the sperm entry point. In most flowering plants, the apical-basal body axis is reflected by the asymmetric division of the polarized zygote. However the underlying mechanisms regulating axis formation remained enigmatic. Here we address which factors establish zygote polarity. We demonstrate that both maternal and paternal factors co-operate in the initiation of the embryo axis.

T4-31: Metabolites in biotic and abiotic stresses

T4-31-01

Evolutionary neighbourhood and climate interact to select for heritable defense phenotypes: anthocyanin and flavonoid contents and inducability

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Under global change, trees face a double pressure from enemies and harsh climate. Both pressures may be modified by the tree neighbourhood such as increased enemy pressure in a closely related neighborhood. Both, local neighbourhood and regional climates may select for adapted genotypes. Adaptations to such pressures include flavonoids and anthocyanins, suggesting that the adaptation to one pressure may interact with the adaptation to the other, either by a trade-off or by mutual facilitation. We studied oak seedlings in evolutionary proximate and distant neighbourhoods as well as adults of genotypes that have been selected by such respective neighbourhoods. We studied anthocyanin and flavonoid contents as well as morphological defenses and the performance of seedlings and trees in terms of growth and survival. We found evidence for a response to both climate and neighbourhood, and heritability of this response. Tentative results are consistent with inducible chemical defenses and (constitutive structural defenses) being selected by an evolutionary proximate neighbourhood. Sharing neighbourhoods with close relatives seems to require particular levels, compositions and inductions of defense compounds.

T4-31-02

Pseudomonas fluorescens G20-18 derived cytokinins determine the biocontrol of *Pseudomonas syringae* infection in *Arabidopsis* - a novel biocontrol concept

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Throughout their life, plants interact with a wide range of pathogenic and beneficial microbes. Beneficial bacteria are described for positive effects such as plant growth promotion, increased tolerance to environmental stress and enhanced disease resistance. Considering future challenges through increasing world population and climate change in combination with restrictions of classic pesticides which urgently demands alternative strategies in plant protection, the biological control of plant diseases by beneficial microbes offers high potential for integrated plant disease management. To facilitate the development of according microbe-based biocontrol strategies, the underlying mechanisms of these effects have to be fully elucidated. Mechanisms contributing to such biocontrol phenomena comprise direct effects on pathogens or virulence factors and strengthening the plant. Recently, the function of the classic growth-stimulating phytohormone cytokinin in modulating plant immunity has been identified in different plant species, complementing the established phytohormone-based defence signaling network. Underlying mechanisms mediating cytokinin-dependent resistance comprise induction of salicylic acid, phytoalexins and reduction of abscisic acid levels in a plant species-specific, cytokinin type-specific, and dose dependent manner. Despite the importance of phytohormones, particularly cytokinins, as essential plant immunity regulators, their microbial production is not yet considered in biocontrol of diseases. We identified the cytokinin production of *Pseudomonas fluorescens* G20-18 as a key determinant of biocontrol of *P. syringae*pv. *tomato* DC3000 infection in *Arabidopsis*. Treatment with this strain strongly suppressed symptom development and spread of the pathogen, thus maintaining tissue integrity, which ultimately sustains biomass yield. While cytokinin-deficient loss-of-function mutants were impaired in controlling the infection, mutants complemented with different cytokinin biosynthetic genes (gain-of-function), exhibiting restored cytokinin production and showed a similar biocontrol effect as the wild-type strain. The efficiency of the biocontrol effect correlated with the cytokinin levels *in planta* resulted from treatments with the different bacterial strains. Analyses of Arabidopsis mutants impaired in defence pathways revealed the necessity of various defence and signalling components. Functional cytokinin perception as well as salicylic acid biosynthesis and signalling were essential to fully establish this biocontrol effect in combination with potential minor involvement of jasmonic acid and ethylene signalling as well as camalexin accumulation. This identification of microbial-derived phytohormones to trigger biocontrol effects offers novel options for the development of successful strategies in plant protection which may be combined, based on cytokinins, with other positive effects such as increased abiotic stress tolerance and plant growth.

T4-31-03

Insect (*Thrips Hawaiiensis* (Morgan)) attacks change the stereochemical configuration of 1-phenylethanol emitted from tea (*Camellia Sinensis*) flowers

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Insect attacks change the quantity of volatiles emitted from plants, but little is known about the effects on the volatiles' stereochemical configurations. 1-Phenylethanol (1PE) is a major internal volatile of tea (*Camellia sinensis*) flowers. In the present study, insect-damaged *C. sinensis* flowers in the field emitted a different (*R*)-/ (*S*)-1PE pattern than that emitted from undamaged flowers. Laboratory experiments showed that changes in the (*R*)-/ (*S*)-1PE ratio were caused by *Thrips hawaiiensis* (Morgan), a pest of cut flowers and vegetable crop, attacks. These attacks led to an increase in jasmonic acid (JA) through the activation of allene oxide synthase, which is involved in JA biosynthesis. Exposing flowers to exogenous JA supplements induced an (*R*)-/ (*S*)-1PE emission pattern that was similar to that induced by a *T. hawaiiensis* attack, suggesting that JA was involved in the changes in the stereochemical configuration of 1PE emitted from the flowers after an insect attack. This study provides new insights into the involvement of volatiles in interactions between plants and insects, showing that not only the quantity but also the stereochemical configuration of plant volatiles is affected by insects.

T4-31-04

Molecular and biochemical analysis of high light induced non-uniform anthocyanin pigmentation in fruits of *BrTT8* transgenic tomato System

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Pigment intensity and pattern are important factors that determinate nutrient and market value of tomatoes. *BrTT8* transgenic tomato plants displayed intense purple anthocyanin pigmentation in leaves, stems, petals and fruits when grown under high-light condition, yet remain acyanic under shade condition. The light-sensitive property of the *BrTT8* transgenic tomato plants makes it a good system that can be used to investigate how light intensity affects anthocyanin biosynthesis and accumulation. The colored phenotypes matched well with anthocyanin production and transcripts abundance of the anthocyanin biosynthetic genes. In *BrTT8* transgenic tomato plants, the constitutive expression of *BrTT8* and *SlTTG1* suggests that high-light induced anthocyanin pigmentation in transgenic tomato plants might be regulated by the temporal and spatial expression of SlAN2. Yeast two-hybrid assay showed that bHLH proteins could interact efficiently with endogenous MYB proteins (SlAN2 and SlMYBL2) and WD40 protein, respectively. Co-expression of *SlAN2* and *BrTT8* induced anthocyanin accumulation significantly in a new set of transgenic plants under normal light condition. These results show that a transcriptional regulation complex consisting of SlAN2, SlTTG1 and exogenous BrTT8 regulates anthocyanin accumulation in *BrTT8* transgenic tomato system under high-light condition. Moreover, a model explaining the non-uniform anthocyanin pigmentation in transgenic tomato fruits was proposed. This work provides more insights into molecular study of accumulation of other secondary metabolites in crops and vegetables with special genotypes under various environmental conditions. Furthermore, the elucidation of the anthocyanin pigmentation pattern in transgenic tomato fruits offers a new angle and cut-in point for the study of non-uniform biological processes in other plant organs.

T4-31-05

Rootstock-specific xylem metabolite signatures in tomato

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Identifying root-derived chemical signals and understanding their specific effects in shoot tissues could open new strategies to improve crop yield stability under abiotic stress conditions (Albacete *et al.*, 2015). Therefore, a commercial tomato cultivar (*Solanum lycopersicum* cv Boludo F1, Monsanto) was either self-grafted (L/L) or grafted onto the interspecific commercial tomato rootstock Maxifort (Monsanto) obtained from a cross between *S. lycopersicum* and *S. habrochaites* (L/H), and onto a recombinant inbred line obtained from a cross between *S. lycopersicum* and *S. pimpinellifolium* from AVRDC (L/P). Grafted plants were cultivated under commercial greenhouse conditions during autumn season in Mazarrón (Spain). Xylem sap, collected by root pressure, was injected into a U-HPLC-MS system (EXACTIVE, ThermoFisher Scientific). The metabolite signature of the '*pimpinellifolium'* L/P graft combination was strongly associated to the first principal component (PC1), which explained 47% of the total chemical variance, while the '*lycopersicum'* L/L combination was linked to PC2, explaining 31% of the variance. The metabolite profile of the '*habrochaites*' L/H plants clustered between the other two combinations. Additionally, we identified 81 putative compounds of both the primary and secondary metabolism. We have shown that the metabolite profile of the xylem sap can be qualitatively and quantitatively modified by changing the root system (rootstock), as a genotypic source of chemical variability. Xylem sap profiling is likely to provide physiological markers to assist root-targeted breeding for resistance to individual or combined biotic and/or abiotic stresses.

T4-32: Stem cell maintenance and cell differentiation in shoot meristem and cambium

T4-32-01

Role of auxin and cytokinin in the secondary vascular tissue regeneration after bark girdling in *Populus*

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The secondary vascular tissues (SVT) can be regenerated after the large-scale bark girdling in trees. However, the mechanisms of SVT regeneration is largely unknown. Here, we explored the roles of auxin and cytokininin in the SVT regeneration process after bark girdling by utilizing an *in vitro* SVT regeneration system in poplar. After the bark girdling, the phloem and cambium were peeled off. With the auxin treatment, the specific callose deposition can be detected at 6-9 days after girdling (DAG) while the expression of phloem specific marker genes is upregulated at the same stage, and the continuous cambium can be observed at 13 DAG confirmed by *pAIL1::UidA* reporter line. With the cytokinin treatment, the phloem can be still regenerated while no cambium regeneration was observed on 13 DAG, suggesting the differential requirement of cytokinin for phloem and cambium regeneration. These results were validated by using transgenetic trees when cytokinin signaling was enhanced. By employing *pDR5*::*UidA* transgenic line, we were able to visualize the auxin redistribution patterns during the SVT regeneration. New auxin maximum appears along with the formation of the continuous cambium on 13 DAG, indicating that auxin redistribution provides the position information for the cambium recovery. Interestingly, the combined application of auxin and cytokinin failed to display the specific auxin distribution pattern as well as the new cambium. This result implied that cytokinin may block the re-establishment of auxin gradients towards paralyzing the cambium regeneration in this *in vitro* system.

T4-32-02

Feedback from leaves controls shoot apical meristem growth

by modulating auxin transport

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Stem cells must balance self-renewal and differentiation; thus, stem cell activities are precisely controlled. In plants, the control circuits that underlie division and differentiation within meristems have been well studied but those that underlie feedback on meristems from lateral organs, such as leaves, remain largely unknown. Here we show that long-distance auxin transport mediates this feedback in a non-cell-autonomous manner. A low-auxin zone is associated with the shoot apical meristem (SAM) organization center, and is required for regulation of SAM size. Using computational model simulations, we show that auxin transport from leaves can inhibit auxin transport from the SAM through an auxin transport switch, and thus maintain SAM auxin homeostasis and SAM size. Genetic and microsurgical analyses confirmed the model's predictions. In addition, the model explains the surprising observation that *yabby* mutants exhibit oscillations of SAM size. Our study shows that plants use a distinct feedback control mechanism for long-distance regulation of stem cell activities.

T4-32-03

Interaction between major regulators WUS and STM controls stem cell specification in shoot apical meristem of *Arabidopsis*

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Plants have evolved a powerful capability to continuously and repetitively generate lateral organs through their meristems. Shoot apical meristem (SAM) gives rise to the aerial part of a plant. Among the regulators identified in SAM of *Arabidopsis*, the homeobox genes *WUSCHEL* (*WUS*) and *SHOOTMERISTEMLESS* (*STM*) encode two major regulators of stem cell specification, yet the molecular mechanisms underlying their function and interaction in stem cell regulation are still under exploration. In this study, we identified STM acting as a direct interacting factor with WUS. STM has synergistic effects with WUS on regulation of their common target *CLAVATA3* (*CLV3*). Moreover, STM synergistically strengthens WUS on *CLV3* transcription, but its function largely depends on WUS. Furthermore, *STM* expression is activated and maintained by WUS, and STM is also critical to maintain *WUS* expression in SAM. Our discoveries thus reveal a new framework for the regulation of stem cell specification during SAM development.

T4-32-04

How do embryos make stem cells?

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A central process during embryogenesis is the formation of stem cells that provide an organism with new cells during the postembryonic phase. Despite considerable progress in elucidating the regulation of postembryonic stem cell homeostasis, however, the regulatory networks responsible for stem cell formation during embryogenesis remain poorly understood in plants and animals. With regard to their developmental potential and plasticity, stem cells resemble early embryo cells and used to be called embryonic initials by the British botanist C. W. Wardlaw. In this study, we investigate the formation of stem cells during *Arabidopsis* embryo development. Our findings reveal a molecular framework combining transcriptional regulators and hormonal components governing this process. These results link stem cell formation to early embryo patterning and revisit classical experiments by Skoog and Miller on plant regeneration.

T4-32-05

Symplastic communication in vascular meristems

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My group is investigating the morphogenesis of phloem, a long distance transporting tissue specified to transport the various products derived from the photosynthesis. Based on identification of the gain-of-function mutations in one of the callose synthase isoforms (responsible for formation of callose, b1,3-glucan polymer that plant uses to regulate symplastic trafficking through plasmodesmata nanochannels) we have recently developed a molecular tool, icals3m, with which we can regulate symplastic molecular trafficking in a time and space specific manner. Using this tool we have identified a family of genes coding for transcription factors that are transcribed in the young sieve element position, and whose protein products move to the adjacent procambial cells to complete procambial/phloem patterning.We are also investigating phloem development (with a focus on sieve element differentiation) in high resolution as a paradigm for how a plant cell progresses from a stem cell to a fully differentiated state. In the *Arabidopsis* root there are some 25 cells from the relatively isodiametric stem cell (touching the quiescent centre of the root) to the elongated cell that loses its nucleus as the final stage of sieve element cell differentiation. Finally, phloem transport is based on the interaction of the sieve element/companion cell unit with their neighbouring cellular domains (in the root culminating at unloading of organic substances to the meristem). We are now analysing other aspects of phloem morphogenesis (and also function) that may involve symplastic communication.

T4-32-06

DWT1 is a key regulator coordinating the panicle growth and culm elongation in rice

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Rice is one of the major staple food plants around the world. The agronomic performance of rice depends largely on desirable plant architecture. Plant height is one the fundamental component of aerial structure of a rice plant. The elongation of uppermost four or five internodes determines the culm (stem) length, which dose not occur until the transition from vegetative growth to reproductive growth. The coordination of the panicle (inflorescence) growth and the culm elongation ensures the timely emergence of the mature panicle from leaves. However, signals and mechanisms responsible for triggering the internode elongation remain largely unknown. We report that *DWARF TILLER1* (*DWT1*) is involved in initiating the culm elongation in rice. The *dwt1* mutant plant develops dwarf tillers with one to several shorter internodes compared with those of the wild type. Shorter internodes results from reduced cell number and cell length, indicating that DWT1 affects cell division and cell elongation. Map-based cloning revealed that *DWT1* encodes a WUSCHEL-related homeobox (WOX) transcription factor homologous to the Arabidopsis WOX8 and WOX9. The *DWT1* gene is highly expressed in young panicles, but undetectable in the internodes, suggesting that DWT1 expression is spatially or temporally separated from its effect on the internode growth. Transcriptomic analysis revealed altered expression of genes involved in cell division and cell elongation, cytokinin/gibberellin homeostasis and signaling in *dwt1* shorter internodes. Moreover, the non-elongating internodes of *dwt1* are insensitive to exogenous gibberellin (GA) treatment, suggesting that the DWT1 activity in the internode elongation is directly or indirectly associated with GA signaling. Our study reveals a genetic pathway synchronizing the development of the panicle and the culm in rice. Interestingly, the *dwt1* main shoot is much less affected and bears a larger panicle compared with *dwt1* tillers, indicating that DWT1 is also involved in balancing the main shoot and tiller growth in rice. Recent progress on understanding how DWT1 coordinates the panicle growth and the culm elongation will be introduced.

T4-33: Male sterility and EGMS

T4-33-01

Molecular mechanism of *Sa*-related reverse thermo-sensitive male sterility in rice

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Manipulation of male fertility is essential for heterosis utilization in crop production. However, hybrid sterility has long been a major barrier in utilization of heterosis. *Sa* is a major locus for *indica-japonica* . We have revealed that *Sa* consists of two adjacent genes *SaF* and *SaM*. In this study, we identified a reverse thermo-sensitive male sterility (rTMS) rice line G392S, which was male sterile under low temperature condition and male fertile under high temperature condition. The fertility of this rTMS line was controlled by an gene, *ORF13*, adjoining to *Sa*, with an average transit temperature at 31 ℃ . When G392S was treated under high temperature (~35 ℃), the expression level of *SaF* decreased dramatically, suggesting that the *SaF* is involved in the regulation of the rTMS trait. Further, using CRISPR/Cas9 gene editing, we knocked out *SaF*, *SaM* and *ORF13* respectively in G392S, and found that the rTMS trait disappeared in resultant knockout lines. Thus, we proposed a three gene/four-component model for the molecular mechanism of rTMS in rice. The application of this rTMS trait in hybrid rice breeding will be discussed in the presentation.

T4-33-02

RNase Z^{S1} mediates Ub_{L40} mRNA decay controlling thermo-sensitive genic male sterility in rice

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Male sterility is an important trait and plays a key role in hybrid breeding in crops. Thermo-sensitive genic male sterility (TGMS) lines, which are male-sterile at high temperatures but convert to male-fertility at low temperatures, have been widely used in two-line hybrid rice breeding. However, the molecular mechanism underlying TGMS remains unclear. Here, we show that a rice (*Oryza sativa* L.) *thermo-sensitive genic male sterile gene 5* (*tms5*) locus confers the TGMS trait through a loss-of-function mutation of *RNase* Z^{S1}, resulting in failing to mediate the mRNA decay of a temperature-responsive *ubiquitin fusion ribosomal protein L40* (Ub_{L40}). RNase Z^{S1} belongs to a short version of the RNase Z family originally involved in tRNA 3'-end processing in nucleus. RNase Z^{S1} retains the endonuclease activity in tRNA processing *in vitro*, however, it does not play a role *in vivo* due to its localization in cytoplasm. RNase Z^{S1} specifically cleaves three Ub_{L40} genes. Defective RNase Z^{S1} in *tms5* plants causes over-accumulation of Ub_{L40} mRNAs and defective pollen development at high but not low temperatures. Finally, we developed a new method of TGMS line breeding using the CRISPR/Cas9-mediated *TMS5* editing system. Our results not only uncover a novel function of the RNase Z family member and reveal the molecular mechanism of rice TGMS through a new post-transcriptional regulation for ubiquitin homeostasis, but also improve the method of TGMS line breeding. Moreover, this study provides applied potential for hybrid breeding not only in rice but also in other crops.

T4-33-03

Towards controlling fertility for hybrid production in temperate cereals; the target of hybrid wheat - applying lessons learnt from other systems.

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Heterosis or Hybrid vigour can result in increased crop yield (3-15%) and enhanced resilience in many crops, however not all crops are able to capitalize upon these potential gains. This is particularly the case for the temperate cereals such as barley and wheat, which as autogamous crops require self-fertilisation to be controlled via the generation of male sterile plants for crossing and for these lines to then be subsequently maintained. Currently the application of hybrid wheat is extremely limited, with only ~200,000 ha planted in Europe, mostly produced using chemical hybridization agents, and about 35,000 ha in China and India, using photoperiodic sensitivity, or the CMS system from *Triticum timosphevii.* Recently, in 2002, a commercial hybrid barley system was commercialised based on a CMS system, however currently only ~200,000 ha are being grown in Europe, nevertheless this is likely to increase due to the reported increased in yield and disease resistance. However, major advances have occurred in other species, typically rice, of which 20 million ha is hybrid, based upon CMS and environmental sensitivity systems. We have been using knowledge from the model systems of *Arabidopsis* and rice to provide knowledge of pollen development and the control fertility in barley and to subsequently apply this to wheat. We have been investigating the use of key conserved transcription factors that are associated with tapetum function as mechanisms to control fertility. Using specific targets we have been able to generate male sterile plants and then by altering the environmental conditions restore fertility. This is providing valuable information on the process of pollen development and the conservation of gene networks in the anther, but is also providing potential switchable systems for controlling fertility for hybrid development and targeted breeding in temperate cereal crops. The progress towards hybrid development of temperate cereals and understanding of tapetum gene networks will be discussed.

T4-33-04

Comparative analysis of cytoplasmic male sterility developed from various rice.

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A cooperative relationship between nucleus and organelle is important for a normal development of plant. In rice, a nuclear substitution sometimes causes defects of male organ development, because of an incompatibility between nuclear and mitochondrial genomes. Such a phenomenon is called cytoplasmic male sterility (CMS) and caused by the expression of mitochondrial unique *orf* termed CMS-associated gene. The function of the CMS-associated gene is prevented by the presence of a particular nuclear gene, called *Rf* (*Restorer of fertility*). We have been studying BT-, CW-, and WA-CMS in rice. Recently, we obtained new type CMS rice, RT98- and RT102-CMS developed from wild rice, *Oryza rufipogon*. Those CMS lines possess the same nuclear genome, because they had been developed by backcrossing with the same cultivar, Taichung 65 (T65). Phenotype comparisons of mature pollen among these five CMS lines revealed that the mitochondrial genotype possibly determines the timings of pollen abortion. In the BT-, CW- and WA-CMS line, the mitochondrial *orf79*, *orf307* and *WA352* are known as CMS-associated gene, respectively. To reveal the CMS-associated gene of RT98- and RT102-CMS, we determined the whole sequence of both types of mitochondria. We found *orf113* and *orf352* respectively, as CMS-associated genes. We have cloned *Rf1* for BT-CMS, *Rf17* for CW-CMS and *Rf4* for WA-CMS. These researches revealed that *Rf1* and *Rf4* encode pentatricopeptide repeat (PPR) protein and *Rf17* encodes an unkonwn protein. To identify fertility restorer genes for RT98- and RT102-CMS, we are performing map-based cloning of these genes. Map-based cloning and complementation tests revealed that an *Rf* gene for RT98-CMS, *Rf98*, also encodes PPR protein. We present recent results of our works.

T4-33-05

Hybrid sterility in rice: From genetics to mechanisms

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Understanding the origin and mechanisms of reproductive isolation is of fundamental importance in evolutionary biology. However, little is known about how genomes evolve to form and establish reproductive barriers in the course of evolution especially during domestication, although such reproductive barriers are widely observed and demand understanding for both biological study of speciation and practical application in crop improvement. Studies have shown that the reproductive barrier regulated by S5 features a killer-protector system encoded by three tightly linked genes (*ORFs3–5*). Typical *indica* and *japonica* varieties contain the haplotypes of *ORF3+ORF4-ORF5+* and *ORF3-ORF4+ORF5-*, respectively. The killer is made of functional alleles of *ORF4+* and *ORF5+*, while the functional allele of *ORF3+* confers protection to the gametes, such that female gametes without the protector *ORF3+* are selectively eliminated, causing hybrid sterility and segregation distortion. We find that such trigenic system of S5 originated by gene duplication after the split of the Oryzeae tribe from other grasses, most likely through *Helitron* transposition. A combination of mutational steps generated incompatible *indica* and *japonica* alleles in pre-differentiated rice groups, giving rise to the trigenic reproductive isolation system. Natural selection in *indica* rice and founder effect associated with domestication in *japonica* populations increased the frequencies of incompatible alleles to form a functional reproductive barrier between the *indica* and *japonica* subspecies, suggesting that domestication can lead to the early stages of speciation in crops, and eventually result in genetic differentiation and restructuring of rice genetic composition. This finding provides an unequivocal example of the origin of a complex system by gene duplication and illustrates the evolutionary processes of establishing a reproductive barrier by actions of combinatorial molecular mechanisms and evolutionary forces. The results may have significant implications for understanding genome evolution and for crop genetic improvement.

T4-33-06

Construction of a male sterility system for hybrid rice breeding and seed production using a nuclear male sterility gene

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The breeding and large-scale adoption of hybrid seeds is an important achievement in agriculture. Rice hybrid seed production uses cytoplasmic male sterile lines (CMS) or photoperiod/thermo-sensitive genic male sterile lines (PTGMS) as female parent. CMS lines are propagated via cross-pollination by corresponding maintainer lines, whereas PTGMS lines are propagated via self-pollination under environmental conditions restoring male fertility. Despite huge successes, both systems have their intrinsic drawbacks. Here we constructed a new rice male sterility system using a novel nuclear gene *OsNP1*. *OsNP1* encodes a putative GMC oxidoreductase protein regulating tapetum degeneration and pollen exine formation. It is specifically expressed in the tapetum and miscrospores. The *osnp1* mutant plant displays normal vegetative growth but complete male sterility insensitive to environmental conditions. *OsNP1* was coupled with an a-amylase gene to devitalize transgenic pollen and the *DsRed* gene to mark transgenic seed, and transformed into the *osnp1* mutant. Self-pollination of the transgenic plant carrying a single hemizygous transgene produced non-transgenic male sterile and transgenic fertile seeds in 1:1 ratio that can be sorted out based on the red fluorescence coded by *DsRed*. Cross-pollination of the fertile transgenic plants to the non-transgenic male sterile plants propagated the male sterile seeds of high purity. The male sterile line was crossed with ~1200 individual rice germplasms available. Approximately 85% of the F1s out-performed their parents in per-plant yield, and 10% out-yielded the best local cultivars, indicating the new technology is promising in hybrid rice breeding and production. This new system is fundamentally advantageous over the current CMS and PTGMS systems. Application of this new technology will greatly enhance the effectiveness and efficiency in hybrid rice breeding and production.

T4-34: Hormone perception and signaling

T4-34-01

AtD14 and ShHTL7 are non-canonical receptors for strigolactone

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Strigolactones (SLs) are a group of carotenoid-derived small molecules synthesized by plants and act as a very special type of plant hormones. As a class of phytohormones, SLs regulate shoot branching, which is an important agronomic trait to determine crop yield. Intriguingly, SLs are also exuded into the soil as rhizospheric signals for communication with arbuscular mycorrhizal fungi (AMF) and root parasitic weed seeds: SLs facilitate the AMF symbiosis with a wide range of land plants, which improves nutrient and water uptake of the host plants, and stimulate seed germination of the root parasitic weeds such as *Striga* for parasitic growth on host plants, which causes agricultural disaster with the loss of billions of dollars' worth of crops every year. Through analysis of a highly branched *Arabidopsis* mutant *d14-5* (Yao *et al.,* 2016), we show that the AtD14^{G158E} mutant maintains enzyme activity to hydrolyze strigolactone, but fails to efficiently interact with D3/MAX2 and loses the ability to trigger strigolactone signaling *in planta,* indicating that the α/β hydrolase AtD14 has dual functions to hydrolyze and perceive SL. We further demonstrate that strigolactone is hydrolyzed by AtD14 into a D-ring derived intermediate, which serves as the novel active form of SL covalently linked with AtD14 (named as the covalently linked intermediate molecule, CLIM). The crystal structure of the strigolactone-induced AtD14-D3-ASK1 complex reveals that CLIM-bound AtD14 undergoes an open-to-closed state transition to interact with D3/MAX2 for triggering strigolactone signaling. Similarly (Yao *et al.,* 2017), we has further defined *Striga hermonthica* HTL7 (ShHTL7, which is not an ortholog of AtD14) as a non-canonical receptor for SL in root parasitic weeds: In parallel with the SL perception by AtD14, ShHTL7 also hydrolyzes SLs into an active intermediate CLIM and covalently binds CLIM to interact with ShMAX2/MAX2 and other downstream signaling components for stimulating seed germination. These findings uncover a mechanism underlying the allosteric activation of AtD14 by strigolactone hydrolysis into CLIM, and define AtD14 as a non-canonical hormone receptor with dual functions to generate and sense the active form of strigolactone.

T4-34-02

A novel function for host jasmonate signaling in viral transmission

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Plant hormones are characterized by the property of serving as chemical messengers, which control cellular activities (division, elongation and differentiation) and processes (pattern formation, sex determination, organogenesis and stress responses). Jasmonate (JA) is a class of plant hormone and ubiquitous in plant kingdom. We previously uncovered that the F-box protein COI1 (Science 280:1091) assembles into the SCF complex (Plant Cell 14:1919; Plant Cell 16:5; Plant Cell 25:486) and functions as a JA receptor (Plant Cell 21: 2220) to regulate diverse plant developmental processes and various plant defense responses (Mol. Cell 50:5014; Plant Cell 27:1634; Plant Cell 27:1620; Plant Cell 26:1118; Plant Cell 26: 263; Plant Cell 23:1795; Plant Cell 23:1000; PLoS Genetics 9:e1003653; PLoS Genetics 5:e1000440). Interestingly, we recently found that hormone signaling pathway in plants was targeted by virus to attract insect vectors for efficient viral transmission. We reveal that cucumber mosaic virus employs viral effector protein 2b to target the host JA signaling pathway for inducing odor-dependent aphid attraction. The recent progress will be discussed.

T4-34-03

Ethylene induced combinatorial effect of histone acetylation in ethylene response

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My research is focus on how plant hormone affect plant growth and plant abiotic stress response. Ethylene is one of the most important plant hormones. It plays pleotropic roles in many aspects of plant life. A model for hormone signaling has emerged in which the perception of ethylene by the receptors alters the activity of CTR1, which in turn, by an unknown mechanism, functions to relieve repression of EIN2, resulting in activation of EIN3/EIL1-dependent transcription and the activation of an ethylene response. Our recent studies have revealed that EIN2 mediates transmission of ethylene signaling that originates at the endoplasmic reticulum membrane to the nucleus and that the EIN2 C-terminus is cleaved and translocated to the nucleus to initiate the ethylene response. However, the molecular mechanism that nuclear translocation of EIN2, the function of EIN2 C-terminal end in the nucleus and how EIN2 C-terminal end communicate with EIN3 are not understood. We recently found that ethylene specifically elevated acetylation of histone H3K14 and the non-canonical acetylation of H3K23. The up-regulation is positively associated with a set of ethylene regulated transcription activation, and the elevation requires EIN2 and partially EIN3/EIL1. Both EIN2 and EIN3 interact with a SANT domain protein EIN2 Nuclear Associate Protein ENAP1 (ENAP1), of which over expression result in elevation of Histone acetylation. These findings reveal that in the presence of ethylene, EIN2 C-terminus is involved in the regulation of the elevation of acetylation at H3K14 and H3K23. In addition, our study reveals that the plant hormone ethylene induces combinatorial effects of H3K9Ac, K14Ac and K23Ac histone acetylation in gene expression genome widely. Further, for a group of ethylene regulated genes, in the absence of ethylene the levels and the covered breadth of H3K9Ac are the preexist markers for distinguishing up- and down- regulated genes, the change in the levels of H3K14Ac and H3K23Ac are required for the alteration of gene expression in the presence of ethylene.

T4-34-04

Degradation of BES1 through autophagy pathway coordinates brassinosteroid-regulated plant growth and stress responses

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Plants need to balance their growth and development in response to various stress conditions. BES1 is a transcription factor that functions as a master regulator in the Brassinosteroid (BR) signaling pathway, directing a transcriptional network that controls the expression of thousands of genes involved in plant growth, development and stress responses. Despite extensive characterization of BES1 in driving BR-related gene expression, the pathways and components regulating BES1 protein levels are not well understood. We found that BES1 interacts with the ubiquitin receptor protein BIP5 and is targeted to the autophagy pathway during stress via the interaction of BIP5 with ATG8, an ubiquitin-like protein directing autophagosome formation and cargo recruitment. Additionally, BIP5 is phosphorylated by the GSK3-like kinase BIN2, a negative regulator in the BR pathway. BIN2 phosphorylation of BIP5 flanking its ATG8 interacting motifs (AIMs) promotes the interaction of BIP5 with ATG8, thereby targeting BES1 for degradation. Accordingly, loss-of-function *bip5* mutants accumulate BES1, have altered global gene expression profiles, and have compromised responses to drought and fixed-carbon starvation stresses. Furthermore, we found that E3 ubiquitin ligase BAR1 can ubiquitinate BES1 under stress conditions and is involved in BES1 degradation through autophagy pathway. Our results thus reveal a new mechanism by which plants coordinate growth and stress responses by targeting a central growth regulator to the selective autophagy pathway via an E3 ubiquitin ligase and a phospho-regulated ubiquitin receptor protein.

T4-34-05

Regulation of master growth repressor DELLA by *O*-Glycosylation in *Arabidopsis*

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Plant development requires constant coordination among complex internal hormonal signaling networks to enhance plant's adaptation to changing environments. The conserved transcription regulators DELLA proteins, originally identified as repressors of phytohormone gibberellin (GA) signaling, play a central role in integrating activities of multiple signaling pathways via direct protein-protein interactions with key transcription factors. We found that DELLA's binding affinity to several interacting proteins is negatively regulated by *O*-linked *N*-acetylglucosamine (GlcNAc) modification by an *O*-GlcNAc transferase (OGT, named SECRET AGENT) in *Arabidopsis*. These results reveal a direct role of OGT in repressing DELLA activity, and indicate that *O*-GlcNAcylation of DELLAs provides a fine-tuning mechanism in coordinating multiple signaling activities during plant development. Interestingly, SPINDLY, a paralog of SEC, plays an opposite role in regulating DELLA function. The biochemical function of SPINDLY will be discussed.

T4-34-06

ABA promotes leaf senescence and bud dormancy to improve plant survival under extreme drought

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Drought stress is a condition that in specific climate contexts, results in insufficient water availability and often limits plant productivity through reducing plant growth, development and survival of plants. Plants use senescence of old leaves and dormancy of buds and seeds to survive extreme environmental conditions. The plant hormone abscisic acid (ABA) is key to drought tolerance. ABA is sensed by the PYR/PYL/RCAR proteins. In the presence of ABA, PYLs interact with and inhibit clade-A protein phosphatase type 2Cs (PP2Cs). The inhibition of PP2Cs leads to the activation of the SNF1-related protein kinase 2s (SnRK2s). The activated SnRK2s phosphorylate transcription factors such as ABF/AREBs to regulate expression of ABA-responsive genes, and other substrates related to stomatal movement, production of ROS, cytoskeleton dynamics, RNA binding, miRNA and epigenetic regulation, chloroplast function and many other processes. ABA increases plant survival by inducing quick responses such as stomatal closure, and long-term responses such as extended growth inhibition, osmotic regulation, accumulation of cuticular wax, senescence, abscission, and dormancy. Leaf senescence and abscission of older leaves reduce total plant transpirational water loss and increase the transfer of nutrients to meristems and to some storage tissues. Osmotic regulation favors water consumption in sink tissues, and accumulation of cuticular wax helps to seal the plant surface and limits non-stomatal water loss. ABA is also an important plant hormone controlling plant growth and development. The extreme ABA-insensitive mutants are wilty-dwarf plant with reduced fertility in soil. The senescence and abscission of flower organs are also delayed in these plants. These results reveal ABA is essential for normal plant growth and development under unstressed condition.

T4-35: Signaling by RAC/ROP GTPases and G-proteins

T4-35-01

Regulation of G protein complex in plants

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The heterotrimeric G protein complex, composing of three subunits: Gα, Gβ and Gγ, emerged in a eukaryotic ancestor. Exchange of GDP for GTP on the Gα subunit leads to G-protein complex dissociation to transmit signals to downstream effectors. While animal G-proteins become activated by G protein-coupled receptors, the regulatory mechanisms differ greatly in plants. Unlike in animals where the nucleotide exchange on Gα requires G protein-coupled receptors (GPCRs), G protein spontaneously activates without the aid of GPCRs in plants. The activation of G proteins in plants is controlled by a Regulator of G protein Signaling (RGS) tethered with seven transmembrane helices (7TM-RGS). This form of regulation is present in most vascular plants but excluded in some cereals and bryophytes that lack the 7TM-RGS gene. Additionally, the Gβ and Gγ genes in plants have duplicated and diversified to produce multiple repertoire of G protein complex, conferring signaling complexity to the G protein network. I will talk about (1) the fundamental biochemical property and regulatory mechanism that differ between animal and plant G proteins, (2) cataclysmic evolution of G protein genes and regulatory systems within the plant kingdom, and (3) the current issues in plant G protein science and our current approaches using three model species.

T4-35-02

Heterotrimeric G proteins regulate panicle branching in rice

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The Green Revolution of the second half of the 20th century, which was based on the adoption of semi-dwarf cereals which had an increased harvest index, was responsible for worldwide crop yield increases. However, introduction of semidwarf genes *sd1* and *Rht* into rice and wheat caused the reduction of panicle (or ear) branching. Here we show that a quantitative trait locus *DEP1* is associated with the increases in panicle branching and grain yield in rice. The *DEP1* gene encodes a non-canonical Gβ subunits, which have an N-terminal GGL (G protein gamma-like) domain, a weakly predicted transmembrane helix near in the central region, and a long C-terminal cysteine-rich domain that consists of the vWFC (von Willebrand factor type C) and TNFR (tumor necrosis factor receptor)/NGFR (nerve growth factor receptor) motifs. Loss-of-function mutation at the *DEP1* locus exhibits decreased number of grains per panicle, whereas gain-of-function mutation confers increased number of grains per panicle and grain yield, indicating that *DEP1* is a positive regulator of panicle branching and grain yield. To uncover the role of the DEP1 protein in control of panicle branching, we performed a yeast two-hybrid screening to identify DEP1-interacting proteins. We found that the DEP1 protein physically interacts with both Gβ (RGA1), and Gβ (RGB1), and reduced RGA1 or enhanced RGB1 activity represses panicle branching. Thus, the modulation of G-protein signalling represents new strategies to increase panicle branching and grain yield in crops.

T4-35-03

FERONIA receptor kinase: a multi-tasking cell surface regulator of RAC/ROP GTPase signaling

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Originally identified as a major regulator of female fertility, FERONIA receptor kinase is in fact an almost ubiquitous cell surface regulator with major roles throughout growth, development, and mediating plant-environment interactions. Our earlier work established that FERONIA functions as an upstream regulator of RAC/ROPs, the RHO GTPases and major molecular switch in plants. We demonstrated that FERONIA interacts directly with ROPGEFs, guanine nucleotide exchange factors that activate RAC/ROPs, recruiting NADPH oxidase to produce reactive oxygen species and regulating at least two of its prominent functions, one in supporting polarized root hair growth the other in inducing pollen tube rupture and sperm release. We also demonstrated that FERONIA interacts directly with two related GPI-anchored proteins LORELEI and LORELEI-like GPI-anchored proteins. In seedlings, LLG1 functions as a chaperone to facilitate delivery of FERONIA to its proper functional location in the cell membrane, where they function as a receptor-coreceptor pair, interacting with the RAC/ROP signaling complex to mediate downstream processes. Use of a co-regulator on the cell surface, and use of RAC/ROPs and reactive oxygen species as downstream mediators might underlie how FERONIA manages at least a subset of its multi-faceted biological roles. Here we discuss recent work that provides insights on how FERONIA responds to various signaling cues to mediate diverse downstream processes.

T4-35-04

POLLEN RECEPTOR-LIKE KINASE family and RAC/ROP GTPase signaling in directional tip growth of the pollen tube

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Pollen tube guidance for successful reproduction involves complex cell-to-cell communication (Annu. Rev. Plant Biol. 2015; Plant Physiol. 2017). We have been working on pollen tube guidance in living materials by using two model plant species, *Torenia* and *Arabidopsis*. Defensin-like LUREs are pollen tube attractant peptides (cysteine-rich peptides; CRPs) of these species working in a short distance which are secreted by two synergid cells (Nature 2009; PLoS Biol. 2012). Recent semi in vitro studies lead to discovery of novel intercellular signaling molecules involved in pollen tube guidance. POLLEN RECEPTOR-LIKE KINASE 6 (PRK6) of *Arabidopsis* is a leucine-rich repeat (LRR) receptor-like kinase, which is critical in sensing of AtLURE1 peptide (Nature 2016). PRK6 localizes at the tip surface of the pollen tube and transduce species-preferential LURE signal to the cytoplasm of the pollen tube with other PRKs. As already shown for other

PRKs working in pollen germination and growth, PRK6 interacts with ROPGEFs (guanine nucleotide-exchange factors), suggesting PRK6 is in the ROP1 Rho GTPase-dependent signaling network of the pollen tube. PRK family has been suggested to work as receptors for CRP ligands in pollen germination and growth. Various CRPs derived from pistil tissues might control appropriate pollen tube germination, growth, and guidance spatiotemporally by regulating the ROP1 Rho GTPase-dependent signaling network with PRK family receptors of different functions. Not only PRK6 but other LRR receptor-like kinases, MDIS-MIK heterodimers, have been reported to be involved in pollen tube guidance. I will discuss how multiple ligands and receptors control precise directional pollen tube growth.

T4-35-05

ROP2 signaling to root hair tip growth

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Root hairs adopt a typical tip growth for cell morphogenesis and proper function. The establishment and maintenance of the polar site are important for root hair tip growth, and are precisely regulated. The Rop GTPase ROP2 is a well known positive regulator of both root hair initiation and elongation in *Arabidopsis thaliana*. We previously reported that *Arabidopsis* MICROTUBULE-ASSOCIATED PROTEIN18 (MAP18) functions in controlling the direction of pollen tube growth and root hair elongation. Interestingly, both loss-of-function of *ROP2* or knock-down of *MAP18* lead to a decrease in root hair length, whereas overexpression of either *MAP18* or *ROP2* causes root hairs with multiple tips or branching hair phenotype. Analyses of root hair phenotypes of mutants and transgenic seedlings demonstrate that MAP18 and ROP2 interact genetically and functionally. MAP18 interacts physically with ROP2 in vitro and in vivo, and preferentially binds to the inactive GDP-bound form of ROP2 protein. MAP18 promotes the ROP2 activity during root hair tip growth. Further investigation reveal that MAP18 competes with RhoGTPase GDP dissociation inhibitor 1 (AtRhoGDI1)/SUPERCENTIPEDE1 (SCN1) for binding to ROP2. The association with MAP18 interferes with the interaction between the inactive form of ROP2 and AtRhoGDI1/SCN1, in turn affecting the localization of active ROP2 in the plasma membrane of root hair tip. Our results reveal a novel regulatory mechanism of ROP2 signaling during root hair growth.

T4-35-06

A control loop in ABA signal transduction pathway

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The phytohormone abscisic acid (ABA) is critical to plant development and stress responses. Abiotic stress triggers an ABA signal transduction cascade, which is comprised of the core components PYL/RCAR ABA receptors, PP2C-type protein phosphatases, and protein kinases. Small GTPases of the ROP/RAC family act as negative regulators of ABA signal transduction. However, the mechanisms by which ABA controls the behavior of ROP/RACs have remained unclear. Our results show that an *Arabidopsis* guanine nucleotide exchange factor protein RopGEF1 is rapidly sequestered to intracellular particles in response to ABA. GFP-RopGEF1 is sequestered via the endosome-prevacuolar compartment pathway and is degraded. RopGEF1 directly interacts with several clade A PP2C protein phosphatases. Interestingly, RopGEF1 undergoes constitutive degradation in *pp2c* quadruple *abi1/abi2/hab1/pp2ca* mutant plants, revealing that active PP2C protein phosphatases protect and stabilize RopGEF1 from ABA-mediated degradation. Our findings point to a PP2C-RopGEF-ROP/RAC control loop model that is proposed to aid in shutting off ABA signal transduction. Further new findings will be reported showing a key role for Ca^{2+} and Ca^{2+} binding protein in regulation of the GEF1-ROP-PP2C control loop.

T4-36: Structure, function and regulation of photosynthetic systems

T4-36-01

A pentatricopeptide repeat protein is required for quality controlling of the di-cistronic atpH/F transcripts in chloroplasts

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Chloroplast gene expression is strictly controlled by a large number of nuclear gene products at various steps. Here, we report that a pentatricopeptide repeat (PPR) protein, BFA2, is essential for accumulation of the *atpH/F* transcripts in Arabidopsis chloroplasts. BFA2 protein belongs to a member of the P subfamily and localized to the chloroplast stroma. Absent of BFA2 leads to a significant reduction in accumulation of the chloroplast ATP synthase, and this phenotype is due to the loss of di-cistronic *atpH/F* transcripts. EMSA analysis demonstrated that the recombinant BFA2 protein binds to the consensus sequence in the *atpH* 3'-UTR and *atpA* 5'-UTR in a sequence-specific manner. We conclude that BFA2 protein binds to the *atpF/H* 3'-UTR and protects it from degradation by 3'-5' exoribonucleases.

T4-36-02

Crystal structure of plant PSI-LHCI supercomplex and its energy transfer mechanism

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In higher plants, the photosystem I (PSI) core is surrounded by a large light-harvesting complex I (LHCI), forming a PSI-LHCI supercomplx to capture and transfer light energy efficiently. In order to reveal the mechanism of energy harvesting and transfer within this large pigment-protein complex, it is essential to solve its crystal structure. We have succeeded in improving the crystal quality of the PSI-LHCI supercomplex from pea significantly, and analyzed its structure at 2.8 Å resolution. Our studies showed that the PSI-LHCI supercomplex contains 16 different subunits (including

12 core subunits PsaA-L and 4 LHCI subunits Lhca1-4) and 205 cofactors (143 chlorophylls *a*, 12 chlorophylls *b*, 26 *β*-carotenes, 5 luteins, 4 violaxanthin, 10 lipids), with a total molecular mass of 600 kDa. Our results identified chlorophyll *a*, chlorophyll *b*, and some carotenoids in the 4 LHCI subunits for the first time, and revealed the differences in the structure of the 4 LHCI subunits, their interactions, and the interactions between them and the PSI core subunits. In particular, the binding sites for violaxanthins in LHCI were identified for the first time, suggesting their possible roles in photoprotection of PSI. Characteristic red chlorophylls were found in the interfaces between LHCI and the PSI core, implying their possible roles in mediating energy transfer. Based on the structure resolved, 4 plausible energy transfer pathways from LHCI to the PSI core complex were deduced. Taken together, this structure provides a solid structural basis for elucidating the energy transfer and photoprotection mechanisms within the PSI-LHCI supercomplex.

T4-36-03

Mechanism of photosynthetic water oxidation based on atomic structure of photosystem II

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Photosystem II (PSII) catalyzes light-induced water-splitting leading to the evolution of molecular oxygen indispensible for aerobic life on the earth. PSII is a membrane-protein complex consisting of 20 subunits and a number of cofactors, with a total molecular mass of 350 kDa for a monomer. We have solved the crystal structure of dimeric PSII from a thermophilic cyanobacterium *Thermosynechococcus vulcanus* at a resolution of 1.9 Å using synchrotron radiation (SR) X-rays, which revealed a clear picture of Mn_4CaO_5-cluster, the catalytic center for water-splitting. The structure of the Mn_4CaO_5-cluster resembles a distorted chair form, with 3 Mn, 1 Ca and 4 O (oxygen) atoms forming a distorted cubane core, and the 4th Mn connected to the core by 2 oxo-bridges. Some of the inter-atomic distances within the metal cluster, however, were shown to be slightly longer than those obtained by EXAFS and theoretical studies, presumably due to radiation damage caused by the SR X-rays. In order to avoid possible radiation damage and eliminate the uncertainties in the inter-atomic distances, we used femtosecond X-ray pulses from an X-ray free electron laser (XFEL) facility SACLA, Japan, to solve the structure of PSII. In order to obtain a high resolution structure, we used large PSII crystals, and adopted an approach where every point of the crystal was illuminated by 1 XFEL pulse, and every two XFEL pulses were separated at least by 50 μm on the crystal by moving the crystals. This approach required a huge number of large, isomorphous PSII crystals, but allowed us to collect damage-free, high resolution diffraction data, enabling us to solve the PSII structure at 1.95 Å resolution. This structure showed that most of the Mn-Mn and Mn-ligand distances were 0.1-0.3 Å shorter than those observed in the previous SR structure. However, the bond distances of O5, a unique oxo-bridged oxygen, with two of its nearby Mn ions Mn1 and Mn4, are still unusually longer compared with typical Mn-O bond distances, giving rise to the distorted shape and flexible nature of this catalyst. We further used PSII small crystals and serial femtosecond crystallography (SFX) by XFEL to collect diffraction data from some of the reaction intermediates of the catalyst, and gained information on the structural changes of the catalyst during the water-splitting reaction. Based on these information, I will discuss the possible mechanisms for photosynthetic water-splitting. This work is a collaboration of many colleagues, and I thank them for their contributions.

T4-36-04

Regulation of photosynthesis via proton motive force

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Photosynthetic electron transport through the thylakoid membrane forms proton motive force (pmf) consisting of ΔpH and membrane potential. Both components of pmf contribute to ATP synthesis. ΔpH also down-regulates photosynthetic electron transport by inducing thermal dissipation of excessively absorbed light energy from photosystem II (a qE component of NPQ) and down-regulation of rate of electron transport through the Cytochrome b_6f complex. Cyclic electron transport around PSI generates pmf without accumulating reducing power in the stroma and regulates the size of pmf. Components of pmf are optimized by regulating ion movement across the thylakoid membrane. Thus, the regulation of size and components of pmf fine-tunes photosynthesis by optimizing the tradeoff between ATP synthesis and down-regulation of photosynthesis. We will discuss the molecular mechanism of this regulation by focusing on cyclic electron transport around PSI and the H^+/K^+ antipoter KEA3 localized to the thylakoid membrane.

T4-36-05

What limits the photosynthetic energy efficiency in nature -Lessons from the oceans

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Solar radiation absorbed by both terrestrial and marine plants during photosynthesis can follow three possible paths, i.e. photochemistry, fluorescence and/or thermal dissipation, but constraining photosynthetic energy conversion efficiency in nature is still challenging because in principle, at least any two yield measurements of these three paths must be made simultaneously. We constructed two different, extremely sensitive and precise active fluorometers for phytoplankoton, i.e. photosynthetic microscopic organisms in upper oceans and fresh water on Earth: one measures the quantum yield of photochemistry from changes in variable fluorescence, the other measures fluorescence lifetimes in the picosecond time domain (the lifetime is capable of being converted to actual quantum yield of fluorescence by dividing chlorophyll a's natural lifetime, 15 ns). By deploying the pair of instruments on eight transoceanic cruises over six years crossing almost the global oceans, we obtained over 200,000 measurements of fluorescence yields and lifetimes from surface waters in five ocean basins. Our results revealed that the average quantum yield of photochemistry was ~ 0.35 (i.e. the average photosynthetic efficiecy in the global ocean was ~ 35%) while the average quantum yield of fluorescence was ~ 0.07. Thus, closure on the energy budget suggests that, on average, ~ 58% of the photons absorbed by phytoplankton in the world oceans are dissipated as heat. This extraordinary inefficiency is associated with the paucity of nutrients in the upper ocean, especially dissolved inorganic nitrogen and iron. Our results strongly suggest that, in nature, most of the time, most of the phytoplankton community operates at approximately half of its maximal photosynthetic energy conversion efficiency because nutrients limit the synthesis or function of essential components in the photosynthetic apparatus.

T4-36-06

Evolution of C_4 photosynthesis in succulent lineages: the role of C_3-C_4 intermediate species

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In terrestrial plants, including grasses, sedges and dicots, different variants of C_4-Kranz anatomy and biochemistry have evolved independently from C_3 plants at least 66 times. There are 37 families in the eudicot order Caryophyllales (APG III system, 2009), which includes 23 C_4 eudicot lines. C_4 species acquired a C_4 cycle and leaf structure with enlarged chlorophyllous bundle sheath cells surrounded by palisade cells (Kranz anatomy). Stepwise evolution is proposed from C_3 to C_3-C_4 intermediates to C_4 plants. Currently, only ~50 species have been identified as intermediates (based on structure, biochemistry, function) with properties intermediate to C_3 and C_4 (structural, biochemical and functional); 18 intermediate species are in Caryophyllales. Aridization is considered one of the main driving forces in C_4 evolution. Two general paths are proposed for evolution of Kranz anatomy from C_3 ancestors having planar versus succulent leaves. For species with planar leaves, increased vein density is the best supported preconditioning to date, leading to multiple Kranz units surrounding individual veins. There has been less focus on the evolutionary path from C_3 to C_4 in succulent plants. There are many succulent species in Chenopodiaceae and Portulacaceae which develop water-storage tissue in leaves, with 7 structural types where formation of Kranz anatomy is not linked to vein density. We have identified structural and biochemical diversity in Salsoleae which provides a model for the path of evolution from C_3 to C_4 in succulent species. Phylogenetic analysis of the path to C_4 in different lineages is limited by identification of C_3-C_4 intermediates.

T4-37: Regulatory mechanism of flowering time

T4-37-01

Natural Variation of FLOWERING LOCUS T in *Arabidopsis thaliana*

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Flowering time is a complex trait that is crucial for fitness and regulated by seasonal changes and internal cues. In the annual plant *Arabidopsis thaliana,* two life styles have been described; winter accessions flower in response to long exposure to cold temperature (vernalization) and single season accessions flower in a short period of time chiefly in response to long-day (LD). Control of these two lifestyles converges at the level of transcriptional regulation of *FLOWERING LOCUS T (FT),* a florigen encoding gene. Indeed *FT* expression, which is repressed prior vernalization in winter accessions, strongly accelerates flowering mainly in response to LD but also to elevated temperatures in short-day. The photoperiodic induction of *FT* expression requires a 5,7 kb region upstream of the start codon of *FT*. This region contains a proximal core promoter and a distal enhancer with open chromatin structure; both regions are highly conserved within the Brassicaceae.
However, the distal enhancer is not required in the regulation of *FT* expression in the siliques, which has been proposed to influence related traits like floral reversion and seed dormancy.
While most of the flowering time quantitative trait loci (QTL) identified in *Arabidopsis* have been associated with variation at coding regions, QTL linked to *FT* are exclusively mapped to its promoter. To test whether *FT* could be a target of selection to modulate flowering time, we dissected Illumina paired-end sequences from the 1001 genomes project for single nucleotide polymorphisms and structural variations at the photoperiod control regions. We identified rare *FT* haplotypes defined by natural variation in *FT* regulatory regions previously characterized in our group. In the rosette leaves, prior the floral transition, allele specific expression (ASE) of *FT* in F1 hybrids revealed non-neutral *FT* haplotypes, which were less responsive to both LD and ambient temperature inductive conditions. Complementation analysis of the *ft-10* mutant with different *FT* promoter accessions constructs revealed *FT* promoter variants that significantly delay flowering time in LD compared to the reference promoter of the Col-0 accession. Interestingly ASE of *FT* in developing siliques of F_1 hybrids remained stable indicating that *FT* haplotypes are specifically affected for the floral transition. Less responsive *FT* haplotypes were enriched in Sweden and coincide with winter accessions. This last observation, led us to investigate the effect of *FT* after extensive vernalization. Surprisingly, extensive vernalization abolished the late flowering effect of the *ft-10* mutant allele compared to wild-type Col-0. These findings redefine the impact of *FT* on flowering time after extensive vernalization. We propose that an accumulation of less responsive *FT* haplotypes could be due to relaxed purifying selection of *FT* in natural population seasonally exposed to long vernalization periods.

T4-37-02

Co-option of shoot maintenance centroradialis and photoperiod regulated FT module on floral reversion and bulbils formation in *Titanotrichum oldhamii* (Gesneriaceae)

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Floral reversion allows flowers to switch from determinate growth back to indeterminate growth, thus avoiding senescence and promoting ongoing growth, a trait with agronomic value to adjust vegetative and reproductive growth. Naturally occurred *Titano-*

trichum oldhamii (Gesneriaceae) involves reversion of floral primordia to indeterminate inflorescence and subsequently vegetative bulbils in late flowering season when day length becomes short for reproductive assurance. Here we show the expression of inflorescence meristem identity gene and flowering pathway integrator for photoperiod signals, *T. oldhamii* homologues of *CENTRORADIALIS* (*ToCEN*) and *FLOWERING LOCUS T* (*ToFT*), correlated to floral reversion into bulbiliferous shoots, the modified inflorescences arouse from lateral flowers. The expression level of *ToCEN* reached its highest peak during the reversion; and *ToCEN* mRNA localized in inflorescence apices (maintenance of shoot for flowering) and lateral bulbiliferous shoots meristems (floral reversion into shoots) but not in floral buds. Ectopic expression of *ToCEN* in *Arabidopsis* showed enhanced inflorescence architecture with extended growth of lateral shoot (coflorescence) branching and delayed bolting, a phenotype greatly resembling bulbiliferous shoots. Detection of *ToCEN* mRNA in the scions of wild type *Arabidopsis* grafted onto *35S::ToCEN* stocks suggested *ToCEN* acting as transmittable signals. Thus, *ToCEN* not only maintains inflorescence architecture but also a new role on floral reversion to bulbiliferous shoots. Duplication of *ToFT* also seems to play a contributing role to this floral reversion module. While *ToFT2* mainly expressed in leaves and its expression dynamic displayed oscillation in long-day; by contrast, *ToFT1* has high expression level in short-day induced reverting bulbiliferous shoots and gradually declined expression toward leaves, although the phenotypes of transgenic *Arabidopsis* over-expressing *ToFT2* suggest that the *ToFT2* can functionally substitute for *ToFT1* in promoting early flowering. Further works on how *ToFT1* integrate photoperiod signals and induce *ToCEN* for floral reversion and phase transition will help to clarify their interacting roles as a photoperiod regulated module. Also, it would be important to examine whether both *ToFT1* and *ToCEN* are transmittable in *T. oldhamii* when responding to photoperiodic changes. Nonetheless, our results demonstrated that *ToCEN* and *ToFT1* perhaps evolved to constitute a new regulation module in triggering floral reversion into formation of bulbiliferous shoots in *T. oldhamii*.

T4-37-03

Shifting thresholds: the evolution of developmental plasticity to seasonal cues in *Mimulus*

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Diverse taxa have adapted to use day length as a cue to promote floral induction because it serves as a reliable indicator of seasonal timing. Indeed, reproduction is obligately dependent on experiencing long days responses in many plants. In such species, floral development is only initiated after calendar dates when photoperiod exceeds a critical minimum day length. Because annual patterns of temperature and precipitation vary regionally, the yearly timing of the growth season varies across latitude and elevation gradients, producing spatially varying selective regimes that maintain variation in flowering time and its plasticity to photoperiod. We have investigated the geographic diversity of critical photoperiod, flowering time, and their genetic basis in annual populations of the common monkeyflower *Mimulus guttatus*. Although critical photoperiod is strongly correlated with elevation and growth season start date, flowering time in inductive conditions is instead correlated with season duration. That these two components of an integrated seasonal phenology can separately evolve not only indicates that different agents of selection drive their evolution, but also predicts that unique genetic architectures govern divergence of each trait. Genetic mapping by multiplex shotgun genotyping in multiple high x low elevation crosses confirms this prediction: critical photoperiod loci do not co-localize with flowering time loci. Surprisingly, the loci contributing to clinal divergence in critical photoperiod are nearly all transect-specific, in contrast to many recent demonstrations of convergence at the genetic level and contradicting a classic prediction about how plasticity evolves. Even when the same genomic region is implicated in critical photoperiod divergence along multiple elevation gradients, patterns of paralog-specific expression divergence at the underlying floral regulatory genes indicate region-specific genetic changes are involved. We have complemented our pursuit of the underlying candidate genes with common garden studies at high and low elevation to understand how allelic variation at two critical photoperiod loci impacts flowering and fitness in native seasonal environments. Notably, our field studies reveal locus-specific patterns of selection. The low elevation allele of one locus is favored in both locations, potentially highlighting this as an allele that will respond to selection as the climate warms and dries through time, while the other locus exhibiting a signal consistent with local adaptation.

T4-37-04

Flowering on time without *CO* and *FLC* in the temperate-llimate legume model *Medicago*

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Flowering time is a major adaptive trait for plants and agriculture and is optimised using environmental cues such as vernalisation (extended exposure to cold) and long day photoperiods. Integration of these signals ensures that plants do not flower and set seed until after winter is past. In eudicots, the flowering-time gene network is best studied in *Arabidopsis*. In *Arabidopsis*, long day floral induction depends on interactions among the circadian clock, photoreceptors and flowering time genes such as *CO*. CO is activated by long days and directly triggers expression of the powerful florigen, the floral activator *FT*. However, in winter-annual *Arabidopsis*, vernalisation is needed for stable silencing of the strong floral repressor *FLC* by Polycomb group proteins to relieve its inhibition of *FT* and *SOC1*, enabling these genes to be upregulated in spring. However, *CO* does not appear to function in the temperate-climate legumes pea and *Medicago* and neither is there a FLC clade. In contrast, the photoreceptor PHYA regulates flowering in *Arabidopsis*, pea and short-day soybean. Work in soybean has also uncovered legume-specific responses downstream of PHYA. For example, in non-inductive long day conditions, soybean PHYA promotes expression of the legume-specific *E1* gene that represses flowering. We are using the powerful genetic and genomic resources that enable reverse genetics in the legume model *Medicago*, to investigate how flowering time is regulated in this reference legume. We will present data indicating that

MtPHYA and *MtE1* both promote *Medicago* flowering. PHYA is a major player that positively regulates *E1* and *FT-like* transcript accumulation and modulates expression of some clock genes. We will also discuss results indicating that *MtVRN2*, a *VRN2-like* Polycomb Group gene, inhibits the transition to flowering via repressing *FTa1* expression, thus having a different function from *Arabidopsis* VRN2 which represses FLC.

T4-37-05

Polycomb-based epigenetic switching

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John Innes Centre

We are studying the chromatin dynamics underlying cold-induced epigenetic silencing of *Arabidopsis FLC*. This determines when plants flower and how they respond to different winter conditions. The epigenetic silencing involves a Polycomb-based epigenetic switching mechanism. *FLC* expression is bistable and cold exposure promotes a switch in individual cells form a transcriptional active (marked by H3K36me3) to a Polycomb-silenced state (marked by H3K27me3). The memory of this silenced state is then inherited in cis through many cell divisions until the plant has flowered, after which point the switch is reversed. The talk will describe our latest understanding of this switching mechanism. Our recent work reveals the switching can be separated to mechanistically distinct two phases of Polycomb silencing. Nucleation of specific Polycomb factors holds a metastable memory of cold in the short-term with subsequent transfer to a long-term histone-modification-based memory via general Polycomb factors. This mechanistic understanding is likely to be general relevant to Polycomb targets in all organisms. This mechanistic understanding is likely to be general relevant to Polycomb targets in all organisms.

T4-37-06

Regulation of developmental transitions by microRNAs

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In plants the changes in morphological and physiological traits serve as markers for the developmental transitions. Mutant characterizations and genetic analyses in *Arabidopsis thaliana* delineate an evolutionarily conserved, *microRNA156 (miR156)*-guided timing mechanism that temporally regulates many aspects of biological processes during development. Recent studies now reveal that sugar metabolites, the products of photosynthesis, feed into this developmental timer by regulating *miR156* levels, thereby ensuring that each developmental transition occurs under favorable conditions. We will present our recent progress in understanding how *miR156* and its targets regulate vegetative phase transition, flowering time and regenerative capacity.

T4-38: Fruit physiology and quality regulation

T4-38-01

Tomato *SlVIF* regulates fruit ripening via modulating sucrose metabolism and pigment synthesis

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Fruit ripening is a complex process that involves a series of physiological and biochemical changes to ultimately influence fruit quality traits. Sugar and pigment contribute to flavor and color, and represent the two most important fruit quality traits. Unraveling the interacting mechanisms between sugar metabolism and pigment synthesis is important to understanding the multiple signaling crosstalk of fruit ripening process, but the direct evidence for sugars in regulating pigment biosynthesis and fruit ripening is still lacking. Here, we identify and analyze the expression profiles of all the 36 genes involved in sucrose metabolism during tomato fruit ripening. By using chromatin immunoprecipitation and gel mobility shift assays, we show that *SlVIF* encoding vacuolar invertase inhibitor and *SlVI* encoding vacuolar invertase are directly regulated by the global fruit ripening regulator RIN (RIPENING INHIBITOR). Moreover, we discover that SlVIF physically interacts with SlVI to control sucrose metabolism. Repression of *SlVIF* by RNA interference (RNAi) delay tomato fruit ripening, while overexpression of *SlVIF* accelerate ripening, with concomitant changes in lycopene production and ethylene biosynthesis. An iTRAQ (isobaric tags for relative and absolute quantification)-based quantitative proteomic analysis indicated that a set of proteins involved in fruit ripening changed abundance in the *SlVIF* RNAi fruit, including proteins associated with lycopene generation and ethylene synthesis. These findings provide strong evidence for the direct role of sucrose in promoting pigment biosynthesis and establish meaningful information of *SlVIF* contributed to fruit quality and RIN-mediated ripening regulatory mechanisms, which are of significant agricultural value.

T4-38-02

CmOr is the master switch to trigger beta-carotene accumulation in melon fruits

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Beta-carotene is the most potent precursor for vitamin A biosynthesis and determines the nutritional quality of many fruits including melon. *CmOr* is defined as the single master gene that governs melon fruit b-carotene accumulation. Two *CmOr* haplotypes (*CmOr*$^{\text{Orange}}$ and *Cmor*$^{\text{Nonorange}}$) distinguish melon fruit flesh color in a broad germplasm collection. Functional analysis of *CmOr* identifies a single 'golden SNP' being responsible for b-carotene accumulation in various orange-flesh melon fruits. This 'golden SNP' alters the evolutionarily highly-conserved arginine^{108} to histidine in CmOR. Recently, an EMS induced melon mutant was identified, which dramatically lowers b-carotene level in melon fruit. This mutant was found to be caused by a *CmOr* nonsense mutation (*Cmor*-lowβ). *Cmor*-lowβ has minimal effect on the expression of carotenogenic genes, but dramatically reduces phytoene synthase (PSY) protein level and enzymatic activity, indicating that an active *CmOr* is required to maintain PSY level for carotenogenesis in melon fruit. While CmOR is a major posttranscriptional regulator of PSY, the high b-carotene accumulation in *CmOr*$^{\text{Orange}}$ melon is not due to its enhanced capacity in activating PSY as both PSY protein level and carotenoid metabolic flux are similar in orange and non-orange melon accessions. Instead, *CmOr*$^{\text{Orange}}$ was found to stabilize b-carotene and inhibit its turnover, resulting in b-carotene accumulation in chromoplasts. The elucidation of the basis of *CmOr*$^{\text{Orange}}$ action broadens our fundamental understanding of mechanisms underlying carotenoid accumulation in plants. The discovery of the 'golden SNP' in the melon *CmOr* gene precisely defines the site for genome editing as a new approach toward β-carotene biofortification of crops.

T4-38-03

Glucose sensor MdHXK1 phosphorylates and stabilizes MdbHLH3 to promote anthocyanin biosynthesis in apple

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Glucose is considered as a major regulatory molecule in addition to being essential metabolic nutrients and structural components in higher plants. As is well known, hexokinase1 (HXK1) is a glucose sensor that integrates diverse signals to govern gene expression and plant growth in response to environmental cues. Previously, it is reported that the nuclear HXK1 forms a glucose signaling complex core with the vacuolar H+-ATPase B1 (VHAB1) and the 19S regulatory particle of proteasome subunit (RPT5B), which influences the transcription of target genes. However, it is yet unknown if and how HXK1 directly targets TFs to modulate their function in the nucleus in plants. Glucose induces anthocyanin accumulation in many plant species; however, the molecular mechanism involved in this process remains largely unknown. Here, we found that apple hexokinase MdHXK1, a glucose sensor, was involved in sensing exogenous glucose and regulating anthocyanin biosynthesis. *In vitro* and *in vivo* assays suggested that MdHXK1 interacted directly with and phosphorylated an anthocyanin-associated bHLH transcription factor (TF) MdbHLH3 at its Ser361 site in response to glucose. Furthermore, both the hexokinase_2 domain and signal peptide are crucial for the MdHXK1-mediated phosphorylation of MdbHLH3. Moreover, phosphorylation modification stabilized MdbHLH3 protein and enhanced its transcription of the anthocyanin biosynthesis genes, thereby increasing anthocyanin biosynthesis. Finally, a series of transgenic analyses in apple calli and fruits demonstrated that MdHXK1 controlled glucose-induced anthocyanin accumulation at least partially, if not completely, via regulating MdbHLH3. Overall, our findings provide new insights into the mechanism of the glucose sensor HXK1 modulation of anthocyanin accumulation, which occur by directly regulating the anthocyanin-related bHLH TFs in response to a glucose signal in plants.

T4-38-04

Silencing of *PL*, which encodes a pectate lyase in tomato, confers enhanced fruit firmness, prolonged shelf-life, and reduced susceptibility to Gray Mold

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Pectate lyase genes have been documented as excellent candidates for biotechnological improvement of fruit firmness in strawberry and tomato, two typical non-climacteric and climacteric, respectively. However, the application of pectate lyase in regulating fruit postharvest deterioration is still not fully explored. In this report, 22 individual pectate lyase genes were identified in tomato, one *PL* gene (Solyc03g111690), showed dominating expression during fruit maturation and was regulated by ripening related phytohormone. RNA interference (RNAi) of Solyc03g111690 resulted in enhanced fruit firmness and changes of pericarp cells. More importantly, transgenic tomato fruit strengthened the anti-rotting and pathogen-resisting ability. Compared to WT, transgenic tomato had higher content of cellulose and hemicellulose, whereas lower content of water soluble pectin. In line with that, peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) activity were higher in transgenic fruit, where less malondialdehyde (MDA) level was detected. RNA-Seq results showed numerous differentially expressed genes (DEGs) involving in hormone signaling, cell wall modification, oxidative stress, and pathogen resistance. Collectively, these data demonstrate that pectate lyase play a role in both fruit softening and pathogen resistance, advancing our insights of postharvest fruit preservation in tomato as well as in other fleshy fruits.

T4-38-05

Identification of citric acid and vitamin C associated genes in *Citrus* by genome approach

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High-quality genomes of basic citrus species were built up by the third generation sequencing technology, single molecule sequencing data (PacBio long reads) in combination with the second generation sequencing technology short-read (Illumina), i.e. 3+2 strategy. All the genome sequence, annotation files and raw data will be deposited from our website http://citrus.hzau.edu.cn/orange/. We also sequenced wild citrus, semi-domesticated forms and cultivated citrus. Transcriptomes of fruit, leaf, of wild citrus and cultivated citrus were further included to narrow down the genes involved in the citric acid genes. We also find that there are footprints of introgressions of wild citrus in the mandarin population. Recently, we collected several spontaneous mutants of acidless citrus from all over the world and included for genome comparison analysis. A strategy combined genome and bioinformatics to identify the mutated locus was applied and succeeded. A similar approach was also used to identify vitamin C related genes in different citrus types. This approach provides some new insights into the citrus genetic loci affect citric acid metabolism.

T4-38-06

SlBHL1 gene regulates the synthesis of lycopene and chlorophyll in tomato fruit

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During the fruit ripening process, the decomposition of chlorophyll and the accumulation of lycopene were closely related to the quality of fruits. It is of great significance to understand the mechanism of fruit quality formation by the synthesis of chlorophyll and lycopene regulated by transcription factors. We used the virus-induced gene silencing (VIGS) method to screen 30 transcript factors with high expression level during fruit ripening; the results show that *SlBHL1* gene silencing led to the chlorophyll accumulation at mature green stage and lycopene reduction at the red stage in tomato fruit. We also obtained *SlBHL1* RNAi tomato plant, which was consistent with VIGS phenotype. We used RNAi tomato fruits to do RNA-Seq, metabonomics, and Chip-seq analysis, The result shown that the *SlBHL1* gene negative regulate chlorophyll accumulation by directly acting the promoter region of *SlTKN2* genes, but also regulate the ethylene synthesis by directly acting the promoter region of *SlACO* genes in tomato fruit. The functional identification of the *SlBHL1* gene provides a method for regulating the synthesis of lycopene and chlorophyll in fruits.

T4-39: Novel insights into wood research– from evolution to developmental genetics and functional traits

T4-39-01

Evolution of woodiness within flowering plants: Where, why and how?

Frederic Lens

Naturalis Biodiversity Center

Insular floras typically harbour species with a remarkable degree of woodiness. This so-called insular woodiness was described and interpreted correctly by Darwin, but many years later we still do not have a global overview (1) in which flowering plant groups transitions from herbaceousness towards derived woodiness have taken place, (2) why these evolutionary transitions have happened, and (3) what genetic mechanism is causing these convergent shifts. Based on an ongoing review study of derived woodiness, it is evident that this phenomenon is concentrated in the later diverging lineages of flowering plants, and many of these woody taxa are native to islands or island-like regions such as tropical mountain peaks. Surprisingly, this transition has also occurred much more on continents than expected, especially in dry continental regions facing at least a number of consecutive dry months per year, such as savannas or (semi-)deserts. This suggests that increasing wood development and increasing drought stress resistance may go hand in hand, which has not been suggested before. We have obtained experimental evidence in *Arabidopsis* (Brassicaceae) and daisies (Asteraceae), showing that woody species are able to better avoid air bubble formation in their conductive xylem conduits due to drought stress than their closely related herbaceous relatives. However, drought stress is not involved in all derived-woody lineages, and environmental factors driving wood formation are probably taxon specific. As a third item, I want to touch on how herbaceous plant groups develop into woody shrubs and trees during evolutionary time. The massive convergent number of transitions towards derived woodiness suggests that the genetic mechanism should be relatively simple, which is supported by a shrub-like woody mutant of Arabidopsis in which two genes are knocked out, but we do not know what are the key regulatory genes that turn on the wood pathway. Is there only one master gene or are there several regulatory genes within a shared interaction network?

T4-39-02

Forensic timber identification: the authenticity diagnostics of species and geographic provenance using genetic methods

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Deforestation represents a massive threat to global biodiversity with illegal logging and the associated trade in illegally sourced wood products. Timber identification of tree species and their origins is crucial and essential for monitoring and control of illegal activities in timber production and thus enforcement of species trade regulations. The traditional identification relies on diagnostic anatomical features, either macroscopic or microscopic. Examination of wood anatomy generally facilitates identification to genus level, as wood characters tend to be highly conserved within genera. However, it is difficult and even impossible for precise timber discrimination at the species level or tracking the origin of wood. The newly developed genetic methods might overcome these limitations and provide effective information with high resolution. DNA barcoding, a genetic approach based on a short DNA sequence from a standard part of a genome, may be the most appropriate for identifying to the species level or higher. Moreover, population genetics and phylogeography are very powerful tools for geographical traceability. In the paper, we focus on CITES-listed or national protected timber species and provide our recent exploration in relevant fields. Additionally, potential diagnostic applications and novel insights of genetic methods are contributed for forensic timber identification.

T4-39-03

Intervessel pit membranes provide key determinants of embolism resistance in angiosperm xylem

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Xylem sap in angiosperms is typically transported under negative pressure through vessels that are interconnected by bordered pit pairs. The intervessel pit membrane, which develops from a modified primary wall and middle lamella after cell hydrolysis, is known to provide a major contribution to xylem hydraulic resistance, while limiting at the same time the spread of micro-organisms and embolism. Pit membranes play a crucial role in drought-induced embolism formation via air-seeding, which traditionally assumes that the movement of gas between two vessels is limited by the high surface tension of water and the nanometre-scaled pores of a wetted pit membrane. However, recent findings challenge this view and highlight that air-seeding remains poorly understood because of our limited knowledge of (1) the surface tension of xylem sap, (2) the three-dimensional characteristics of the tortuous pore pathways in wet pit membranes, and (3) the contact angle at the air-water-pit membrane interface. This talk will focus on the ultrastructure of intervessel pit membranes, especially their thickness and porosity. Transmission electron microscopy (TEM) shows a 10-fold variation in pit membrane thickness for fresh samples. Pit membrane thickness is significantly correlated with embolism resistance ($r = 0.78$, $P < 0.01$, n = 37 species), with species from dry environments typically showing thicker pit membranes than those growing in mesic conditions. Assuming that cellulose microfibrils in pit membranes show an equal spatial density, pit membrane thickness is suggested to affect the length of the entire pore pathway, but not the actual pore size. In fact, it is unknown if pit membrane pore sizes show considerable variation across angiosperm species. Perfusion experiments with colloidal gold particles of known size indicate that pit membrane pores are typically 5 to 10 nm wide, which is much smaller than observations based on scanning electron microscopy. This is explained by the finding that dried wood shows shrunken pit membranes with much larger pores than wet, undamaged pit membranes. Depending on the drying method, the thickness of intervessel pit membranes shrinks by 30% to 50% compared to the thickness in fresh samples. Moreover, this shrinkage is irreversible, may occur under natural conditions in the field, and explains air-seeding fatigue. Therefore, TEM observations on pit membrane thickness should be based on fresh samples to avoid preparation artefacts. The shape of pit membrane pores is geometrically highly variable, with many pores showing a slit-like outline, but little cylindrical pores. This means that the air-seeding pressure is typically reduced by ca. 50% compared to all pores being perfectly circular. Also, there is growing evidence that bubble snap-off occurs in a tortuous pore pathway, especially if an air-water meniscus passes a pore volume that has < ½ the diameter of the adjacent pore volume. Future attention should be paid to the role of surfactants that are found in pores of intervessel pit membranes, and their potential role in the coating and stabilisation of nanobubbles after a snap-off event.

T4-39-04

Paving the road to land: gene diversification and arise of water-conducting cells in land plants

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Colonization of land by early land plants coincided with an increase in cellular and morphological diversities. Advances in genome sequence data suggested this increase of diversities was associated with transcription factor duplication and subsequent functional diversification, which played critical roles in innovation of novelties for survival on continental surface. One novelty is related to develop specialized cells, water-conducting cells, for long distance water transport. Recent studies revealed a NAC transcription factors VNS (VND/NST/SND)-based gene regulatory network orchestrating the xylem cell formation in vascular plants. However, it still remains unclear on genetic origin of these cells. Here we investigated in non-vascular plant moss *Physcomitrella patens*, an early land plant lineage, seeking to understand the functional conservation and diversification of VNS family with regard to innovation of water-conducting cells. Eight VNS proteins were identified in *P. patens* and further divided into two groups. By comparing the function of these two groups, it showed that a group of *PpVNS* genes shared the conserved function in water-conducting cell formation with their homologues in vascular plants. *P. patens* mutants with abnormal water-conducting cells tended to a wilting phenotype when exposed to the condition with lower humidity. Our findings indicated a conserved VNS-based genetic network governing the development of water-conducting cells across land plants, and suggested that the diversification of NAC proteins to induce highly specialized cell formation to facilitate water transport was a pivotal event for early plants to adapt to terrestrial environment.

T4-39-05

Tracheid cell wall structure and chemical composition in the earliest woody plants

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The evolution of tissues composed of cells with robust lignified walls was fundamental to the early diversification of vascular plants, giving rise to forest ecosystems by the Middle Devonian (ca 387 Ma). These developments caused large quantities of recalcitrant organic matter to enter the environment, with consequences for the carbon cycle that are still poorly understood. How was this material decomposed in the environment and what organisms were involved? Here we develop an approach to investigate the wall structure and composition of the tracheids in the earliest fossil wood with the aims of characterizing the nature of decomposition of the cell wall when wood is attacked by fungi. Axes of *Armoricaphyton chateaupannense* (Early Devonian, ca 407 Ma from the Armorican Massif, western France) were imaged using propagation phase contrast X-ray synchrotron microtomography (PPC-SRµCT). The axes of this small plant are preserved as both thin coaly compressions and permineralizations or partial permineralizations in pyrite (FeS2). Tomographic datasets where imported into volume rendering Drishti v2.6 software for visualization, allowing the fine details of the tracheids cell walls (pits, bars, and wall membranes) to be examined in three-dimensions. This was realized through the creation of a three-dimensional reconstruction showing a single (virtually sectioned) tracheid from which digital animations were obtained. High fidelity images of the cell wall and pit structure (p-type) were obtained to a spatial resolution of 0.551 µm per voxel. Further ultrastructural and chemical investigations were performed at the submicrometre scale on focused ion beam (FIB) ultrathin sections extracted from freshly fractured specimens. Scanning and transmission electron microscopies (SEM and TEM) were employed to document the composition of the tracheids. In parallel, synchrotron-based scanning transmission X-ray microscopy (STXM), coupled with X-ray absorption near edge structure (XANES) spectroscopy, was used to determine the carbon speciation of the tracheid to investigate possible lignin signatures. Analyses were realized on both a three-dimensional pyritized specimen and on a specimen preserved in compression allowing comparison between the two samples. Results show that this combination of analytical methods can be highly effective for imaging the cell wall structure and chemical composition of the earleist wood cells. Using these approaches will enable effective comparisons of Devonian Period fossil woods with other fossil and modern woods that have undergone various types of fungal attack (e.g., white rot, brown rot) to shed light on the nature of wood decomposition in early terrestrial ecosystems.

T4-39-06

Diversity of microscopic bark structure revealed and classified

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The importance of bark in plant biology, including structure, physiology, diversity and evolution can hardly be overestimated. Yet its microscopic structure is poorly documented in comparison with wood. The International Association of Wood Anatomists (IAWA) therefore commissioned the production of an IAWA List of microscopic bark features, which sets a new international standard for the definition and recognition of anatomical features of barks in woody angiosperms and gymnosperms. Codes are given for 173 anatomical character states to facilitate databasing the huge diversity of bark structure in trees, shrubs and lianas. It is richly illustrated with 169 colour micrographs. The "IAWA Bark List" is not only indispensable for any future work on comparative bark anatomy and microscopic bark identification, but can also be used as a first inventory of functional traits in this complex tissue. Here we will present some of the enormous diversity present in barks, how the list is organized and can be used both in teaching and in phloem and periderm studies.

THEME V: GENETICS, GENOMICS & BIOINFORMATICS

T5-01: Polyploid genomics

T5-01-01

Genomic analysis of transgressive segregation in autopolyploid sugarcane

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Sugarcane is a dual-purpose crop for energy and sugar production. It has the highest biomass production reaching 1.9 billion tonnes in 2014 worldwide. To improve sugarcane biomass accumulation, we developed an interspecific cross between *Saccharum officinarum* 'LA Purple' and *Saccharum robustum* 'MOL5829'. The selected F1 individuals were self-pollinated to generate a transgressive F2 population with a wide range of biomass yields. Leaf and stem internodes of fourteen high biomass and eight low biomass F2 extreme segregants were used for RNA-seq to decipher the molecular mechanism of rapid plant growth and dry weight accumulation. Gene Ontology terms involved in cell wall metabolism and carbohydrate catabolism were enriched among 3,274 differentially expressed genes between high and low biomass groups. Specifically, up-regulation of cellulose metabolism, pectin degradation and lignin biosynthesis genes were observed in the high biomass group, in conjunction with higher transcript levels of callose metabolic genes and the cell wall loosening enzyme expansin. Furthermore, UDP-glucose biosynthesis and sucrose conversion genes were differentially expressed between the two groups. A positive correlation between stem glucose, but not sucrose, levels and dry weight was detected. We thus postulated that the high biomass sugarcane plants rapidly convert sucrose to UDP-glucose, which is the building block of cell wall polymers and callose, in order to maintain the rapid plant growth required for biomass accumulation. The gene interaction of cell wall metabolism, hexose allocation and cell division contributes to biomass yield, expanding our understanding at the molecular level required for energy cane breeding and engineering.

T5-01-02

Genome sequencing supports a multi-vertex model for Brassiceae species

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The economically important *Brassica* genus is a good system for studying the evolution of polyploids. Comparative genomic analysis of *Brassica* genomes has revealed that these have undergone whole genome triplication (WGT), which can be explained by a two-step theory. The diploid progenitor genome of *Brassica* before WGT was deduced to have seven chromosomes that resemble the translocation Proto-Calepineae Karyotype (tPCK). Subgenome dominance phenomenon such as biased gene fractionation and dominant gene expression were observed in *Brassica*. Furthermore, WGT and gene dominant expression were determined to contribute to, or be associated with, morphotype diversification and trait development during domestication and breeding improvement of *Brassica* crops. In addition, the genome of radish (*Raphanus sativus*), which is another important crop of the tribe Brassiceae, was derived from the same WGT event and shows similar subgenome dominance. These findings and molecular dating indicate that radish has a similar position from the evolutionary aspect as that of *Brassica* species. Finally, we extended the *Brassica* "triangle of U" model to an open system as a multi-vertex model to include radish and other Brassiceae species that share the same WGT event. As many Brassiceae species are known to be closely related to *Brassica*, this multi-vertex model describes the relationships or potentials of applying more Brassiceae paleohexaploids in the creation of new allotetraploid oil or vegetable crop species. Finally, further extensive work confirming the relationships of Brassiceae species is warranted.

T5-01-03

Genome evolution in newly formed polyploids: Insights from *Tragopogon*

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Polyploidy (whole-genome duplication; WGD) is a major force in eukaryotic evolution, and ancient and recent WGDs are well documented in many lineages, including vertebrates, fungi, and, most extensively, angiosperms. Despite repeated WGDs, most eukaryotes are functionally diploid, yet little is known about the earliest stages of polyploidy and the processes that transform a duplicated genome into a diploid one. *Tragopogon* is a textbook model for studying polyploidy with two recently (~80 years old; 40 generations in these biennials) and repeatedly formed natural allotetraploids (*T. mirus*, *T. miscellus*) and their diploid parents (*T. dubius*, *T. pratensis*, *T. porrifolius*). Our cytogenetic, genetic and genomic data suggest that patterns of gene loss, silencing, and chromosomal change in the young polyploids *T. mirus*, *T. miscellus* are repeated across multiple origins of these polyploids in nature, a well as in synthetic lines. These changes are ongoing--we have caught evolution in the act. Furthermore, the ongoing changes observed in these newly formed polyploids appear to be fixed in the older polyploid *T. castellanus*. Thus, patterns of genetic and genomic change may be predictable within just a few generations in young polyploids. With its range of ages, *Tragopogon* affords research opportunities not available in other systems (e.g., cotton, soybean, maize).

T5-01-04

Cytonuclear evolution in allopolyploid plants

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Allopolyploid speciation, faces the challenge of stoichiometric disruptions resulted by merging bi-parental nuclear genome yet with only one (always maternal) of the two sets of progenitor cytoplasmic genomes. Rubisco (1,5-bisphosphate carboxylase/oxygenase) is composed of nuclear-encoded small subunits (SSUs) and plastome-encoded large subunits (LSUs), which is an ideal enzyme to explore the evolutionary cytonuclear accommodation process. By studying gene composition and diversity as well as gene expression in natural and synthetic allopolyploid lineages, including *Arabidopsis*, *Arachis*, *Brassica*, *Nicotiana* and *Oryza*, we characterized the cytonuclear co-evolution/coordination in terms of its variation pattern and evolution pace. It is demonstrated that paralogous nuclear-encoded *rbcS* genes within diploids evolved in a "balanced mutation and concerted homogenization" model. Within the hybrids, upon the genome merger, no genomic coordinative responses of allelic *rbcS* gene conversions are made. However, transcriptional coordination could be conditionally achieved via biased expression of maternal *rbcS* nuclear genes; at the initial stage after genome doubling, a pool of allopolyploid individuals harboring variable coordination patterns are generated, in which neither transcriptional nor genomic coordination is established. During afterwards evolution, the functional constraint from the cytoplasmic partner genes and/or other organellar factors could exert selection pressure to the nuclear *rbcS* genes, which could achieve the eventual well-established cytonuclear coordination such that *rbcS* maternal homoeologs are preferentially biased utilized and maternal-like *rbcS* paternal copies carrying the maternal-to-paternal conversions preferentially occur. These results shed lights on the unexplored cytonuclear coordination in plant allopolyploid speciation and evolution, which could be a novel focus for future polyploidy researches.

T5-01-05

Genome organization of autotetraploid potato (*Solanum tuberosum* L.)

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Potato (*Solanum tuberosum* L.), the most important tuber food crop in the world, has been facing many difficulties in its genetic improvement during the past hundred years. Genome sequence of a haploid potato was available, but we know little about the genome organization of cultivated potato, an autotetraploid. Here we reported a chromosome-scale 2.6 gigabase draft genome of a potato cultivar, Cooperation-88. The assembly covered ~87% of the tetraploid genome and was anchored onto 48 pseudochromosomes using a genetic map generated from 1000 S1 progeny from Cooperation-88 . We identified ~1.6 gigabase repeated sequences accounting for 65% of the assembly and annotated 133,733 protein-coding genes. The analyses of the RNA-seq data and Methyl-seq data uncovered the fates of multiple alleles in the autopolyploid genome. The genome landscape of Cooperation-88 provides insights into evolution and domestication of autoplolyploid crop.

T5-01-06

Genetic variation in diploid and polyploid vascular plants

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Although polyploids often arise from multiple origin events, polyploid speciation is likely a severe bottleneck. We thus expect genetic variation to be lower in polyploids than in diploids. To test this hypothesis, we assembled from the literature an array of expected heterozygosity (He) statistics for vascular plants. This data set includes observations from 54 families and 32 orders representing lycophytes, ferns, gymnosperms, and angiosperms. We made comparisons between diploids and polyploids, between spore- and seed- dispersed plants, and among different methods (AFLP, isozyme, RADseq, RAPD, and microsatellite). We also evaluated the influence of paleopolyploidy on genetic variation in diploids. Contrary to our expectations, we observed a similar level of genetic variation in polyploids and diploids. Spore-dispersed vascular plants have a slight but statistically significant deficit of heterozygosity relative to seed-dispersed plants. Among methods, microsatellite markers consistently estimated higher levels of heterozygosity than the other methods. We observed a slight negative trend between *He* and chromosome number; plants with high chromosome numbers tend to be polyploid ferns with *He* near or below 0.2. Finally, we found higher heterozygosity in diploids with more recent whole genome duplications than in those with more ancient polyploidies; around 5% of the variation in expected heterozygosity can be explained by the age of the most recent paleopolyploidy. These results suggest that whole genome duplication casts a “long shadow” lasting millions of years, with profound, long-lasting effects on the evolution of vascular plant genomes.

T5-02: Gymnosperm genomes and comparative genomics

T5-02-01

DNA methylome of the 20-gigabase Norway spruce

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DNA methylation plays important roles in many biological processes, such as silencing of transposable elements, imprinting, and regulating gene expression. Many studies of DNA methylation have shown its essential roles in angiosperms (flowering plants). However, few studies have examined the roles and patterns of DNA methylation in gymnosperms. Here, we present genome-wide high coverage single-base resolution methylation maps of Norway spruce (Picea abies) from both needles and somatic embryogenesis culture cells via whole genome bisulfite sequencing. On average, DNA methylation levels of CG and CHG of Norway spruce were higher than most other plants studied. CHH methylation was found at a relatively low level; however, at least one copy of most of the RNA-directed DNA methylation pathway genes was found in Norway spruce, and CHH methylation was correlated with levels of siRNAs. In comparison with needles, somatic embryogenesis culture cells that are used for clonally propagating spruce trees showed lower levels of CG and CHG methylation but higher level of CHH methylation, suggesting that like in other species, these culture cells show abnormal methylation patterns.

T5-02-02

Genetic and epigenetic features of gymnosperm genomes. Are the gymnosperms really slow in evolution?

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Seed plants today comprise the angiosperms and the gymnosperms. Whilst much attention has been devoted to angiosperm genome structure and evolution, rather little has been expended on gymnosperms. Gymnosperms typically have large genomes exhibiting reduced frequencies of a number of processes (e.g. polyploidy) that have shaped the genomes of other vascular plants. Here we studied genome organization focusing on major chromosome domains (mostly rDNA and telomeres) in gymnosperm lineages including Coniferales, Ginkgoales, Gnetales and Cycadales using combined molecular cytogenetic and genomic (NGS) approaches. While overall genome organization (e.g., retroelement content) is similar to angiosperms wide differences exist in individual genomic components. For example, genus *Cycas* is unique in having extraordinary diversity of rDNA paralogs, high level of DNA methylation and extremely amplified telomeric repeat variants. The unusual linked arrangement of 35S and 5S rDNA has evolved repeatedly in several groups. We also searched for epigenetic genes potentially involved in genome size control discovering that members of a dicer family (*dcl1-4*) underwent multiple losses and gains in evolution of gymnosperms. In conclusion, individual gymnosperm lineages exhibit many unique features not generally found in angiosperms. Thus, gymnosperms genomes may not be as static as previously thought.

T5-02-03

Lack of DNA removal following polyploidization drives genome sizes in the gymnosperm genus *Ephedra*

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Most gymnosperms are remarkably stable as far as chromosome number, karyotype and genome size are concerned. In this study on *Ephedra*, we investigated the latter three features together with state dependent diversification and species environmental niche breadth in a phylogenetic framework to (1) evaluate the impacts of genome size and polyploidy on speciation rate, (2) identify where shifts in genome size and polyploidy have taken place during diversification both temporally and spatially, and (3) determine whether polyploid taxa have wider ecological niche breadths than their diploid progenitors. To achieve this, we generated four new chromosome counts and measured genome size data for 84% of *Ephedra* species across the phylogenetic tree; used phylogenetic modeling to infer chromosome number, genome size evolution, and ancestral states; tested for state dependent diversification under a BiSSE model, and compared ecological niche breadth using phylogenetic generalized least squares (PGLS) regressions. Our results showed that *Ephedra* is chromosomally highly variable. Chromosome numbers range from $2n = 14$ to $2n = 56$ with rampant polyploidization ($4x$, $6x$, $8x$) affecting 28 taxa (68%). Most of these polyploid taxa (78.6%) were tetraploid. Genome sizes are also very variable with 1C-values ranging nearly 5-fold from 8.09-38.34 pg making Ephedraceae the most variable of all gymnosperm families. *Ephedra* is also notable as it includes the largest genome size so far reported for any gymnosperm (*E. antisyphilitica*, $2n = 8x = 56$). In contrast to angiosperms and most gymnosperms, genome size and chromosome number are strongly correlated in *Ephedra* and C-values can thus be used to predict chromosome numbers and ploidy levels. Consequently, monoploid (1Cx) genome sizes at different ploidy levels are relatively uniform suggesting that polyploidy is not accompanied by significant genome downsizing, as commonly reported in angiosperms. Polyploidy in *Ephedra* is associated with higher speciation rate and is inferred mostly at more recent internal nodes, while the backbone is inferred to be consistently diploid with an ancestral chromosome number of $n = 7$ and ancestral 1C-value of 9.66 pg. PGLS regression results did not find support for polyploid species having wider niche breadths than their diploid counterparts.

T5-02-04

Drifting towards genome gigantisms in seed plants

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Most angiosperm genomes are small (modal 1C Genome Size = 0.6pg, mean 1C Genome Size = ~6pg), but some are enormous, indeed the largest known diploid genome in plants is *Fritillaria amabilis* (1C=100.6pg) and largest known genome for a eukaryote is the chromosomal octoploid *Paris Japonica* (1C=152.3 pg). In contrast gymnosperm genomes are typically larger than angiosperms (modal 1C Genome Size = ~10 pg and mean 1C Genome Size = ~ 18.6 pg) with the largest known diploid gymnosperm being *Pinus ayacahuite* (1C = 36 Gbp). Genetics reveal remarkable similarities between large genomes in angiosperms and those of gymnosperms (e.g. diverse heterogeneous repeats) that may be influenced by divergence in the epigenetic machinery. But environmental factors, small population sizes and drift may also play important roles in genome size divergence too. This talk explores genetic, epigenetic, and environmental factors that may contribute towards the drift towards genome giganticism.

T5-02-05

Repetitive elements in the genomes of gymnosperms

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Extant gymnosperms comprise four distinct lineages, the conifers, cycads, *Ginkgo* and Gnetales. Most species have large genome sizes (1C DNA values mean=18.8pg, mode=10.0pg), in comparison with angiosperms (1C DNA values mean=5.9 pg, mode=0.6pg). These large genome sizes have meant that few studies have addressed genome structure and composition in gymnosperms. Nevertheless, with the advances in next generation sequencing (NGS) technologies, coupled with developments in analytical techniques we can now start to shed light on the genomic composition of the major gymnosperm lineages. Here we use the RepeatExplorer pipeline (www.repeatexploer.com/) to characterize and compare the most abundant repeats comprising the genomes of representative species in each of the major gymnosperm lineages. The analyses show that the repeats are typical of other land plants that have been analyzed comprising abundant satellite sequences and LTR retrotransposons and derivatives, (e.g. *Copia*- and *Gypsy*-like elements). Astonishingly, the repeats in cycads, whose eight genera are estimated to have diverged c. 150 million years ago, are still sufficiently similar that we can group the cycads as a distinct clade from other gymnosperms. However, an analysis of individual gymnosperm repeat families reveals that they can be highly diverse and degraded. Indeed so stable are the repeats in gymnosperms that we can use them to similarly resolve *Welwitschia*/*Ephedra* and Pinaceae/*Ginkgo* lineages. Patterns of slow repeat divergence contrasts with the fast repeat turnover that is typically reported for angiosperms with small genome sizes (1C DNA values <10Gb). Indeed, similar analyses using the phylogenetic signal contained in repeat abundance cannot in our experience group angiosperm taxa above the genus level due to the rapid divergence of the repetitive DNA sequences. Nevertheless, ancient diverse repeats are also observed in the early diverging angiosperm *Amborella*, and fast repeat turnover is therefor likely to be a derived character that evolved after *Amborella* diverged from the rest of the angiosperms. In conifers there is evidence that the large genomes have expanded considerably through the accumulation of repeats, but there is not a proliferation of one or a few repeats that often dominate the genomes of many angiosperms with small genome sizes. Amongst gymnosperms, the Gnetales and in particular *Welwitschia* and *Gnetum* have small genomes, which are likely to have arisen through genome downsizing following their divergence from *Ephedra* (Gnetales), and the other gymnosperm lineages.

T5-02-06

The *Gnetum* genome reveals new insights into seed plant evolution

Tao Wan
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The dearth of genomic data from Gnetophytes is preventing resolution of conifer-specific from gymnosperm-specific genomic features. This is inhibiting understanding of evolutionary divergence across major seed plant lineages. Whilst partial assemblies for conifers are available, none exist for Gnetophytes. Here we report the 4.07 Gb fully sequenced and assembled genome of *Gnetum montanum* and find an enigmatic genome, which is distinct from all other seed plants. The contrasting genome dynamics represent a mosaic of characters, including (i) an absence of recent whole genome duplications in contrast to angiosperms and conifers, (ii) a diverse assemblage of retrotransposons (RT), as in conifers but not in most angiosperms, and (iii) a small, dynamic genome, with elevated rates of RT excision and higher rates of gene sequence divergence compared with conifers. Overall, the assembly enables the better resolution of ancestral genomic features within seed plants, the identification of genomic characters that distinguish gymnosperms from angiosperms, and reveals new dynamics in gene family evolution.

T5-03: State-of-the-art phenotyping: deep, high-throughput and field (two sessions)

T5-03-01

CyVerse tools for managing plant data

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CyVerse, formerly known as the iPlant Collaborative, is an US National Science Foundation (NSF) funded initiative "to design, deploy, and expand a national cyberinfrastructure for life sciences research, and to train scientists in its use" (http://www.cyverse.org/about). As part of this mission, CyVerse houses over 2 petabytes of data. Much of that data is genetic or genomic, but with the growth of high throughput plant phenotyping, we expect phenotypic and environmental data, including images and geospatial data, to be a rapidly growing part of our catalog. CyVerse includes platforms for managing and analyzing data, including the Discovery Environment (a web GUI), Atmosphere (cloud computing), Bisque (image management and analysis) and multiple APIs (application programming interfaces. The Data Commons (DC) is a new service that strives to build a space where data can live as a searchable, discoverable, and reusable resource. DC development builds on foundational CyVerse infrastructure, while expanding into new areas such as metadata and ontologies, data publication, and federation with external collaborators and repositories. Key components of the DC are the data portal at http://datacommons.cyverse.org/, and newer functions such as metadata templates, permanent identifier requests, data submissions to NCBI, and a Projects Interface (under development). This presentation will introduce the various CyVerse platforms and services available to support plant phenotyping and botanical research more generally.

T5-03-02

LiDAR based platforms for 3D high-throughput crop phenotyping

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Molecular designer breeding has been considered as an efficient and effective solution to solve the food crisis caused by the growing population and limited arable land worldwide. However, the inconsistent development of genotype and phenotype delayed the breeding process seriously, which really means that phenotypes cannot be non-destructively measured by high throughput quantification during the whole growth period of crops, especially in the field conditions. Recent progresses in phenomics show that a suite of new technologies, including Light Detection and Ranging (LiDAR) technology, intelligent robot technology, unmanned aerial vehicle (UAV) remote sensing technology, could become the main impetus to accelerate the progress in understanding gene function and environmental responses. Supported by "the Strategic Program of Molecular Module-Based Designer Breeding Systems" and "the Instrument Developing Project of the Chinese Academy of Sciences", we developed a series of phenotyping platforms to characterize agriculturally relevant traits used in greenhouse and field conditions, which were named as Crop 3D. By integrating LiDAR, high-resolution camera, thermal camera and hyperspectral imager, Crop 3D platforms were capable of acquiring multi-sources data through whole growth period automatically and synchronously along single-population-regional crop scales. The platform in greenhouse adopted "sensor-to-plant" working style to avoid interference on plant growth. For single plant in greenhouse, phenotype parameters such as plant height, crown, leaf length and width, leaf angle, *etc.* were extracted. Canopy height, canopy cover, plant area density and other relevant traits were measured to enhance our understanding of relationship between genotype and environment. Comparing the height derived by Crop 3D with the data measured by Electronic Distance Measuring Device, the R^2 was as high as 0.94. Field-based platform precisely measured the expression of physiological traits at canopy level under heterogeneous and fluctuating environment. In large region, UAV platform covered crops phenotype under different growing stages was applied to estimate crop progress, condition and yield. Phenotyping platforms still need to be further optimized in the application process according to the user demands. Transdisciplinary cooperation among breeder, agronomists and engineers will provide a broad stage for the application of phenotyping platforms to play its advantages.

T5-03-03

Remote estimation of leaf area index in wheat using multi-angular imagery acquired from an Unmanned Aerial Vehicle (UAV)

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Leaf area index (LAI) is a critical structural parameter for assessing crop growth status and an important trait in crop phenotyping. Spectral vegetation indices (VIs) represent a common method to estimate LAI from optical remotely sensed data. However, they are known to suffer from saturation when LAI is at moderate to high values (LAI > 5). A possible reason is that the VI-based methods only use spectral data from nadir observations. The flexibility of UAV systems enables the application of multi-angular remote sensing to monitor crop growth, which may mitigate the saturation problem and improve the accuracy of LAI estimation. This study employed a multispectral camera with 5 bands (490 nm, 550 nm, 671 nm, 700 nm, 800 nm) equipped on a multi-rotor UAV to acquire imagery at 7 fixed view angles (0°, ±20°, ±40°, ±60°) in the growing season of wheat at a field station in Rugao (32°16’N, 120°45’E), China. The experiment encompassed 36 field plots representing variations in cultivar, nitrogen fertilization level and sowing density. The UAV was flown at 50m above ground level to acquire multispectral imagery at around a 3 cm resolution, which is high enough to separate soil background from the green vegetation. Concurrent with UAV flights, the field LAI was measured with LI-COR LAI 2200C. A number of VIs selected for LAI estimation from the literature were calculated UAV imagery from different view angles. Empirical models with each VI were established to examine the effect of view angle on LAI estimation. At the end of this study, we draw conclusions on the optimal combination of view angle and VI for estimating LAI and make recommendations for UAV data acquisition strategies for wheat phenotyping applications.

T5-03-04

Combine phenotyping and modeling of canopy photosynthesis to guide breeding for higher crop yield and resource use efficiency

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Canopy photosynthesis, which is the sum of all leaves’ photosynthesis in a field, is a determinator of final yield production as it provides the carbon and energy necessary for plant growth and seeds. However, the development of canopy photosynthesis research is stagnant due to the difficulty to measure it in field. Optimizing canopy photosynthesis is even harder as its susceptibility to many factors, such as plant architecture, micro-climate in the canopy and photosynthetic features of all leaves. To tackle these problems, we developed a new canopy photosynthesis and transpiration system (CAPTS), which can automatically measure gas exchange of plant canopy or soil, for phenotyping canopy photosynthesis and a integrated 3D canopy model, which incorporated a comprehensive three-dimensional (3D) representation of canopy architecture, a ray tracing algorithm and a mechanistic dynamic model of leaf photosynthesis, for optimizing canopy photosynthesis. First, the CAPTS measurement correlated with the calculated canopy photosynthesis using the model, which was detailed parameterized and we showed the previous models using averaged light in each layer of canopy may over estimate canopy photosynthesis up to 25%. Secondly, we theoretically studied the impacts of different plant structures, locations and planting strategies to canopy photosynthesis with validations using CAPTS. Thirdly, we dissected the contributions of environmental and physiological factors to the increasement of canopy photosynthesis under future global change senario, which can hardly be assessed by experiments. Finally, we systematically studied the impact of chlorophyll content in leaves with our model and predicted that proper decrease of leaf chlorophyll content can increase canopy photosynthesis rate and nitrogen use efficiency. This was also confirmed with transgenic experiment. In conclusion, we developed a new equipment for measuring canopy photosynthesis and a new 3D model for studying canopy photosynthesis theoretically. With the two methods combined, we provided a new platform to optimize canopy photosynthesis for higher crop yield and resource use efficiency.

T5-03-05

Exploiting the multidimensional aspect of the radiance measurements for more efficient high-throughput field phenotyping

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Field high-throughput phenotyping is recognized as a major component needed to foster genetic improvement. It is achieved using non-destructive radiance measurements recorded by a number of sensors. Radiance measurements are sampling the total radiance field that extends over at least 6 dimensions, namely the 3 dimensions of the space, time, wavelength and directions. High-throughput field phenotyping measurements should therefore sample optimally the total radiance field of the micro-plots to retain most of the relevant information used to derive the structural and functional traits of interest. This presentation shows examples of the optimal sampling for several traits including the plant density, the green area index, the fraction of light intercepted by the canopy, the canopy height and the leaf chlorophyll content. It involves a number of sensors including RGB cameras, multispectral cameras, spectrophotometers and LiDARs that can be mounted aboard Unmanned Aerial (UAV) working in passive mode (the sun as radiation source) or Ground (UGV) Vehicles allowing to work in active mode with artificial illumination. Emphasis is put on the selection of the optimal spatial resolution, wavebands and directions (view and source) for each of these traits. Further, the temporal sampling is a key element for deriving higher level traits more closely related to the plant functioning. Conclusions are finally drawn on the typical experimental design best suited to extract the most informative traits from high-throughput field phenotyping platforms.

T5-03-06

In-vivo beloground phenotyping using computed tomograpy

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During the last years, X-ray computed tomography (CT) has been applied for the non-destructive visualization of belowground structures in pots. Formerly, this technology was only used for medical imaging. Nowadays, CT is used as a standard tool in industrial applications for material analysis. With X-ray CT the 3D volume information of objects can be reconstructed using X-ray projections of the object from different points of view. The scanning geometry we used is the axial 3D-CT, where a conical X-ray beam projects the plant pot on a 2D flat panel detector. The resulting spatial sampling frequency of this setup is about 85 µm using a high-power X-ray source to reduce the measurement time. Due to the non-destructive nature of CT it is possible to track the growth of plant organs such as cassava tubers, potato tubers or root systems in general. Thus, the below-ground development of an individual plant can be observed time dependently in natural soil. To virtually extract the root system out of the soil, new algorithms are used for the automatic segmentation. This allows to search for new traits with an increased throughput compared to manual volume segmentation. We will present the results of time resolved measurements for Cassava storage roots, potato tubers in heat and drought stress and root architectures of two different maize varieties. Within the timeframe of the experiments we periodically analyzed the below ground growth and applied an automated segmentation algorithm. Thus, we are able to characterize the volumetric change of the belowground organs time resolved and track them already at an early stage in a reliable and reproducible manner. For example, this approach allows to calculate the total volume as a function of the depth for each of the different varieties. Due to the time resolved experiment this data can be used to calculate the biomass growth rates, as well. Using this approach, we are able to phenotype for new traits and therefore breed distinct varieties under the aspect of root development.

T5-03-07

Describing the wheat canopy structure dynamics by combining ADEL-Wheat model with green fractions observations

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Monitoring the dynamics of canopy architecture during the early stages is highly desirable for breeders to select cultivars of high drought-tolerance, resistance to weed infestation, improved water-use efficiency and potentially high grain yield. These desired traits may be grouped into early vigor trait. A dedicated in silico study was completed to evaluate the feasibility and interest of a phenotyping data assimilation approach. A sequential assimilation scheme was developed to limit the space of canopy realization since the simulations of the 3D canopy architecture as well as the rendering of the phenotyping measurements is computer demanding. The early growth was decomposed into 2 consecutive stages. For each stage, 5 main influential parameters were identified. The phenotyping measurements were consisting into RGB images taken at several dates along the growth cycle and from several view inclination angles. For the first stage, the 5 influential parameters were estimated using a neural network machine learning technique. For the second phase, the 5 additional influential parameters were adjusted using the same neural network based technique while exploiting as prior information the posterior values of the 5 parameters defined during the first phase assimilation process. Results show that the parameters defining the size and rate of change of the green canopy crop elements were estimated with a relatively good accuracy. Conversely, the parameters driving the orientation and inclination of the elements were retrieved with a poor accuracy. However, some important emerging properties were very well retrieved, including the GAI and the number of tillers with more than 3 leaves at the beginning of stem elongation. Further detailed sensitivity analysis highlights the importance of getting enough dates of measurements covering the growth cycle, while dates separated by less than a phyllochron do not provide significant additional information. The sensitivity analysis highlighted also the importance of the inclined observations. The assimilation approach proposed appears very general and could be applied to a number of phenotyping measurements completed along the growth cycle. Nevertheless, because our results were achieved using in silico experiments, further validation is necessary using actual field measurements.

T5-03-08

Plant phenotyping reveals genetic and physiological factors of plant performance

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Knowledge of the structural and functional genetic architecture of agricultural traits is a prerequisite for the systematic exploration and utilization of plant genetic resources in plant breeding. To uncover mechanistic links between genetic variation, physiological factors and whole plant performance for development of novel crop improvement strategies, Arabidopsis, maize, and rapeseed are investigated by integrating genotyping, transcript and metabolite profiling, and automated plant phenotyping using dedicated platforms. The three high throughput plant phenotyping facilities at IPK support automated whole plant analyses for small, medium, and large plants including cultivation, transport (plant-to-sensor) and imaging of plants in climate controlled phytotron/glasshouse cabins. They are equipped with camera and illumination systems for visible, fluorescence, and near infrared imaging using top view and side views. Furthermore, 3D laser scanners and LED panels and CCD cameras for functional chlorophyll fluorescence detection and a broad range of environmental sensors are installed. The value of repeated non-invasive/non-destructive monitoring of large plant populations is highlighted by results of the analysis of a collection of 261 maize dent lines using a specifically optimized cultivation and phenotyping setup characterized for biomass accumulation and water consumption, and thus, for water use efficiency. Combined with 50k SNP information, these data have been used to identify QTL of these traits and of the growth dynamics by genome-wide association testing. 12 main effect QTL and 6-pairs of epistatic interactions were detected that displayed different patterns of expression at various individual developmental time points. A subset of them also showed significant effects on relative growth rates in different intervals. Using nonparametric functional mapping and multivariate mapping approaches four additional QTL affecting growth dynamics were detected. Our results demonstrate that plant biomass accumulation is a complex trait governed by many small effect loci most of which act at certain restricted developmental phases. This highlights the need to detect and investigate stage-specific growth control genes operating at different developmental phases. Integrated metabolome analysis and whole plant phenotyping performed in Arabidopsis revealed direct links between a promoter InDel polymorphism of the *FUM2* gene, its mRNA expression, fumarase enzyme activity, and fumarate to malate ratio in leaves. The promoter InDel that coincided with a fumarate QTL found in Arabidopsis Col-0/C24 RILs and ILs was also significantly associated with the fumarate to malate ratio, with malate and fumarate levels, and with dry weight in 174 natural accessions at 15 days after sowing (DAS) and was associated with biomass production in another 251 accessions (at 22 DAS). This supports a role of the cytosolic FUM2, which specifically occurs in *Brassicaceae*, in diurnal carbon storage and point to a growth advantage of accessions carrying the *FUM2* Col-0 allele preferentially occurring in colder climates.

T5-03-09

RhizoChamber-Monitor: A compact, automatic root growth monitoring platform with stretchable shading system

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Roots play a key role in resource acquisition and perception of environmental signals. However, compared with the aboveground, the phenotypic information on root system has been largely neglected due to the inaccessibility of the soil and the complexity of its structure. Here, we provide a novel root growth monitoring platform integrating customized rhizoboxes, rhizobox-loaders (RL), an imaging system, a precise irrigation system and a stretchable shading system as a whole, and a matched software. The rhizobox is assembled as a sandwich leaving two sheets of narrow space between cloth and transparent plastic for root growing and a small groove in the top of rhizobox for mounting irrigation tube. The operational principle of imaging system is similar to the mobile racking system frequently applied in logistics with RL analogous to the storage rack, rhizoboxes to the objects and sensor to the lift. The push-and-pull motion of RL and the three-dimensional and rotational motion of DSLR camera is conducted by one "H" shape mechanical system. The mechanical system crosses the RLs at the bottom of RLs. The cooperation of these two types of motion completes the imaging of both faces of all rhizoboxes. Unlike to the mode - one container with one suit of shading apparatus – that frequently used in HT root phenotyping system, in this platform, neighboring RLs are connected by one long suit of flexible organ shields and thus all rhizoboxes share one shading system. The advantage of using this shading system is that the system does not act as a block between sensor and root during imaging and the change in the size and number of rhizoboxes does not require the modification of the system. The Irrigation system allows the deployment of different water regimes on given groups of rhizoboxes in the same platform simultaneously. Software based on matlab is also developed for processing the time-series images and extracting global and local root traits. However, root crossing and paralleling makes topology analysis an issue. Instead, some specific local traits such as the surface area and number of lateral roots in successive equidistance segment of primary root can be extracted automatically using our software. Root growth of cotton plants under different water treatments was monitored and analyzed to demonstrate the performance of the platform and the software. As cultivation and imaging system are both in the same space and the height space is utilized as traffic aisle, this increases the space utilization ration and allows the deployment of the platform in limited space. The application of the shading system allows the control of root growing temperature independently. The root monitoring platform combined with the capacity for environmental simulation provides a possibility to assess the interaction between genotypes and environment dynamically.

T5-03-10

Plant water stress remote sensing system using UAV with RGB, multiple-spectral and thermal cameras

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Remote sensing technology has been used for determining crop water requirements and irrigation scheduling in recent 20 years. Efficient irrigation scheduling tools and management are based on high spatially and temporally resolution images obtained by remote sensing platforms. Unmanned Aircraft Vehicle (UAV) sensing systems with RGB, multiple-spectral and thermal cameras to obtain high quality images were developed. Two fixed wing and 14-rotors UAVs were developed using open source pilot software. The control system was designed to trigger the camera independent of the pilot system of UAV. The GPS coordinates were obtained and saved in the log file of the camera. The ground resolution can be calculated based on the parameters of camera and the height of UAV. The UAV remote sensing systems were used to obtain the images of crops in 2013,2014 and 2015 with the combination of ground data collection like soil and leaf water contents. Soil water content models were developed using linear regression methods based on the UAV remote sensing images and ground measurements.

T5-03-11

Machine learning methods in plant image phenotyping

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The importance of high-throughput and automatic phenotyping of crops has grown in recent years as a way to generate larger and denser datasets to analyze complex plant biological systems. While the automated acquisition of plant image data is becoming more straightforward, extracting interesting features remains a crucial bottleneck. Approaches to increase the accuracy and efficiency of feature extraction, as well as to expand the number and type of phenotypes which can be extracted from complex multimodal image datasets are a critical need for downstream biological analysis. Machine learning methods are promising tools for analyzing large complex data and have great success in applications involving images such as disease diagnosis from CT/PET/MRI, face recognition, pixel segmentation, object labeling and applications not involving images such as voice recognition, language translation and artificial intelligent. This project intends to evaluate and compare the performance of different machine learning tools in plant phenotyping based on matched hyperspectral images and RGB images. The target plant phenotypes include both morphological variables and measures of the plant ionome, including the abundance of nitrogen (N), phosphorus (P) and potassium (K). The raw data come from two experiments conducting in the UNL automated phenotyping greenhouse. The first employed 196 maize (corn) plants, imaged each day from Oct. 10, 2015 to Nov. 9, 2016 and the second includes 504 corn plants imaged every other day from May 16, 2016 to the mid of July 2016. Ground truth manual phenotypic measurements were collected at the end of each experiment. We consider different machine learning algorithms including random forest, supporting vector machine and combinatorial approaches. We implement each algorithm with different settings of kernel functions and data normalization. Our research may assist and serve as a reference for researchers in plant phenotyping.

T5-03-12

Leaf chlorophyll content estimation from multispectral imagery acquired from unmanned aerial vehicle (UAV) over wheat crops

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Estimates of leaf chlorophyll content (LCC) is a highly-desired trait to be characterized in field phenotyping experiments. Current methods are mostly based on tedious destructive sampling or based on leaf-level indirect methods using leaf transmittance or fluorescence measurements achieved by clipping individual leaves. Alternative methods based on canopy reflectance measurements have been proposed. They allow much higher throughput but have been limited in accuracy because of the confounding effect of canopy architecture. This study investigates a new approach allowed by the high spatial resolution images and the inclined views accessible from unmanned aerial vehicle (UAV) observations. A joint experiment between China (Rugao, 32°16'N, 120°45'E) and France (Gréoux, 43°45'N, 5°51'E) was conducted over wheat plots showing a range of nitrogen level fertilization, sowing density, growing conditions and genotypes. Multispectral images were acquired with the AIRPHEN camera aboard a UAV at end of tillering and booting stages of wheat growth. The camera is equipped with 6 bands sampling the chlorophyll absorption features both from nadir and inclined (45° inclination perpendicular to the rows) observations. The UAV was flown at low altitude to acquire high spatial resolution images allowing the separation of the green elements from canopy background. It was flown three times during the day to account for potential influence of the solar position. Ground measurements of LCC, green fractions at 0° and 45° inclination, and green area index (GAI) were concurrently collected. Three types of empirical methods were compared to estimate LCC from the multispectral images acquired over wheat crops at the Rugao and Gréoux sites. The first (respectively second) one is based on the use of vegetation indices (VI) computed with the average reflectance values derived from nadir (respectively inclined) observations. The third one is based on VIs computed with the average reflectance values derived from a fraction of the green pixels observed from nadir. Performances of the three methods are compared with due attention paid to the sensitivity to the green fraction and time of acquisition during the day. Conclusions are finally drawn on the optimal method that would provide the best performances while being practically easy to implement within phenotyping experiments.

T5-04: Evolution of plant reproductive systems: from ecology to genomics

T5-04-01

Identification of causal heterostyly genes in *Primula*

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Heterostyly is a wide-spread floral adaptation to promote outbreeding, yet its genetic basis and evolutionary origin remain poorly understood. In *Primula* (primroses), heterostyly is controlled by the *S*-locus supergene that determines the reciprocal arrangement of reproductive organs and incompatibility between the two morphs. However, the identities of the component genes remain unknown. We have recently identified the *Primula CYP734A50* gene, encoding a putative brassinosteroid-degrading enzyme, as the *G* locus that determines the style-length dimorphism.

CYP734A50 is only present on the short-styled S-morph haplotype, it is specifically expressed in S-morph styles, and its loss or inactivation leads to long styles. The gene arose by a duplication specific to the Primulaceae lineage and shows an accelerated rate of molecular evolution. Additional genes with analogous characteristics, such as being present only on the dominant S-morph haplotype, have been identified, representing candidates for other causal heterostyly genes. Their functional characterization is ongoing. Thus, our results provide a mechanistic explanation for the *Primula* style-length dimorphism and begin to shed light on the evolution of the *S*-locus as a prime model for a complex plant supergene.

T5-04-02

Heritability and fitness consequences of architectural effects within inflorescences

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Production of multiple flowers allows the reproductive phenotypes of individual angiosperms to include systematic among-flower variation, the details of which could be adaptive in particular environments. Systematic trait variation within inflorescences could arise from resource competition among a plant's flowers, or be a developmentally determined feature of flower position, regardless of resource dynamics. The latter, architectural effect is obvious in individual plants that produce distinct floral morphs (e.g., various types of monoecy, peripheral sterile flowers), but manifests more often as continuous floral variation within inflorescences. For architectural effects to be adaptive, differences in the relations of floral traits to flower position among individuals must both cause consistent variation in reproductive performance and be heritable. We assess systematic positional variation within inflorescences for natural populations *Delphinium glaucum* (Ranunculaceae), which produces vertical racemes with protandrous bisexual flowers. In this species, features of flower size, flowering phenology and female resource allocation (ovule number) and time allocation (female-phase duration) decline from bottom to top flowers. In contrast, anther number is constant among flowers and male-phase duration increases distally. Consequently, upper flowers emphasize relative male function compared to lower flowers. A manipulative field experiment demonstrated that at least the number of new flowers opened per day on inflorescences (anthesis rate) depends on their position, rather than resource competition with lower (earlier) flowers. This trait is an essential control on the number of flowers displayed simultaneously (floral display size), and so contributes to pollinator attraction and among-flower self-pollination. The existence of architecture effects suggests that gradients in floral traits within inflorescences are products of selection and so have functional benefits and are heritable. We examine gradient function by quantifying the association of among-plant variation in overall seed production, siring success and outcrossing rates to gradients of flower size, ovule and anther production and anthesis rate. We specifically characterize phenotypic gradients as spline functions and analyse them as function-valued traits using the developing body of statistical methods known as functional data analysis. We also use SNP-based approaches to evaluate and compare the heritabilities of individual trait means, variances and gradients. These results illustrate that architectural effects within inflorescences affect reproductive performance and are partially genetically determined, rather than simply representing phenotypic plasticity or developmental instability. Such effects demonstrate that the reproductive phenotypes of angiosperms are functional mosaics shaped by adaptation.

T5-04-03

A peculiar marriage arrangement - Evolutionary processes in tristylous populations

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Populations of tristylous plants are reproductively subdivided into three mating types that differ in the reciprocal arrangements of their sex-organs. The mating types are referred to as the long-, mid- and short-styled morphs (hereafter L-, M- and S-morphs). This floral polymorphism is maintained by frequency-dependent selection resulting from disassortative mating. Ronald Fisher first demonstrated that with this mating system a 1:1:1 ratio of morphs (isoplethy) is predicted in large equilibrium populations. Here, we consider the ecological, demographic and reproductive factors that can cause deviations from the Fisherian isoplethic equilibrium, particularly in the context of frequent colonization and biological invasion. We draw examples from the three well known tristylous families (Lythraceae, Oxalidaceae, Pontederiaceae), with a focus on recent comparative investigations of *Lythrum salicaria* (purple loosestrife) in its native European and introduced North American ranges. Founder events, limited sexual recruitment and prolific clonal propagation have played key roles in determining the representation and frequency of style morphs in invasive populations of *Oxalis pes-caprae* (Bermuda buttercup) and *Eichhornia crassipes* (water hyacinth). Until recently, it was widely thought that introduced populations of *O. pes-caprae* were exclusively composed of a sterile pentaploid clone of the S-morph. However, recent studies in the Iberian Peninsula indicate that tetraploid L- and M-morphs capable of sexual reproduction also occur in some populations, and molecular evidence suggests that they have arisen from separate introductions from the native South African range. In the introduced range of *E. crassipes,* the M-morph dominates globally with the L-morph occurring sporadically where sexual reproduction is permitted. Molecular evidence indicates that a striking genetic bottleneck accompanied invasive spread from the New to the Old World. The S-morph is restricted to lowland South America and thus is entirely absent from the introduced range. In contrast to clonal *O. pes-caprae* and *E. crassipes, Lythrum salicaria* reproduces almost exclusively by seed and the majority of populations in both the native and introduced ranges are tristylous. However, recent surveys indicate that invasive populations can often be dimorphic, with ~25% lacking the S-morph. Two large-scale surveys conducted 25 years apart in Ontario, Canada involving >100 sampled populations indicate that despite the rapid increase in abundance of the species, genetic drift and founder events still play a dominant role in governing patterns of morph-frequency variation. In contrast, a survey of 96 populations along a latitudinal gradient in the Iberian Peninsula found that despite increasingly smaller populations towards the more arid southern range margin, 95% of populations were trimorphic. The maintenance of tristyly in European native populations may be aided by the genetic connectivity of populations in the agricultural landscapes where they reside, owing to gene flow by pollen and seed dispersal. Our studies demonstrate that tristyly can be used to provide insights into the relative importance of founder events, genetic drift and frequency-dependent selection in governing patterns of variation at a conspicuous genetic polymorphism of known adaptive significance.

T5-04-04

Integrating micro- and macro-evolutionary perspectives on heterostyly: Case studies from primroses

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The evolution of flowers opened unprecedented opportunities for complex interactions with pollinators, enabling new types of reproductive barriers, generally favoring outcrossing, and promoting diversification. Outcrossing increases genetic diversity, potentially mitigating the detrimental effects of environmental change and favoring long-term resilience to extinction. One of the best-studied floral syndromes enforcing outcrossing is heterostyly. Repeatedly appearing in angiosperm evolution and extensively studied in *Primula* L. (Primulaceae), heterostyly is a genetically-controlled floral polymorphism, whereby individuals in a population produce complementary types of self-incompatible flowers with anthers and stigmas in spatially matching positions. Conversely, homostylous species produce monomorphic, self-compatible flowers. Comprising ca. 90% heterostylous and 10% homostylous species that differ markedly in floral morphology, reproductive strategies, and size of distributional ranges, Primulaceae (ca. 730 species) represent an ideal group to investigate fundamental questions on how specific floral syndromes affect reproductive strategies, evolutionary dynamics, and biogeographic processes. By combining evidence from experimental pollination studies, quantitative morphological analyses of floral traits, different types of modeling approaches, phylogenomics, and biogeographic/demographic analyses, we integrate micro- and macro-evolutionary perspectives on the effects of heterostyly in primroses. It has been observed that several heterostylous groups appear to be especially species-rich, thus we wanted to know whether and how heterostyly might be implicated in explaining this phenomenon. We started to address this question by investigating how heterostyly works at the intra-specific level in the florally dimorphic *Primula* system. We confirmed the general predictions of the disassortative pollination hypothesis, but found that long-styled flowers export more pollen to non-reciprocal than reciprocal stigmas. We then switched to the inter-specific level and asked whether distyly might contribute to reproductive isolation via mechanical barriers. We discovered that distyly supports weak mechanical isolation, but only between high sexual organs, thus documenting, for the first time, the existence of morph-dependent, in addition to species-dependent asymmetries in reproductive barriers. Finally, we moved to the macro-evolutionary level[1] and asked whether the evolution of heterostyly in Primulaceae is associated with an increase of diversification rates. We found that heterostyly accelerates diversification not via spurring speciation, but via decreasing extinction, possibly because it enforces outcrossing, thus favoring lineage survival in varying environmental conditions. Finally, we tested whether repeated switches from heterostyly to homostyly in *Primula* are associated with the so-called selfing syndrome. We discovered that floral traits involved in pollinator attraction and interaction are indeed smaller in homostylous, self-compatible species than in distylous, obligatorily outcrossing species. Most recently, we employed whole-genome analyses to study how the distributional ranges of distylous primrose species respond to climatic oscillations and to generate the first draft genome of any heterostylous species, a tool that has been crucial to progress on the molecular characterization of the heterostyly supergene. Current research exploits the newly available genomic resources to reconstruct a data-rich phylogeny of Primulaceae, the evolutionary steps leading to the assembly of the heterostyly supergene and repeated switches to homostyly, and how hybridization and introgression affect different parts of the genome in heterostylous vs. homostylous primroses.

T5-04-05

Flower number - flower size relations in Montiaceae in the context of reproductive adaptation across environments

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Many plant traits do not evolve independently of other traits, rather, suites of traits often evolve in concert. A particularly striking example is the frequently negative relation between the number of flowers a plant produces and the investment in each, leading to flower number-flower size "trade-off"-like relations. Although common across angiosperms, their ecological relevance and environmental drivers remain poorly understood. In this talk, we present results from Montiaceae (Caryophyllales) and compare these to patterns across angiosperms. We employ a baited-phylogenomic marker system to elucidate the phylogeny of Montiaceae and reveal shifts in evolvability of floral traits: in some clades, carpel and stamen numbers have greatly evolved among species and correlate with flower number, in other clades, gynoecium and androecium structure are conserved. We analyse the ecological context of correlated evolution of flower number and size traits and find that evolution of large- (but few-) flowered species is associated with transitions into extremely seasonal environments, such as high-elevation deserts. We compare these results to other taxonomic groups to investigate whether reproductive adaptation at the inflorescence level across environments is a common evolutionary trend.

T5-04-06

Biogeography and ecology of style polymorphisms across spatial and temporal scales in the Mediterranean

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Style polymorphisms are characterized by the presence of two or more discrete morphs within plant populations and have been interpreted as a mechanism to promote disassortative mating. Like most of breeding systems, they have a context-dependent nature and their evolutionary success is variable depending on the shifting ecological conditions in time and space. We will focus here on heterostyly and related polymorphisms, such as stigma height dimorphism, in a specific geographic and ecological setting, the Mediterranean basin, where environmental changes have been the rule due to its transitional stage between temperate and tropical biomes and its strong historical constraints. Particularly, both long- and short-term changes have left a mark in the distribution of these polymorphisms. The geomorphological and climatic scenario, dated back to the late Tertiary, is associated with the evolution of style polymorphism across species in some Mediterranean lineages. More recently, the climatic heterogeneity and oscillation during the Pleistocene has provoked changes in the populations of related style-polymorphic species, expressed in their morph ratios. These changes are associated to deviations from typical heterostyly (perfect reciprocal placement of anthers and stigmas linked to heteromorphic incompatibility and ancillary traits). However, heterostyly in these Mediterranean lineages is rarely broken down to homostyly, which is usually linked to hybridization, polyploidy and selfing in northernmost latitudes. We review the available evidence based on several studies of Mediterranean plant lineages where style polymorphism is present at variable frequency in different conditions. We will focus on both at macro- and microevolutionary levels. *Narcissus* is an old pre-Mediterranean genus that underwent several pulses of diversification in critical periods of the Mediterranean history. This affected differently the geographic and ecological distribution of clades and the representation of style polymorphisms. Species that have experienced considerable range shifts are quite labile in their polymorphism expression at population level. Thus, morph ratios are extremely variable, including the complete loss of polymorphism, due to concomitant changes in pollinator identity and frequency. Boraginaceae is a plant family with a high diversity of breeding systems, which are well exemplified in Mediterranean and European lineages. Studies in *Anchusa*, *Pulmonaria*, *Glandora* and *Lithodora* showed a high diversity of conditions, whose dynamic nature does not fit with traditional models of heterostyly evolution. Despite this fascinating diversity, we are far from understanding its ultimate and proximate causes. *Linum*, flax species in Linaceae, is a sub-cosmopolitan genus with its highest diversity in the Mediterranean. Although it was one of the paradigmatic cases where Darwin put his interest in the topic, recent studies have revealed that this genus includes more variation in the expression of the polymorphism than formerly presumed. As far as we know, the variation affects morphological aspects more than incompatibility systems. This offers an opportunity to study the implications of variations in sex organ deployment affecting reciprocal placement of sex organs and thus the functioning of these style polymorphisms.

T5-05: Plant genome evolution from the very beginning (two sessions)

T5-05-01

How plastids shaped (land) plant genomes and physiology

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The endosymbiotic origin of plastids is well known as having afforded eukaryotes the ability to harness the energy of the sun. However, the evolutionary impact of plastid evolution extends far beyond photosynthesis. At its origin, thousands of genes were transferred from the cyanobacterial endosymbiont to the nuclear genome of the host, cementing the relationship between host and cyanobiont. While maintaining the plastids' biochemical processes rests upon a tight communication between plastid and nucleus, little is known about this 'cross-talk' beyond land plants. We recently reported that higher branching streptophyte algae share with land plants the lack of a few essential plastid genes that have been transferred to the nucleus. Compared to those of other algae, the plastids of higher streptophytes ought to be more submissive to nuclear control. These organelle-associated changes, we proposed, allowed for an elaborate plastid-nucleus signaling network to evolve. The evolution of such a network aided in dealing with plastid-associated stressors such as high irradiances. Overcoming such stressors might have been a major selective advantage during plant terrestrialization. Ultimately, the increase of nuclear control over plastid function along the trajectory of streptophyte evolution made the plastid of vascular plants plastic; thanks to this plasticity, vascular plant plastids can differentiate into an unparalleled number of different types within a single species. What this suggests is that the embryophytic control over plastid function evolved within the streptophyte algae. Using comparative functional genomics across the diversity of streptophyte algae, we found an increased repertoire of retrograde signaling factors in higher streptophyte (algae). This advanced plastid-nucleus communication has implications beyond the regulation of photosynthesis. Plastids are not only photosynthesis machines; they are at the centre of a vast range of land plant secondary metabolic processes. Many of the canonical phytohormones, such as abscisic or jasmonic acid, are derived from biochemistry occurring within the plastid. The genetic potential to synthesize and sense them is found among streptophyte algae. This prompted us to design a large-scale transcriptomic comparison of both higher and lower streptophyte algae to study how the regulation of streptophyte plastid stress signaling evolved. Our data improves our understanding of their responsiveness to plastid-relevant treatments. Plastids are at the heart of land plant biology. For a comprehensive picture of what defines land plants and what made their success on land possible, it is critical to develop a comprehensive understanding of the lineage-specific adaptations of their plastids and those of their closest relatives.

T5-05-02

The prasinophyte - land plant connection: Opportunities for insights into evolutionary processes within the green lineage

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Progress in sequencing genomes of photosynthetic microbial eukaryotes in recent years, from model chlorophytes (e.g., *Chlamydomonas reinhardtii*) to ecologically relevant marine phytoplankton (e.g., *Micromonas pusilla* and *Micromonas commoda*), has improved our understanding of algal evolution, biological processes and ecology. Here, we will present highlights from lab and field based studies of marine green algae as well as genomic comparisons to land plants and other algae. The prasinophyte *Micromonas* reveals 'new' suites of genes that now appear to be ancestral among green lineage taxa although lacking in model chlorophytes. In addition to several transcription factors, these include the photosensory protein phytochrome, a master regulator in land plants. Additionally, patchy loss patterns in the peptidoglycan pathway (derived from the cyanobacterial endosymbiont) suggest that plastid division and structure differs among closely related green lineage taxa. Here, we will discuss these and other recent discoveries as well as RNA-seq and proteomic functional studies of prasinophyte algae.

T5-05-03

Insights into land plant evolution garnered from the *Marchantia* genome project

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The origin of a land flora had profound consequences on the global environment and altered the evolutionary trajectories of other major clades of life. Land plants evolved from an ancestral charophycean alga from which they inherited numerous developmental, biochemical, and cell biological features. Characteristics of extant charophycean algae suggest that at least some of the physiological adaptations to land evolved prior to the origin of land plant. For example, several lineages can occupy terrestrial habitats, tolerate UV radiation and respond to desiccation similarly to land plants. In addition, many gene families found in land plants, but not in the largely marine Chlorophyta algae, have their origins in the charophycean algal grade. However, the origin and diversification of land plants involved dramatic evolutionary changes in life history, physiology, and body plan. The three earliest diverging lineages of extant land plants (liverworts, mosses, hornworts) are likely a paraphyletic assemblage, collectively called the bryophytes, which lack vascular tissues and true roots but collectively acquired all key features associated with land plant evolution (1) the origin of a multicellular diploid sporophyte, (2) a gametophytic apical meristem with an apical cell that produces 3-dimensional tissues, a sporophytic shoot apical meristem (SAM) with a capacity for branching, and (3) cell fate specializations providing morphological and physiological adaptations to life on land. The phylogenetic relationships of the bryophytes remain contentious, with nearly every possible topology proposed based on different molecular datasets. However, fossils assigned to liverworts (Ordovician-Silurian) predate those described as either mosses (Carboniferous) or hornworts (Cretaceous) and evidence from systematics, and phylogenetic studies suggest that the first plants that colonized terrestrial environments possessed attributes of liverworts, and it appears that extant liverworts retain a larger suite of ancestral land plant characters than any other lineage. Due to its ease of growth and genetic manipulation in the laboratory and its extensive historical literature, we have chosen *Marchantia polymorpha* as a representative liverwort and present an analysis of its genome, in comparison to those of charophycean algae and other land plants, including the moss *Physcomitrella patens*.

T5-05-04

Hornworts, a missing link to study fundamental questions of plant biology

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The monophyletic group of hornworts is believed to represent the immediate sister group of all vascular land plants. However, this traditional view is still debated and cannot be satisfactorily resolved owing to the lack of detailed knowledge on the general biology and genomic features of hornworts. Until now, advancement in this field was primarily hindered by the lack of genomic resources for a hornwort model species. Here we provide an update on the efforts towards a high-quality genome draft of the model hornwort, *Anthoceros agrestis*, and some of its relatives. We show that *A. agrestis* has a remarkably small genome, with few recent paralogs, which makes it approriate for genetic analysis. We also provide an overview of the *A. agrestis* gene space and a preliminary gene expression atlas which shed light on the regulation of morphological and developmental traits that are either shared with other embryophytes or unique to hornworts. Furthermore, we report our first achievements on the genetic transformation of *A. agrestis* using various techniques. Finally, we summarize our achievements and provide a list of issues that need to be resolved in the future.

T5-05-05

Unfurling monilophyte genomics: how polyploidy, transposable elements, and the alternation of independent generations drive fern evolution

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Ferns (Monilophyta) are notorious for possessing large genomes (average 1C = 12 pg) and numerous chromosomes (average n = 59). Historically, these features, which characterize all homosporous ferns, were attributed to repeated rounds of ancient polyploidy (whole-genome duplication - WGD). However, this explanation for the origin of large genomes and many chromosomes, long considered emblematic of ferns, is unsubstantiated due to the absence of a sequenced fern genome. The lack of this crucial resource has not only hindered investigations of the evolutionary processes underlying the unusual genome characteristics of ferns, but has also impeded synthesis of genome evolution across land plants. Using the model fern species *Ceratopteris richardii*, or

C-Fern, we obtained the first sequenced and assembled nuclear genome for a homosoporous fern. With this novel resource, we evaluated the possible roles of WGD and the expansion of transposable elements (TEs) in shaping fern genome evolution. In addition, we investigated the ramifications of having two independent life phases (gametophyte and sporophyte generations) at the genome, gene, and transcript level. Not only do these results address the evolutionary processes and genomic changes that have shaped the genomes of ferns, but the assembled data set will also facilitate new insights into evolutionary genomics of land plants as a whole.

T5-05-06

The contributions of hybridization and polyploidization to hybrid establishment in natural populations of homoploid and allotetraploid *Selaginella arizonica* x *eremophila*

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At least 13% of plant species are allopolyploids formed via hybridization and genome duplication. Despite the frequency of allopolyploidy, the impacts of hybridization versus genome duplication in shaping allopolyploid genomes remain poorly understood. A major limit to understanding the different impacts of hybridization and polyploidy is the absence of a study system with diploid and polyploid hybrids from the same parental species cross. Here, we describe such diploid and polyploid hybrids from the lycophyte genus *Selaginella* [Selaginellaceae]. In the Sonoran desert of the Southwestern U.S., we analyzed diploid hybrids and allopolyploids formed between crosses of *Selaginella arizonica* and *S. eremophila*. Both types of hybrid populations occupy drier, hotter climatic niches than either of their parents and have different ecophysiological strategies related to water use efficiency and relative growth rate. Using a combination of next-generation sequencing techniques including Illumina, PacBio, and ddRADseq, we dissect the ancestry of these hybrid taxa, the relative contributions of hybridization and polyploidization to the respective genomes, and highlight the evolutionary dynamics of their resurrection phenotypes that may have aided in their establishment and persistence in novel ecological niches.

T5-05-07

Primitive mechanisms to adapt land environments revealed by the genome of a charophyte alga *Klebsormidium flaccidum*

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We have reported the draft genome sequence of *Klebsormidium flaccidum* (Hori et al 2014). Comparative genome analysis revealed that the genome encodes more than one thousand of land plant type genes which may be associated with its adaptation to harsh land environments. Here, we will introduce their unique intra and extracellular lipid components. By comparing genes involved in the lipid biosynthetic pathways of *Arabidopsis thaliana* with *K. flaccidum* genomes, we identified wax-related genes in this alga. A simple and easy extraction method was developed for the recovery of the surface lipids. Although this alga has wax components, its surface lipids were largely different from those of land plants. We also investigated aliphatic substances in the cell wall fraction of *K. flaccidum*. Many of the fatty acids were determined to be lipophilic monomers in *K. flaccidum*, and a Fourier transform infrared spectroscopic analysis revealed that their possible binding mode was distinct from that of *A. thaliana*. We propose that *K. flaccidum* has a cuticle-like hydrophobic layer composed of lipids and glycoproteins, with a different composition from the cutin polymer typically found in land plant cuticles. We will also introduce our recent study in primitive cellular systems in this microorganism.

T5-05-08

Lycophyte evolution and development: A transcriptomic and genomic approach

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Genomic research in plants have been focused on angiosperms with 43 sequenced genomes currently available. However, genomic data outside the angiosperms are limited to 3 species: *Selaginella moellendorffii*, *Marchantia polymorpha* and *Physcomitrella patens* where the majority of the studies have been dedicated to *P. patens*. In order to expand the understanding of the genetic mechanisms that underlies the evolution of land plants our research has been concentrated on Lycophytes which occupy a key phylogenetic position within the land plants as sister to all other vascular plants. Features such as vasculature and a sporophyte dominant life cycle arose first in the common ancestor of all vascular plants and by investigating this lineage we will be able to better understand how these innovations arose. Lycophytes evolved several features in parallel to the remainder of the vascular plants, including roots, leaves (microphylls), the tree habit (wood formation), heterospory and endosporic gametophytes. Thus, it is necessary to elucidate the molecular genetic networks underlying the development of these key innovations such as the leaves in Lycophytes because they are integral to understanding the evolution and development of vascular plants-the dominant component of the land flora-and economic botany. In Lycophytes, the genome of *S. moellendorffii* is available and we have sequenced the genome of *S. apoda*. In addition to the whole genome sequence of *S. apoda*, we have transcriptome data from leaves, shoots, roots, cones and rhizophores. Transcriptome analyses will allow us to make comparisons between the two different species of lycophytes but also to investigate the genes specific to root, cone, or rhizophore development. With whole genome analyses in lycophytes we have investigated gene families known to be important for body plan specification such as the MADS-box family of transcription factors. We studied the evolution and expression patterns of MADS-box genes to assess large scale and local duplications and evaluate protein motifs likely related with functional changes in lycophytes. We have found that the MADS-box genes have only 2 independent duplications in lycophytes one for Selaginellales and the other in Lycopodiales. Analyses of the conserved domains show that the lycophytes share the M and K domains with angiosperms and outside those regions we have been able to identify 5 new motifs specific to lycophytes where one is exclusive to Lycopodiales and the other to Selaginellales suggesting that even between lycophytes MADS-box genes are likely involved in different functions. Expression analyses by *in situ* hybridization of the three MADS-box genes in *S. moellendorffii* showed differential expression patterns for each gene, and we are performing similar expression analyses in additional lycophyte species. Genomic and transcriptomic analyses in lycophyte species have allowed us not only to investigate the development of lycophytes but also to provide unparalleled insight into the evolution and development of all vascular plants.

T5-05-09

Exploring algal phylogenomics: Genome sequencing and analysis in the araphid pennate diatom *Psammoneis japonica*

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The hyper-diverse and ecologically important diatoms (Bacillariophyta) are unicellular phytoplankton with chimeric genomes derived from two photosynthetic (red and green algae) and one heterotrophic precursor lineages. Complete genome sequences are currently available for three diatom species, representing two of four major morphological types (polar centrics and raphid pennates). Here, we present the genome sequence and analyses for *Psammoneis japonica*, which is a member of a critical third diatom clade, the araphid pennates. The *Psammoneis* assembly comprises approximately 25× PacBio and 100× Illumina coverage and is stringently filtered for contaminant sequences. With a resulting total assembly size of 91.2 Mbp (N50 = 390 kbp) and over 15,000 annotated genes, the *Psammoneis* genome is the largest diatom genome sequenced thus far. Sequencing of this genome enables a more comprehensive, comparative genomic view of diatom ecological (pelagic/benthic), morphological (centric/pennate, araphid/raphid) metabolic (carbon/nitrogen cycles) and reproductive (oogamy/anisogamy/isogamy, dioecy/monoecy) character evolution. General comparisons, including orthologous protein divergence, intron conservation and repetitive element content also provide further insight into rates and patterns of evolution that have shaped genome size and architecture across the diatoms.

T5-05-10

Molecular and evolutionary conservation of ABA signaling for stomatal closure

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Evolutionary trajectories of land plants have led to structurally complex and functionally active stomata for terrestrial life. The unique morphology, development and molecular regulation of stomata enable their rapid environmental response. Evolution of the molecular mechanism behind stomatal development and membrane transport has clearly drawn on conserved and sophisticated signaling networks common to stomata of all vascular plants and some mosses. ABA-driven stomatal regulation reportedly evolved after the divergence of ferns, during the evolution of seed plants around 360 Mya based on stomata of certain fern species being unresponsive to ABA, but exhibiting passive hydraulic control. However, growing evidence has shown that ABA signaling controlling stomatal closure may have evolved in some species of mosses. Here, we observed that a number of ABA signaling, membrane transporter, and ABA reception protein families diversified over the evolutionary history of land plants. Phylogenetic analysis of the key ABA signaling proteins indicates to an evolutionarily conserved stomatal response to ABA. Moreover, comparative transcriptomic analysis has identified a suite of ABA responsive differentially expressed genes in terrestrial fern species. These genes encode proteins associated with ABA biosynthesis, transport, reception, transcription, signaling, and ion and sugar transport, which fit the general ABA signaling pathway constructed from *Arabidopsis thaliana* and *Hordeum vulgare*. Furthermore, our stomatal assays have shown the first evidence for ABA-induced stomatal closure on epidermal peels of two terrestrial fern species. We thus propose that, despite the advantages of passive hydraulic control for fern stomata, the molecular mechanisms of ABA-induced stomatal closure may not have been lost in all fern species. Understanding this evolutionary trend will inform predictive modeling and functional manipulation of plant productivity and water use and will benefit future efforts towards food security and ecological diversity.

T5-05-11

Conservation and divergence of small RNA pathways and microRNAs in land plants

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As key regulators of gene expression in eukaryotes, small RNAs have been characterized in many seed plants, and pathways for their biogenesis, degradation, and action have been defined in model angiosperms. However, both small RNAs themselves and small RNA pathways are poorly known in other land plants such as lycophytes and ferns, preventing a comprehensive evolutionary perspective on small RNAs in land plants. We selected 25 representatives from major lineages of lycophytes and ferns, most of which lack sequenced genomes, and characterized small RNAs and small RNA pathways in these plants through RNA sequencing. We identified homologs of *DICER-LIKE* (*DCL*), *ARGONAUTE* (*AGO*) and other genes involved in small RNA pathways, predicted over 2,600 conserved microRNA (miRNA) candidates and performed phylogenetic analyses on small RNA pathways as well as miRNAs. We predicted targets of miRNAs and performed degradome sequencing to examine the transcript-cleavage activities mediated by miRNAs. Pathways underlying miRNA biogenesis, degradation, and activity were established early in land plant evolution, but the 24-nt siRNA pathway that guides DNA methylation was incomplete in early-divergent land plants, especially lycophytes. We show that the functional diversification of key gene families such as *DCL* and *AGO* as observed in angiosperms occurred early in land plants followed by parallel expansion of the *AGO* family in ferns and angiosperms. We uncovered a conserved *AGO* subfamily absent in angiosperms. Our phylogenetic analyses of miRNAs in bryophytes, lycophytes, ferns, and angiosperms refined the time of origin for conserved miRNA families in land plants.

T5-05-12

Functional ecology of mycobiome shifts associated with plant senescence - linking environmental and experimental re-synthesis metatranscriptomics

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The same fungi are often found in healthy and dead tissues of the same plant species. Because plant tissues at different stages of senescence are usually separated by time and space, tracking functional transitions of their mycobiomes is often confounded with extraneous abiotic factors. The perennial moss *Dicranum scoparium* has a growth form with a continuous senescence gradient, making it an ideal system to study fungal trophic transitions. Our goal is to understand the function of fungi associated with tissues spanning from healthy photosynthetic tissues to advanced states of decomposition. A metatranscriptomic approach was first applied to obtain the metabolic (rRNA) and functional (mRNA) activities of fungi associated with top (photosynthetic), middle (senescent), and bottom (decomposing) layers for the naturally occurring *D. scoparium*. By looking at the top nutrient-related genes expressed in each layer, we found that the highly expressed genes in the top layer are mostly related to carbon exchange, whereas some highly expressed genes in the middle and bottom layers were involved in nitrogen and phosphate transport. In parallel to our metatranscriptomic study of environmental samples of *D. scoparium*, we isolated fungal strains from the three layers of this moss and grow *D. scoparium in vitro* from spores. By comparing and integrating the fungal rRNA sequences generated by metatranscriptome and culture-based approaches, we assigned fungi into three types of association categories: 1) High activity in photosynthetic tissues, 2) High activity in decomposing tissues and 3) Low activity throughout the gametophyte but abundant when using culture-based detection. Based on these association categories, seven fungal strains representing distinct fungal lineages (four Ascomycota, two Basidiomycota, one Mortierellomycotina) were selected to establish fungus-plant re-synthesis pairs in the laboratory with both living and dead gametophytes. For every fungus-plant pair, growth rates were monitored for two months after fungal colonies reached the moss. Metatranscriptomes of these fungus-plant pairs were obtained at the end of this period.

T5-06: Ontologizing botanical knowledge (two sessions)

T5-06-01

Bottom-up phenotype ontology building using OTO and ETC text capture and ontology building tools

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Vast amount of valuable phenotypic character information has been published in the past, but due to the natural language nature of the character descriptions, they are not directly useful for biological computation. Domain ontologies, as a form of controlled vocabulary, have been recognized as a promising approach to integrate data from different sources, but in the realm of phenotypic characters, the majority of the biodiversity domains never had a standard vocabulary established, leaving authors tremendous freedom of describing phenotypic characters in various ways, for example "[shape] resembling a children's comic idea of a hand-grenade". Such issues have been widely recognized.While recognizing the large variations in terminology usage in published character descriptions, we emphasize that the primary descriptions are the ultimate source that must be covered by phenotype ontologies if we hope to bring these published knowledge to life. Towards this goal, we developed a set of web-based, public-accessible applications: OTO (Ontology Term Organizer) and ETC (Explorer of Taxon Concepts) toolkit, including Text Capture and Ontology Building. ETC Text Capture tools parse morphological descriptions on organisms of various taxon groups and extract phenotypic terms (such as anatomical structures, characters, and character states) in a semi-automated fashion. These terms can be exported to OTO directly from the ETC website. OTO provides a simple drag-and-drop user interface that allows multiple domain experts to categorize various types of terms to reach consensus, which is crucial for ontology building, because for an ontology to be a stable infrastructure for a domain, it must reflects the consensus of the domain. OTO provides a suite of features to support consensus making, including (1) accompanying terms with their source sentences to aid the user in discerning the meaning of a term, (2) visual highlighting of conflicting user decisions, (3) supporting user comments on terms and categorization decisions, (4) referencing definitions in other dictionaries/ontologies (e.g., PATO), (5) recording full user categorization history, and (6) basing the final decisions on input from all users. With categorization decisions being finalized on the terms, the ETC Ontology Building tool can be used by biologists to build a new ontology using the terms, or to add the terms to an existing ontology. A set of design patterns for constructing phenotype ontologies are built into the Ontology Building tool. When users invoke a pattern, a set of assertions will be added to the ontology automatically by software. In this talk we will present the bottom-up phenotype ontology building pipeline and the ontologies built using the pipeline. Through this talk, we hope to generate discussions on ways to improve tools for biologists/biology students to use to create ontologies as a computation infrastructure for systematics biology.

T5-06-02

Merging data across continents and scales with the plant phenology ontology

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Plant phenology — the timing of plant life-cycle events, such as flowering or leafing-out — has cascading effects on multiple levels of biological organization, from individuals to ecosystems, and is crucial for understanding the links between climate and biological communities. Plant phenology data are collected and used by many different types of researchers, from plant breeders to ecosystem ecologists. Today, thanks to data digitization and aggregation initiatives, phenology monitoring networks, and the efforts of citizen scientists, more phenologically relevant data is available than ever before. Unfortunately, combining these data in large-scale analyses remains prohibitively difficult, mostly because the individuals and organizations producing the data are using non-standardized terminologies and metrics during data collection and processing. Lack of standardization remains particularly problematic for historical datasets, which are crucial for time-based analyses. Furthermore, phenology data are often recorded as a type of trait, such as whether or not a plant has young leaves or flowers present, making it difficult to associate the data to development stage terms in ontologies like the Plant Ontology (PO). The Plant Phenology Ontology (PPO) is a collaborative effort to help solve these problems by developing the standardized terminology, definitions, and term relationships that are needed for large-scale data integration. The PPO builds on the widely used PO and Phenotype and Trait Ontology (PATO) to promote broad reuse of phenological data. This talk will provide an overview of the PPO, including the history of how it came to be, design patterns used in the ontology, examples of annotating real phenological data, and plans for future development.

T5-06-03

Challenges presented by biodiversity ontologies in the context of building a botanical knowledge portal

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We will describe the role of ontologies in our botanical knowledge portal, and will discuss some of the ontology-related challenges that we have overcome, and some that we continue to struggle with. Our portal uses Semantic Mediawiki (SMW) to store and display structured data extracted from the Flora of North America (FNA), and to integrate this data with an open biodiversity knowledge graph. SMW provides users with a familiar interface for browsing, filtering, and querying semantic data without the need to learn (or even be aware of) semantic web technologies such as RDF (the Resource Description Framework) and SPARQL (the SPARQL Protocol and RDF Query Language). In the case of the portal, this allows users to query taxonomic treatments based on: the characters and character states of taxa, including phenology and habitat; invasiveness and conservation status; occurrences; and any other information contained in the knowledge graph. Broadly, the ontology-related issues we have faced deal with representing, integrating, and reasoning over data about phenotypes, habitats, phenology, and establishment means (native vs. introduced). They fall into a number of categories, including: inter-curator variation in ontology construction; inter-curator variation in the use of ontologies to represent data; idiosyncrasies of applications that consume RDF; and, most broadly, heterogeneous (and sometimes incompatible) approaches to building the semantic web that have been pursued by different communities whose data products we want to integrate. We will describe these in the context of the portal, and will also describe the structure of our biodiversity knowledge graph, demonstrate how human and software agents (such as the portal) can interact with it, and invite collaboration in its continued construction.

T5-06-04

Towards consolidating the plant trait data domain

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Plant traits-the morphological, anatomical, physiological, biochemical and phenological characteristics of plants and their organs-determine how primary producers respond to environmental factors, affect other trophic levels, influence ecosystem processes and services and provide a link from species richness to ecosystem functional diversity. Trait data thus represent the raw material for a wide range of research from evolutionary biology, community and functional ecology to biogeography and vegetation modeling. However, so far the value of plant traits is obscured by two roadblocks: ecological research produces a tremendous amount of trait data, but the diversity in topics covered and the ways in which studies are carried out result in large numbers of small, idiosyncratic data sets using heterogeneous terminologies. Such heterogeneity can be attributed, in part, to a lack of standards for acquiring, organizing and describing data. We here present recent progress in consolidating the plant trait data domain by standardized terminology (TOP thesaurus of plant characteristics: top-thesaurus.org) and data integration (TRY database: try-db.org). In combination with other activities these two initiatives may provide a relevant contribution towards ontologizing the plant trait data domain and providing consolidated trait data.

T5-06-05

FLOPO: A tool for integrating morphological traits and phenotypes of vascular plants, and bridging the link to genetics

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The systematic analysis of a large number of comparable plant trait data can support investigations into phylogenetics and ecological adaptation, with broad applications in evolutionary biology, agriculture, conservation, and the functioning of ecosystems. Floras, i.e., books collecting the information on all known plant species found within a region, are a potentially rich source of such plant trait data. Floras describe plant traits with a focus on morphology as well as other traits relevant for species identification in addition to other characteristics of plant species, such as ecological affinities, distribution, economic value, health applications, or traditional uses. However, a key limitation in systematically analyzing information in Floras is presented by the lack of a standardized vocabulary for the described traits as well as the difficulties in extracting structured information from free text. A similar problem arises with molecular biology datasets where traits and phenotypes are recorded for a variety of studies including mutant plant model organisms experiment, or plant case-control experiments. To address the data integration problem, the molecular biology community developed a number of ontologies, i.e., controlled, formal vocabularies that capture the basic concepts within a domain and their interrelations. These ontologies are used for data annotation and integration across databases. Combining the information about plant traits and phenotypes resulting from molecular biology studies with the information found in biodiversity texts, such as Floras, would render a large volume of trait data amenable to integration and computational comparative phenomics analyses that can provide insights into the genetics of wildtype plants. Currently such an endeavour is hindered by the lack of a similar ontology for plant traits as well as a method to combine it with the ontologies used in molecular biology. We have developed the Flora Phenotype Ontology (FLOPO), an ontology for describing traits of plant species found in Floras. Similarly to the majority of phenotype ontologies in molecular biology, we used the Plant Ontology (PO) and the Phenotype And Trait Ontology (PATO) to extract entity-quality relationships from digitized taxon descriptions in Floras. We have used a formal ontological approach based on phenotype description patterns and automated reasoning to generate the FLOPO. The FLOPO is primarily intended as a framework based on which plant traits can be integrated computationally across all species and higher taxa of flowering plants. Importantly, it is not intended to replace established vocabularies or ontologies, but rather serve as an overarching framework based on which different application- and domain-specific ontologies, thesauri and vocabularies of phenotypes observed in flowering plants can be integrated. We will outline the current uses of FLOPO in data integration and annotation, and will also showcase our preliminary work on using FLOPO to integrate phenotypes observed in a biodiversity and ecology context with mutant model organism phenotypes. In particular, the combination of genetically well studied crop plants with the ecological and trait diversity of crop wild relatives would be a possible field of application.

T5-06-06

Reasons and effects of the duality of term “woody”

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The term "woody" have being used in many botanical disciplines. But special reviews show that this term has a duality of usage (Gatzuk, 1976; Dulin, Kirghoff, 2010; Schweingruber, Büntgen, 2013). 1. Woody plants from ecologo-morphological point of view. This notion has long history, but the main modern criterion of classification is the duration of life of aboveground shoot, positing the plants of the seasonal climates can be determined as woody, semi-woody and herbaceous. 2. From anatomical positions, under woody commonly understand plants with the secondary xylem. At the first look, it correlates with previous proposition (soft herbs without secondary xylem and hard trees and shrubs with secondary xylem). However, detail analyses shows that many herbaceous plants have cambium and secondary xylem. More other the whole ring of the xylem - a common trait of annuals. Under those two topics we will discuss: How the duality of term "woody" inhibits the theoretical generalizations in plant anatomy. How the wood of herbs looks like. What is the best way to use the term "woody".

T5-06-07

IPNI – future vision for providing Plant & Fungal names in a digital world

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For more than a century IPNI has been globally recognized as the primary source for plant name data. The proliferation of plant databases and the advent of electronic publication have radically changed the environment in which IPNI operates. As a response to these technological changes the IPNI partnership (Royal Botanic Gardens Kew, Harvard University Herbaria & Libraries and the Australian National Botanic Gardens), sets out its vision for the future of IPNI ensuring that we continue to be a globally recognized authority on names. IPNI is already widely used as a source of unique identifiers (LSIDs) and names data. In the future we will facilitate automatic updates across platforms for anyone who has linked their data through these identifiers. We will be improving the download and search options over broader ranges of fields with more choices for viewing and sorting results. IPNI will provide a user friendly registration service to speed up the rate at which published and unpublished names are entered. We will also create the possibility within registration to allow for micro-publication of nomenclatural novelties. We aim to improve the completeness of IPNI by integrating data which at the moment reside in separate databases, many of which are at Kew; in particular fungal names and infraspecific plant names. IPNI will then be in a prime position to provide a names backbone, initially for Kew's Plants of the World Online and subsequently for external services. The IPNI partnership want to improve its relationship with users by creating an interactive feedback system and disseminating knowledge on nomenclature by providing online tutorials. We will work more closely with publishers to automate the entry of names from online publications. In formulating this vision of IPNI's future we will be consulting our users as well as the broader community to create a user friendly system fit for the challenges of the digital world.

T5-06-08

The Planteome project: Common reference and application ontologies for plant biology

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The Planteome project (www.planteome.org) features a suite of reference ontologies for plants, associated with a growing corpus of genomics data in a centralized online plant informatics portal. The species-neutral references, the Plant Ontology, Plant Trait Ontology, and Plant Environment Ontology, (developed by the project), along with those developed by collaborating groups, such as the Gene Ontology and the Phenotype and Trait Ontology, and others, are mapped to species-specific controlled vocabularies for crop plant traits and phenotypes. Traditional plant breeding methods for crop improvement may be combined with next-generation analysis methods and automated scoring of traits and phenotypes to develop improved varieties. Linking these analyses to the growing corpus of genomics data generated by phylogenomics, taxonomy, high-throughput sequencing, transcriptomics, proteomics, phenomics and genome annotation projects requires common, interoperable, reference vocabularies (ontologies) for the description of the data. Data annotations to ontology terms link phenotypes and accessions/germplasm to genomics resources, and provide semantic integration of widely diverse datasets with the goal of plant improvement. Analysis and annotation tools are being developed to facilitate studies of plant traits, phenotypes, diseases, gene function and expression and genetic diversity data across a wide range of plant species. The project database and the online resources provide researchers tools to search and browse and access remotely via APIs for semantic integration in annotation tools and data repositories, providing resources for plant biology, breeding, genomics and genetics.

The project is supported by the National Science Foundation award IOS #1340112

T5-06-09

First steps to build an ontology for flagellate plants using natural language processing tools

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Novel Natural Language Processing (NLP) software has been designed to parse text written in the abbreviated language (i.e., telegraphic syntax) prevalent in taxonomic descriptions. The software greatly facilitates the extraction of phenotypic traits from taxonomic descriptions. Our aim is to create a comprehensive phenomic dataset for the flagellate plants (i.e., bryophytes, lycophytes, ferns, and gymnosperms) using the NLP technology. We are interested in building a formal ontology for flagellate plants, as incorporating a formal ontology to the parsing analyses will increase the number of usable characters in the matrices we build and the precision of the data extracted. We are using the Explorer of Taxon Concepts (ETC) pipeline (http://*etc*.cs.umb.edu/etcsite/start.htm) to parse taxonomic descriptions of gymnosperms and fern taxa. The ETC "Text Capture" tool enables users to load the taxonomic descriptions into the system. The ETC software then transforms the descriptions into XML format, segments the sentences, and identifies terms that describe structures or descriptors. The users must place unrecognized terms into discrete predefined categories, which allows the system to establish "is_a" relationships (e.g., blue is a color, leaf is a structure). The result of this step is downloadable as a .csv file. Once this task is completed, the system parses the text, and generates output files that have detailed-semantically annotated text. The output is then input into the ETC "Matrix Generation" tool to assemble a taxon by character matrix. Using the "Term Review" option of ETC's "Text Capture" tool, we have gathered terms that are not represented in Ontologies. We present the data gathered and discuss how we can integrate our work with other ontology building initiatives.

T5-06-11

An evolutionary continuity principle for evolutionary system of organism divisions

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Evolutionary research is guided by the scientific basis of organism evolution. To overcome the limitations of being partial and subjective in the tree of life or phylogenetic system, the authors proposed a basic principle of organism evolution, evolutionary continuity principle. Based on this principle, the evolutions can be divided into two basic forms: vertical evolution and horizontal evolution. The vertical evolution is that evolution of the structures and features of organisms from non-existent to entirety, from simple to complex, or from primitive to advanced. The evolutionary course of vertical evolution can be divided into two or three significantly different phases, such as plants: the non-vascular → the vascular, non-seed → Gymnospermophyta D. L. Fu & H. Fu, phyl. nov. → Fructophyta D. L. Fu & H. Fu, phyl. nov., and the animals: Proenteratozoa D. L. Fu & H. Fu, phyl. nov. → Coelenteratozoa → Euenterata, non-chordata and non-gnatha → Chordato-gnathozoa D. L. Fu & H. Fu, phyl. nov. → Vertebrognathozoa D. L. Fu & H. Fu, phyl. nov. The horizontal evolution is that evolution of structures and features from loose to compact, from inefficient to efficient, or from primitive to advanced, which can also cause significant organism changes, such as Nudembryophyta D. L. Fu (Plantae) → Proenteratozoa D. L. Fu & H. Fu, phyl. nov. (Animalia), Bacterophyla D. L. Fu & H. Fu, phyl. nov. → Acytophyla D. L. Fu. Based on the continuity principle, the new evolutionary taxonomical system of divisions was established and the evolutionary diagram was drawn. The new system includes 20 divisions in three kingdoms, and 11 of those divisions are new. These are: I. Regnum Microbia D. L. Fu: 1.Cyanoalgophyla D. L. Fu & H. Fu, phyl. nov., 2. Bacterophyla D. L. Fu & H. Fu, phyl. nov., 3. Acytophyla D. L. Fu, 4. Monoalgophyla D. L. Fu & H. Fu, phyl. nov., 5. Monomycophyla D. L. Fu & H. Fu, phyl. nov., 6. Eualgophyla D. L. Fu & H. Fu, phyl. nov., 7. Fungophyla D. L. Fu & H. Fu, phyl. nov.; II. Regnum Plantae Haeck.: 8. Nudembryophyta D. L. Fu, 9. Bryophyta, 10. Pteridophyta, 11. Gymnospermophyta D. L. Fu & H. Fu, phyl. nov., 12. Fructophyta D. L. Fu & H. Fu, phyl. nov.; III. Regnum Animalia L.: 13. Proenteratozoa D. L. Fu & H. Fu, phyl. nov., 14. Coelenteratozoa, 15. Nematodozoa, 16. Annelidozoa, 17. Arthropodozoa, 18. Echinodermatozoa, 19. Chordato-gnathozoa D. L. Fu & H. Fu, phyl. nov., 20. Vertebrognathozoa. D. L. Fu & H. Fu, phyl. nov. The new evolutionary theory, new evolutionary taxa and new evolutionary system can provide scientific bases for Evolutionomy, a new science of organism evolution.

T5-07: Extremophyte genomics

T5-07-01

Return to the sea: A rare evolutionary event for angiosperms

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The genome of *Zostera marina,* the first marine angiosperm to be fully sequenced (*Nature* 18 Feb 16) reveals unique insights into the genomic losses and gains involved in achieving the structural and physiological adaptations required for its fully marine lifestyle, arguably the most extreme habitat shift ever accomplished by flowering plants. Seagrasses returned to the sea some 75-100 Mya. *Zostera* underwent whole genome duplication (WGD) near the Cretaceous-Tertiary boundary thus opening new ecological opportunities in moving from freshwater to fully marine conditions. In returning to the sea, seagrasses found a vast new habitat free of terrestrial competitors, insect pests and drought stress; but they also had to adapt to high salinity, develop flexible leaves able to take up nutrients, and perform photosynthesis in the spectrally reduced light of the submarine environment. Here we present a number of highlights including modified cell walls that are more algal-like than plant-like, loss of all stomata and all of the genes, reduced plant defense genes and volatiles, and modifications for underwater pollination involving exineless pollen. The genome resource will advance a wide range of functional ecological studies from adaptation of marine ecosystems under climate warming, to unraveling the mechanisms of osmoregulation under high salinities that may further inform our understanding of the evolution of salt tolerance.

T5-07-02

Adaptations towards low phosphate soils: Insights from the extremophyte *Eutrema salsugineum* (aka *Thellungiella salsuginea*)

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Model plants, including *Arabidopsis*, have provided an abundance of information about how plants assimilate and manage phosphate, particularly under phosphate deprivation. However, plants adapted to extreme environmental conditions will likely offer novel insights into mechanisms underlying nutrient assimilation given the challenges to which they have adapted. We applied physiological, lipidomic, and transcriptomic approaches to the study of *Eutrema salsugineum* plants exposed to phosphate-sufficient and phosphate-deficient growing conditions. *Eutrema* is a close relative of *Arabidopsis* but by comparison, *Eutrema* is highly tolerant to extremes in soil salinity, temperature, and water deficits. The Yukon *Eutrema* ecotype is also adapted to highly alkaline soils where phosphate content is typically low and its availability likely further compromised by an unfavorable soil cation composition. We found that Yukon *Eutrema* plants display a remarkable capacity to tolerate low phosphate conditions in that plants showed the same biomass and relative growth rates whether treated with 0 or 2.5 mM Pi. Factors including the capacity to acidify the rhizosphere, exude organic anions, and remodel metabolism, including through altered membrane lipid composition, were examined and found to diverge from mechanisms reported for *Arabidopsis* in showing fewer phosphate starvation-inducible traits. Interestingly, comparisons between the phenotypic features and transcriptomes of phosphate-deprived and sufficient plants suggests that *Eutrema* has a comparatively fixed, and inherently strong constitutive capacity to tolerate low phosphate and that this species likely only invokes “fine-tuning” to its baseline defenses in order to calibrate its response to an actual low phosphate stress. The baseline suite of traits and comparatively modest adjustments under phosphate stress could inform our selection of nutrient efficiency traits for crops as we move towards a challenging future shaped by global climate change. The traits displayed by *Eutrema* have already withstood testing under the challenging conditions of its native habitat and have likely been indispensable for the acclimation and survival of this species on low nutrient soils.

T5-07-03

Life on earth's poorest soils: Applying genomics and transcriptomics to unravel P and N nutrition in *Hakea prostrata* (Proteaceae)

Patrick Finnegan, *Hans Lambers, Albina Ilyasova, Ricarda Jost, Thirumurugen Kuppusamy, M. Asaduzzaman Prodhan, Kosala Ranathunge*
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The flora of south-western Australia has evolved on the world's most P-impoverished soils. Proteaceae feature prominently, particularly on the most severely P-impoverished soils. We are using genomic and transcriptomic approaches to explore the molecular basis of adaptations in Proteaceae that allow them to thrive in low-nutrient environments, using *Hakea prostrata* as a model species. Proteaceae typically have very high P-acquisition efficiencies. They are able to mine P and other nutrients from soil through cluster roots. These dense clusters of short determinant lateral roots exude high quantities of carboxylates, as well as enzymes, to mobilise nutrients, particularly phosphate, from soil particles in the root zone. The need for the Proteaceae to focus on acquiring phosphate from P-poor soils may have led to a nutrient-acquisition selectivity that is unique among plants. That is, *H. prostrata* appears to be unable to down-regulate its P acquisition as effectively as other plants and easily becomes intoxicated. On the other hand, it seems to very tightly regulate the acquisition of nitrate and sulfate, which is again in stark contrast to most plants. Furthermore, increasing internal P in an artificial hydroponics experiment does not translate into an increased demand for nitrate or sulfate. This lack of response to internal P suggests that evolution has given *H. prostrata* the ability to maintain a low and set requirement for N and S that aligns with the extremely low level of P available in its habitat. Once P, N and S are within the plant, they are used with exquisitely high efficiency. Leaf development in *H. prostrata* displays delayed greening, where young leaves are brownish red or red, instead of green. During delayed greening, P and N investment in ribosomal RNA and protein is spread out over time. Early in leaf development, cytosolic ribosomal RNA predominates, and ribosomes are primarily dedicated to producing proteins for building leaf structure. After full expansion, the leaf greens, and the ribosomal RNA and protein pools are reconfigured to produce an active photosynthetic apparatus. During leaf maturation in Proteaceae, the lipid pool also changes dramatically, decreasing the use of phospholipids and increasing that of sulfolipids and galactolipids. Mature leaves, then, have a remarkably low P and N concentration, while photosynthetic rates are similar to those in plants with much higher leaf nutrient concentrations. This situation translates into very high photosynthetic P-use and N-use efficiencies. Finally, at the end of their organ life, P is highly efficiently and proficiently recycled during senescence. At this time, we have very little knowledge about the molecular mechanisms underlying these remarkable adaptations to a severely nutrient-impoverished environment. We have found that *H. prostrata* has fewer, and perhaps even lacks, genes whose expression responds to variations in nutrient availability in other species. Instead, constitutive orthologues predominate. From these considerations, it becomes apparent that the Proteaceae are ideal models for learning how plants cannot only cope with, but thrive in, nutrient-poor environments.

T5-07-04

Can *Schrenkiella parvula*, an extremophyte relative of *Arabidopsis*, help determine a genomic blueprint in adapting to boron toxicity?

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Boron toxicity is a worldwide problem for crop production and significantly limits crop yield especially in Australia, Africa, and Asia. The genetic mechanism behind boron toxicity, as well as genomic signatures for adapting to high borate soils, are largely unknown. *Schrenkiella parvula* (syn. *Thellungiella parvula)* is an extremophyte, naturally adapted to the high borate soils in the shores of Lake Tuz, Turkey. *S. parvula* can survive high borate at levels toxic to most plants including *A. thaliana*. However, the *S. parvula* genome shares remarkable macro-synteny with the closely related genome of *A. thaliana*. Both species also share similar lifecycles. Therefore, *S. parvula* provides an ideal system to study the genomic blueprint for adapting to high borate levels in the soil. To identify the potential key genetic contributors for boron tolerance, we have compared RNA-Seq profiles of borate-treated and control samples of the shoot and root tissues from *S. parvula* and *A. thaliana*. Our goal was to explore the transcriptomic adaptations underlying boron tolerance with the integrated insight from genome comparisons. We observed a significantly reduced level of global expression changes in *S. parvula* to borate treatments compared to the response observed for *A. thaliana*. Clusters of differently expressed genes (DEGs) in *S. parvula* illustrated the stress readiness of the extremophyte transcriptomes compared to *A. thaliana* in a tissue-specific manner. One of the most highly expressed genes at the basal level expression in *S. parvula* was annotated as a putative boron transporter (*SpBOR5*) due to its sequence similarity to the Arabidopsis *AtBOR5*. *AtBOR5* is an uncharacterized gene in the family of putative borate transporters in *A. thaliana*. We found that *SpBOR5* could functionally complement the native yeast BOR1 transporter when expressed in a yeast *bor1* mutant background and SpBOR5 indicated strong boron transporter activity compared to closely related homologs in *A. thaliana* and *S. parvula*. The basal level expression of *SpBOR5* was > 500-fold higher than that of the ortholog, *AtBOR5*, and was even more up-regulated upon borate treatment in *S. parvula*. Interestingly, one of the key genomic structural variations identified from the genome comparisons indicated a 15 kb genomic insertion upstream of the *SpBOR5* gene that is absent in other closely related genomes. This may contribute to the significantly high expression of *SpBOR5*. We have developed loss of function mutants of *SpBOR5* via the CRISPR-Cas9 genome editing system to test whether *SpBOR5* plays a significant role in the borate tolerance exemplified by *S. parvula*. We plan to present the underlying genetic mechanisms of boron tolerance inferred from transcriptionally differently regulated pathways and the functional significance of putative borate transporters using targeted transgenic lines in *S.*

parvula.

T5-07-05

Transcriptome analysis of the *Arabidopsis* Negev desert relative, *Anastatica hierochuntica* (True Rose of Jericho), reveals stress tolerance and developmental determinants that might facilitate an opportunistic extremophyte lifestyle

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The search for novel stress tolerance determinants has led to increasing interest in plants native to extreme environments – so called “extremophytes.” One successful strategy has been comparative studies between *Arabidopsis thaliana* and its extremophyte Brassicaceae relatives. Here, we present the Negev desert species, *Anastatica hierochuntica* (True Rose of Jericho), a member of the poorly investigated lineage III Brassicaceae. We show that *A. hierochuntica* has a genome approximately 4.5-fold larger than *Arabidopsis*, divided into 22 diploid chromosomes, and demonstrate that *A. hierochuntica* exhibits tolerance to heat, low nitrogen and salt stresses that are characteristic of its habitat. To investigate molecular mechanisms of *A. hierochuntica* stress tolerance, we generated a high quality *A. hierochuntica* reference transcriptome, and performed a phylogenomic analysis, based on 13,927 orthologous sequences of 17 Brassicaceae plant species including various crop plants, extremophytes and *A. thaliana*. We identified positively selected genes that may have been crucial for adaptation to abiotic stresses, and uncovered evidence suggesting that selection pressures leading to adaptation and speciation focus on common biological processes although the identity of the positively selected genes is specific to each species. We also identified positively selected genes that are specific to *A. hierochuntica*, and may underlie some of the morphological and life history characteristics that allow an opportunistic existence in the harsh desert environment. Lastly, we compared *A. hierochuntica* and *A. thaliana* responses to single and combined, low nitrogen and heat stresses using parallel metabolomic and transcriptomic analyses. Preliminary transcriptome analysis of 16,266 orthologous gene pairs between *A. thaliana* and *A. hierochuntica* revealed species-specific transcriptomes responses, which will be further discussed.

T5-07-06

Local adaptations of neotropical mangrove species discovered through comparative ecophysiology, transcriptomics and genomic scan for selection

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Mangrove trees inhabit intertidal zones spanning wide ranges of latitude, climate, salinity, nutrients and fresh water availability. The varying environmental conditions to which species are exposed confer different selective pressures, favoring the emergence of locally adapted populations. Recent population genetics studies revealed strong genetic structure in mangroves from the Atlantic coast of South America. However, these investigations used only neutral genetic markers. The variability of functional traits in these populations had never been studied, despite their importance in determining plant survival in a rapidly changing world. Here, we used public data from weather stations of the Brazilian National Meteorological Institute, *in situ* RNA sequencing data (RNA-Seq), and a common-garden experiment for the comparison of environmental pressures and variations in adaptive characters between contrasting natural populations of Neotropical species of the genus *Avicennia*. Additionally, we have combined RNA-Seq and Genotyping by Sequencing data (Next-Seq) for searching for transcribed regions with signatures of selection - i.e. that showed significantly higher or lower genetic differentiation between populations than it would be expected under neutrality. We observed substantial differences in anatomical and physiological traits related to water and carbon balances between individuals from two genetically different populations grown in common-garden. Also, we have found differential expression of transcripts involved in the response to functionally relevant environmental variables contrasting between populations. Genetic markers showing evidence of selection were mapped to putative genes related to the distinct abiotic conditions observed in both sampling sites. Our multi-disciplinary approach revealed an existing overlap between environmental variation and adaptive genetic diversity in the studied species, shedding light on the species’ potential to bear novel climate conditions.

T5-08: Chromosome evolution and introgression: past, present and future

T5-08-01

The nature, evolution and large-scale organization of repetitive DNA on the chromosomes of *Chrysanthemum*

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Tandemly repeated DNA and transposable elements make up the majority of most higher plant genomes, and can be identified in clones of sequence assemblies by their structure and similarities to known repetitive DNA motifs. High-throughput sequencing allows survey of all the DNA in a genome, but whole-genome assembly leaves a substantial fraction of highly repeated sequences which cannot be assembled. *Chrysanthemum nankingense* (2n=2x=18; genome size c. 2,500 Mbp) in the Asteraceae is an interesting species with value for breeding and a high-value food and considered as an ancestral species in the genus. We aimed to characterize in detail the major repetitive DNA motifs in *Chrysanthemum* unprocessed raw reads from sequencing using bioinformatics and fluorescent *in situ* hybridization. A combination of graph-based clustering using the program RepeatExplorer and frequency analysis of DNA motifs k-bases long (k-mer analysis where k ranged from 16 to 128 bases) was used to identify repetitive motifs in 12.3 Gb of 250 bp paired-end Illumina reads. Apart from rDNA, a few microsatellites, and telomeric sequences, no highly abundant tandem repeats were identified from the raw reads. There were many transposable element sequences: 40% of the genome had recognizable domains related to TEs. The retroelements showed widespread distribution over chromosomes, while each family had characteristic features such as abundance or exclusion from centromeric or sub-telomeric regions. Another group of very abundant repetitive sequence motifs, including the most abundant families, showed no similarity to known sequence motifs or tandemly repeated elements. Preliminary analysis is suggesting most are related to degenerate and presumably ancient LTRs (Long Terminal Repeats) of retroelements. Better approaches to characterization of repetitive DNA in all species is critical to generate complete genome sequence assemblies. Identifying the presence, nature and genomic organization of major genome fractions, enables inference of evolutionary history, including degeneration and loss, critical to understanding biodiversity and diversification processes in plants.

T5-08-02

Revealing *Brachypodium* chromosome structure and evolution as well as stability after mutagenic treatment using multicolour FISH and BAC-FISH-based comparative chromosome barcoding

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Modern molecular cytogenetics amalgamates various methodological approaches of cytology, molecular genetics and advanced digital image acquisition, processing and analysis. It focuses on the study of nuclear genomes at the microscopic level. Many plants, including economically and ecologically important cereals and forage grasses, usually have large genomes saturated with repetitive DNA, which hampers detailed molecular cytogenetic analyses. Therefore, the cytomolecular organisation of the vast majority of plant genomes is still rather poorly understood, compared to that of many animals, mammalian genomes in particular. Model organisms possess a set of features, which makes them more amenable to scientific investigation than others. One of relatively recent and rapidly developing model systems contains representatives of the *Brachypodium* genus, particularly *B. distachyon*. This genus comprises 15-20 species with small, and in some cases, sequenced and resequenced genomes with a low repeat content, different basic chromosome numbers, size, morphology and ploidy levels. These species also have interesting phylogeny, short life cycles and simple growth requirements, and are subject to a rapidly and continuously growing repertoire of experimental tools. Here we focus on chromosome rearrangements that have shaped the structure of *Brachypodium* karyotypes, using comparative chromosome barcoding by BAC-FISH (fluorescence *in situ* hybridisation with chromosome-specific bacterial artificial chromosomes as probes). The karyotypes of selected *Brachypodium* species were compared to the model grass *B. distachyon*. Single-locus BAC clones derived from *B. distachyon* genomic libraries were selected from the assemblies of FingerPrinted Contigs that had previously been assigned to the chromosomes of *B. distachyon*. This comparative chromosome barcoding approach can be used to study the organisation of karyotypes and reconstruct mechanisms of the chromosome rearrangements that have shaped the genome structure of extant grasses. Due to its unique cytogenetic properties and well-developed research infrastructure, *B. distachyon* is also a convenient system for analysing ‘hot spots’ (and ’cold spots’) of DNA damage in nuclear genomes, and consequently could be used for environmental monitoring. Chromosome rearrangements are commonly identified using classical cytogenetic techniques. However, combining whole genome sequence information and BAC-FISH-based mapping technology enables comprehensive analysis of mutagenic effects at the chromosomal level and extends our understanding of the mechanisms responsible for chromosomal aberrations. We present the visualisation of mutagen-induced genome changes, including micronuclei formation and alterations of chromosome territories in interphase nuclei using fluorescence *in situ* hybridisation (FISH) with selected chromosome-specific BAC clones, as well as ribosomal DNA and chromosome region-specific (centromeric and telomeric) probes. Financial support from the National Science Centre Poland (grants no. 2012/04/A/NZ3/00572,2014/14/M/NZ2/00519 and 2015/18/M/NZ2/00394) is acknowledged.

T5-08-03

Extraction of the constituent subgenomes of the natural allopolyploid rapeseed (*Brassica napus* L.)

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As the dynamic nature of progenitor genomes accompanies the speciation by interspecific hybridization, the extraction of the constituent subgenome (s) from a natural allopolyploid species of long history and then restitution of the progenitor (s) provides the unique opportunity to study the genome evolution and interplay. Herein, the A subgenome from the allotetraploid oilseed rape (*Brassica napus* L., AACC) was extracted through inducing the preferential elimination of C-subgenome chromosomes in intertribal crosses and the progenitor *B. rapa* was restituted (RBR). Then by crossing and backcrossing RBR with *B. napus* donor, the C subgenome was in situ dissected by adding each of its nine chromosomes to the extracted A subgenome and establishing the whole set of monosonic alien addition lines (MAALs, C1~C9). RBR from spring-type *B. napus* genotype “Oro” expressed a phenotype resembling some type of *B. rapa* never observed before, but showed a winter-type flowering habit. This RBR had weaker growth vigor and suffered more seriously from biotic and abiotic stresses compared with Oro. This RBR had weaker growth vigor and suffered more seriously from biotic and abiotic stresses compared with Oro. The phenotypes specific for these MAALs showed the location of the related genes on the particular C-subgenome chromosomes. These MAALs exhibited obviously different frequencies in homeologous pairing and transmission of additional C-subgenome chromosomes, which were associated with the distinct degrees of their relatedness, and even with the possible genetic regulation for meiotic pairing evolved in *B. napus*. Using MAALs, large scaffolds undetermined for sequence assembly of *B. napus* were anchored to specific C-subgenome chromosomes. In different addition lines, varied portions (1.54%-6.52%) of A genome genes were changed significantly in comparison with that of RBR and more genes were down-regulated in all addition lines except in C6. Those genes which are expressed at high and moderate level in RBR were biased to be down-regulated (except in C7) while low expressed genes were substantially up-regulated in MAALs.

T5-08-04

Polyploidy-driven diversification and diploidization: The origin of the Australian and New Zealand crucifers

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We showed previously that three endemic Australian crucifer species (tribe Microlepidieae, Brassicaceae) have undergone a whole-genome duplication (AUS-WGD) followed by diversification and species-specific diploidization, generating some of the lowest chromosome numbers (n = 4 - 7) known for the crucifers (Mandáková *et al.,* 2010, *Plant Cell*). The New Zealand genus *Pachycladon* has undergone either the AUS-WGD or an independent WGD followed by less extensive genome reshuffling towards n = 10 genomes (Mandáková *et al.,* 2010, *BMC Evol Biol*). To further elucidate the origin and fate of Australian/New Zealand mesopolyploid genomes, we analyzed 12 species (9 genera) with variable chromosome numbers (n = 4, 5, 6, and 7) by comparative chromosome painting and gene sequencing. We concluded that all members of the Microlepidieae tribe originated through the AUS-WGD. This allopolyploid WGD most likely spurred the diversification of the group on the Australasia. Cytogenetic signatures and multi-gene (*CHS*, *PHYA*, *ndhF)* phylogenies suggest that the ancestral allopolyploid genome had most likely 30 chromosomes (n = 15) and was formed through an inter-tribal hybridization (tribe Crucihimalayeae and Smelowskieae/Descurainieae), dated to c. 10.6 million years ago, followed by a long-distance dispersal from Asia to Australia. The AUS-WGD was followed by independent, multi-speed and massive genome re-diploidizations towards the extant diversity of quasi-diploid genomes (n = 4 - 10) of the Microlepidieae taxa.

T5-08-05

Retrotransposon heterogeneity and their evolution in higher plants

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Repetitive DNA comprises large quantities in all plant genomes studied, with proposed roles in genome structural integrity, as evolutionary motor and transcriptional regulator. Although advances of 'omics' datasets enable unique views on the plant repeat landscape, their assembly and analysis remains challenging. In sugar beet (*Beta vulgaris*), only 567 Mb of its 758 Mb genome are integrated into the reference genome sequence, neglecting a quarter of the genome-retrotransposons and satellite DNAs. Using a combination of next-generation sequencing, comparative genomics and cytogenetics, we identified and characterized the most abundant repeats within the sugar beet genome, generated a FISH karyotype, and derived principles applying to higher plants. In order to survey the sugar beet retrotransposon landscape, we extracted their mobility key enzyme reverse transcriptase (RT). Out of nearly intact 6,000 RTs, 4,476 are typical for long terminal repeat (LTR) retrotransposons of the Ty1-*copia* and Ty3-*gypsy*-type belonging to eight lineages. Prominent clades harboring additional putative envelope ORFs and chromodomains, were investigated in greater detail across the genus *Beta*. We highlight LTR retrotransposon evolution by amplificational bursts, reshuffling, recombination and targeted integration. Additional 1,471 intact long interspersed nuclear element (LINE) RTs form 17 L1 families and one RTE family, differing in sequence, amplification timepoints, heterogeneity and type of structural domains. An increasing species spectrum enables a view into plant LINE evolution: The comparison of 59,390 intact LINE RTs from 23 higher plant genomes indicates a limitation to L1- and RTE-clade-LINEs in higher plants. Whereas RTE LINEs show high homogeneity, even across taxonomical borders, L1 LINEs are highly dissimilar. L1 LINEs are subdivided into seven subclades, reflecting sequence similarity and structural aspects of full-length LINEs. We define these subclades, explain their modular organization and show that each of the plants investigated harbors LINEs of at least two subclades, whereas each subclade occurs in at least five species. In the genus *Beta*, retrotransposons without coding capacity, such as short interspersed nuclear elements (SINEs), are present in high copy numbers (> 34,000) and form 22 families. SINEs often associate with genes, contribute start and stop codons, splice sites, or exon variants, making them strong candidates for gene regulators.

T5-08-06

Phylogenetic models of chromosome number evolution and chromosomal speciation

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Chromosomal changes play a fundamental role in evolution. Dysploid gains and losses in chromosome number, as well as polyploidization events, may drive reproductive isolation and lineage diversification. The recent development of probabilistic models of chromosome number evolution in the groundbreaking work by Mayrose *et al.,* (2010) have enabled the inference of ancestral chromosome numbers over molecular phylogenies and generated new interest in studying the role of chromosome changes in evolution. However, these models assume all changes occur anagenetically (along branches), and do not model events that are specifically cladogenetic and may be expected if chromosomal changes result in reproductive isolation. Here I present a new class of models of chromosome number evolution that incorporate both anagenetic and cladogenetic change. These models allow one to determine the mode of chromosome number evolution; is chromosome evolution occurring primarily within lineages, primarily at lineage splitting, or in clade-specific combinations of both? Furthermore, one can estimate the location and timing of chromosomal speciation events over the phylogeny. I test the model's accuracy with simulations and re-examine chromosome evolution in Aristolochia, Carex section Ovales, Helianthus, Mimulus sensu lato, and Primula section Aleuritia.

T5-09: Plant organellar genomics and phylogenomics, driven by the NGS approaches (two sessions)

T5-09-01

Chloroplast phylogenomics of green algae

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The green algal phylum Chlorophyta has six diverse classes, but the phylogenetic relationship of the classes within Chlorophyta remains uncertain. In order to better understand the ancient Chlorophyta evolution, we analyzed a chloroplast genome data set including new chloroplast genomes from class Ulvophyceae (Chlorophyta), and applied a site pattern sorting method to study compositional heterogeneity and the model fit in the green algal chloroplast genomic data. We show that the fastest-evolving sites are significantly correlated with among-site compositional heterogeneity, and these sites have a much poor fit to the evolutionary model. Our phylogenomic analyses suggest that the class Chlorophyceae is a monophyletic group, but the classes Ulvophyceae, Trebouxiophyceae and Prasinophyceae are paraphyletic groups. Our proposed phylogenetic tree of Chlorophyta will offer new insights to investigate ancient green algae evolution, and our analytical framework will provide a useful approach for evaluating and mitigating the potential errors of phylogenomic inferences.

T5-09-02

Development of a reference standard library of chloroplast genome sequences, GenomeTrakrCP

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An increasing number of people have turned to herbal dietary supplements for preventing diseases, staying healthy, and as an alternative to medicines after getting sick. In 2015, the total sales of herbal dietary supplements in the United Stated reached $6.92 billion, a 7.5% increase from the year before, and demand for botanicals have increased for 12 consecutive years. Many different chemical techniques have been used to monitor authenticity of supplements and more recently DNA based tools have been included. While DNA barcoding has been found to be a powerful identification tool for many species, questions remain about its utility in plants, especially for processed products and closely related species. Therefore, developing methods targeting smaller diagnostic regions and reference libraries for rapid species identification of plants in foods and dietary supplements would be useful and complementary to chemical methods. In the past, the United States Food and Drug Administration (FDA) has been able to develop species specific assays targeting plant species of interest by utilizing chloroplast genome sequences. Presented here are the details for FDA's whole chloroplast genome sequencing effort and database, known as GenomeTrakrCP. Targeted species include plants found in foods and dietary supplements as well as plants known as toxin producers. Additionally, contaminants or adulterants and closely related species to these targeted species were sequenced. All data will be publically available through a bioproject in GenBank, e.g., PRJNA325670 derived from authenticated specimens and fully annotated. Currently there are 40 complete chloroplast genomes in the database from authenticated specimens. These data can be used by FDA and other government agencies, industry and any other researcher as complete chloroplast genomes or to design species specific assays to target plant species of interest.

T5-09-03

Mitochondrial phylogenomics of vascular plants: Evolutionary relationships and genomic diversity

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The mitochondrial genomes of angiosperms are remarkably variable in size, structure and content. Much less is known about the mitogenomic diversity in the other vascular plant lineages, for which few genomes are available. To gain insight into the extent of mitogenomic diversity across vascular plants and the evolutionary changes that have generated such diversity, we have assembled complete or draft mitogenomes for dozens of species of angiosperms, gymnosperms, ferns, and lycophytes. Taxa were chosen to span the breadth of taxonomic diversity in each major lineage, with a particular focus on species from underrepresented groups. We found that each major lineage exhibits a wide range of features, only some of which are shared across lineages. We also performed a comprehensive phylogenomic analysis of vascular plants using mitochondrial genes, and explored the effects of RNA editing on these results.

T5-09-04

Holoparasitism breaks the evolutionary stasis of mitochondrial genome evolution in the broomrape family (Orobanchaceae)

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The parasitic lifestyle in plants has brought about extreme genomic modifications, including dramatic functional and physical reductions to the plastid genome (plastome), accelerated rates of molecular evolution across apparently all genomic compartments, losses of essential mitochondrial genes for respiratory complex I, as well as massive horizontal gene transfers. While the reductive evolution of plastomes is well understood as a consequence of relaxed selective constraints on photosynthesis, we still have little insight into the reorganizations pertaining to mitochondrial genomes (mitogenomes) in parasitic lineages. To infer the evolutionary trajectories and underlying causes of mitogenome reconfiguration in parasites, we completely sequenced and assembled the mitogenomes of 25 species from 17 genera covering all trophic transitions in the broomrape family (Orobanchaceae). We find that mitogenome structure varies greatly in Orobanchaceae, where mitochondrial DNA ranges in size from 316 kb to 2.73 Mb and can be organized into one or more (mini-) circles, several linear fragments with multiple recombination points, or any combination of these. Mitogenome inflation coincides with the transition to a holoparasitic lifestyle, most notably in the broomrape (*Orobanche*) clade. Mitochondrial gene losses in Orobanchaceae are confined to several ribosomal proteins (e.g. *rpl2*, *rps10*, and *rps13*) and the *sdh3* gene, which encodes a cytochrome subunit for mitochondrial complex II. Multiple independent pseudogenizations and differing degrees of degradation of those genes indicate that their losses from the mitogenome likely are the result of ancient functional transfers to the nucleus. Correlating with the degree of plastome reduction, plastid-derived DNA is most abundant in the mitogenomes of photosynthetic parasites. The nature and conservation of plastid inserts suggest that they originated from many independent intracellular transfer events, with such transfers being either more recent or more frequent in hemiparasites. The expansion of mitogenomes is mostly due to an increase of lineage-specific non-coding DNA, whose origin has yet to be determined. However, several lines of evidence suggest that a fraction of these novel DNA segments have been obtained from host plants, implying that recent reports of the sporadic incorporation of foreign genes merely touch the tip of the iceberg. Molecular evolutionary analyses involving trait-rate fusion models show that lifestyle alone has only limited effect on rate elevation in mitochondrial genes of Orobanchaceae and that genomic traits also contribute significantly. Our comparative analysis of completely sequenced mitogenomes of Orobanchaceae species spanning the transition from autotrophy to holoparasitism contributes significantly to our understanding of the genetic reconfigurations in parasitic plants in general, and provides first evidence for a linkage of the structural and molecular evolution between organellar genomes as parasitism unfolds.

T5-09-05

Towards a meta-analysis of plastid genome evolution in heterotrophic flowering plants

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Plastid and mitochondrial genomes (plastomes and mitogenomes, respectively) are rewarding objects for molecular evolutionary studies and useful tools in plant phylogenetics and phylogenomics. Our understanding of the evolutionary trajectories of organellar genomes has benefitted considerably from the broad availability of NGS technologies with which any number of plastomes and mitogenomes can be generated from essentially any group of interest, including the most extreme ones like heterotrophic plants. Available now are the complete plastome sequences from representatives of at least 15 independent transitions to an obligate heterotrophic lifestyle, thus allowing to test (molecular) evolutionary hypotheses in an unprecedented wide manner within and across the various transitions in flowering plants. However, such a “meta-analysis” bears many potential pitfalls. For example, different sequencing technologies or bioinformatic pipelines may leave behind signature errors, taxonomic depths and coverage often differs within and between lineages, and comparing and categorizing trophic specializations across the various heterotrophs could introduce researcher biases. Here, I review our current knowledge of plastid genome evolution in heterotrophic plants and discuss open questions regarding the physical and functional degradation of plastomes under relaxed selective constraints. Using simulations with controlled errors relating to plastome annotations, phylogenetic effects, and trait data allows evaluating the accuracy and robustness of different phylo-statistical approaches. The analyses show that annotation errors are among the most problematic issues, which to correct requires either thorough (manual) re-annotation or solutions that avoid automated parsing of pre-annotated sequence flatfiles. Integrating complete plastome information rather than restricting analyses to (annotated) coding sequences further enables the identification of conserved non-coding sequence elements. Beyond setting the framework for a meta-analysis of plastome degeneration in heterotrophic plants, the results of this study may contribute to better assessing the potential functionality of genes in the absence of both expression data and documented guidelines how to categorize pseudogenes.

T5-09-06

Variable plastid genomic architecture within the *“Adenocalymma-Neojobertia”* clade (Bignonieae, Bignoniaceae) and its phylogenetic implications

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The chloroplast is one of the most important organelles of green plants, due to its crucial role in photosynthesis. This organelle ranges in size between 25 to 217 kb in flowering plants, and contains a circular DNA with approximately 130 genes. Plastid genomes typically include a quadripartite structure that consists of a small single copy region (SSC), a large single copy region (LSC), and a pair of inverted repeats (IRs). This plastid structure, its gene content and organization is usually highly conserved when compared to plant mitochondria. The use of plastid genomic data in phylogenetic and evolutionary studies became possible with high-throughput sequencing methods, which allowed us to rapidly obtain complete genomes at a reasonable cost. Here, we use high-throughput sequencing to study the “*Adenocalymma-Neojobertia*” clade (Bignonieae, Bignoniaceae), a large lineage (ca. 75 spp.) of Neotropical treelets, shrubs and lianas, that is sister to the “Core Bignonieae” clade. More specifically, we used HiSeq Illumina technology to sequence the plastomes of 46 species. Overall, 12 plastomes were fully sequenced and used in this initial study. For the plastome assembly, we used a pipeline that included Bowtie 2 to select plastome reads, and SPAdes 3.9 to assemble the plastomes using a *de novo* approach. We then used Dogma to annotate contigs, and Mauve 2.4 to compare the genomic structure of all sequenced plastomes with those from closely related taxa (i.e., *Olea europea*, *Crescentia cujete*, and *Tanaecium tetragonolobum*). We further used the complete plastomes to estimate relationships among major clades within the “*Adenocalymma-Neojobertia*” lineage using PhyloBayes 1.7. For the phylogenetic analyses, five different data partitions were used, including the following plastome regions: (1) All genic and intergenic regions; (2) Genic regions exclusively; (3) Intergenic regions exclusively; (4) Intergenic regions excluding poorly aligned portions; and (5) All genic and intergenic regions excluding poorly aligned portions. The analyses of all datasets shows an expansion of the IRs and compression of the SSC region within the "*Adenocalymma-Neojobertia*" clade with the inclusion of the ycf1 and part of the rps15 genes in the IR regions. Major genomic translocations are observed at the LSC and IRs, shuffling gene order among plastomes. However, only small changes in gene content are observed and only two putative pseudogenes (i.e., *acc*D and *ycf*4) are found in the genomes of some species. Phylogenetic analyses of all datasets led to the same topology. Relationships were generally strongly supported, with the highest support values obtained through the analysis of dataset (5), and the lowest support values recovered from the analysis of dataset (3). The "*Adenocalymma-Neojobertia*" clade shows an unusual pattern of plastid structure variation, challenging the current view of conserved plastid genome architecture in terms of gene order, even when closely related species are considered. This variable architecture complicates genomic assemblies using reference genomes and sequence alignments using whole plastomes. This plastic architecture also complicates the alignment of intergenic regions, where rearrangements are most common. Therefore strategies that employ *de novo* assemblies and evaluate sequence alignments are required to prevent assembly and alignment errors.

T5-09-07

The complete chloroplast genome sequence of the CAM epiphyte Spanish moss (*Tillandsia usneoides*, Bromeliaceae) and its comparative analysis

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Spanish moss (*Tillandsia usneoides*) is an epiphytic bromeliad widely distributed throughout tropical and warm temperate America. This plant is highly adapted to successfully inhabit its extreme environmental conditions. Striking features of this species include specialized trichomes (scales) covering the surface of its shoots aiding the absorption of water and nutrients directly from the atmosphere and a specific photosynthesis using crassulacean acid metabolism (CAM). Here we report the plastid genome of Spanish moss and present the comparison of genome organization and sequence evolution within Poales. Our study shows that early diverging lineages of Poales did not experience mutation rate acceleration as compared to grasses and plastid genomes of bromeliads show structural features considered to be ancestral in graminids, for example loss of *ycf*, and *accD*. We found that single-nucleotide variation and insertion/deletion frequencies are clearly higher than average in *rps7* revealing that this gene have been positively selected in *Tillandsia usneoides*. The same pattern can be found in the pineapple (*Ananas comosus*), another bromeliad with CAM photosynthesis. The *rps7* gene is encoding the ribosomal protein S7 that is crucial for ribosome assembly and synthesis; processes which have complex regulatory function in the ribosome reprograming pathway in water stress environments. This might suggest that the positively selected *rps7* is subjected to a number of ecological selective pressures and the gene may also be involved in stress acclimation potentially contributing to adaptation of bromeliads in drought conditions.

T5-09-08

Resolving the systematic positions of fence-riding taxa: Manipulating the chloroplast genome data of Paeoniaceae and its alliances

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Accurately resolving the phylogeny of fence-riding taxa is always a challenge in phylogenetic inference. Such uncertainties could be due to systematic errors (long branch attraction, and compositional heterogeneity) or non-phylogenetic signal artifacts (model violations). Here, we provide an example demonstrating how these factors affect the positioning of Paeoniaceae within Saxifragales based on chloroplast genome data. We newly assembled 14 chloroplast genomes from Saxifragales, and by combining these genomes with those of 63 other angiosperms, three datasets were assembled to test different hypotheses proposed by recent studies. These datasets were subjected to maximum parsimony, maximum likelihood and Bayesian analyses with site-homogeneous/heterogeneous models, different data partitioning strategies, and the inclusion/exclusion of weak phylogenetic signals. These analyses indicated that the phylogenetic position of Paeoniaceae in Saxifragales is conflicting, i.e., site- and substitution model-dependent. Accommodating the site-heterogeneous model after removing the fastest-evolving site yields support for the optimal resolution of Paeoniaceae as a sister to the woody group (A group), which suggests that the robust inference of Paeoniaceae in the core Saxifragales (B or C group) may be due, in part, to evolutionary rate biases among sites. We conclude that although genomic data significantly increase the phylogenetic resolution of fence-riding taxa with high support, phylogenetic results inferred from such data might be analytical or signal dependent. The analytical pipeline outlined here combines phylogenomic inference methods with evaluating the lineage-specific rate of substitution, model section, and assessing systematic error. It would help resolve similar difficult questions in the tree of life.

T5-09-09

Assessing genomic diversity via whole genome resequencing in bean cultivars (*Phaseolus* L. spp.) from Brazil, a putative secondary center of diversity

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Pulse crops, such as the lima bean (*Phaseolus lunatus*) and the common bean (*Phaseolus vulgaris*), are critical components of human health and the global economy. With the designation of 2016 by the United Nations as the International Year of Pulses, much research has centered on understanding the evolutionary, nutritional, and agronomic impacts of beans. Both the common bean and lima bean were domesticated by indigenous peoples of the New World and are said to each have two centers of domestication: a Mesoamerican origin and an Andean origin. However, genetic studies have suggested Brazil as another center of diversity. This is supported by anthropological studies of the Canela indigenous culture of northeast Brazil who cultivate an astonishing diversity of beans, with over 60 vernacular-named varieties. To assess the genetic diversity within Brazilian cultivars, we re-sequenced the complete genomes of 28 *P. lunatus* and 18 *P. vulgaris* accessions (including one wild-sourced) as well as the wild species *P. filiformis* collected from Brazil and *P. polystachios* collected from Virginia. All *lunatus* and *vulgaris* accessions were from small garden plots or local markets from areas northern Brazil proximate to Canela lands, with the exception of one proximately collected wild accession of *P. vulgaris*. Single nucleotide polymorphisms were called against the *P. vulgaris* complete nuclear and chloroplast genomes. Genetic diversity of Brazilian accessions was compared against SNPs from each species' two gene pools that represent centers of domestication and population structure as examined for each species. In addition, estimates of divergence times between gene pools and species were calculated. Brazilian germplasm presents a high level of genetic diversity and should be considered for preservation and conservation to help mitigate loss of genetic diversity.

T5-09-10

Phylogenetic relationships within *Lactuca* L. (Asteraceae), based on chloroplast genomics

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Lactuca L. belongs to the subtribe Lactucinae, tribe Cichorieae of the family Asteraceae. The genus has been the difficult and controversial part in the phylogenetic studies of Asteraceae family for a long time, due to their complex and diverse morphological characters and similar traits to the species from related genera. Domesticated lettuce (*L. sativa* L.) is an important leaf vegetable and consumed by people worldwide, especially in Asia, North and Central America, and Europe. Therefore, the study of *Lactuca* species is of great scientific and economic importance. In recent years, the next generation sequencing (NGS) technologies have been developing very fast, providing an opportunity to study deep phylogenetic relationships within *Lactuca*. This project aims to study the phylogenetic relationships within *Lactuca* by NGS, including *Lactuca* species that have not been sequenced and species from related genera as outgroups. The chloroplast genome sequences will be assembled, annotated and used for constructing phylogenetic trees. Then some important morphological characters, chromosome number and geographical distribution will be plotted on the phylogenetic trees to investigate their patterns on the evolutionary trees. The results of this study will contribute to the circumscription of *Lactuca* and its subgeneric classification and origin. In the meantime, the results will also shed some light on new genetic resources for lettuce breeding.

T5-09-11

The evolution of liverwort mitochondrial genomes

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The evolution of the mitochondrial genomes of vascular plants is highly dynamic in terms of structure and composition, resulting in numerous distinct gene orders. By contrast the so-called early land plant lineages show less variation, and in fact the mitogenome of mosses exhibits no change across the moss tree of life. Liverworts may be characterized by similarly stable mitochondrial genomes, considering the synteny among three early diverging liverwort lineages. To better understand the evolution of liverwort mitochondrial genomes, we sampled ~20 liverworts representing members across the liverwort phylogeny. Based on next-generation sequencing (NGS) approach, we have assembled mitochondrial genomes from these liverworts. Preliminary analyses indicate that the structure and genic content of mitochondrial genomes in liverworts are not strictly conserved throughout liverwort evolution, as some genomic rearrangements and gene losses are detected. In this study, we also performed RNA editing analyses based on transcriptome sequence data.

T5-09-12

Plastome evolution in Bignonieae (Bignoniaceae)

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Bignonieae is the largest tribe of Bignoniaceae, embracing ca. 400 species and 21 genera of Neotropical lianas. A phylogeny that includes 25% of the species known to date is available for the whole tribe. While this phylogeny defined major generic-level clades, the relationship among clades is still moderately supported. In order to improve our knowledge of deeper relationships in the tribe and further understand patterns of plastome evolution, we sequenced whole chloroplast genomes of selected species from most genera in the tribe using genomic pair-ended libraries and an Illumina HiSeq platform. Plastomes were assembled using *de novo* approach in Velvet and the final sequences were annotated using DOGMA and Geneious. Annotated plastomes were aligned using Progressive MAUVE along with plastomes from other Lamiales. Overall, the plastomes of 25 species, representing 16 genera, were completely assembled, with lengths varying between 157 and 181 kbp. In all species, the inverted repeat regions (IRs) extend toward the Small Single-copy region, resulting in the duplication of *ycf1*. Another expansion of the IR, toward the large single-copy region, was observed in most members of the "Multiples of four" clade (M4) examined (i.e., *Amphilophium*, *Anemopaegma*, *Bignonia* and *Pyrostegia*). Fifteen locally collinear blocks (LCB) that ranged from 400 bp to 57 kbp were identified, with variable positions and orientations, leading to two local inversions and four reallocations. One inversion was found in *Bignonia uleana* (1.8 kbp) and another in the most recent common ancestor of the "*Anemopaegma* + *Pyrostegia* clade" (ca. 8 kbp). Given the complementarity of the sequences found flanking the inversions, it seems that both inversions may have resulted from hairpins. Reallocations were found in *Bignonia capreolata* and *Mansoa* (also members of the M4 clade). These reallocations may have resulted from the expansion of the IR in the MRCA of the M4 clade, with two subsequent losses of the duplicated regions. Two other translocations were observed in *B. magnifica* and in *Neojobertia* but the molecular mechanisms the led to these translocations remain unclear. These structural changes in Bignonieae indicate that plastomes may be more variable than previously thought, highlighting the importance of using *de novo* approaches, instead of reference guided approaches, for plastome assembly. An improved knowledge of plastome evolution is crucial for phylogenetic reconstructions, given their impact on rates of molecular evolution in different portions of the plastome.

T5-10: The genomes of orphan crops

T5-10-01

Discovery of genomic and cellular components conditioning pod length provides insights into grain-vegetable cowpea (*V. unguiculata* L. Walp) diversification

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Cowpea (*V. unguiculata* L. Walp) is a climate resilient legume crop important for food security. Cultivated cowpea (*V. unguiculata* L) generally comprises the bushy, short-podded grain cowpea dominant in Africa and the climbing, long-podded vegetable cowpea popular in Asia. How selection has contributed to the diversification of the two types of cowpea remains largely unknown. In the current study, a novel genotyping assay for over 50 000 SNPs was employed to delineate genomic regions governing pod length. Major, minor and epistatic QTLs were identified through QTL mapping. Seventy-two SNPs associated with pod length were detected by genome-wide association studies (GWAS). Population stratification analysis revealed subdivision among a cowpea germplasm collection consisting of 299 accessions, which is consistent with pod length groups. Genomic scan for selective signals suggested that domestication of vegetable cowpea was accompanied by selection of multiple traits including pod length, while the further improvement process was featured by selection of pod length primarily. Pod growth kinetics assay demonstrated that more durable cell proliferation rather than cell elongation or enlargement was the main reason for longer pods. Transcriptomic analysis suggested the involvement of sugar, gibberellin and nutritional signalling in regulation of pod length. This study establishes the basis for map-based cloning of pod length genes in cowpea and for marker- assisted selection of this trait in breeding programes.

T5-10-02

Whole genome and transcriptome sequencing unravel the differences between Shum and Gilo groups of the African Eggplant

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The African eggplant (*Solanum aethiopicum*) is a phenotypically and morphologically diverse species, divided into four recognized cultivar groups, namely Gilo, Shum, Kumba and Aculeatum. The Gilo and the Shum groups are the most popular and most widely cultivated groups in Africa. Although some previous taxonomic studies have reported the complexity within and across these defined groups of *S. aethiopicum*, there have been no focused genomic studies aimed at enhancing our understanding of the specific groups. We generated whole genome sequences of an inbred landrace, Shum303, belonging to the Shum group using Illumina sequencing technologies. We additionally generated transcriptome sequences from leaves, roots, flowers and fruits of Shum303, Shum307, Gilo524 and Gilo610. The transcriptome sequences were assembled both *de novo*, and aided by the new draft genome of Shum303 as a reference. The draft assembly is 1.03 Gb in size, comprising ~88% of the genome (1.17Gb, estimated using K-mer analysis), with N50 of scaffold and contig of 340 Kb and 21 Kb, respectively. We generated 183,201 and 144,450 unigenes for Shum and Gilo groups respectively. Both Shum and Gilo fruits generated the most unique transcripts in comparison to leaves, flowers and roots. Disease resistance genes were some of the most highly expressed genes in both Shum and Gilo groups suggesting the likely exposure of both genotypes to a disease prior to transcriptome analysis. A comparison of Shum and Gilo with other Solanaceae crops revealed a higher similarity of the African eggplant with tomato than potato. Further comparison between Shum and Gilo revealed that the Shum group was more closely related to tomato than the Gilo group. The Gilo group also showed differential gene expression of a pleiotropic drug resistance gene, known to play an important role in the prevention of serious adverse drug reactions (ADRs). Our work reveals unique group and tissue specific expression patterns in Shum and Gilo and provide excellent opportunities for medical research and resistance breeding.

T5-10-03

AOCC research aided by large scale plant genomics study

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Plants play a crucial role in ecosystems, food and nutritional security and resources of medicine. Understanding plant genetics and biodiversity require integrated efforts ranging from distribution of germplasm, germplasm resources collection and storage, genotypic and phenotypic data analysis, transformation and agricultural application. In the past one and half decade, series of innovative technologies especially NGS (Next-Generation Sequencing) technologies have been developed and widely applied in plant research, which has accelerated and reshaped the researches in biodiversity and practices in food and nutrition development. AOCC (African Orphan Crops Consortium) associates a panel of scientists, anthropologists, nutritionists and plant breeders and all aim to make high-nutritional value crops grown by African famers and available to rural and urban consumers. In this consortium, 101 African orphan crops genomes are selected to be sequenced, assembled and annotated. Whole genome sequence, transcriptome sequence, SNP marker arrays/panels can access genetic diversity and also support breeding programs. *De novo* genome sequencing or re-sequencing, as well as transcriptome sequencing of more than 20 crops have been performed to date. The genomics efforts will benefit to accelerate crops domestication, diversify farming landscapes and agribusiness, conserve germplasm, and enhance crops yields for the entire Africa continent with ambition to diversify diets to support healthy nutrition.

T5-10-04

Ecology and community genomics of an important crop wild relative as a prelude to agricultural innovation.

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Florida International University

Domesticated species are impacted in unintended ways during domestication and breeding. Changes in the nature and intensity of selection impart genetic drift, reduce diversity and increase the frequency of deleterious alleles. Such outcomes constrain our ability to expand the cultivation of crops into environments that differ from those under which domestication occurred. We address this need in chickpea, among the world's most important pulse legumes, by conserving, characterizing and harnessing the diversity of wild crop relatives. We document an extreme genetic bottleneck between modern breeding lines and the wild progenitor species, while also deciphering the genetic history of wild populations. We provide evidence of ancestral adaptations in wild populations for seed coat color crypsis and demonstrate variation between wild and cultivated accessions for agronomic properties including flowering time, seed shattering, seed nutrient density, nitrogen responsiveness and drought adaptation. The assembled germplasm will serve as an essential resource for future chickpea breeding, while our methods may inform collection of other wild crop progenitor species.

T5-10-05

Grapevine rootstock species (*Vitis* spp.): diversity, comparative transcriptomics, and impacts on shoot system phenotypes in grafted plants

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Perennial, woody crops including grapevines and many fruit and nut trees are often cultivated using the ancient horticultural technique of grafting. Grafting fuses plant organs together, creating plants where the lower stem and root system is genetically distinct, often a separate species, from the shoot system that produces harvested material. Grafting separates the breeding of shoot from root, permitting the development of domesticated rootstocks selected for superior performance in specific climates and soil types. For many grafted plants, even for well-known economically important crops with extensive genomic resources, species used as rootstocks represent "the lesser known half of the perennial crop equation." For example, grapevines are among the most widely planted berry crops in the world, with total worldwide production exceeding 77M tonnes in 2013. Grafting in grapevine dates to the mid-1800's when the North American aphid *Phylloxera* was inadvertently introduced into Europe where it devastated the roots of cultivated grapevines. Starting with this crisis, native North American grapevine species have been used for their *Phylloxera*-resistant rootstocks. Today the bulk of the global grape industry relies on traditional European grape varieties grafted to North American grapevines and their hybrid derivatives. Mounting concerns about the effects of a changing climate on vineyard locations and crop quality have re-focused attention on the potential contributions of diverse rootstocks for grape crop sustainability. Here we present data from two related studies: 1) morphological, genomic, and transcriptomic diversity of two North American *Vitis* species used as rootstocks; and 2) impacts of hybrid derivatives of these species on shoot system phenotypes in grafted plants. In the first experiment, we established a common garden of *V. riparia* and *V. rupestris* in the Kemper Center for Home Gardening at the Missouri Botanical Garden. Three clonal replicates of three genotypes for each of the two species were grown ungrafted. Leaf and ion concentrations were measured for each individual. We characterized gene expression patterns of each individual at two different time points. These data demonstrate differences in leaf shape, ion concentrations, and gene expression among *V. riparia* and *V. rupestris*. In the second experiment we leveraged an experimental vineyard where a common scion 'Chambourcin' is growing on its own roots and grafted to three different rootstocks. This combination of four individuals is replicated 72 times. Phenotypic data collected in the shoot systems demonstrate significant effects of rootstock on leaf shape, ion concentration, and vine physiology, among other traits. These data underscore the importance and potential of rootstock species and crop wild relatives in general that play an underappreciated role in agriculture and crop improvement.

T5-10-06

Exploring the evolutionary history of *Mangifera* and its domesticated species using ddRAD-seq

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Among the many domesticated species of the poison ivy family (Anacardiaceae), including pistachio, cashew, pepper tree *(Schinus)* and jocote *(Spondias),* the mango *(Mangifera indica* L.) provides a novel system in which to study perennial crop domestication. Wild *M. indica* is thought to have originated in the foothills of the Himalayas in what is now northeastern India, Myanmar, Bangladesh, and Nepal. The mango was originally domesticated in India around 4,000 years ago, and today, is one of the world's most important tropical fruits. In addition to *M. indica*, some 26 species of *Mangifera* have edible fruits; these species range from incipiently-domesticated to cultivated landraces, and many exhibit traits of interest to breeders, such as resistance to mango anthracnose, salinity tolerance, and reduced fiber content. *Mangifera* is one of the largest of the Anacard genera, encompassing some 69 species. The native range of *Mangifera* stretches from eastern India to the Solomon Islands, with the highest diversity present in Borneo, Sumatra, and the Malay Peninsula. The most recent taxonomic revision of *Mangifera*, based on morphology, distinguishes between two subgenera, Mangifera and Limus, and six sections, but does not hypothesize species-level relationships. This monograph of *Mangifera* remains the standard for species identification and classification, as no systematic analysis of the genus has been published. Previous work based on morphological evidence proposed the delimitation of *Mangifera* into two subgenera and six sections, but published gene trees have called into question this classification. An understanding of the evolutionary relationships between *Mangifera* species will inform cultivation techniques and could lead to mango cultivars with increased tolerance to both disease and harsh environmental conditions. Here, we present the first multilocus phylogeny of *Mangifera*. Leaf samples were collected from 280 individuals representing 40 taxa grown in living collections in Indonesia, Singapore, Malaysia, and the U.S. Reduced representation genomic data were obtained by double digest restriction site associated DNA (ddRAD) sequencing. Reads were quality filtered, then processed with the software ipyRAD to conduct denovo assembly and call single nucleotide polymorphisms. Phylogenetic reconstruction was performed using Bayesian and Maximum Likelihood frameworks along with SVDquartets. The phylogeny was time-calibrated using a previously published phylogeny for the families Anacardiaceae and Burseraceae. This work challenges some of the previously proposed infrageneric classifications for *Mangifera*, and reveals the evolutionary relationships between the highly domesticated mango and its wild and semi-domesticated relatives.

T5-11: Genomics and domestication of economic crops

T5-11-01

Temporal and spatial variation in a large collection of chickpea (*Cicer arietinum*) landraces.

Eric Von Wettberg

Florida International University

Landraces of crops are important genetic resources, reflecting traditional cultivation practices and potential adaptation to diverse climates and agroecologies. We examine landrace diversity in chickpea, a unique crop with a wild ancestor with an extremely narrow geographic distribution in Southeastern Anatolia but landrace cultivation spanning six continents and conditions from subtropical South Asia and East Africa, to Mediterranean climates worldwide, and to temperate prairie conditions. By assembling a diverse collection of landraces from multiple genebanks spanning nearly century of germplasm collections and the three primary centers of diversification for chickpea, we examine changing patterns of diversity. We find little evidence of declining landrace diversity from the oldest collections in the Vavilov Institute for Research to contemporary collections. We document shifts in diversity in the center of domestication in Turkey to secondary diversification centers in South Asia and East Africa. Comparison to diversity in a recently assembled large collection of the wild relatives allows us to build a demographic model of multiple bottlenecks and diversifications impacting the diversity of chickpea.

T5-11-02

Re-sequenced genomes reveal the genetic diversity of polyploid cotton

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By deep-sequencing many accessions of cotton, we have revealed detailed genetic information and phylogeographic patterns demonstrating the diversity in the cotton genome. We have re-sequenced nearly 600 accessions, including 431 accessions of *G. hirsutum*, 165 accessions of *G. barbadense*, and other cotton species. We detected the variation among these accessions and identified genetic variation between cultivars and wild species, also between the different species. This variation is interpreted against the background of new *de novo* cotton genome sequences. This wealth of sequence information will allows us to identify which specific gene or genome region have been selected during domestication of cotton. Understanding the location of these loci will allow us to further improve the cotton genome for agricultural improvement.

T5-11-03

Domestication origin and breeding history of the tea plant (*Camellia sinensis* (L.) O. Kuntze) in China and India

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China and India are the largest tea producing countries yet, the breeding history of the tea plant in these countries still unclear. Three distinct lineages of the tea plant, China tea, Chinese Assam tea and Indian Assam tea were defined recently, and nuclear microsatellites (nSSRs) revealed independent domestications in China and India. However, the domestication events need further confirmation and the timing of tea domestication remained unknown. In this study, a combination of 23 nSSRs and three cpDNA were used for genotype of 402 and 101 samples of the tea plant and its wild relative. Multiple analyses of Demographic modeling, NewHybrids and Neighbour joining tree were performed to investigate the domestication origin and breeding history of the tea plant. Three independent domestication centers were confirmed by both nSSR and cpDNA data, and two origins of domestication for Chinese Assam tea were further revealed in Southern and Western Yunnan of China with cpDNA. China tea and Assam tea was firstly diverged 22k year ago during the last glacial maximum (LGM), and subsequently split between Chinese Assam tea and Indian Assam tea in 2770 year ago, corresponding with the early use of tea in Yunnan, China. The three tea types underwent different breeding history and showed a complex breeding patterns. Hybridization may be the most important approach for tea cultivar breeding, and high proportion of the hybrids are F2 and backcross generation. Priority conservation strategy of Chinese Assam tea germplasm, especially the landraces should be implemented, which are very valuable resources for future tea breeding.

T5-11-04

Phylogenomics of the genus *Rosa*: proposal to solve a complex evolutionary scheme

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The rose bush is the most economically important ornamental plant and the model organism for many studies about ornamental traits such as flowering, fragrance or disease resistance. Some of these traits were retrieved from Asian wild *Rosa* which represent the main gene pool, counting for about half of the genus species. The genus *Rosa* has a complex evolutionary history with interspecific hybridization events and polyploidizations. Most of current *Rosa* phylogenies are based on chloroplastic markers despite they are known for being maternally inherited. In addition, such phylogenies show low supports on internal nodes, which may be explained by low mutation rate of these markers.The objective of this project is to identify simultaneously a large number of nuclear and highly variable markers. Recently, as part of an international consortium, co-coordinated by the *Genetic and Diversity in Ornamentals* team from the French Research Institute for Horticulture and Seeds, the first version of the genome sequence of a rose cultivar (*Rosa* 'Old Blush') has been obtained. In addition, other genomic sequences have been recently published or acquired such as *R. damascena*, *R. roxburghii* or *R. palustris*. These sequences provide an unrecorded access to gene variation and organization in *Rosa* species. From comparisons between molecular sequences, we selected the best suitable low copy nuclear genes to reconstruct the phylogeny of the genus as suggested by phylogenies performed with Rosaceae Conserved Orthologous Set of data (RosCOS). About 150 genes have been already identified corresponding to nearly 600Kbp of phylogenetic information. Currently, these genes are tested on an additional representative sample of the four commonly accepted *Rosa* sub-genera. Once this step done, the markers showing the higher sequence variations will be amplified on a broader sample representing about 100 *Rosa* species. We thus expect to obtain more robust phylogenies, with higher supports for internal nodes, making them more accurate to study traits evolution through the genus. Indeed, both current cultivated roses and their wild counterparts show hybrid specimen with mixed characteristics. In this way, a well-resolved phylogeny offers the prospect of understanding how natural and horticultural diversities were generated among the genus. Knowing phylogenetic relationships between roses may also help increasing the cultivated diversity, by introducing new traits through interspecific crosses with wild material.

T5-11-05

Evolution and domestication of the flax (*Linum ustitatissimum*) genome and its further improvement through reverse genetics

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Flax (*Linum usitatissimum*) has been used for millennia as a source of fiber or oil and is now recognized also as a source many biologically active compounds (e.g. lignans). Flax cultivation decreased dramatically after the middle of the 20th century, however new resources for flax research are supporting renewed interest in this crop, especially in relation to biocomposite materials. These new resources include: a whole genome and transcriptome assemblies from multiple genotypes and wild relatives, high density SNP maps, and mutant populations. The situation in flax therefore presents an interesting opportunity to test whether genomics can effectively accelerate basic and applied research in a previously neglected annual crop. Although flax has great potential to be a dual purpose crop, current varieties are cultivated either for seed (i.e. linseed) or for stem fiber (used traditionally in linen). One of our objectives is to make the stem fiber of linseed available as a feedstock for biodegradable composites and other advanced materials, whereas linseed straw is now seen only as a nuisance. Industrial use of linseed straw is limited by the yield and extractability of the fibers and by their chemical and mechanical properties. We have therefore used the newly available resources for flax to investigate the development of its strong, cellulose rich bast fibers, which have much in common with the specialized cell walls of tension wood produced in trees. To this end, we have characterized the following gene families of flax: beta-galactosidases (BGAL, GH35), cellulose synthases (CesA) and CesA-like proteins, chitinase like proteins (CTL, GH19), pectin methylesterases (PME), and trichome birefringence-like (TBL) proteins. We are using three complementary reverse genetics strategies to identify mutations in genes of interest. Our short term objective is to identify mutants with improved fiber separability (by targeting pectin and metabolism), and improved seed qualities (e.g. taste and meal quality). In the first approach, 10 amplicons, each containing 200 bp of a targeted coding region, are amplified individually from 768 independent M4 individuals, and are combined in 3D-pools for barcoding and subsequent sequencing using an Ion-Torrent 314-chip. In the second approach, a custom olignucleotide library was used to capture exomes of over 30,000 targeted genes. Capture-enriched genomic DNA fragments from individual M4 lines were sequenced using an Illumina HiSeq. Finally, we have also resequenced entire genomes of mutant lines and varieties to discover variants associated with useful traits. Results show that all three approaches are effective in identifying mutations in flax genes of interest, although there are significant differences in cost per-run and cost per-mutation.

T5-11-06

The cotton β-galactosyltransferase 1 (GalT1) that galactosylates arabinogalactan-proteins participates in controlling fiber development

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Arabinogalactan-proteins (AGPs) are highly glycosylated proteins which play pivotal roles in diverse developmental processes in plants. Type II AG glycans, mostly O-linked to the hydroxyproline residues of the protein backbone, account for up to 95% w/

w of the AGP, but their functions are still largely unclear. Cotton fibers are extremely elongated single-cell trichomes on the seed epidermis. However, little is known of the molecular basis governing the regulation of fiber cell development. Here, we characterized the role of a CAZy glycosyltransferase (GT) family 31 member GhGalT1 in cotton fiber development. Fiber length of the transgenic cotton overexpressing *GhGalT1* was shorter than that of wild type, whereas in the *GhGalT1*-silenced lines there was a notable increase in fiber length, compared with wild type. The carbohydrate moieties of AGPs were altered in fibers of *GhGalT1* transgenic cotton. The galactose:arabinose ratio of AG glycans was higher in *GhGalT1* overexpression fibers, but lower in *GhGalT1*-silenced lines, compared with that in wild type. Overexpression of *GhGalT1* upregulates transcript levels of a broad range of cell wall-related genes, especially the fasciclin-like AGP (FLA) backbone genes. An enzyme activity assay demonstrated that GhGalT1 is a β-1,3-galactosyltransferase (β-1,3-GalT) involved in biosynthesis of the β-1,3-galactan backbone of the type II AG glycans of AGPs. We also show that GhGalT1 can form homo- and hetero-dimers with other cotton GT31 family members to facilitate AG glycan assembly of AGPs. Thus, our data demonstrate that GhGalT1 influences cotton fiber development via controlling glycosylation of AGPs, especially FLAs.

T5-12: Arabidopsis: genomics and natural variations

T5-12-01

The ecological genetics of seed size and number and adaptive differentiation in *Arabidopsis thaliana*

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Local adaptation can be expressed through differences between local and non-local genotypes in several fitness components, and the magnitude of adaptive differentiation will thus depend on which fitness components are considered. Life-history theory predicts that within a given environment, adaptive evolution will be constrained by tradeoffs between allocation to different life-history functions. Reciprocal transplant experiments have demonstrated strong adaptive differentiation between an Italian and a Swedish population of *Arabidopsis thaliana* have shown that differences in fruit production of reproducing plants contribute to the advantage of the local genotype at the Italian, but not at the Swedish site. Here we use a field experiment conducted over five years that involved the parental genotypes and a RIL population derived from a cross between the two populations to examine whether differences in seed production per fruit, and size of individual seeds add to any reproductive advantage of the local genotype arising from differences in number of fruits per reproducing plant, whether any tradeoffs can be detected among these components of reproductive allocation, and to map QTL for the different components of reproductive success at the native sites. In Italy, the local genotype consistently produced more seeds per fruit than did the non-local genotype, whereas in Sweden this was true in only two of five years with no difference recorded in the other three years. Seed production per reproductive plant was 16- to 160-fold higher in the local compared to the non-local genotype in Italy, but only 1- to 9-fold higher in Sweden. In two years in Italy and one year in Sweden, the local genotype produced larger seeds than did the non-local genotype. Seed number per fruit showed weak correlations with seed mass (mean r = 0.07), moderate correlations with fruit number (mean r = 0.18) and strong correlations with seed production per reproductive plant (mean r = 0.50). Alleles at QTL for fruit number favour the local genotype, consistent with observed differences in fecundity between the parental lines. In contrast, alleles influencing seed number per fruit and seed number per reproductive plant show uniform effects at both sites and were just as likely to increase as to decrease seed number. At 10 QTL for seed number per plant, alleles show positive correlations with both seed number per fruit and fruit number among RILs, reflecting the positive relationships between phenotypes. We also identify four QTL where the local allele increases one component of fecundity, but decreases the other, suggesting a trade-off between phenotypes at individual loci.

T5-12-02

Rapid adaptation of *Arabidopsis thaliana* to the Yangtze River basin

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Global climate change has tremendous effect on the human health, food security and biological diversity. To keep pace with a changing environment, organisms need to rapidly adapt. However, how organism could rapidly adapt to the climate change is a crucial biological question to address but largely unknown. An excellent system to study such adaptive processes is the plant *Arabidopsis thaliana*. Its origin is in Europe, but it has spread to the Far East, including the most southeastern edge of its native habitats - the Yangtze River basin, where the climate is very different. We sequenced 91 *A. thaliana* strains from the region flanking the Yangtze River basin, and found that this population is genetically uniform, having been dispersed eastwards recently about 3000 years ago. Here we integrated population genomics and genome wide association study (GWAS) for the both climate parameters and phenotypic trait flowering time together, and found out that genes correlated with responses to stress and flowering time could have contributed to the rapid adaptation to the Yangtze River basin. Especially, two genes, PP2C and ACS8, both involved in either stress response or flowering time, could have been the most important determinant genes during the rapid adaptation to the Yangtze River basin. Overall, our results shed light on how a species can rapidly adapt to locales with a very different climate.

T5-12-03

Exploring *Arabidopsis* natural variation

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The genetic variation of *Arabidopsis thaliana* in nature provides a useful resource for the functional analysis of genes. The genetic complexity of this type of variation requires Quantitative Trait Loci (QTL) analysis, using various types of mapping populations. In addition to the analysis of gene function, natural variation studies may reveal signatures of selection in nature that may explain local adaptation. To demonstrate the power of natural variation, examples on the analysis of plant architecture (plant length and branching and vein density patterns) will be presented. For plant length, we found ample functional variation for the GA 20 oxidase (*GA5*) gene of which gene mutants have been exploited to generate modern short straw varieties in barley and rice. For branching an example on how to analyse such variation up to the gene level in *Arabidopsis* is the finding of the *AGL6* gene to be involved in this process. The complexity of the genetic interactions among natural variants and its consequences for evolution is shown by genetic incompatibilities that arise in certain combinations of genotypes.

T5-12-04

1001 Genomes Plus (1001G+): From single-reference mapping to the integration of multiple genome assemblies

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The 1001 Genomes Consortium, an informal international collaboration, has recently marked an important milestone with the publication of 1135 Illumina short-read resequenced genomes of *Arabidopsis thaliana* from throughout the global range of the species. In that analysis, only an average of 100 Mb (84%) per line were called against the Col-0 TAIR10 reference genome (119 Mb). The missing positions differ greatly between lines, because they are in the vicinity of structural variants (SVs), which are difficult to assess with short read technology. In the next phase, the consortium is building multiple platinum-standard *de novo* genome assemblies, primarily with PacBio long-read technology and BioNano optical mapping, as a further step towards a species-wide pan-genome and to discover a core set of common structural variants in *A. thaliana*. The variants discovered from a comparison of these platinum-standard genome assemblies will then be typed in the 1135 Illumina short-read set to describe the global distribution of SVs. This knowledge will further inform Genome Wide Association Studies (GWAS) in this species, and advance our understanding of how SVs such as gene duplications, transpositions, deletions and transposable elements (TEs) shape the genetics and epigenetics of *A. thaliana*.

T5-12-05

High throughput phenotyping of photosynthesis to identify relevant quantitative trait loci and underlying genes in *Arabidopsis thaliana*

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Natural genetic variation in plant photosynthesis efficiency is hardly studied even though breeding for photosynthesis would be interesting to maintain increases in crop yields. One of the reasons this is not studied is the notorious difficulty in adequately phenotyping photosynthesis parameters for genetic research. We designed a versatile plant phenotyper, the Phenovator, capable of multispectral imaging of 1440 plants per experiment. Imaging includes chlorophyll fluorescence to determine the light use efficiency of photosystem II electron transport (Φ_{PSII} or Fq'/Fm'), and near infrared reflection to measure projected leaf area. This system has been used to phenotype *Arabidopsis thaliana* recombinant inbred line populations as well as around 350 genetically diverse accessions for genome wide association analysis. Plants were phenotyped at optimal conditions and in response to cold treatment (5 °C) or a switch from 100 to 550 μmol m^{-2} s^{-1} irradiance. The observed genotypic variation was used to identify quantitative trait loci (QTL). Our latest progress in identification and confirmation of QTL for Φ_{PSII}, plant growth and epinastic leaf movement will be presented, including detailed analysis of the allelic variation at the nucleotide level of some of the genes underlying these QTL. Our work has shown that there is sufficient genetic variation for PSII efficiency in Arabidopsis amenable for gene identification, which suggests the same will be the case for crop species. Such would offer interesting opportunities for future crop photosynthesis improvements.

T5-12-06

Populations of *Arabidopsis thaliana* from Tibet, a unique ecotype at high elevation

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Arabidopsis thaliana is found in different geographic regions with different climatic conditions. Six populations were collected from Tibet, the upper reaches of the Yangtze River, China. They are found in habitats at 3,800-4,400 meters above sea level, and they formed a monophyletic clade based on chloroplast DNA. These populations have distinct morphological and physiological characters such as very short, sometimes multi-branched, low germination rate, very sensitive to freezing temperature, and *etc.* The low tolerance to freezing temperature is partially due to the malfunctioning of a group of C-repeat binding factor (*CBF*) genes which have been found playing an important role in responding to low temperature in plants. The expression level of *CBF1~3* is lower in all 6 populations than that of Col ecotype at 4°C, and a loss-of-function mutation was found in *CBF2* and/or *CBF3* of these populations. An RNA-seq analysis was performed on the plants treated at 4°C for 3 h and 12h. It was found that at 3 h, the 6 populations shared 98 exclusive up-regulated genes which were mainly involved in responses to various phytohormones such as ABA, auxin, and JA, and to cold and salt stress; while Col had 198 exclusive up-regulated genes involving in regulation of gene expression, protein autophosphorylation, ethylene signaling pathway, and systemic acquired resistance. At 12 h, the 6 populations had a totally different set of up-regulated genes from Col. We will discuss about the adaptation significance of these variations, together with the genetic and molecular characterizations of some other interesting traits in this unique ecotype from Tibet.

T5-13: Extremophyte: transcriptomes and beyond

T5-13-01

Transcriptomic analysis reveals molecular mechanisms underlying salt adaptation in *Sonneratia alba*

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Salinity in soil is increasing in many areas and presents a widespread and serious environmental risk for most plants. Mangroves are a group of unique plants that well adapt to extreme environments with high salinity in tropical and subtropical intertidal zones. In this study, we performed transcriptomic analysis to investigate the transcriptional regulation underlying salt adaptation in *Sonneratia alba*. Seedlings of *S. alba* were treated by 0 mM, 250 mM and 500 mM NaCl for 7 days, and transcriptome profiles were obtained from leaf and root tissues, respectively. Gene expression pattern analyses suggested an earlier salt-response in roots than in leaves. With the whole genome dataset of *S. alba* as background, we conducted functional analyses for differentially expressed genes (DEGs). Gene Ontology enrichment analysis indicated that GO term “response to stimulus” was overrepresented in up-regulated genes in both leaf and root, suggesting the expression of most known salt-tolerant genes were elevated in response to salt stress. Furthermore, the KEGG pathways of “sulfur metabolism” and “Flavone and flavonol biosynthesis” were significantly enriched in up-regulated genes in leaf tissue. They were both involved in synthesizing osmosis-related metabolites in plants, suggesting the accumulation of osmotic substances might play a major role in salt tolerance in mangroves. Moreover, there were higher percentages of transcription factors (TF) in DEGs than the proportion in the whole genome, and the expression of salt tolerance related TF families were elevated under salt stress, suggesting that TFs could also play a curial role in salt adaptation of mangroves. Our study sheds light on the transcriptional regulation of mangroves to high salinity in the intertidal zones, and provides some useful information for plant genetic improvement.

T5-13-02

Transcript profiles of genes involved in increasing sugar content in leaves and salt exclusion in roots during salt stress in sweet sorghum

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Sweet sorghum (*Sorghum bicolor* (L.) Moench) is a variation of traditional grain sorghum, a C_4 crop, with the characteristics of high photosynthesis and high biomass accumulation. It has been considered as energy and forage crop of high-yield and salt-resistant, which is most suited to grow in the saline-alkali land of Yellow River Delta. However, little is known about sweet sorghum resisting salt stress and increasing its sugar content in response to salt stress. In this study, the transcript profiles of both leaves and roots of two inbred lines of sweet sorghum (salt-tolerant M-81E and salt-sensitive Roma) were analyzed in the presence of 0 mM or 150 mM NaCl. In leaves, the majority of differential expressed genes are involved in photosynthesis, carbon fixation, and starch and sucrose metabolism. Genes important for maintaining photosystem structure and for regulating electron transport were less affected by salt stress in the M-81E line compared to the salt-sensitive Roma line. In addition, expression of genes encoding NADP$^+$-malate enzyme and sucrose synthetase was up-regulated and expression of genes encoding invertase was down-regulated under salt stress in M-81E. In contrast, the expression of these genes showed the opposite trend in Roma under salt stress. In roots, many differentially expressed genes were involved in pathways related to salt exclusion, such as apoplastic barriers, ion transport, aquaporins and ROS scavenging. These results revealed that the salt-tolerant genotype M-81E have a better ability to limit root absorption of Na$^+$ and to maintain ion homeostasis under salt stress which may be caused by the combination of physical barrier effect of root apoplastic barriers and the transportation of Na$^+$ out of the xylem into the xylem parenchyma cells by HKT1; 5. Additionally, M-81E leads to increased sugar content under salt stress by protecting important structures of photosystems, by enhancing the accumulation of photosynthetic products, by increasing the production of sucrose synthetase and by inhibiting sucrose decomposition. All these may be regulated by stress-related transcription factors such as bHLH, MYB, ERF, WRKY and NAC.

T5-13-03

Genome, transcriptome and gene function of *Thellungiella salsuginea*

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Thellungiella salsuginea, a close relative of Arabidopsis, represents an extremophile model for abiotic stress tolerance studies. We obtained the draft sequence of the *T. salsuginea* genome, assembled based on ~134-fold coverage to seven chromosomes with a coding capacity of at least 28,457 genes. This genome provides resources and evidence about the nature of defense mechanisms constituting the genetic basis underlying plant abiotic stress tolerance. Comparative genomics and experimental analyses identified genes related to cation transport, abscisic acid signaling, and wax production prominent in *T. salsuginea* as possible contributors to its success in stressful environments. Especially we will present the detail analysis an only copy of a small C2 domain protein exhibit a great stress tolerance ability once it is expressed in vs, both in model and crop plants.

T5-13-04

Genome and transcriptome analysis in the desiccation tolerant grass *Oropetium thomaeum*

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Limited water availability has resulted in evolution of mechanisms to survive periods without water supply. As a result of this a small group of vascular angiosperm plants termed “resurrection plants” have evolved unique mechanisms to survive desiccation to an air-dried stage. Desiccation tolerance of vascular tissues may have evolved through rewiring seed desiccation pathways. *Oropetium thomaeum*, originally collected in India, is an emerging model for understanding desiccation tolerance. The recently sequenced genome of *Oropetium thomaeum* is the smallest among the grasses. Transcriptome and targeted metabolome analyses demonstrate the dominant presence of seed specific pathways in vegetative tissues. Recently evolved gene duplications are observed for genes encoding protective components. Pathways involved in programmed cell death inhibition, ABA signalling, synthesis of stress protective proteins and seed specific-transcription factors are activated during desiccation. Oleosins and oil bodies are highly abundant in desiccated leaves and may contribute to membrane stability and storage of reserves. Orthologs of seed-specific LEA proteins from other grasses have neo-functionalized in *Oropetium thomaeum* with high expression during desiccation. Accumulation of sucrose, raffinose, and stachyose in leaves during dehydration are similar to those in maturing seeds. The overall transcript analysis suggests that vegetative desiccation tolerance is closely related to seed desiccation. The understanding of desiccation tolerance will provide novel biotechnological targets for improving growth of agricultural plants in environments with restricted water supply.

T5-13-05

Gene expression reaction norms of *Alternanthera philoxeroides* in contrasting hydrological conditions

Ji Yang

Fudan University

Alternanthera philoxeroides is an amphibious invasive weed that can colonize both aquatic and terrestrial habitats. Individuals growing in different habitats exhibit extensive phenotypic variation but little genetic differentiation. Little is known about the molecular basis underlying environment-induced phenotypic changes. Variation in transcript abundance in *A. philoxeroides* was characterized throughout the time-courses of pond and upland treatments using RNA-Sequencing. Seven thousand eight hundred and five genes demonstrated variable expression in response to different treatments, forming 11 transcriptionally coordinated gene groups. Functional enrichment analysis of plastically expressed genes revealed pathway changes in hormone-mediated signaling, osmotic adjustment, cell wall remodeling, and programmed cell death, providing a mechanistic understanding of the biological processes underlying the phenotypic changes in *A. philoxeroides*. Both transcriptional modulation of environmentally sensitive loci and environmentally dependent control of regulatory loci influenced the plastic responses to the environment. Phenotypic responses and gene expression patterns to contrasting hydrological conditions were compared between *A. philoxeroides* and its alien congener *Alternanthera pungens*. The terricolous *A. pungens* displayed limited phenotypic plasticity to different treatments. It was postulated based on gene expression comparison that the interspecific variation in plasticity between *A. philoxeroides* and *A. pungens* was not due to environmentally-mediated changes in hormone levels but to variations in the type and relative abundance of different signal transducers and receptors expressed in the target tissue.

T5-13-06

Dehydration tolerance in a tropical liverwort: Ecological and genomic patterns

Rose A Marks

University of Kentucky

Our work stems from an interest in understanding the strategies by which plants respond to limited and variable water availability. Adaptive mechanisms to cope with water stress are highly relevant in light of current and future climate change. Dehydration tolerance (DhT, also dehydration tolerant) is a relatively rare plant strategy for coping with water shortage that allows tissues to survive substantial cellular drying. This adaptation to water shortage provides a unique opportunity to identify key genes, physiological, and ecological traits that will inform biotechnological advances and management practices aimed at reducing crop and species loss due to drought. Our recent studies indicate that *Marchantia inflexa*, a tropical liverwort, exhibits moderate and variable levels of DhT. Furthermore, *M. inflexa* has the capacity to acclimate to different climatic moisture levels, suggesting that there is a plastic component to DhT in this species. *M. inflexa* is currently undergoing range expansion and colonizing novel habitats, likely facilitated by its ability to acclimate to these novel conditions. However, older populations also exist across a variety of habitat types and these populations could harbor genetic adaptations in DhT. Plants that exhibit adaptive differences in DhT, provide a valuable genetic resource for identifying key genes that contribute to increasing DhT. To test for adaptive differences between *M. inflexa* populations, plants were collected from two habitat types, which differ in their relative water availability. The DhT of these plants was tested immediately after collection to assess acclimation to each habitat. Plants were then cultured in a common garden for four months, and assayed to determine the innate level of DhT in these groups. Plants displayed acclimation to contrasting water regimes. In addition, we recently demonstrated that males of this species are significantly less DhT than females, and that the vegetative meristem survives dehydration more often than the remaining vegetative tissue. Based on these two observations we designed and conducted an RNA sequencing study to characterize changes in gene expression during dehydration and rehydration in these tissues in male and female plants. Consequently, we were able to identify genes with different expression profiles across samples. These genes were implicated as candidate genes that contribute to underlying differences in DhT. By taking a within species approach, we were able to minimize background differences and identify a high-resolution set of candidate DhT genes. Furthermore, the resulting transcriptome provides a valuable resource for other researchers wanting to compare sensitive species or highly desiccation tolerant species to the more intermediate phenotype of a DhT plant.

T5-14: Epigenetics I: histone modifications

T5-14-01

The influence of histone modifications and variants on *Arabidopsis* meiotic recombination

Ian Henderson
University of Cambridge

Meiotic recombination varies extensively in plant genomes, which has important implications for both genome evolution and plant breeding. We are using the model species *Arabidopsis thaliana* to investigate genetic and epigenetic control of meiotic recombination patterns. In this talk I will present our work mapping meiotic DNA double strand breaks, and crossover events, comparing wild type with mutants that have altered histone modifications or histone variants. I will present evidence for both positive (H2A.Z, H3K4me3) and negative (H3K9me2) roles for epigenetic information controlling recombination patterns. Understanding these mechanisms has the potential to modulate meiotic recombination in the context of crop breeding, for example, by releasing heterochromatic recombination which limits improvement of many agriculturally important species.

T5-14-02

Meiotic chromosome remodeling driven by germline-specific Argonaute in rice

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2. SOKENDAI/Graduate University for Advanced Studies

Phased small RNA (phasiRNA) is a distinct class of small RNAs in plants, and in monocots, abundantly expressed in reproductive organs. We discovered that 24-nt phasiRNA biogenesis was triggered by a transcription factor exclusively expressed in anther tapetum in rice. In addition, a subset of the 24-nt tapetum-of-origin phasiRNAs were bound by germline-specific Argonaute protein, MEL1, suggesting a mobile nature of phasiRNAs from somatic tapetal cells to male germ cells. Downstream of the MEL1 regulatory cascade involves large-scale meiotic chromosome remodeling (LMR), accompanying dynamic alteration of histone H3 lysine-9 dimethylation. The epigenetic relation of LMR to reproductive small RNAs will be discussed.

T5-14-03

Bigdata epigenomics reveals hierarchical regulation of DNA methylation in *Arabidopsis*

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Genome-wide characterization by next-generation sequencing has greatly improved our understanding of the "landscape" of epigenetic modifications. Since 2008, whole-genome bisulfite sequencing (WGBS) has become the gold standard for DNA methylation analysis and a tremendous amount of WGBS data has been generated by the research community. However, methods for the systematic comparison of DNA methylation profiles to identify novel regulatory mechanisms have yet to be established. Here we developed a standardized pipeline and re-analyzed over five hundred publicly available *Arabidopsis* WGBS libraries from various mutant backgrounds, tissue types, and stress treatments. In total this collection included more than 3,700 Gb (Giga base-pairs) of sequencing data and a large number of wild-type controls. This enabled us to identify "high-confidence Differentially Methylated Regions" (hcDMRs) with high reliability by comparing each non-wild-type library to each of the 54 controls. We developed two statistical methods, Statistical Measurements on Overlapping of DMRs (S-MOD) followed by Quantitative Measurements on Overlapping of DMRs (Q-MOD), to analyze the hcDMRs, and identified a hierarchical network regulating non-CG methylation, as well as novel connections between methylation pathways. Our bigdata-methylome approach has established a framework for extracting biological insights via large-scale comparison of methylomes, and can also be adopted for other omics datasets. Together, the results demonstrate the effectiveness of data-driven, hypothesis-generating epigenetic research.

T5-14-04

Establishing and interpreting DNA methylation in *Arabidopsis thaliana*

Julie Law
Salk Institute

DNA methylation is a highly conserved epigenetic modification associated with gene silencing in eukaryotic organisms. Although pathways controlling the establishment, maintenance and removal of DNA methylation have been identified, relatively little is understood about how the DNA methylation machinery is targeted to transposons and repeats or how DNA methylation influences gene expression. Using the plant model *Arabidopsis thaliana*, we have recently identified several protein complexes involved in these key aspects of DNA methylation. Using a combination of genetic, genomic and biochemical approaches were are beginning to uncover the mechanisms through which these proteins function, which will provide insight not only into normal biological processes but also into how alterations in chromatin modifications can lead to aberrant gene expression and developmental defects.

T5-14-05

DNA replication-coupled histone deposition and modification maintains polycomb silencing

Danhua Jiang, *Frédéric Berger*
Gregor Mendel Institute

The developmental fate of a cell is specified by its transcriptional program, which is regulated by chromatin-associated mechanisms. Maintenance of repressive transcriptional states by Polycomb group (PcG)-mediated histone H3 lysine 27 trimethylation (H3K27me3) is conserved in animal and plant development. H3K27me3 is deposited by Polycomb repressive complex 2 (PRC2) and recognized by Polycomb repressive complex 1 (PRC1) in a feedback loop that maintains silencing. When cell fate is established in cycling cells, H3K27me3 needs to be properly restored after the dilution caused by DNA replication to maintain transcriptional repression. Recent studies in different animal models have described distinct mechanisms for H3K27me3 inheritance, suggesting a complexity in H3K27me3 transmission. Here we show that histone incorporation and H3K27me3 deposition are coupled at the DNA replication fork during the plant cell cycle. We find that PcG proteins localize at the DNA replication sites and directly interact with the chaperone that deposits replication-dependent histone H3 variant H3.1. Deposited H3.1 at the replication fork serves as a substrate for trimethylation at K27, and safeguards faithful inheritance of H3K27me3 after DNA replication. Moreover, incorporation of H3.1 facilitates H3K27me3 dynamics during developmental transitions. Our results demonstrate coordination between DNA replication and the deposition and modification of histones, shedding light on how epigenetic marks are transmitted in cycling cells.

T5-14-06

Chromatin signatures at distal regulatory regions in plant genomes

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2. University of Minnesota

A handful of long-distance transcriptional regulatory regions have been described in plants (e.g. the enhancers located ~60 kb upstream of the maize *tb1* gene). However, the prevalence, characteristics and functional importance of long-distance regulatory regions in plant genomes remain poorly understood. By profiling and comparing genome-wide patterns of chromatin accessibility, DNA methylation, RNA polymerase activity as well as histone modifications and variants, we identified thousands of putative regulatory regions in the intergenic space in maize with an average size of ~1.2 kb and average distance to the nearest gene of ~30 kb. These regions are devoid of heterochromatic features such as DNA methylation or H3K9me2, but are associated with distinct sets of chromatin signatures that resemble those found at proximal transcription factor binding sites. The identification, characterization, large-scale functional validation and evolutionary analyses of these long-distance regulatory regions will be presented.

T5-15: Biological studies of mosses and algae

T5-15-01

Molecular phylogeny and morphological diversity of inland *Cladophora* (Cladophorales, Ulvophyceae) from China

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Cladophora has a global distribution and provides habitat and food for numerous organisms. However, the taxonomy of *Cladophora* remained confused and poorly defined. In this study, 69 *Cladophora* freshwater and brackish water samples from inland China were collected and their morphological and phylogenetic analysis were performed. The Concatenated molecular phylogenies based on rDNA sequences revealed that these inland samples belong to two groups: freshwater *Cladophora* and non-freshwater *Cladophora*. The phylogenetic results showed that there were 8 clades in the freshwater group. The results of morphological observation showed characteristic similarities among clades and samples in each clade exhibited different degrees of phenotypic plasticity. The prediction model for ITS2 secondary structure showed that freshwater *Cladophora* contains one ring structure with three main helices (Helix I–III), in which Helix III-2 region is most variable. The high phenotypic plasticity and lack of diagnostic characters of freshwater *Cladophora* group made delineating species difficult and challenging. Further sampling from a broader range and combining with more efficient molecular markers would be necessary to clarify the taxonomy of *Cladophora* in the future.

T5-15-02

The application of large datasets to understanding the ecological and evolutionary diversification of diatoms

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Despite their extraordinary diversity and global importance as primary producers, anchors of aquatic food webs, and biogeochemical cyclers of carbon, oxygen, and silica, efforts to understand basic questions about the phylogeny and evolution of diatoms have lagged behind those of many other groups. We present results of two parallel efforts to better understand the phylogeny, diversification, and ecological history of diatoms. The first effort builds upon a growing genomic and transcriptomic dataset for diatoms, leveraging hundreds of molecular markers to resolve parts of the diatom tree that have presented a challenge for analyses based on fewer characters. A complementary effort that compiled all existing sequence data for 11 genes from 1100 diatom taxa was used to to better understand patterns of character evolution, habitat shifts, and species diversification from a much denser sample of diatom diversity. Results from these projects are providing new insights into the historical importance of transitions between the plankton and benthos and marine and freshwaters. They further highlight that shifts in the mode of sexual reproduction and the evolution of active motility set in motion a radiation that produced the majority of present-day diatom diversity.

T5-15-03

The biosynthesis and transcriptional regulation of a novel sunscreen compound in desiccation-tolerant terrestrial cyanobacteria

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The mycosporine-like amino acids (MAAs) have strong UV absorption and protect cyanobacteria against UV-B damages. However, the molecular mechanisms linking the UV-B signal transduction and MAA biosynthesis remain largely unknown. Here, a gene cluster with five putative members (*mylA-mylE*) for MAA biosynthesis in *Nostoc flagelliforme* was identified and their expression levels were significantly increased under UV-B exposure. A LuxR family protein OrrA was identified as a positive UV-B responsive regulator binding to the promoter region of *mylA-mylE* gene cluster. OrrA functions as a UV-B activator mediating the UV-B induced *mylA-mylE* gene cluster expression and the MAA biosynthesis in *N. flagelliforme*. Heterologous expression of this gene cluster in *Anabaena* PCC 7120 produces the same MAA as that in *N. flagelliforme*. The assigned MAA structure of mycosporine-2- (4-deoxygadusolyl-ornithine) with molecular formula of $C_{34}H_{52}O_{15}N_4$ is confirmed based on proton and carbon NMR of purified MAA from transformant of *Anabaena* PCC 7120. This compound has a molecule weight (M+H) of 757 Da, the largest MAA compound reported to date. The common precursor of MAA (4-deoxygadusol) was synthesized from sedoheptulose-7-phosphate and catalyzed by the products of the first two genes (*mylA-mylB*) in the cluster. The final product of MAA, mycosporine-2- (4-deoxygadusolyl-ornithine), is catalyzed by the three proteins encoded by genes of *mylC-mylE* in the same gene cluster. The new transcriptional mechanisms and novel MAA molecular structure found in the desiccation-tolerant cyanobacterium *N. flagelliforme* suggest the common physiological significance of MAAs and their chemical modifications associated with evolutionary UV protection strategies.

T5-15-04

Insight on the genomics and epigenetics of *Microcystis*

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Microcystis blooms occur in freshwater lakes, estuaries and rives worldwide. They present major challenges in ecosystems, social economy and water resources management, for the accumulation of vast biomass, as well as the potent cyanotoxins they release. The genomic evolutionary strategy of this cyanobacterium is not well-deciphered, which is mainly due to the low syntenic value each isolate' genome shares and the lack of sufficient complete genomic data. In this work, the genome of *Microcystis* model strain PCC 7806 was integrated using Single Molecule, Real-Time (SMRT) sequencing technology. Accompanied with three more newly released high quality *Microcystis* genomes data, comprehensive genomic comparison was conducted and the evolutionary strategy of this genus emerged. We suppose that the *Microcystis* genomes evolution is a process of genome expansion. That is, strains with small genome sizes are closer with ancestral *Microcystis*, expansion process is driven by transposases mediated gene rearrangement accompanied with frequent gene loss and gain through horizontal gene transfer (HGT), and then increased-genomic size strains emerges. We wish that this finding could help in the taxonomy of *Microcystis* and further set global eyes on the cosmopolitan distribution of this genus. Since SMRT can provide us accurate and intact genomic DNA methylation modification profiles (methylomes) as by-product in genome sequencing, DNA methylation modification survey was also carried out in this cyanobacterium. According to our results, *Microcystis* occupies vast and diversified methylomes, as well as restriction-modification (R-M) systems. A total of 200 methyltransferases were found in five strains, while only 20 were shared by all. And NIES-843 strain contains the largest methyltransferases amount when compared with 3,861 prokaryotic collections. We also concluded that the tremendous R-M systems in *Microcystis* mainly caused by 1) intra-genome replication and mutation, 2) insertion and substitution through HGT-mediated mechanisms. At last, the potential regulative role of DNA methylation modification emerged when we conducted RNA-seq investigation in NIES-843 and PCC 7806 under series of nutrient stresses. This study is anticipated to provide epigenetic clues on some critical bloom questions.

T5-15-05

Proteomic analysis of post translational modifications in cyanobacteria

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Cyanobacteria are a diverse group of Gram-negative bacteria and the only prokaryotes capable of oxygenic photosynthesis. Recently, cyanobacteria have attracted great interest due to their crucial roles in global carbon and nitrogen cycles and their ability to produce clean and renewable biofuels. To survive at various environmental conditions, cyanobacteria have developed a complex signal transduction network to sense environmental signals and implement adaptive changes. The post-translational modifications (PTMs) systems play important regulatory roles in the signaling networks of cyanobacteria. Mass spectrometry (MS)-based proteomic technologies have been applied to identify PTMs in a systematic manner. Although the proteomic studies of PTMs carried out in cyanobacteria were limited, these data have provided clues to elucidate their sophisticated sensing mechanisms that contribute to their evolutionary and ecological success. We will summarize the current status of PTM studies and recent publications regarding PTM proteomics in cyanobacteria, and provide the novel developments and applications for the analysis of PTMs in cyanobacteria. The results of functional studies of different PTMs in cyanobacteria suggested that these PTMs may affect photosynthesis and carbon metabolism in cyanobacteria as well as other photosynthetic organisms.

T5-15-06

Bioaccumulation of selenium and selenoprotein gene cloning in *Haematococcus pluvialis*

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Selenium (Se) is one of the most interesting trace elements because it is both essential and toxic for most species, with a very narrow range between deficiency and toxicity. Algae can uptake and accumulate Se, which can be either essential or toxic to algal growth depending on the dosage and species. In green alga *Haematococcus pluvialis*, an important industrial algal resource for natural astaxanthin, we investigated the effects of Se on this unicellular organism. The results showed that EC_{50} for the algal growth rate was 24 mg/L, and that a low dosage of selenite (3 mg/L) may not hinder *H. pluvialis* cell growth, but selenite at levels higher than 13 mg/L do restrain cell growth. Bioaccumulation experiments showed that *H. pluvialis* accumulated up to 646 g/g total Se and 380 g/g organic Se, dry weight. However, treatment with high concentrations of selenite significantly increased intracellular hydrogen peroxide levels, antioxidant enzyme activity, and the production of astaxanthin. Thus, *H. pluvialis* is an ideal organism for Se bioaccumulation and organic Se transformation. Microalgae assimilated Se can incorporated in the form of SeCys as the backbone of essential selenoproteins. Among the selenoenzymes in *H. pluvialis*, a thioredoxin reductase (TR) has been identified, which is a mammalian-type NADPH TR containing a SeCys residue and type I *cis*-acting Sec insertion sequence (SECIS) element of eukaryotes in the 3′-UTR region. TR as a selenoprotein is involved in many cellular processes and mainly serves as a key member of the thioredoxin system, which is a crucial antioxidant system in defense against oxidative stress. Molecular properties of HpTR1 were analyzed. The full-length cDNA of HpTR1 consists of 2506 base pairs encoding a putative 535-amino acid product. The conformation of HpTR1 has been characterized via sequence analysis and protein structure modelling. A comprehensive phylogenetic tree of TR1 in animals and algae was constructed, which provided an evolution history of selenoprotein from aquatic to terrestrial environment. The expression pattern of TR1 mRNA and TR activity assay indicate that Se is an important regulator of HpTR1 and plays different roles in a dosage dependent manner.

T5-16: New frontiers in genetics and molecular biology

T5-16-01

R-loop is a general chromatin feature in *Arabidopsis* genome

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A three-stranded nucleic acid structure is universally formed in the process of transcription, as the nascent RNA molecule will hybridize with the template DNA strand forming a DNA:RNA hybrid and leaving the non-templated DNA single stranded. Such structures are termed R-loops and have been found in various organisms. However, we still have limited understanding of formation and preferences of R-loops in plant genome. By using the newly developed sDRIP-seq, an efficient, unbiased, high throughput and strands-specific method, we have been able to identify thousands of R-loop loci in *Arabidopsis* genome. Comprehensive analysis showed that R-loops are strongly correlated with many chromatin modifications. Our data suggests R-loop is the common feature of *Arabidopsis* genome organization. We will present our results in this meeting about genome wide R-loops characterization, and discuss their biological functions in plants.

T5-16-02

Hybrid seed failure among wild tomato lineages: Perturbed genomic imprinting and expression in the developing endosperm

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Endosperm misdevelopment leading to hybrid seed failure (HSF) is a frequent cause of postzygotic reproductive isolation in angiosperms. This phenotype is found in interploidy and homoploid crosses between closely related species. In a variety of studies, histological evidence points to impaired endosperm development at early stages of seed development in crosses yielding inviable mature seeds. Parent-of-origin-dependent expression (that is, genomic imprinting) is thought to be necessary for normal endosperm development. The HSF phenotype might thus be impacted by differences in patterns of genomic imprinting between parental species. The parental conflict theory posits that imprinting is driven by divergent parental interests in outcrossing lineages. Imprinting status may evolve relatively rapidly, thus creating incompatibilities between nascent species. To date, published studies have not yet addressed the potential correlation between imprinting divergence and HSF. The tomato clade (*Solanum* section *Lycopersicon*), encompassing at least 13 closely related species with partial-to-complete HSF phenotypes and diverse mating systems, provides outstanding opportunities to study these issues. For the current study, we employed intra- and interspecific crosses of three diploid, self-incompatible tomato lineages. *Solanum arcanum* var. marañón, *S. chilense*, and *S. peruvianum* were chosen for their recent divergence and partial to near-complete reciprocal HSF. Based on transcriptome sequencing of laser-extracted endosperm, we quantified the extent of shared imprinting among the three reciprocal intraspecific crosses. Candidate imprinted genes have higher mean expression levels compared to non-imprinted genes, implying that specific mechanisms contribute to their apparent upregulation. In the two hybrid crosses with strong HSF, maternal proportions are increased and imprinting is systematically perturbed. Such marked changes were not detected in the hybrid cross resulting in partial HSF (*S. arcanum* var. marañón, *S. chilense*), suggesting that the extent of misexpression covaries with the degree of HSF. Future inclusion of self-compatible tomato species may reveal whether imprinting dynamics is driven by parental conflict. This can be addressed by quantifying the proportions of imprinted genes across a wider range of species, and by studying the molecular evolution of 'conserved' imprinted genes among both self-compatible and self-incompatible lineages.

T5-16-03

Coordinate sister chromatid segregation during pollen development

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Asymmetric cell divisions play a crucial role during development, for instance to set up distinct cell lineages. The polar localization of internal cellular factors or external signals has often been implied as a mechanism underlying asymmetric cell division.

However, processes affecting sister chromatid segregation during mitosis, generally thought to be random, could also be a decisive factor. For instance, the sister chromatids could carry distinct epigenetic marks or associated proteins, such that their non-random or directed segregation would set up a difference between the resulting daughter cells. Although the directed segregation of marked sister chromatids has been proposed to underlie a variety of biological phenomena, the methods to study such a mechanism has been limited because sister chromatids are genetically identical and mitotic recombination complicates cell biological analyses. We developed a generally applicable method based on labeling sister chromatids, isolating individual nuclei of daughter cells, and single cell genomic sequencing to investigate directed sister chromatid segregation. We applied this method to study whether directed sister chromatid segregation occurs during pollen development, which is characterized by an asymmetric division that gives rise to vegetative and generative cell, the latter producing the two sperm cells and thus contributing to the next generation. During the synthesis phase of the cell cycle both DNA strands (referred to the Watson and Crick strand) of a chromosome serve as a template for the synthesis of the two new DNA strands. Of the resulting sister chromatids, one has an old Watson but a new Crick strand, the other has the old Crick but a new Watson strand. For instance, the immortal strand hypothesis postulates the directed segregation of the replication-error-free template strands into the daughter cell retaining stem cell fate to prevent stem cells from accumulating replication errors. We used strand-specific single cell genomic sequencing of DNA isolated from vegetative and sperm cells, respectively, allowing us to track sister chromatid segregation during pollen development. While the segregation of the old strand to either vegetative or generative cell was random, we observed the old strands of all chromosomes to segregate coordinately into one of the daughter cells. The unexpected finding of coordinate sister chromatid segregation was confirmed using an independent, fluorescence-based method and seems to be evolutionarily conserved in plants and animals.

T5-16-04

Transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA, RNA or RNP

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Crop improvement requires the constant creation and use of new allelic variants. Conventional breeding can be limited in providing the genes and alleles required to meet the agricultural challenges. In the past decade, Genome editing can accelerate plant breeding by allowing the introduction of precise and predictable modifications directly in an elite background. The most promising utilization of both the CRISPR/Cas9 system and TALENs can be used to generate targeted genome modifications including mutations, insertions, replacements and chromosome rearrangements. Typically, TALEN or CRISPR expression cassettes are delivered to plant cells and expressed, which cleaves chromosomal target sites and produces site-specific DNA double-strand breaks (DSBs), leading to genome modifications during the repair process. We developed simple and efficient genome editing approaches in which wheat plants are regenerated from callus cells transiently expressed with CRISPR/Cas9 reagents introduced as DNA, RNA or RNP. The effectiveness of the three methods in yielding specifically targeted, transgene-free mutants in T_0 generation was validated using six different wheat genes. This approach may be widely applicable for producing genome edited crop plants and has a good prospect of being commercialized.

T5-16-05

Using Structure-seq to probe RNA structure-function relationships *in vivo* and genome-wide in *Arabidopsis* and rice

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Due to the single-stranded nature of RNA, a given RNA molecule can fold into different structures depending on the surrounding physico-chemical environment. We recently developed a method, Structure-seq, which combines chemical probing of RNA structure with high throughput sequencing to allow characterization of RNA secondary structure genome-wide and *in vivo*. Structure-seq relies on covalent modifications by dimethyl sulfate at the Watson-Crick face of unpaired As and Cs which are read out as stops to reverse transcription. This information is then used to restrain structure prediction based on free energy minimization algorithms. Application of Structure-seq to Arabidopsis reveals significant correlations between DMS reactivity and alternative polyadenylation, alternative splicing, and gene function. We are currently applying Structure-seq to both Arabidopsis and rice with the goal of uncovering general principles that govern changes in RNA secondary structure induced by abiotic stresses, particularly temperature and salinity, and their functional consequences.

T5-16-06

Genome-wide fishing expeditions: chromatin features of regulatory regions in *Arabidopsis thaliana* and *Zea mays*

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One fundamental question remains how eukaryotic cells sharing the same genome sequence develop distinct transcriptional responses to external and internal cues. The expression of distinct sets of transcription factors is one part of the answer. Furthermore, the propensity of genes to respond to transcription factors is altered between cell types due to epigenetic variation at regulatory regions, which may be located at considerable distance from their target genes. Recent progress in the characterization of chromatin features allows an approach to pinpoint candidate regulatory regions by making use of conserved chromatin features. I will present examples of distal plant enhancers in *Arabidopsis thaliana* and *Zea mays*. To predict transcriptional enhancers in the crop plant maize, we integrated available genome-wide data on DNA methylation with newly generated maps for chromatin accessibility and Histone 3 Lysine 9 acetylation (H3K9ac) enrichment in young seedling and husk tissue and classified 1,500 intergenic regions as enhancer candidates. Enhancer candidates were linked to putative target genes based on tissue-specific chromatin and expression patterns. Last, I present data illustrating that siRNA induced methylation can be a versatile method to validate enhancer candidates *in vivo*.

T5-17: Genomics and domestication of food crops

T5-17-01

The origin of economically important species of *Cucumis*, *Citrullus*, and *Momordica* – combining Chinese genomics know-how and old European herbaria to find wild relatives

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China is World-leading in Cucurbitaceae genomics, having produced the draft genomes of cucumber, *Cucumis sativus* (Huang *et al.,* Nature Genetics 2009; also Qi *et al.,* Nature Genetics, 2013; Shang *et al.,* Science 2014; Zhou *et al.,* Nature Plants 2016) and watermelon, *Citrullus 'lanatus'* (Guo *et al.,* Nature Genetics 2013). Old-fashioned Sanger sequencing over the past 10 years, however, has revealed numerous misclassifications of material of Cucurbitaceae, from simple misidentification of germplasm to serious misplacements of species in unnatural genera, meaning that names do not reflect evolutionary relationships and cannot be trusted to guide sampling for genomic projects. The family Cucurbitaceae is underrepresented in herbaria because wild cucurbits often are climbers in disturbed habitats or desert plants that are hard to find at the right stage. Fruits of the family are difficult to preserve. Only with DNA barcoding has it become possible to figure out what is what (simply put). In the talk I will illustrate how molecular data are revolutionizing our knowledge about the wild relatives of *Cucumis sativus* (in India, Thailand, and tropical China), *Cucumis melo* (in India and Australia), *Citrullus lanatus* (in West or Northeast Africa), *Momordica charantia* (in Africa) and *Luffa* (in India and Australia), using examples of recent and ongoing work in my lab, in collaboration with genomics experts from China.

T5-17-02

Meiotic and epigenetic behaviour of alien chromatin enabling exploitation of novel variation

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Exploitation of the Triticeae gene pool through chromosome engineering and introgression has been the most effective way to introduce novel traits to wheat breeding lines. Transfer and expression of alien chromatin in wheat is critical to access more genetic variability. Many wheat varieties carrying rye or *Thinopyrum* chromatin show increased resistance to biotic and abiotic stresses. Genomic *in situ* hybridization (Schwarzacher *et al.,* 1992) identifies alien chromatin in hybrid and introgression lines and, combined with repetitive DNA probes, we have identified novel translocations, chromatin segments and multiple alien introgressions. The wheat variety 'Mace' shows wheat streak mosaic virus resistance and carries *Th. intermedium* chromatin as a translocation. Sister lines of 'Mace' were found to carry additionally small segments of rye on the short arm of 1B (Ali *et al.* 2016). Other lines investigated carry various intercalary *Th. bessarabicum* translocations involving different recipient wheat chromosomes, wheat-barley addition lines and different rye translocations. Alien chromatin transfer uses wheat x alien hybrids followed by back crossing and loss of most alien chromatin. Loss processes are not controllable but large numbers of lines can be screened by phenotype, markers, and/or chromosomal constitution. It is important to generate recombinant wheat-alien chromosomes to introduce small segments of the alien chromatin so only small numbers of genes carrying desirable traits are transferred. We studied mitotic and meiotic processes to understand stable inheritance of chromosomes to the next cell generation through mitosis; and pairing, recombination and segregation at meiosis. Centromere behaviour is critical for meiotic events and we have investigated epigenetic modification of centromeres using immuno-staining with the centromere specific histone variant CENH3 (Sepsi *et al.,* 2016). We studied DNA methylation and histone modifications to understand the behaviour and epigenetic interactions of alien chromatin in a wheat background. To exploit the diversity of alien species in wheat breeding, we need not only be able to identify alien chromatin segments and specific genes, but also control chromosome stability, meiotic behaviour and gene expression.

T5-17-03

The domestication and modern breeding in rice

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The domestication of cultivated rice is one of the most important developments in history. However, its origins and domestication processes are have long been debated. We collected a large number of diverse rice accessions from wild rice, landraces and modern cultivars. In the search for signatures of selection, we identified 55 selective sweeps that have occurred during domestication. In-depth analyses of the domestication sweeps revealed that japonica rice was first domesticated from Oryza rufipogon in southern China, and indica rice was subsequently developed from crosses between japonica rice and local wild rice in South East and South Asia. Furthermore, through population genetics approaches we identified 20 selective sweeps during modern breeding in rice, the pattern of which was quite different from that in rice domestication. These works provide an important resource for rice breeding and functional genomics studies.

T5-17-04

Genomic history of vegetable domestication

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The majority crop today is domesticated from their wild ancestors. Aid by analyses of 360 tomato genomes, we identified 111.0 Mb sweep regions and re-built the genomic history of tomato. A total of 13 fruit weight QTLs were subjected to domestication and play important parts in a more than 100-fold fruit weight increase in modern tomato than its wild ancestor. As an important resource of vitamins, minerals and other nutritious in daily diet, how breeding have changed metabolome of tomato fruits was largely unknown. By multiple-omics integration of variome, transcriptome and metabolomes, we identified 3,426 mGWAS (metabolites genome-wide association study) signals for 514 metabolites and 42.26% signals were supported by eQTLs (expression quantitative trait locus). We found the genetic basis of how steroidal glycoalkaloids (SGAs) were domesticated and identified a UDP-transferase centered genes clusters functioning during fruit ripe process. Fruit mass is an important domesticated trait, and we take fruit weight *fw11.3* as an example to illustrate how selection for bigger fruits hitchhike neighboring genes that altered multiple metabolites. Color-targeted selection produced pink tomato divergence, and more than 100 metabolites were identified to underlie this phenotype. Gene editing of the transcription factor *Myb12* reveals the comprehensive metabolic and transcriptional networks involving 261 metabolites and 869 genes. To tap the genetic potential and elite traits from wild resource, exotic genomic fragments were introgressed into elite cultivars. We found many metabolic evidences and unexpected changes in fruits through the case of Tm-^{2} resistance genes from *S. peruvianum*, and the changed chemicals might contribute to its pathogen resistance. Wild cucumber plants bear extremely bitter fruits, a trait conferred by the dominant *Bt* gene. Non-bitterness of cultivated cucumber fruit is conferred by *bt*, a domestication gene. We previously mapped the *Bt* locus to a domestication sweep on chromosome 5, and further fine mapping delimited it to a 442-kb region that harbors 67 predicted genes. By RNA-seq, we considered *Csa5G157230* to be a good candidate for *Bt*, given that it is specifically expressed in the fruit of the wild line PI 183967, consistent with the distribution of cucurbitacin C in wild cucumber. In addition, clear positive correlations were observed between the expression levels of *Csa5G157230* and *Bi*, and between fruit bitterness and gene expression, especially in 5 ancient lines, whose fruits are extremely bitter. A local association analysis was next performed using all variants within the 442-kb region to identify further genetic variants associated with the extremely bitter phenotype. We identified two key mutations within the regulatory region of *Csa5G157230*, one structural variant SV-2195 and an SNP-1601. SV-2195 mainly exists in five extremely bitter ancient lines, leading to a dramatic decrease in fruit bitterness. However, we discovered that SNP-1601 is essential for conditional bitterness in cucumber fruits, a phenomenon in some cucumber lines whereby fruits become bitter under stress conditions. When the mutation corresponded to SNP-1601 is ‘A’, fruits were non-bitter even under low temperature conditions.

T5-17-05

Subgenomic loss, morphorlogical and expression changes during wheat polyploidization events

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Polyploidization has been recognized as a major force in driving and shaping the evolution and speciation of vascular plants. The nascent polyploidy organisms have to reconcile immediate challenges of divergent subgenomes in one nucleus to secure an exclusive intra-genome pairing and orchestrated inter-genomic gene expression in the initial generations to adapt to the environment. However, to what extent polyploids modifying genomic changes and by what molecular means polyploids accommodating environmental constraints are still a challenging question. Hexaploid wheat (BBAADD) is a typical allopolyploid species with three distinct subgenomes and has undergone two separate allopolyploidization events, providing an ideal model for studying polyploid plants. By exploiting three sets of synthetic hexaploid wheats and whole exome capture coupled with RNA-sequencing methods, we observed that the proportion of genomic sequence loss was significantly higher in the inter-genetic region than in other regions during polyploidization events. In addition, subgenome D experienced more sequence loss compared with subgenome A and B. Furthermore, different chromosomes exhibited distinct genomic modification features during wheat hexaploidization scenario, and the sequences with high similarities among wheat subgenomes are preferentially lost. In addition, we also examined gene expression trends of wheat homeologs during polyploidization, and found that up to 20% of genes showed non-additive expression patterns in leaf and root. Based on the high-throughput transcriptome sequencing of hexaploid seedlings under normal and stressed conditions, we presented that *c*. 68.4% of homeologous genes exhibited expression partitioning, which possibly contributes to the wide adaptability and distribution of hexaploid wheat in various environments. 1) The homeologs of *TaEXPA1-A* and *TaEXPA1-D* was expressed while *TaEXPA1-B* was silenced in wheat seedling leaves. Chromatin immunoprecipitation assays revealed that the transcriptional activation of *TaEXPA1-A* and *TaEXPA1-D* was correlated with increased levels of H3K4 trimethylation and H3K9 acetylation, and decreased levels of H3K9 dimethylation in their promoters, respectively. Moreover, a higher level of cytosine methylation was detected in the promoter region of *TaEXPA1-B*, which may contribute to its silencing in leaves; 2) The increased root hair length in allohexaploid wheat originated in the allotetraploid progenitors, and subgenome A biased expression of *TaRSL4* gene by genome interplay shapes root hair length in allopolyploid wheat and consequently lead to enhanced shoot fresh biomass under nutrient-poor conditions; 3) Under salt stress, synthetic hexaploid exhibited higher fitness than both of its parental genotypes, including fresh weight of aerial part, chlorophyll content and K^{+}/Na^{+} ratio. Moreover, the expression of the *TaSOS1* gene, a plasma membrane Na^{+}/H^{+} antiporter, showed an upregulated transcription in the synthetic allohexaploid wheat than its tetraploid and diploid parents. Among of this, *TaSOS1-D* homeolog showed a high expression than A and B homeologs, suggesting that the salt stress induces enhanced expressivity of the D-subgenome homeologs in the synthetic hexaploid wheat.

T5-18: Bioinformatics resources for comparative functional and phylogenetic analysis of plant genomes, pathways and diversity

T5-18-01

Gramene Ensembl Browser: A resource for browsing & comparing plant genomes

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The Gramene database (www.gramene.org) is an integrated resource for comparative genomics and pathways in plants. The Ensembl software powered genome browser is the comparative genome portal of Gramene, and is built collaboratively with Ensembl Plants. As of Jan 2017, Gramene Genome Browser hosts 44 complete reference genomes spanning across a wide range of plant kingdom, while maintaining a deep coverage on agronomic importance species, such as grasses. For each genome, Gramene provides a standard set of baseline annotations including protein-coding genes, Interpro domains, GO, cross-reference to other sources, repeats, DNA features such as ESTs. Depends on the data availability, resources to support integration and community interest for some genomes, Gramene also hosts variation data such as SNP and genetic markers (SSR, RFLP), population data with genotype/phenotype, noncoding genes, expression data (RNAseq/microarray), and epigenomic data (methylation). To elucidate evolutionary history of these genomes, Gramene performs genome-wide comparisons at both the protein and DNA level. For protein comparisons, Gramene provides phylogenetic gene trees predicted by EnsemblCompara GeneTrees pipeline. The gene trees provide orthologs/paralogs reference standards for the community. Derived from ortholg prediction, Gramene conducts syntenic regions mapping between a subset of the genomes, using rice, grape and arabidopsis as reference for monocots and dicots. These gene and chromosome comparisons provide a standard reference for cross-species comparisons and characterization conservation of structural and functional features. At the DNA level, Gramene employs Ensembl's Whole Genome Alignment (WGA) pipeline to generate genomic scale alignments between selected genome pairs with the promise to help identify large-scale structure variations and conserved non-coding regulatory features. In addition to serving as a data resource for the community, Gramene also provides many useful tools/functions to help scientists conduct their research. The Gramene browser allows users to add their own data under views such as Location and Karyotype to display it along with other Gramene data; Variation Effect Predictor (VEP) tool allows users to predict the effect of their SNPs in a reference genome; AssembyConverter enables user to transform genomic coordinates across different assembly versions of the same species; BLAST tool serves as an entry point for browsing the region of interest given a pieces of sequence; BioMart tool enables complex queries of sequence, annotation, homology, and variation data, and provides an additional gateway into the genome browsers; Gramene search tool, a recent development from Gramene, provides an unified search against integrated databases of both genomic data and pathway data, results shown interactively in aggregate and gene-context views. Gramene is supported by NSF grant IOS-1127112, and partially from USDA-ARS 1907-21000-030-00D.

T5-18-02

Gramene's Plant Reactome portal: A resource for comparative plant pathway analysis

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Gramene's pathway portal Plant Reactome (http://plantreactome.gramene.org/) features *Oryza sativa* (rice) as a reference species for the curation of cellular-level pathway networks by employing the Reactome data model that organizes metabolites, proteins, enzymes, small molecules, and various macromolecular interactions into reactions and pathways in the context of their subcellular location within a plant cell. This reference set of curated rice pathways is being used to generate gene-orthology based pathway projections for 66 additional species, including plants and photosynthetic microbes, with sequenced genomes and/or transcriptomes. Currently, the Plant Reactome hosts various types of pathways, including metabolic, transport, genetic/regulatory, developmental and signaling pathways. Users can compare reference rice pathways with the projected pathways from any plant species from the available list. The pathway clustering across the broad phylogenetic spectrum of photosynthetic organisms shows distinct gene-pathway association patterns reflecting evolutionary history and ploidy levels. Plant Reactome also provides analysis tools for the visualization and analysis of omics data, links to external sources that provide detailed information on various pathway entities (e.g., Gramene's species-specific Ensembl Genome browser and gene pages, UniProt, ChEBI, PubChem, PubMed, GO *etc.*). Users can access data programmatically via APIs or download it in various standard formats. The Gramene project is supported by an NSF award (IOS-1127112). The Plant Reactome database is produced with intellectual and infrastructure support provided by the Human Reactome award (NIH: P41 HG003751, ENFIN LSHG-CT-2005-518254, Ontario Research Fund, and European Bioinformatics Institute (EBI) Industry Programme).

T5-18-03

Population genomic analyses of resequenced *Amborella trichopoda* individuals reveal chromosomal regions of selection and genetic diversity associated with local environmental conditions

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Amborella trichopoda, an understory shrub native to wet tropical forests in New Caledonia, is the sole living species in the sister lineage to all other extant angiosperms. A draft genome of *Amborella* (*Amborella* Genome Project, 2013) has served as a guide to infer the genome structure of the most recent common ancestor of extant angiosperms. As part of the *Amborella* Genome Project, the genomes of 12 individuals from the native range in New Caledonia were resequenced. Population genomic analyses of the 12 individuals revealed high levels of genetic diversity for such a geographically restricted species. Several population genomic metrics were computed across the scaffolds constructed as part of the original draft genome assembly. Some metrics varied greatly across the genome (e.g., some scaffolds were highly polymorphic and had variation in Tajima's *D* between positions on the scaffold, whereas other scaffolds were virtually invariant and had negative values of *D*), yet the fragmentary nature of the draft genome assembly made it unclear how these population genetic metrics and selection measures varied across chromosomes. The *Amborella* genomic assembly has now been improved; 13 chromosomal assemblies are now available to use as references for mapping reads of the 12 resequenced individuals. We used Bowtie 2 to map the reads from the 12 individuals to each of the chromosomal references and identified variants with SAMtools and FreeBayes. The software package MAPGD was used to estimate various population genomic statistics, such as allele frequencies, heterozygosity and *F*-statistics, across all chromosomes to locate hotspots in the genome that have unusually high or low variation among individuals. The R package PopGenome was used to calculate Tajima's *D* for each position on every chromosome to identify regions of the genome under especially low or high selection. We used locality data from digitized collection records (available from iDigBio; www.idigbio.org) and Bioclim environmental data (available from www.worldclim.org) to build ecological niche models using the software MAXENT to quantify suitable habitat throughout New Caledonia. We then tested for correlation between genomic regions under strong selection or genomic hotspots of variation and modeled environmental conditions in each of the sampling localities. Our analyses will identify regions on each of the 13 chromosomes that have unusually strong or weak selection, and we will pinpoint regions of chromosomes that are hotspots of genetic variation. By modeling environmental conditions, we can test for correlation between local environment and signatures of selection or high genetic variation in the genome. In addition to improving our understanding of selection and genetic variation across each chromosome, our results will serve as a resource for future investigations of the genomic origins of phenotypic variation in *Amborella*.

T5-18-04

Transcriptome single-molecule long-read sequencing of *Amborilla trichopoda*, and a genome-reference sequence independent method for detecting alternative splicing

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Alternative splicing (AS) is a major source of mRNA transcript diversity, proteome diversity, and functional innovation, but examining AS in species without well-annotated reference genomes is difficult. Thus, AS studies in non-model plants (e.g., naturally occurring species), which contribute to a majority of diversity, are very rare. We applied Iso-Seq™ to investigate AS in *Amborella trichopoda,* a phylogenetically pivotal species that is sister to all other living angiosperms. Our data show that, compared to RNA-Seq, the Iso-Seq™ platform provides better recovery on large transcripts, new gene locus identification, gene model correction, especially for AS detection. Reference-based AS detection with Iso-Seq™ data identifies AS within a higher fraction of multi-exonic genes than observed for published RNA-Seq analysis (45.8% vs. 37.5%). Using the Iso-Seq-defined transcript collection in *Amborella* as a reference, we also describe a pipeline for detection of AS isoforms from PacBio Iso-Seq™ without using a reference sequence (*de novo*). Results using this pipeline show a 66-76% overall success rate in identifying AS events. This *de novo* AS detection pipeline provides a means to accurately characterize and identify bona fide alternatively spliced transcripts in any non-model plant species that lacks a reference genome sequence. Hence, it has important genetic/genomic implications to the broader plant biology community.

T5-18-05

Biotaphy--Connecting resources to enable large scale biodiversity analyses

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Extracting biological knowledge from detailed, complex datasets such as those now being compiled requires integrated, powerful tools. Recent developments in phylogenetics coupled with emerging cyberinfrastructure and new data sources provide unparalleled opportunities for mobilizing and integrating massive amounts of biological data, driving the discovery of complex patterns and new hypotheses for further study. We have developed a cyberinfrastructure framework (Biotaphy) linking diverse data (phylogeny, morphology, ecology, fossils, geography, and climate). We are making these linkages across several data-driven community research tools, including Open Tree of Life (and other tree resources), providing a framework to which all other biological data-traits, genes, genomes, and especially specimens are linked, as well as Lifemapper (geospatial data, modeling and analyses), and iDigBio (~1 billion museum specimens that carry locality data and their implicit ecological information). These linkages provide researchers the opportunity to rapidly synthesize rich datasets and use them to perform detailed hypothesis tests addressing diverse evolutionary questions. Biotaphy tools and infrastructure can connect phylogeny with species distribution models, climate projections, genes and traits. As test cases (presented here) we have used: 1) a well-sampled phylogeny of vascular plants of Florida and 2) the hyper-diverse plant order Saxifragales provide excellent test cases for integrative study. Using complete (or nearly so) datasets that cover diverse traits (e.g., morphology, ecology, geography, fossils, and climate) provide motivating biological test cases for tool refinement and their integration. Such integration will be transformative for studies of biodiversity and provides one vision for how a global integration of powerful tools will lead to data-driven discovery in "next generation" biodiversity science.

T5-18-06

Phylogenetic structure (Rc, Rc, fix, Rc, fix (S)) and micro-structural evolution of organellar markers

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Non-coding plastid DNA sequences can be confidently used to infer evolutionary relationships at far deep level among angiosperms than previously assumed (e.g. Borsch 2003). In comparison with multi-gene approaches, the results even suggested that fast-evolving, non-coding regions are more effective in reconstructing phylogenies at high taxonomic levels. Subsequently this idea was tested by Mueller *et al.,* (2006) who showed that regions evolving close to neutrality are indeed more effective than slowly evolving genes. Later, Barniske *et al.,* (2012) provided more insight into the capability of noncoding regions to resolve phylogenetic backbones (in this case eudicots): spacer and intron regions clearly outperformed coding regions except for *matK* which ranked highest. Two questions remain: 1) does the neutrality assumption hold true for other evolutionary old lineages as well? 2) is this phenomenon also present in mitochondrial data? So far, all studies have been solely based on plastid data. To address these points, the divergence of the major moss lineages was chosen as further test case, as i) mosses represent another evolutionary old clade, and in fact older than angiosperms and ii) various non-coding mitochondrial markers are accessible for all lineages. We used extensions of the phylogenetic structure (R) framework in order to a) correct R for staggered sequence alignments (R_C), b) calculate Rc for a given topology ($R_{C,\ \text{fix}}$) and c) characterize the distribution of R on a subset of nodes. ($R_{C,\ \text{fix}}$ (S)). Our analyses imply that i) high functional constraints generally lead to low phylogenetic structure that is concentrated in phylogenetically old nodes, ii) evolutionary constraints of organellar non-coding regions of both compartments are similar, iii) plastid markers seem to be more informative than mitochondrial markers in general, iv) an unexpected high number of long SSRs, especially tri-, tetra-, and penta- SSRs are observed, and v) indels are less homoplastic than substitutions.

T5-19: Pan genome studies: Technologies, strategies and beyond

T5-19-01

Advances in genomic big data analysis to boost breeding of more productive crops: Wheat as an example

Gil Ronen
NRGene

Doubling crop plant productivity over the next 30 years is crucial to meet the world's future food requirements. Despite of the vast annual investment in breeding, the year-over-year increase in yield rates is half of that. A shift in breeding from random trial-and-error to a predictive process of defining and selecting favorable DNA combinations, is expected to accelerate yield gain. PanMAGIC is the first ever computational platform that can analyze complex plant genomes and mine DNA data of millions of individual plants in order to find the favorable DNA makeup leading to yield gains. PanMAGIC organizes the broad genomic diversity of any given crop into a pangenome database and uses it to impute the full genomic content of plants from very low-cost genotyping data. Genomic information is then matched with field performance data to discover the set of genes that favorably affect yield. The bread-wheat genome is a hexaploid, 16 Gb genome (five times larger than the human genome) with over 85% repeated sequences, whose assembly and analysis was impossible prior to PanMAGIC. This talk describes the first ever successful construction of the full reference sequence of bread wheat, and its immediate uses to create the wheat pangenome and support active breeding programs of wheat across the globe.

T5-19-02

Histone modifications of diploid and tetraploid cottons reveal global insight into gene activities and genome evolution

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In the whole processes of eukaryotic cell development and differentiation, functional genes are widely regulated in precise pattern, through various regulatory elements such as promoters, enhancers and suppressors. Histone modifications are highly correlated with the regulatory elements; H3K4me3, H3K27ac and H3K27me3 specifically label promoters, enhancers and suppressors, respectively. Genome wide regulatory elements are unraveled via high through-put Chip-seq experiments and systematic bioinformatics. We carried out 4 histone modification and 2 transcription factor Chip-seq experiments in tetraploid upland cotton Gossypium hirsutum (AADD) wild type and a fiberless mutant, and in its two diploid ancestors Gossypium arboreum (AA) and Gossypium raimondii (DD). We compared the differences of histone modifications in AADD wild type and mutant, found that 678 genes were specifically expressed in wild type ovules while 455 genes were specifically expressed in mutant ovules. We investigated the histone modification signals and transcription factor binding signals in the two diploid cotton (AA and DD) and compared with the tetraploid cotton AADD, found that Chip-seq signals were even stronger in D subgenomes than A subgenomes. Considering that Gossypium raimondii cotton fiber abortion happens before elongation stage, the combination of D genome expressions and A genome expressions enhances upland cotton fiber length and quality. This phenomenon indicated that there were extensive interactions and regulations between D subgenome and A subgenome in the tetraploid.

T5-19-03

De Novo Whole Genome Assembly in Allo-Octoploid Strawberry

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Strawberry (Fragaria × ananassa) is an allo-octoploid (2n=8X=56) and allogomous species. The whole genome sequence of strawberry was published by Hirakawa *et al.,* (2014). Although the data has contributed genetic analysis in strawberry, the assembled sequences consisted of large number of sequences with short N50 length, suggested the necessary of further improvement. Therefore we performed *de novo* whole genome assembly again by using DenovoMAGIC which was developed in NRGene. A Japanese variety, 'Reikou', was used for construction of paired end (PE), mate pair (MP) and 10X genomics libraries. Illumina reads with 250 nt (PE) and 160 nt (MP and 10X) were obtained by HiSeq platforms, and assembled by DenovoMAGIC3.0. The total length of the assembled sequences was 1,406 Mb, consisted of 32,715 sequences and N50 of 3.9 Mb. Meanwhile, a high-density genetic linkage map was constructed with an S1 mapping population of 'Reikou' by mapping SNPs on the IStraw 90K Axiom® Array and previously published SSR markers. The 'Reikou' linkage map was consisted of 11,608 loci spanning 2,827.4 cM of 31 linkage groups. A total of 62 (31 × 2 haplotypes) pseudomolecules were developed based on the linkage map in a total length of 1,125 Mb. By comparison with the linkage map, 40 mis-assembly was found in the scaffolds. The mapping result of the pseudomolecules on the genome of F. vesca, a possible diploid ancestor of strawberry, clearly indicated the different structure of the four homoeologous genomes in strawberry. This is the first report on the pseudomolecules developed in strawberry, which is expected to accelerate genomics, genetics and molecular breeding for strawberry.

T5-20: Non-coding RNAs: the long and the short of it

T5-20-01

Role of long noncoding RNA in RNA-directed DNA methylation

Andrzej Wierzbicki
University of Michigan

RNA-mediated transcriptional gene silencing is a conserved process where small RNAs target transposons and other sequences for repression by establishing chromatin modifications. A central element of this process is long noncoding RNA (lncRNA) which has been proposed to serve as a binding scaffold for small RNAs and associated proteins. In *Arabidopsis thaliana*, this lncRNA is produced by a specialized RNA polymerase known as Pol V. We performed genome-wide identification of Pol V transcripts using RNA immunoprecipitation followed by high throughput sequencing. We show that noncoding transcription by Pol V is controlled by preexisting chromatin modifications located within the transcribed regions. The vast majority of Pol V transcripts is associated with AGO4 but are not substrates for slicing by AGO4. lncRNA-binding proteins remain in close proximity to the Pol V complex and siRNA is likely to base pair with lncRNA exiting Pol V. Finally, Pol V preferentially transcribes into silenced transposable elements. We propose a model explaining several key events surrounding noncoding transcription by Pol V and predicting that determination of heterochromatin boundaries is an important function of noncoding transcription.

T5-20-02

Custodian of RNA induced silencing complexes in plants

Xiuren Zhang
Texas A&M University

RNA silencing is implemented through RNA-induced Silencing Complex (RISC) that is composed of microRNAs (miRNAs) and Argonaute (AGO) proteins. AGOs use miRNAs as guides to slice target mRNAs with highly complementary sequences to produce truncated 5' and 3' RNA fragments. The 5' cleaved RNA fragments are marked with uridylation for degradation to accelerate RISC recycling. However, key enzymes responsible for such degradation and whether the clearance of cleaved targets affects miRNA metabolism remain poorly understood. Here, we identified a novel cofactor of Arabidopsis AGOs, named RICE1 (RISC Implanted Clearing 3'- 5' Exoribonuclease 1). RICE1 and its closest paralog, RICE2, specifically degraded single-strand RNAs but positively regulated miRNA accumulation in vivo. RICE1 exhibited a DnaQ-like exonuclease fold and formed a homohexamer with the active sites located at the interfaces between RICE1 subunits. Whereas dimerization-impaired RICEs were unstable in vivo, ectopic expression of catalytically inactive RICE1 significantly reduced miRNA levels and the retaining of miRNAs in RISC; notably, catalytically inactive RICE1 also increased 5' cleavage RISC fragments with extended uridine tails resulting from RISC activity. Our results suggest that RICEs are engaged in the clearance of the uridylated cleavage products to maintain functional RISC and to secure miRNA sheltering in RISC in vivo. Thus, this study provides new insight into the coordination between decay of cleaved target mRNAs and miRNA stability in eukaryotes.

T5-20-03

DiRNAs reach out for DSB repair

Mingming Liu, Ba Zhaoqing, ***Yijun Qi***
Tsinghua University

Repair of DNA double-strand break (DSB) is critical for the maintenance of genome integrity. We have previously shown that a class of DSB-induced small RNAs (diRNAs) facilitates homologous recombination (HR)-mediated DSB repair in *Arabidopsis thaliana*. How diRNAs mediate DSB repair remains largely unknown. We find that INVOLVED IN DE NOVO 2 (IDN2), a double-stranded RNA (dsRNA) binding protein involved in small RNA-directed DNA methylation, is required for DSB repair in *Arabidopsis*. We find that IDN2 interacts with the heterotrimeric replication protein A (RPA) complex. Depletion of IDN2 or the diRNA-binding ARGONAUTE 2 (AGO2) leads to increased accumulation of RPA at DSB sites and mislocalization of the recombination factor RAD51. These findings support a model in which IDN2 interacts with RPA and facilitates the release of RPA from ssDNA tails and subsequent recruitment of RAD51 at DSB sites to promote DSB repair.

T5-20-04

miRNA-mediated flowering control in temperate grasses

Wu Liang

Zhejiang University

The transition from vegetative growth to flowering stage is crucial for seed propagation in plants. The highly conserved florigen encoded by FLOWERING LOCUS T (*FT*) functions at the core of the flowering pathways. Extensive studies have examined the transcriptional regulation of *FT*; however, other layer of *FT* regulation remains obscure. We identify miR5200, a Pooideae-specific microRNA, in *Brachypodium distachyon*, a model plant of temperate grasses, and find that miRNA is specially expressed in leaves and targets *FT* orthologs for mRNA cleavage. miR5200 is dramatically accumulated in plants grown under short-day (SD) conditions, but is dramatically repressed in plants transferred to long-day (LD) conditions. We also find that the epigenetic chromatin status, specifically the levels of histone methylation marks, at miR5200 precursor loci are changed in response to day-length. Artificial interruption of miR5200 activity by target mimicry in *B. distachyon* alter flowering time in SD but not in LD conditions, indicating a role of miR5200 in photoperiod-mediated flowering time onset. Furthermore, we find that regulation of miR5200 by day-length is highly conserved in temperate grasses. Together, our results show a posttranscriptional regulatory mode of *FT* by miRNA, and provide insights into understanding of the multiple concerted pathways for flowering time control in plants.

T5-20-05

MicroRNA functions in *Arabidopsis* embryos

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Gregor Mendel Institute

MicroRNAs (miRNAs) are a class of small regulatory RNAs that repress key developmental regulators and are essential for plant embryo development. Previously we found that miRNA-deficient embryos exhibit widespread differentiation defects and precociously express maturation-phase genes. This indicates that miRNAs are required for both embryonic pattern formation and the timing of the morphogenesis-to-maturation phase transition. Because plant miRNAs typically repress transcription factors and other key developmental regulators they likely have a large influence on the gene regulatory networks that control plant embryogenesis. Our major goal is to understand how miRNAs shape the gene regulatory networks that govern plant embryogenesis, and I will present our progress towards this aim.

T5-20-06

Epigenetic regulation of the plant micro RNA machinery turnover.

Pablo Manavella

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Micro RNAs (miRNA) are 21 nucleotides molecules essential for post-transcriptional gene silencing. These molecules are generated from a structured RNA precursor and then incorporated into the RNA induced silencing complex (RISC). DICER-LIKE 1, HYPONASTIC LEAVES 1 and SERRATE are the main proteins involved in miRNA biogenesis but several new cofactors have being described recently. After processing of the miRNA precursors, ARGONAUTE 1 (AGO1) binds the mature miRNAs and inhibits targeted-mRNA translation or induces its cleavage. Using a forward genetic approach we have isolated novel mutants deficient in miRNA activity, from which several cofactors of the pathway, such as CPL1, RCF3 and the THO/TREX complex, have been recently described. Here we describe *MIRNA-SILENCING SUPPRESSED 47 (mss47*), another miRNA deficient mutant identified in our genetic screening. Small RNA blots and RT-qPCR showed that *mss47* mutants present altered level of miRNAs and targets' mRNAs. *MSS47* encodes a protein with a histone-methyltransferase domain. This protein does not directly regulate the transcripts levels of *MIRNA* genes or its targets. Our experiments show that MSS47 epigenetically regulates the abundance of essential factors of the miRNA pathway by modulating their degradation. Such depletion in the miRNA machinery impairs the production and activity of miRNAs and, therefore, the gene silencing and plant development.

T5-21: Epigenetics II: DNA modifications

T5-21-01

Encyclopedia of plant methylomes - from patterns to mechanisms and functions

Robert Schmitz

University Of Georgia

Advances in DNA sequencing technologies have led to the first single-base resolution maps of cytosine DNA methylation. These maps are revealing a diverse set of mechanisms that genomes utilize to control genome stability, transposon silencing, and gene expression. Our previous studies in natural and experimental *Arabidopsis thaliana* populations have uncovered errors in maintenance of DNA methylation that lead to the existence of spontaneous epigenetic alleles. The rates of formation of single methylation polymorphisms are about five orders of magnitude greater than the spontaneous mutation rate, whereas the formation of differentially methylated regions that affect gene expression occurs at a frequency that is similar to the spontaneous DNA mutation rate. To better understand the mechanisms governing maintenance of DNA methylation over longer evolutionary timescales, we sequenced and used publicly available DNA methylomes of 34 flowering plant species and the basal flowering plant Amborella trichopoda. Additionally, we have developed a novel high-throughput analysis method for profiling DNA methylation levels for any species regardless of the availability of a reference genome. We are unraveling the mechanistic underpinnings that evolved to establish and maintain DNA methylation by using this base modification as a molecular phenotype in combination with phylogenetic and molecular analysis of enzymes involved in DNA methylation.

T5-21-02

Critical role of a DNA demethylase in tomato fruit ripening

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DNA methylation is a conserved epigenetic mark important for genome integrity, development and environmental responses in plants and mammals. Active DNA demethylation in plants is initiated by a family of 5mC DNA glycosylases/lyases (i.e. DNA demethylases). The role of DNA demethylases in transposon regulation, pathogen responses, and gene imprinting and other developmental processes have been studied extensively in the model plant *Arabidopsis thaliana*. Recent studies suggested a role of active DNA demethylation in fruit ripening in tomato. In this study, we generated loss-of-function mutant alleles of a tomato gene, *SlROS1/SlDML1/2*, which is a close homolog of the *Arabidopsis* DNA demethylase gene *ROS1*. In the fruits of the tomato mutants, increased DNA methylation was found in thousands of genes. These genes include not only hundreds of ripening induced genes but also many ripening repressed genes. Our results show that *SlROS1* is critical for tomato fruit ripening, and suggest that active DNA demethylation is required for both the activation of ripening induced genes and inhibition of ripening repressed genes.

T5-21-03

Hybrid and hybrid mimics in *Arabidopsis*

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Hybrids in *Arabidopsis* and crop species are uniform and high yielding. In contrast, the F_2 generation shows a great deal of phenotypic heterogeneity and does not maintain the hybrid yield advantage. The reduced yield and heterogeneity of the F_2 as well as the increased yield of the F_1 are the basis of the hybrid seed industry as farmers have to buy fresh seed each planting season. To address the molecular mechanisms of both the F_1 and F_2 features we carried out recurrent selection using *Arabidopsis* hybrids combination C_{24} and Landsberg *erecta* (L*er*) in an attempt to "fix "the hybrid vigour phenotype. We generated pure breeding Hybrid Mimic lines with similar properties to the F_1 hybrids and we also selected pure breeding Small Phenotype lines. By comparing the transcriptomes of the selected F6 lines, both Hybrid Mimic and Small Phenotype lines, we determined which changes in gene expression were shared in the mimics and the hybrid, but not shared with Small plant line, these genes presumably are associated with the large rosette phenotype. Basal defence pathways, hormone biosynthesis and response pathways are major pathways altered in the Hybrid Mimic lines and F_1 hybrids. A number of auxin responsive genes promoting leaf growth were upregulated in the F1 hybrids and Hybrid Mimics. The transcription factor PHYTOCHROME-INTERACTING FACTOR (PIF4), may be responsible for these altered activities of auxin-associated genes by targeting the auxin biosynthesis gene *YUCCA8* and regulating auxin signalling through *IAA29*. The Hybrid Mimics are essentially homozygous with chromosome segments from each parent. This finding indicates that the vigour phenotype does not require heterozygosity throughout the genome. 12 conserved chromosomal segments each sourced from one particular parent were present in all of the Hybrid Mimic lines whereas in the F_6 Small Phenotype line the 12 segments were derived from the alternative parent. Loci critical for promoting growth vigor may be contained in each of these conserved segments. In the hybrids segments from the chromosomes of both parents are combined in the one nucleus leading to alterations in gene expression involving trans-interactions. These can be genetic or epigenetic interactions. *Arabidopsis* ecotypes used as parents for hybrids often are more different epigenetically than genetically. In F_1 hybrids Trans Chromosomal Methylation (TCM) and Trans Chromosomal deMethylation (TCdM) occur, resulting in gain or loss of methylation at loci where two parental alleles are differentially methylated. Changed methylation patterns in the F_1 hybrids can be inherited in Hybrid Mimics which though impacting gene expression may contribute to their phenotypes. TCM is dependent upon the RNA directed DNA Methylation (RdDM) pathway and 24 nt siRNAs; in the *RNA polymerase IV* (*pol IV*) mutant TCM is decreased. The *Pol IV-V* pathway is not critical for the hybrid vigor phenotype but the *DECREASED DNA METHYLATION 1 (DDM1)* methylation pathway is required for the full level of hybrid vigor.

T5-21-04

Transcriptomic dynamics after perturbing maize methylome

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DNA methylation is an important regulator of the expression of both genes and transposons. We previously collected mutant alleles for maize genes that play important roles in controlling DNA methylation. Here RNA sequencing was used to investigate the transcriptomic changes in seedling tissue of plants with altered maize DNA methylation. Expression levels of the majority of genes are not changed in most mutant alleles. However, plants containing two alleles, one loss-of-function allele for chromomethylase (*CMT*) gene and one for *DDM1* gene exhibit strong expression changes in more than 1000 genes compared to their wild-type controls. While no consistent patterns between DNA methylation and gene expression were observed for genes that are down-regulated in the mutant plant, genes with up-regulated expression in the mutants generally have high CHG methylation across gene body and this CHG methylation was greatly lost in the mutants. Consistent with a role of DNA methylation in response to environmental stress, many of the genes with altered expression in the methylome mutants also display expression changes under various stress conditions. In an attempt to study the prevalence of DNA methylation in regulating gene expression in natural populations, we profiled DNA methylation in near 20 diverse maize inbred lines. Interestingly, many of the differentially expressed genes in the methylome mutants have variation in DNA methylation among these inbred lines, many of which also affect natural variation in gene expression. This suggests that many targets of epigenetic regulation in maize are naturally varying, providing a possibility to exploit these epigenetic changes in maize breeding.

T5-21-05

Identification and characterization of DNA methylation readers in *Arabidopsis thaliana*

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Methylation of cytosine bases in genomic DNA (mC) provides an additional layer of information that has the potential to alter transcription and chromatin state. In plants, mC plays an important role in the transcriptional repression of transposable elements, and is established, maintained and removed by a variety of "writer" or "eraser" factors, depending on local DNA sequence context (CG, CHG and CHH, where H = A, C or T). mC can be interpreted by "reader" proteins, some of which are known to be involved in feedback loops with other modifications such as histone methylation. While some plant mC reader proteins have been documented, a comprehensive survey of the proteins that can bind mC in plants is required to identify the range of factors that can interpret this DNA modification. To screen for potential mC readers, we performed a DNA affinity pull-down assay combined with quantitative mass spectrometry, using short methylated DNA probes for each sequence context. We identified 37 nuclear proteins that show a significant enrichment in methylated probes in at least one sequence context, including 12 previously characterized mC readers such as VIM proteins, SUVH2/6/9 and MBD5/6/7. Approximately half of the identified candidates have not previously been reported as potential mC binding proteins. To further explore the function of these mC reader proteins, we are utilizing ChIP-seq to study their DNA binding sites genome-wide, tandem affinity purification coupled with mass spectrometry to identify interacting proteins, and analysis of the transcriptome and DNA methylome in mutant plants to assess their potential role in gene regulation and the epigenome. This work constitutes the first comprehensive assay to identify potential mC readers in plants, and a initial steps toward understanding their molecular functions.

T5-21-06

Epigenetic silencing-mediated alternative polyadenylation (APA) regulates CHG methyaltion by promoting the proper expression of histone demethylase IBM1

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DNA methylation-dependent heterochromatin formation is a conserved mechanism of epigenetic silencing of transposons and other repeat elements in many higher eukaryotes. Genes adjacent to repetitive elements are often also subjected to this epigenetic silencing. Consequently, plants have evolved anti-silencing mechanisms such as active DNA demethylation mediated by the REPRESSOR OF SILENCING 1 (ROS1) family of 5-methylcytosine DNA glycosylases to protect these genes from silencing. Some transposons and other repeat elements have found residence in the introns of genes. It is unclear how these intronic repeat elements-containing genes are regulated. Our recent work identified several new anti-silencing factors via genetic screen and biochemical methods. Genome-wide DNA methylation analysis reveals that these proteins has a similar role to that of the histone H3K9 demethylase INCREASE IN BONSAI METHYLATION 1 (IBM1) in preventing CHG methylation in the bodies of thousands of genes. We found that these protein ensure the proper expression of IBM1 full-length transcript by promoting the distal polyadenylation. Our research also found that the intronic heterochromatin region is required for the usage of alternative polyadenylation (APA). Through mRNA sequencing, we identified many genes containing intronic transposon elements that require these proteins for proper expression. Our study identifies several new regulators of CHG methylation and reveals a unique mechanism by which higher eukaryotes deal with the collateral effect of silencing intronic heterochromatin.

T5-22: Plant genome editing (two sessions)

T5-22-01

A high-efficiency CRISPR/Cas9 system for multiplex genome editing in plants and a sequencing decoding software DSDecode

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CRISPR/Cas9 genome targeting systems have been applied to a variety of species. We have developed a robust and versatile CRISPR/Cas9 vector system for high efficiency multiplex genome editing in plants. We designed PCR-based procedures to rapidly generate multiple sgRNA expression cassettes, which can be assembled into the binary CRISPR/Cas9 vectors in one round of cloning by Golden Gate ligation or Gibson Assembly, for editing of a number of target sites by single plant transformation. Average 85% or higher rates of mutations were generated in rice. We also developed a web-based tool 'DSDecode' and its updated version 'DSDecodeM' (skl.scau.edu.cn) for decoding sequencing chromatograms with superimposed peaks derived from heterozygous and biallelic mutations of targeted sites. These systems have provided a usable toolbox for studying functions of multiple genes and gene families in plants for basic research and genetic improvement.

T5-22-02

Evidence of high-frequency off-target mutagenesis induced by CRISPR/Cas9 in *Arabidopsis* and improvement of the specificity

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lege of Biological Sciences, China Agricultural University, Beijing 100193, China.

We presented evidence that high frequency off-target effects of CRISPR/Cas9 existed in *Arabidopsis* and thus very possibly in other plants. To improve the specificity, we examined six mutant SpCas9 variants: two eSpCas9 reported in human cells and named for their enhanced specificity, one SpCas9-HF1 reported in human cells and named for its high fidelity, and three not reported combinatorial forms of the two types. We demonstrated that a few variants worked well in plants: showed much higher specificity and maintained on-target activities comparable to wild-type SpCas9. We also demonstrated that on-target activities of the high specificity Cas9 variants were dependent on expression levels of sgRNA/Cas9 complexes. We tested some strategies for improving the expression level of the sgRNA/Cas9 and found some of them useful.

T5-22-03

Double-strand break induced genome engineering in plants

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Sequence-specific nucleases can be used to induce double-strand breaks (DSBs) in plant genomes. In the past we could show that thus gene targeting (GT) by homologous recombination (HR) can be enhanced and targeted mutagenesis can be achieved by error-prone non-homologous end joining (NHEJ). Moreover, by inducing several DSBs, sequences can be deleted out of the genome or chromosome arms exchanged. In the last years CRISPR/Cas became the major tool for targeted mutagenesis. We were able to demonstrate *Streptococcus pyogenes* (Spy)Cas9 nuclease induced, NHEJ mediated, heritable targeted mutagenesis in *Arabidopsis thaliana* as well as homology dependent *in planta* GT. Off-target effects might be avoided using two sgRNAs and a Cas9 protein that was transformed from a nuclease to a nickase, to induce adjacent single strand breaks (SSBs) in opposite strands. This “paired nickase” strategy has a mutagenic potential at the target site comparable to the nuclease. Interestingly; sequence duplications are a prominent outcome of this approach, hinting to the possibility that the repair of adjacent SSBs is a major cause of sequence duplications during genome evolution of plants. Recently we applied the Cas9 orthologues from *Streptococcus thermophilus* (Sth1Cas9) and *Staphylococcus aureus* (SauCas9) for NHEJ-mediated targeted mutagenesis in *A. thaliana* with efficiencies at least comparable to those of SpyCas9. We were also able to show that the SauCas9 and SpyCas9 proteins only work in the presence of their species-specific single guide (sg) RNAs and show no inter-species interference. Thus, the Cas9 proteins of *S. pyogenes* and *S. aureus* should be appropriate for simultaneously addressing different sequence motifs with different enzyme activities in the same plant cell. The simultaneous use of different Cas9 orthologues will offer the opportunity to control genetic information of plant cells on more complex levels than before and will lay the basis for future synthetic approaches in plant biology.

T5-22-04

A CRISPR-Cpf1 system for efficient genome editing and transcriptional repression in plants

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Clustered regularly interspaced short palindromic repeats (CRISPR)-Cpf1 has emerged as an effective genome editing tool in animals. Here we compare the activity of Cpf1 from *Acidaminococcus sp. BV3L6* (As) and *Lachnospiraceae bacterium ND2006* (Lb) in plants, using a dual RNA Polymerase II promoter expression system. LbCpf1 generated biallelic mutations at nearly 100% efficiency at four independent sites in rice T0 transgenic plants. Moreover, we repurposed AsCpf1 and LbCpf1 for efficient transcriptional repression in Arabidopsis, and demonstrated reduction of *miRNA159* transcription >10-fold. Our data suggest promising applications of CRISPR-Cpf1 for editing plant genomes and modulating the plant transcriptome.

T5-22-05

Optimizing gene targeting strategies in plants

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The ability to precisely modify plant genomes through homologous recombination (HR) promises to advance both basic and applied plant biology. However, even with the use of sequence-specific nucleases, which stimulate HR by creating targeted DNA double-strand breaks, there are only a handful of studies that report precise editing of endogenous plant genes. Our group has been focusing on two efforts to more effectively modify plant genomes through HR. In one, we are developing new vectors to deliver sequence-specific nucleases and DNA repair templates to plant cells. Specifically, we have been using geminivirus replicons, which function in both monocots and dicots, to amplify nuclease-encoding cassettes and DNA repair templates. In a second effort, we are attempting to achieve HR by either genetically manipulating DNA repair pathways or delivering nucleases and repair templates to cells proficient in HR. Progress on our efforts to optimize gene targeting strategies will be reported.

T5-22-06

Precision mutagenesis in plants via NHEJ or HR pathway

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There are two types of genome editing technique, non-homologous end-joining (NHEJ) mediated targeted mutagenesis and homologous recombination (HR) mediated gene targeting. In/del and substitution types of mutation can be introduced at the cleavage site of sequence-specific nucleases (SSNs) via imprecise NHEJ. Therefore, targeted mutagenesis is useful if we want to knock-out a targeted gene. The number of papers reporting plant targeted mutagenesis with CRISPR/Cas9 has increased markedly. However, widely used SpCas9 protein from Streptococcus pyogenes uses guanine-rich PAM sequence (NGG). So, it is difficult to mutagenize thymidine-rich sequences. The PAM sequence of Cpf1 from Fransisella novicida is TTN. We have shown that targeted mutagenesis had occurred in transgenic plants expressing FnCpf1. Furthermore, Cpf1 generates DNA ends with 5' overhang, whereas Cas9 creates blunt DNA ends after cleavage. Sticky DNA ends should increase the efficiency of insertion of a desired DNA fragment into the Cpf1-cleaved site using complementary DNA ends via precise NHEJ. In the case of a targeted mutagenesis in seed-propagated crops, once integrated into the genome as a transgene, sequence-specific nucleases (SSNs) genes can be segregated out by self-pollination or back-crossing. However, in the case of crops that are propagated vegetatively, we would like to remove transgene without using genetic segregation. We found that piggyBack transposon can be used as a reversible transgenesis vector in plants to put SSNs IN and OUT without leaving any footprints. Gene targeting enables precise modification of the targeted gene using a specific template DNA which must be supplied exogenously. Usually, a positive selection marker gene is left at the targeted gene after positive/negative-selection-mediated gene targeting. Recently, we succeeded in removing the positive selection marker gene completely using a piggyBack transposition. We can modify any sequence in the rice genome precisely using this system.

T5-22-07

Efficient and heritable genome engineering in barley

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Genome engineering is a breakthrough technology that facilitates site-directed modifications of genomic DNA in planta. It offers versatile novel possibilities for both the experimental elucidation of gene functions and the improvement of crop performance. Aiming to establish site-directed mutagenesis in *Triticeae* cereals, target gene-specific transcription activator-like effector nucleases (TALENs) were designed and barley mutant plants generated via transfer of a pair of TALEN-coding expression units into haploid cells from which transgenic doubled haploid plants can be produced. Owing to the haploid founder cells and whole genome duplication taking place in the course of the regeneration process, some of the resultant primary mutants proved instantly homozygous, as was indicated by non-segregating progeny. In a second approach, we produced two types of plant lines each carrying only one of the two required TALEN-units. TALEN cleavage activity was then induced in hybrid progeny derived from crossings of complementary pairs of these lines. By this principle, many independent and heritable mutations can be produced even if only a few TALEN-transgenic lines are available, which is a particularly valuable option, especially in plant species that are not readily transformable. Whereas primary mutants are typically chimeric, we demonstrated that chimera dissolution and genetic fixation can be greatly facilitated by generating doubled haploid progeny, i.e. plants which, by definition, inherit no more than one allele each. To allow for optimization of endonuclease construct design and routine prevalidation of target-specific expression units before investing in the laborious creation of stable transgenic plants, a transient expression test was established which indicates cleavage activity via frame shift reconstitution of a reporter gene. More recently, we have also established site-directed mutagenesis using customized RNA-guided Cas9 endonuclease, which derives from the microbial CRISPR/CAS immune system. This genome engineering platform has proved more reliable in generating barley mutant plants as compared with current TALEN technology. We also showed that the proportion of plants carrying genetic modifications at the target site can be as high as 84 percent of the gRNA/Cas9 transgenics produced. Examples will be shown demonstrating targeted mutagenesis using the model cultivar Golden Promise followed by mutant fixation via haploid technology as well as genome engineering of elite breeding material. In addition, homology-dependent genome editing was exemplified at the cellular level using a customized DNA-repair template, which allows to precisely predefine not only the genomic target locus but also the resultant DNA sequence.

T5-22-08

Targeted base editing in plants by Target-AID, the CRISPR/Cas9 deaminase fusion.

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DNA deamination is the process by which deoxyribonucleotides turn into other type of bases (ex. deoxycytidine to deoxyuridine). Although most deaminated nucleotides are repaired properly, excess deamination or compromised repair may results in mutagenesis. The activation-induced cytidine deaminase (AID) is responsible for generating genetic variation at the immunoglobulin locus, an essential process for the adaptive immune system in vertebrates. By tethering the DNA deaminase activity to nuclease-deficient CRISPR/Cas9 system, we have developed a genome editing tool that enables targeted point mutagenesis. An AID ortholog PmCDA1 was attached to nuclease-deficient mutant of Cas9 (D10A and H840A). This hybrid system, termed Target-AID, induced cytosine point mutation in 3-5 bases range at the distal site within target sequence. Plant-optimized Target-AID was developed and applied to rice and tomato by agrobacterium mediated transformation to produce base-edited plants to induce loss- and gain-of function mutations.

T5-22-09

Genome-wide target specificities of CRISPR nucleases and deaminases

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Despite broad interest in RNA-guided genome editing, Cas9 and Cpf1 are limited by off-target mutations. We developed nuclease-digested whole genome sequencing (Digenome-seq) to profile genome-wide specificities of these nucleases in an unbiased manner. Digenome-seq captured nuclease cleavage sites at single nucleotide resolution and identified off-target sites at which indels were induced with frequencies below 0.1%. We also showed that these off-target effects could be avoided by using purified Cas9/Cpf1 ribonucleoproteins (RNPs) and modified guide RNAs. Digenome-seq is a robust, sensitive, unbiased, and cost-effective (< USD 1,500) method for profiling genome-wide off-target effects of programmable nucleases.

T5-22-10

Base editing of major cereals using Cas9 nickase-cytidine deaminase fusion

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A plant base editing method (nCas9-PBE), using a Cas9 nickase-cytidine deaminase fusion, has been optimized and validated for genome editing in rice, wheat and maize. Unlike nuclease-dependent genome editing that induce random insertions and deletions, the fusion induces efficient and targeted conversion of cytosine to thymine in both protoplasts and mutant plants, and some of the resulting amino acid mutations have unique and valuable characteristics. The optimized method was tested with five endogenous rice, wheat and maize genes in protoplasts. The frequency of targeted C to T substitution, as estimated by deep amplicon sequencing, varied from 0.57% to 7.07%. Stable rice, wheat and maize mutants carrying desired C to T substitution were obtained, thus validating the value of nickase Cas9-AID mediated base editing for producing mutants useful to the genetic and breeding research of major cereals. This approach will not only technologically advance plant genome engineering, but may also provide better solution for social acceptance of genome-edited crops as they do not require a donor DNA template or chromosomal cleavage.

T5-22-11

Genome editing in agriculture … an industry perspective on riding the hype-curve for robust outcomes beyond low-hanging fruits

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KWS SAATSE, EINBECK, GERMANY

Recent availability of robust Genome Editing technologies in plants promises to both revolutionize biological research and to facilitate faster and cheaper development of commercial crop varieties with enhanced biotic and abiotic stress characteristics, processing and quality characteristics and yield per se. To date the technology is at the peak of its hype curve … expectations are sky-high but significant breakthrough developments in a number of adjacent biologic research fields will be required for Genome Editing to achieve its full potential in plant breeding and agriculture. This talk will focus on current opportunities and limitations for Genome Editing technology in modern plant breeding programmes and in particular focus on key developments in plant biology required to overcome current technical, regulatory, public perception and intellectual property hurdles limiting the achievement of its full potential. In addition we will outline KWS' key interests and activities in the field of Genome Editing as well as KWS' open innovation based approach to "research & development" and its consequent unique way of engaging with the scientific community in the academic, the start-up and the corporate research scene.

T5-22-12

Targeted gene activation in plants by a potent dead cas9-based synthetic transcription activator

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Targeted activation of gene expression is highly desirable for elucidating and manipulating gene functions in basic and applied plant research. Repurposing the CRISPR/Cas9 technology from a genome editing tool to a targeted transcriptional activation system represents an appealing way to achieve this goal. However, pilot efforts in mammalian cells suggest that the current generation of catalytically dead Cas9 (dCas9)-VP64 transcription activator works with a low efficiency when using a single synthetic guide RNA (sgRNA). Multiple sgRNAs tiling the promoter of target gene need to be used simultaneously to enhance the transcription, leading to increased workload and decreased scalability of the system. Using plant protoplast-based screens, we identified a potent synthetic transcription activator containing dCas9 and a chimeric transcriptional activation module composed of different transcriptional activation domains, each in multiple copies. This improved synthetic transcription activator allows efficient activation of target gene expression using only a single sgRNA in both protoplasts and transgenic plants, which promises multiplex up-regulation of target gene expression in plant cells to rewire regulatory networks.

T5-23: Plant-microbe interactions

T5-23-01

Effectoromics of the citrus Huanglongbing-associated bacteria

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The devastating citrus Huanglongbing (HLB, aka greening disease) has caused billions of dollars of loss in citrus industry worldwide. In the US and China, HLB is dominantly associated with a Gram-negative bacterium *Candidatus* Liberibacter asiaticus (CLas). Transmitted by insect vectors, CLas exclusively colonizes phloem tissues in citrus; importantly, there is no curative treatment after the trees are infected. CLas possesses the Sec secretion system, through which a variety of Sec-delivered effectors (SDEs) could be secreted from the pathogen cells into the citrus phloem. In other bacteria, effectors play an essential role in pathogenicity by suppressing host immunity, facilitating nutrient uptake and causing disease symptoms. We analyzed the genome sequences of ten CLas isolates and determined their SDE repertoire. Each isolate encodes 23-35 SDEs and 13 of them are "core" effectors produced by all the isolates. One of the core effectors, named SDE1, has 40-folds higher expression in citrus than in the insect vector, implicating a possible role in HLB progression. We found that SDE1 directly interacts with several members of papain-like cysteine proteases (PLCPs) in citrus and potentially functions as a broad-spectrum inhibitor. Homologs of PLCPs in Solanaceae plants have been reported to contribute to defense against microbial pathogens. Our proteomic analysis showed that the abundances of specific PLCPs are increased in the phloem of HLB-infected citrus, where the bacteria reside. Furthermore, expression of SDE1 significantly impairs citrus development, supporting a model where SDE1 inhibits PLCP activity to promote HLB. These findings provide key information on HLB pathogenesis and discover promising targets that are subjected to genetic manipulation in order to enhance citrus resistance to this devastating disease.

T5-23-02

RNAi mediated host antiviral defense in rice

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Rice is the most important stable food supply worldwide. RNAi, the evolutionarily conserved mechanism of antiviral defense which is well studied in plants and other eukaryotes, is poorly understood in economically important crops. Rice encodes 19 AGOs, AGO18 is a member of a novel clade of AGOs from rice. We found that AGO18 is highly induced by infections of evolutionary different viruses. Loss-of-function *ago18* mutation abolishes, whereas over-expression of AGO18 increases antiviral activity. Significantly, AGO18 functions antiviral defense through disturbing and sequestering certain miRNAs such as miR168 and miR528 and releasing their targets which are directly involved in antiviral defense. Expression of miR168 resistant AGO1a in *ago18* background rescues rice antiviral activity. Mutated miR528 or expression of miR528 resistant target, *L-ascorbate oxidase* (AO) significantly increases rice antiviral activity. AO localizes in the cell wall and catalyzes apoplastic oxidation of L-ascorbic acid (AsA). We further found that AGO18 sequesters *AO*-targeting miR528 and promotes the role of AO in enhancing antiviral defense response through regulation of basal ROS accumulation. Together with other results, we propose that *miR528* deficiency and AO-mediated plant antiviral defense function in parallel with the AGO18-miR168-AGO1 pathway. While the exact functional mode of ROS in antiviral defense remains to be elucidated, it is well known that ROS are central players in the cellular signaling network in different organisms. To summarize, our study not only reveals a pathway that elegantly regulates the availability of a key defense factor (AO) based on the sensing of virus attack, but also provides strong evidence to support that ROS have an active role in antiviral immunity. We foresee that this unique mechanism could be engineered to improve the antiviral capabilities of crops.

T5-23-03

Interacting calcium pump and BON1 modulate calcium signature, stomatal closure, and plant immunity

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Calcium signaling is essential for environmental responses including immune responses. Here we provide evidence that the evolutionarily conserved protein BONZAI1 (BON1) functions together with autoinhibited calcium ATPase 10 (ACA10) to regulate calcium signals in *Arabidopsis*. BON1 is a plasma membrane localized protein and the *bon1* loss of function (LOF) mutant has increased expression of immune receptor genes and a consequent autoimmune phenotype. We found that BON1 interacts with the autoinhibitory domain of ACA10 and the *aca10* LOF mutants also have an autoimmune phenotype similarly to the *bon1* mutants. Genetic evidences indicate that BON1 positively regulates the activities of ACA10 and perhaps its homologous protein ACA8 as well. Consistent with this idea, the steady level of calcium concentration is increased in both *aca10* and *bon1* mutants. Most strikingly, cytosolic calcium oscillation imposed by external calcium treatment was altered in *aca10* and *bon1* mutants in guard cells. In addition, calcium and pathogen induced stomatal closure was compromised in the *aca10* and *bon1* mutants. Taken together, this study indicates that ACA10 and BON1 physically interact on plasma membrane and function in the generation of cytosol calcium signatures that are critical for stomatal movement and impact plant immunity.

T5-23-04

Actin remodeling during plant innate immunity requires both capping protein and reactive oxygen species

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Plants perceive microbe-associated molecular patterns (MAMPs) and damage-associated molecular patterns (DAMPs) to activate innate immune signaling events, such as bursts of reactive oxygen species (ROS). The actin cytoskeleton remodels during the first five minutes of innate immune signaling in *Arabidopsis* epidermal cells; however, the immune signals that impinge on actin cytoskeleton and its response regulators remain largely unknown. Here, we demonstrate that rapid actin remodeling upon elicitation with diverse MAMPs and DAMPs represents a conserved plant immune response. Actin remodeling requires ROS generated by the defense-associated NADPH oxidase, RBOHD. Moreover, perception of flg22 by its cognate receptor complex triggers actin remodeling through the activation of RBOHD-dependent ROS production. Our genetic studies reveal that the ubiquitous heterodimeric capping protein (CP) transduces ROS signaling to the actin cytoskeleton during innate immunity. Additionally, we uncover a negative feedback loop between actin remodeling and flg22-induced ROS production.

T5-23-05

Argonaute 2 medaited plant resistance to bacteria

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Small RNAs (sRNAs) are 20 to 30 nucleotide (nt)-long noncoding RNA molecules that regulate gene expression in eukaryotes through a process generally termed RNA silencing. RNA silencing machinery plays critical roles in plant resistance to virus and other pathogens. To determine the possible function of sRNAs to plant resistance to bacteria, we studied the accumulation of the miRNAs, nat-siRNAs, NBS-LRR siRNAs and other sRNAs at the genome level and found that the accumulations of some sRNAs are specifically regulated by bacteria infection. All sRNAs are loaded into Argonaute (AGO) protein, the key component of RNA silencing pathway, to perform regulation functions. We showed that the accumulation of AGO2 is specifically induced upon bacterial infection. Further analysis uncovered that miR393* loaded into AGO2 can down-regulate the accumulation of MEMB12, a Golgi-localized SNARE gene, and thus increases the secretion of immunity proteins and plant immunity. We also studied the sorting of sRNA to AGOs and found that AGO2 prefers sRNA duplexes without middle and 15th mismatches, while AGO1 can tolerates middle mismatches. Structure remodeling and mutational analyses showed that some conserved motifs within AGO PIWI domain play important roles in this sorting bias.

T5-23-06

Epigenetic regulation of an antagonistic receptor pair confers broad-spectrum and durable disease resistance with yield balance in rice

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Breeding for broad-spectrum and durable disease resistance with yield balance, i.e. combining high disease resistance and high yield, has been the ideal target of crop breeding. Many resistance (R) genes have been so far identified, however, very few R genes have been adopted for broad-spectrum and durable disease resistance in long-term crop breeding programs due to either resistance breakdown caused by pathogen variation or defense cost with yield penalty. Here we report that the rice *Pigm* locus from an indigenous landrace containing an nucleotide-binding leucine-rich repeat (NLR) receptor cluster confers durable and broad-spectrum resistance to fungal blast (*Magnaporthe oryzae*) without yield penalty. Within the NLR cluster, the NLR PigmR confers broad-spectrum resistance; while another NLR PigmS interacts with and competitively attenuates the homodimerization of PigmR to suppress the PigmR-mediated resistance. The expression of *PigmS* and thus the Pigm-mediated resistance are subjected to tight epigenetic regulation by the RNA-dependent DNA methylation (RdDM) pathway, misregulation of the RdDM pathway altered the PigmR-mediated resistance. Importantly, the yield cost by PigmR-mediated resistance could be counteracted by the PigmS-increased seed production. Therefore, our study reveals a novel mechanism of balancing broad-spectrum resistance and yield through epigenetic regulation of a pair of antagonistic NLRs, providing a tool to develop elite rice with both high disease resistance and yield potential.

T5-24: Genomics and molecular breeding

T5-24-01

A TRIM insertion in the promoter of *Ms2* causes male sterility in wheat

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The male-sterile *Ms2* mutant has been known for 40 years and has become extremely important in the commercial production of wheat. However, the gene responsible for this phenotype has remained unknown. Here we report the map-based cloning of the *Ms2* gene. The *Ms2* locus is remarkable in several ways that have implications in basic biology. Beyond having no functional annotation, barely detectable transcription in fertile wild-type wheat plants, and accumulated destructive mutations in *Ms2* orthologs, the *Ms2* allele in the *Ms2* mutant has acquired a terminal-repeat retrotransposon in miniature (TRIM) element in its promoter. This TRIM element is responsible for the anther-specific *Ms2* activation that confers male sterility. The identification of *Ms2* not only unravels the genetic basis of a historically important breeding trait, but also shows an example of how a TRIM element insertion near a gene can contribute to genetic novelty and phenotypic plasticity.

T5-24-02

GW5 acts in brassinosteroid signaling pathway to regulate grain width and weight in rice

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Grain size is a major determinant of grain yield in cereal crops. Previous studies have shown that qSW5/GW5 exerts the greatest effect on rice grain width and weight and is associated with a 1,212-bp deletion, which was selected during rice domestication for increased grain yield. However, the identity of GW5 has remained mysterious. Here we show that a gene encoding a calmodulin binding protein, located ~5 kb downstream of the 1,212-bp deletion, corresponds to qSW5/GW5. GW5 is expressed in various rice organs with highest expression level detected in young panicles. We provide evidence that the 1,212-bp deletion affects grain width through influencing the expression levels of GW5. GW5 protein is localized to the plasma membrane and can physically interact with and repress the kinase activity of rice GSK2, a homolog of Arabidopsis BIN2 kinase, resulting in accumulation of unphosphorylated OsBZR1 and DLT proteins in the nucleus to mediate Brassinosteroid (BR)-responsive gene expression and growth responses (including grain width and weight). Our results suggest that GW5 is a novel positive regulator of BR signaling and a viable target for genetic manipulation to improve grain yield in rice and perhaps in other cereal crops as well.

T5-24-03

The emergence, evolution, and diversification of the miR390-TAS3-ARF pathway in land plants

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In plants, miR390 directs the production of tasiRNAs from *TRANS-ACTING SIRNA 3* (*TAS3*) transcripts to regulate *AUXIN RESPONSIVE FACTOR* (*ARF*) genes, transcription factors critical for auxin signaling; these tasiRNAs are known as tasiARFs. To understand the evolution of this miR390-*TAS3-ARF* pathway, we characterized homologs of these three genes from thousands of plant species, from bryophytes to angiosperms. We found the lower-stem region of *MIR390* genes, critical for accurate DCL1 (DICER-LIKE 1) processing, is conserved in sequence in seed plants. We propose a model for the transition of functional tasiRNA sequences in *TAS3* genes occurred at the emergence of vascular plants, in which the two miR390 target sites of *TAS3* genes showed distinct pairing patterns in different plant lineages. Based on the cleavability of miR390 target sites and the distance between target site and tasiARF we inferred a potential bidirectional processing mechanism exists for some *TAS3* genes. We also demonstrated a tight mutual selection between tasiARF and its target genes, and characterized unusual aspects and diversity of regulatory components of this pathway. All these data illuminate the evolutionary path of the miR390-*TAS3-ARF* pathway in land plants, and demonstrate the significant variation that occurs in this functionally important and archetypal regulatory circuit.

T5-24-04

New insights into the genetic networks affecting seed lignocellulose content in *Brassica napus*

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Oilseed rape or canola (*Brassica napus*) varieties with reduced levels of antinutritive seed phenolic acid and fibre compounds would considerably improve the value of the seed meal after oil extraction. Dissection of the genetic network for lignocellulose biosynthesis is of great importance for improving seed quality. The genetic basis of lignocellulose biosynthesis in B. napus was investigated via quantitative trail locus (QTL) analysis using a doubled haploid (DH) population from a low oil content of oilseed rape line 'KenC-8' crossed with the high oil content of *B. napus* line 'N53-2' with 219 lines. The phenotypic performance and frequency distribution of fiber components in Wuhan and Dali were analyzed. Seed lignin content (LC), cellulose content (CC) and hemicellulose content (HC) were significantly negatively correlated with seed oil content (OC) in DH population, respectively. A total of 85 QTLs were detected for LC, CC and HC. It is very interesting to found that co-localization between QTL for LC, CC, HC and OC was observed on A9 with contribution ranging from 9.87 to 48.50%. A total of 125 homologous genes of *Arabidopsis thaliana* that are involved in lignin, cellulose and hemicellulose biosynthesis were found within the confidence intervals of 75 QTLs. After the candidate gene analysis, a potential regulatory pathway controlling lignocellulose biosynthesis in *B. napus* was constructed including 52 genes. Subsequently, the interaction between the genes and regulatory factors involved in lignocellulose biosynthesis were analyzed. A potential regulatory pathway controlling the lignocellulose was constructed by QTL analysis. 14 candidate genes were identified, including GAPC1, GAPC2, LAC17 and CSLC08. These results enriched our knowledge of QTLs for lignocellulose biosynthesis and provided useful information for understanding the genetics of key seed quality traits in rapeseed.

T5-24-05

Rice Expression Database (RED): An integrated RNA-Seq-derived gene expression database for rice

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Rice is one of the most important stable food as well as a monocotyledonous model organism for the plant research community. Here we present RED (Rice Expression Database; http://expression.ic4r.org), an integrated database of rice gene expression profiles derived entirely from RNA-Seq data. RED features a comprehensive collection of 284 high-quality RNA-Seq experiments, integrates a large number of gene expression profiles and covers a wide range of rice growth stages as well as various treatments. Based on massive expression profiles, RED provides a list of housekeeping and tissue-specific genes and dynamically constructs co-expression networks for gene (s) of interest. Besides, it provides user-friendly web interfaces for querying, browsing and visualizing expression profiles of concerned genes. Together, as a core resource in BIG Data Center, RED bears great utility for characterizing the function of rice genes and better understanding important biological processes and mechanisms underlying com-

plex agronomic traits in rice.

T5-24-06

Simultaneous identification of multiple causal mutations in rice

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Next-generation sequencing technologies (NGST) are being used to discover causal mutations in ethyl methanesulfonate (EMS)-mutagenized plant populations. However, the published protocols often deliver too many candidate sites and sometimes fail to find the mutant gene of interest. Accurate identification of the causal mutation from massive background polymorphisms and sequencing deficiencies remains challenging. Here we describe a NGST-based method, named SIMM, that can simultaneously identify the causal mutations in multiple independent mutants. Multiple rice mutants derived from the same parental line were back-crossed, and for each mutant, the derived F2 individuals of the recessive mutant phenotype were pooled and sequenced. The resulting sequences were aligned to the Nipponbare reference genome, and single nucleotide polymorphisms (SNPs) were subsequently compared among the mutants. Allele index (AI) and Euclidean distance (ED) were incorporated into the analysis to reduce noises caused by background polymorphisms and re-sequencing errors. Corrections of sequence bias against GC- and AT-rich sequences in the candidate region were conducted when necessary. Using this method, we successfully identified seven new mutant alleles from Huanghuazhan (HHZ), an elite *indica* rice cultivar in China. All mutant alleles were validated by phenotype association assay. A pipeline based on Perl scripts for SIMM is publicly available at https://sourceforge.net/projects/simm/.

T5-25: Phenotyping beyond technology - approaching real world problems (two sessions)

T5-25-01

Phenomics, genomics and crop systems model - Three pillars of future crop breeding programs.

Xinguang Zhu

Chinese Academy of Sciecnes

Improving crop yield potential is a major direction of future crop breeding. The rapid advances of genotyping, systems biology and high throughput phenotyping technologies now offer unprecedented opportunities to rapidly advance our capacity to improve crop yield potential. Together with rapidly increasing of genomics information and maturation of crop systems models, modern high throguput phenotyping technologies can help identify the weakest links in an elite cultivar, design the crop ideal-type under a defined environment given certain agronomic practices, and further to identify the molecular markers that can be used in the breeding. Phenomics, genomics and crop systems models are becoming three pillars of future crop breeding programs.

T5-25-02

Crop phenotyping center in HZAU (CPC): Strategies and challenges

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The functional analysis of the rice genome has entered into a high-throughput stage, and a project named RICE2020 has been proposed to determine the function of every gene in the rice genome by the year 2020. However, as compared with the robustness of genetic techniques, the evaluation of rice phenotypic traits is still subjective, inefficient, destructive and error-prone. In last decade, the key questions of genomics involved why and how to sequence genomes; now, we are faced with a new challenge: phenomics. To relieve the bottleneck, we established a crop phenotyping center (CPC) in Huazhong Agricultural University, in which we developed a rice automatic phenotyping (RAP), a yield traits scorer (YTS), a high-throughput leaf scorer (HLS), a high-throughput hyperspectral imaging system (HHIS), and a micro-CT. Equipped with multidisciplinary techniques, including photonics, automatics, computers, and mechanics, the integrated facility can extract plenty of morphology-relates traits, biomass-related traits, yield-related traits, and physiological traits. And we also demonstrate that combining high-throughput phenotyping tools and genome-wide association study can unearth more treasures hidden within rice genomics. In addition, the phenotyping had been expanded to maize and rape and we discussed the challenges of rice phenotyping in future.

T5-25-03

Development of next generation phenotyping technologies and application for characterization of genetic resources in pre-breeding scenarios

Fabio Fiorani

Forschungszentrum Juelich GmbH, IBG2 Plant Sciences

Imaging-based phenotyping of crop traits has emerged as an important tool for complementing progress in genomics. A few methods based on imaging time courses of shoot development using visible and non-visible portion of the electromagnetic spectrum are well established and their application demonstrated for quantifying non-destructively leaf area growth as a proxy for biomass accumulation, and reflectance properties linked to pigment composition and to photosynthetic activity. This presentation will first focus on a brief overview of these methods and the current applications for large scale screening of genetic resources in a pre-breeding scenario with examples from different crops (wheat, rapeseed, in particular). Next, the field of plant phenomics however still requires adaptation and of available technologies and automation for plant screening and the development of new sensors. Examples based on our work in this area will focus particularly on sensor development for non-invasive measurements of water content of leaves and developing seeds, including methods that provide volumetric data such as Nuclear Magnetic Resonance (NMR). The case for new approaches and next generation phenotyping technologies will be extended to the study of root architecture and root growth dynamics with the presentation of research phenotyping facilities developed at our IBG2 Plant Sciences Institute that encompass large scale paper pouches and automated rhizoboxes setups, and dedicated Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET) for functional studies of root growth and carbon distribution in plants.

T5-25-04

Quantitative information on plants in changing environments - integrated approaches to mechanistic, high-throughput and field phenotyping

__Ulrich Schurr__, Roland Pieruschka, Fabio Fiorani, Uwe Rascher
Forschungszentrum Jülich - IBG-2: Plant Sciences

Plant phenotyping develops rapidly into a bottleneck for progress in basic and applied research. Lack of adequate solutions for quantitative analysis of plant architecture and function as well as their interaction with the dynamic and heterogeneous environment hampers progress in basic sciences as well as in breeding-related research. In recent years significant interdisciplinary approaches have been started to overcome this “phenotyping bottleneck”. Techniques were developed to quantify the dynamics and the heterogeneity of plant structure and function as well as of environmental cues. These mostly non-invasive technologies are developed and implemented into biological concepts that allow novel insights in the dynamic characteristics of plants above- as well as belowground. The technologies include high-resolution analysis for mechanistic understanding (like MRI and PET for structure, growth and activity of roots and shoots), the high-throughput approach for analysis of large numbers of genotypes and environmental conditions as well as field approaches, which are the reference to indicate the relevance. The talk will provide an overview on recent developments in technologies as well as conceptual approaches as the basis for a quantitative understanding of plant-environment-dynamics and its application for plant breeding and plant management. The talk will also present recent developments in infrastructure platforms that have been and will be established in Germany, in Europe and globally.

T5-25-05

New phenotyping tools for drought resistance studies in rice

Zilong Guo, Wanneng Yang, Haifu Tu, __Lizhong Xiong__
Huazhong Agricultural University

Drought severely damages global food production and understanding plant responses to drought benefits drought resistance (DR) breeding. Here, we established a high-throughput phenotyping platform for DR evaluation. More than 50 new image-derived electronic ‘traits’, which have high heritability and repeatability and can monitor plant dynamic responses to drought, were phenotyped in 507 global rice panel containing 507 accessions at three developmental stages. Using these new traits, we discovered an interesting drought-induced trait association phenomenon. A large number of drought-related loci and candidate genes were identified by genome-wide association study. Integration of the new image-derived ‘traits’ and traditional DR-related traits reveals a comprehensive genetic network, which led to some significant findings about DR mechanisms. Importantly, we demonstrated that several image traits could be useful for DR evaluation in the field. This study provides new image-derived traits, which are useful in precise and dynamic phenotyping, and demonstrates that an integration of traditional and new image traits is a powerful approach for dissecting complex traits.

T5-25-06

Prototyping of an integrated field phenotyping platform – University of Tokyo field plant phenotyping research laboratory

__Seishi Ninomiya__, Wei Guo
Graduate School of Agriculture and Life Sciences, University of Tokyo

Field Plant Phenotyping Research Laboratory has launched at Institute of Sustainable Agro-ecosystem Services (formerly the University Farm), the University of Tokyo aiming at prototyping of an integrated high-throughput phenotyping platform which will be a part of a high performance breeding pipeline. The laboratory is composed of not only the scientists from several different faculties of the University of Tokyo but also the scientists from other organizations such as NARO, INRA, UQ, IITH, IITB and private sectors accelerating virtual collaboration among them and open science. The campus is located in a very convenient place rather close to the central Tokyo as well as the main campus of U. Tokyo so that the scientist and students not only from U. Tokyo but also visitors even from foreign countries can easily access there. Phenotyping is, of course, to measure crop characteristics. Crop characteristics are controlled and determined by three factors; genotypes, environmental conditions and human managements under fairly complicated interactions among them. To duly evaluate such crop characteristics, we need to understand the complicated relationship among the factors. Namely, we need to develop a model to explain crop performance (phenotype) by genotype, environment and management based on observations; $y = f(g, e, m)$ where y, g, e, m are crop performance (e.g., yield), genotype, environment and managements, respectively. Regarding the data acquisitions for the modeling, field phenotyping is currently a serious bottleneck compared with other factors. Research and development for high throughput plant phenotyping has been becoming popular in Japan. However, the studies still remain in the development of element technologies such as single traits evaluations by image analyses and evaluations of multispectral electromagnetic wave sensors. Thus, we need a study to optimally integrate those technologies to compose a high throughput breeding pipeline. Key research topics in the laboratory are; (1) aerial sensing, (2) ground sensing, (3) image analysis and 3D reconstruction, (4) artificial intelligence and modeling, (5) high throughput training data set collection, (6) plant physiology sensors, (7) environment sensor network, (8) database and data exchange platform, (9) crop performance prediction modeling which can handle G X E X M (8) prototyping of an integrated high-throughput phenotyping platform which will be a part of a high performance breeding pipeline.

T5-25-07

Field phenotyping: Quantifying dynamic plant traits across scales in the field.

***Onno Muller**, M. Pilar Cendrero-Mateo, Mark Mueller-Linow, Beat Keller, Angelina Steier, Benedikt Janssen, Ines Munoz-Fernandez, Lars Zimmermann, Christoph Jedmowski, Luka Olbertz, Anke Schikling, Andreas Burkart, Anna Van Doorn, Roland Pieruschka, Uli Schurr, Uwe Rascher*
Forschungszentrum Juelich

Phenotyping in the field is an essential step in the phenotyping chain from well -defined and controlled conditions in the laboratory and greenhouse to the heterogeneous and fluctuating environment in the field. Field measurements represent a significant reference for the relevance of the laboratory and greenhouse approaches and an important source of information on potential mechanisms and constraints for plant performance to be tested at controlled conditions. Here we present results of a range of methods that are being deployed within the German Plant Phenotyping Network (DPPN, www.dppn.de) focusing on plant architecture, photosynthesis and water relations. Specialized field platforms are established (a) to test innovative phenotyping technologies (b) to provide access to semi-controlled field installation to support breeding approaches for future CO_2-concentrations (breed-FACE) and (c) to study the translation of phenotypic properties from controlled environments to stands in the field. We show that stereo imaging allows for the quantification of canopy structure; active thermography estimates leaf water content and provides information on transpiration conditions, sun induced (SIF) and light induced fluorescence transients (LIFT) techniques allow for remote estimating of photosynthesis at canopy and leaf-to-plant level respectively . SIF is measured by the next European Space Agency satellite Earth Explorer mission as well as with the other methods is incorporated in (semi-)automated systems positioning sensors in the field. This introduces a promising portfolio to measure plant traits for field phenotyping and to enhance our understanding of relevant traits under natural conditions .

T5-25-08

Developing automated field phenotyping platforms to quantify dynamics between key performance traits and environmental factors for bread wheat

***Ji Zhou**[1], Daniel Reynolds[1], Graham Finlayson[3], Simon Griffiths[2]*
1. Earlham Insititute
2. John Innes Centre
3. University of East Anglia

Automated field phenotyping can provide continuous and precise measures of adaptation and performance related traits that are key to today's GxE crop research, breeding pipelines, and agricultural practices. In the talk, I will introduce our multi-layer automated field phenotyping system at John Innes Centre (JIC) and Earlham Institute (EI), including UAV (unmanned aerial vehicles) and fixed-wing light aircraft, 3D scanning crop phenotyping platform (i.e. Phenospex), cost-effective CropQuant workstations and other novel hardware/software solutions that we have developed to facilitate high-resolution and high-frequency crop phenotyping to study performance related traits for bread wheat. In particular, I am going to talk about how to utilise phenotypic information to empower the assessment of genes controlling yield potential and environmental adaptation. I will introduce CropQuant, a cost-effective IoT (Internet of Things) powered platform that has incorporated networked sensors, single-board computers, in-field wireless communication and open high-throughput analysis algorithms to process field experimentation data. Besides performance and adpative trait analysis, we have also established machine-learning based models to explore and predict the dynamics between genotype, phenotype and environment (GxPxE). A proof-of-principle example based on near-isogenic lines (NILs) of wheat such as *Ppd-1* (loss of function), *Ppd-D1a* (photoperiod insensitivity), *Rht-D1b* (semi dwarfing), stay green induced mutants, and *Lr19* (hypersensitive response to the pathogen), and Paragon wild type will be discussed in the talk.

T5-25-09

Using high throughput dynamic phenotyping to uderstand the influence of heat stress on wheat

Jiwan Han
Aberystwyth University

A key driver in plant breeding is to improve crop resilience to unfavourable weather events. Such events are expected to become more frequent. In this project, we examine the response of wheat to combined water and heat stress, two stresses that often occur together. To address how water stress affects subsequent heat stress we subjected plants to one of 2 water regimes at an early stage of development and subsequently to a period of heat stress at booting (meiosis), a stage thought to be particularly susceptible. Water regimes were based on soil water content and heat treatments were 5 days at 25, 30, 35 and 40℃ , with night time temperatures being 5 ℃ lower. Plant growth was monitored by daily imaging and growth stage recording, physiological parameters were taken before, during and after heat stress and mature plants harvested and grain parameters measured using microCT scanning. Image processing segments plant from background and extracts useful feature information. We manually setup the colour range to avoid including background colours into the plant. For each plant, the directly measured information includes the imaging date, bar code, height in pixel, total pixel, total yellow pixel, total pixel of flag leaf area, yellow pixel of flag leaf area, total pixel of second leaf area, yellow pixel of second leaf area, total pixel of remaining leaf area and yellow pixel of remaining area. The derived information include height in mm, area in mm^2, normalized area, overall yellow area percentage, percentage of yellow area of flag leaf area, percentage of yellow area of second leaf area and percentage of yellow area of remaining area. The height and side view area development are direct features to describe growth and senescence. Physiological measurements included measurement of chlorophyll fluorescence, gas exchange and transpiration. CT scanning of harvested mature spikes provided information on grain set and size. Results indicate that there is an interesting interaction between pre-existing water stress and the effects of a heat treatment on both stem elongation and grain yield. Further experiments are being designed to refine the stage at which heat stress has its main effect and will be discussed.

T5-25-10

Using image-based phenotyping to dissect longitudinal and developmental traits in plants

Karen Askew, Roger Boyle, Jason Brook, Anyela Camargo-Rodriguez, Fiona Corke, Despoina Dadarou, Gina Garzon-Martinez, Alan Gay, Jiwan Han, Candida Nibau, Colin Sauze, Richard Webster, Kevin Williams, ***John Doonan***
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Plants, as sessile organisms, adapt their growth and development to a wide variety of environmental influences, including abiotic, biotic and competition with neighbours. These stresses may change during their life history and also can change that history. Growth, phenology and stress-induced changes in these processes vary across life history and between individuals and these traits, referred to as longitudinal, can be dissected genetically using natural and induced variation. Until recently, such analyses have been demanding in terms of time and resource. Automated and semi-automated image-based across scales from the sub-cellular to whole organism to the field scale, combined with spacially-dispersed time-resolved and intregrated environmental sensing technologies, allow data acquisition across time, while the development of sophisticated genetically characterised populations provide access to the alleles underlying trait variation. Rapid extraction of information from such data demands innovative computational approaches. Experiments involving both model and crop plants will be used to illustrate progress made towards meeting these challenges. We discuss the potential of novel phenotyping approaches to dissect dynamic and complex traits, ranging from plant shape, response to single and multiple stresses to yield and quality associated characteristics. It is expected that a better understanding of dynamic traits will have an impact in the development of plant breeding strategies to improve crop yield and resilience.

T5-25-11

Phenotyping of the plant architecture in maize: Opportunities and strategies

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Biotechnology Research Institute, Chinese Academy of Agricultural Sciences

Maize is one of the most important cereal crops in the world and the largest grain crop in China. The breeding for ideal plant architecture is always important, especially in near future. Fast, accurate and nondestructive phenotyping has become more and more urgent and necessary. However, the traditional manual measurement which is inefficient, time consuming and error-prone, is mainstream in China, and new strategy and technology in phenotype analysis should be developed soon. Right now, a high throughput phenotyping facility with 456 pots capacity which can automated whole plant phenotyping as well as cultivation for maize has been set up in BRI, CAAS. This facility can extract plenty of morphology-relates traits, biomass accumulation and water content information by visible light imaging and near infrared imaging, followed by high capacity computing at the same time. We have obtained morphology characters of a collection of 222 maize lines during these two years. A lot of QTLs of important traits have been identified with the combination with genome-wide association studies. The phenomics technology will strengthen the understanding of the plant architecture in maize.

T5-25-12

Determination of differential pathogen sensitivity of barley cultivars by multi-reflectance and -fluorescence imaging in combination with deep physiological phenotyping

Dominik Grosskinsky*, Jesper Svensgaard, Svend Christensen, Thomas Roitsch*
University of Copenhagen

Whereas great advances have been made in the cost-efficient and high throughput analyses of genetic information and non-invasive phenotyping, the large scale analyses of the underlying physiological mechanisms is lagging behind. The external phenotype is determined by the sum of the complex interaction of metabolic pathways and intracellular regulatory networks that is reflected in an internal, physiological and biochemical phenotype. These various scales of dynamic physiological responses need to be considered and genotyping and noninvasive phenotyping must be linked to the physiology at the cellular and tissue level. Thus a high-dimensional physiological phenotyping across scales is needed that integrates the precise characterization of the internal phenotype into high-throughput phenotyping of whole-plants and canopies. Thus complex traits can be broken down into individual components of physiological traits. Since the higher resolution of physiological phenotyping by wet chemistry is inherently limited in throughput, high throughput non-invasive phenotyping needs to be validated and verified across scales to be used as proxy for the underlying processes. As a case study we have addressed the infection of four barley cultivars by two fungal pathogens with distinctly different lifestyle, the obligate biotroph *Blumeria graminis* or and the necrotroph *Drechslera teres* by amulti-dimensional, holistic phenotyping approach. The non-invasive phenotyping by multi-spectral and -fluorescence imaging in the novel high throughput phenotyping facility PhenoLab was complemented by metabolic fingerprinting via the determination of activity signatures of key enzymes of carbohydrate and antioxidative metabolism, phytohormone profiles and determination of specific defense marker genes. A differential sensitivity of the tested barley genotypes versus both pathogens could be detected by the sensor based imaging techniques which was further validated and verified by distinct physiological responses and defense reactions. This study proofs that in such an interdisciplinary and multi-dimensional phenomics approach, plant physiology, non-invasive phenotyping and functional genomics will complement each other, ultimately enabling the *in-silico* assessment of responses under defined environments with advanced crop physiology models. This will allow to generate robust physiological predictors also for complex traits to bridge the knowledge gap between genotypes and phenotypes for applications in breeding, precision farming and basic research.

T5-26: Woody plants: Genomics, Transcriptomes and Evolution

T5-26-02

Unravelling the complex regulation of xylogenic organelles

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The nuclear genomes of woody plants have evolved over millennia to efficiently sequester atmospheric carbon and deposit it in sink tissues as lignocellulosic biopolymers. In parallel, the plastid and mitochondrial genomes of these plants have had to evolve to function coordinately with the nuclear genome during xylogenesis. Despite being the sites of energy production and important cell wall precursor biosynthesis, the contribution of plastids and mitochondria in wood formation remains almost completely unexplored. We used next-generation sequencing data to analyse the expression and regulation of organellar genes in the main carbon source (mature leaf), sink (developing xylem), and transport (secondary phloem) tissues of *Eucalyptus grandis*, an economically important tree species, in order to better understand how organelles contribute to xylogenesis. We assembled and annotated the plastid and mitochondrial genomes of *E. grandis*, and utilized ribosomal RNA-depleted, polyA-selected, and small RNA sequencing data of leaf, xylem, and phloem, to profile organellar gene expression, with a specific focus on the post-transcriptional regulation of organellar transcripts. We find that organellar gene transcript abundance is regulated at multiple levels between autotrophic and heterotrophic tissues to ensure functional gene products. In plastids specifically, which are primarily geared toward photosynthesis in mature leaf, and towards phenolic precursor biosynthesis in developing xylem, we find that differential gene expression, splicing, and editing contribute to the functional heterogeneity of plastids between tissues. Further, we identify the relationship between nuclear encoded regulators of organellar biology and transcription and the function of organelles during xylogenesis. This study sheds new light on the complex interplay between nuclear and organellar genomes during xylogenesis, opening up new avenues of future research into the regulation and evolution of xylogenesis, as well as the biotechnological improvement of tree crop species.

T5-26-03

Sex in the Salicaceae

Stephen Difazio[1], *Ran Zhou*[1], *Lawrence Smart*[2], *Craig Carlson*[2], *Fred Gouker*[2], *Brian Sanderson*[3], *Sandra Simon*[1], *David Macaya-Sanz*[1], *Ken Keefover-Ring*[4], *Matt Olson*[3], *Gerald Tuskan*[5]
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5. Oak Ridge National Lab

The Salicaceae family contains several primarily dioecious genera, most notably *Populus* and *Salix*. These provide excellent opportunities for studying the evolutionary and ecological dimensions of sex dimorphism due to their shared and contrasting characteristics. *Populus* is comprised of about 30 wind-pollinated tree species. In contrast, *Salix* consists of approximately 500 species, most of which are insect-pollinated shrubs. Both genera have excellent genomic and genetic resources and show high collinearity and sequence conservation, thus facilitating comparative analyses of the genomics of sex determination. *Populus* sex determination is conferred primarily by loci on chromosome 19, apparently coupled with other loci on chromosomes 3,6, 9,14, and 18. Some of these may be assembly artifacts, so these auxiliary loci must be validated by improved assembly coupled with dense genetic mapping. In contrast, sex determination in *Salix* is primarily conferred by chromosome 15, coupled with loci on chromosomes 1 and 19. Furthermore, based on genotype configurations in and around the sex determination region (SDR), *Populus* has male heterogamety and *Salix* has female heterogamety. Using long-read sequencing and dense genetic mapping, we have reconstructed portions of the Y and W chromosome regions of each species and analyzed sex-specific sequence expression and sequence motif enrichment. Surprisingly, there is virtually no overlap in gene content of the SDR between the genera. Furthermore, chromosome 19 in *Salix* shows some hallmarks of sex chromosomes, while chromosome 15 in *Populus* does not. This leads to the hypothesis that chromosome 19 is the ancestral SDR, and that *Salix* has undergone a conversion to a ZW system located on chromosome 15. This multi-locus sex determination system may account for the consistently female-biased sex ratios observed in *Salix* species. Functional and population genetic analyses of these regions are ongoing, and should shed light on the evolutionary and ecological factors favoring male and female heterogamety in plants.

T5-26-04

Adaptive landscape and population genomics of silver birch

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Silver birch (*Betula pendula*) is a pioneer tree species prevalent in boreal forests across Eurasia. In contrast to most other trees, which have generation times from several years to decades, birch can be induced to flower within one year. This, together with a small 440 Mb genome and advanced breeding material, makes birch an attractive target for modern genomics-based biotechnology. We assembled the nuclear genome sequence of a fourth generation self-pollinated individual and anchored it by genetic linkage to chromosomes. Deep-time coding sequence evolution was analyzed by partitioning duplicate genes based on their evolutionary origins. Polyploid duplicates, dating back to the pan-core-eudicot paleohexaploid event, were enriched for transcription factor functions, whereas tandem duplicates were over-represented with environmental responses and secondary metabolism. Population genomic analyses based on 80 resequenced individuals representing most of the geographic species range showed effective population size crashes at well-known points of climatic upheaval during geologic history. Selective sweeps, shallow-time adaptive events, were enriched for genes originating from the ancient polyploidy event, and included genes involved in receptor kinase signaling, cytokinin and light perception, and sexual reproduction. Several candidates showed signatures of recent natural selection correlating with environmental variables. For example, selective sweeps in *PHYC* and *FRS10*, two tightly linked genes encoding light-response proteins, correlated with latitude/longitude and temperature, and in the case of *PHYC*, also with precipitation. Similar associations were also detected for *ARR1*, which acts in growth-promoting cytokinin perception, and for *KAK* and *MED5A*, which are involved in wood development. Overall, swept genes were enriched for key developmental or physiological triggering functions, suggesting that at least in birch, selection has acted by tuning the timing and crosstalk between fundamental plant processes.

T5-26-05

Genomes and evolutions of the endangered and widespread ironwood species

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Ironwood species, known for their hard woods, provide an especial resource in our life. But the mechanism of the hard wood formation remains unknown. One of the ironwood species with extremely small population with only five wild individuals also serves as the ideal system to examine genetic consequences of the endangered ironwood species. Here, we report the high-quality genome assemblies of the endangered *Ostrya rehderiana* (366.2 Mb, scaffold N50 = 2.3 Mb) and the widespread *O. chinensis* (386.6 Mb, scaffold N50 = 762.5 Kb). Both assembled genomes cover more than 94%/96% of the estimated genomes (386 Mb/400 Mb) and harbour 27,831/31,152 predicted protein-coding genes respectively. A total of 756 and 1,310 gene families were respectively expanded in *O. rehderiana* and *O. chinensis*. These gene families were mainly associated with the interaction with their local environments, such like signal transduction and ion channel. 606 gene families were expanded in the ancestor lineage of *O. rehderiana* and *O. chinensis*, some of which are related to the lignin metabolic process underlying the ironwood formation. The endangered *O. rehderiana* shows a lower heterozygosity and sequence diversity (π) and higher levels of deleterious mutations and inbreeding than the widespread *O. chinensis*.

T5-26-06

Identification of the molecular processes needed for secondary vascular development in woody plants through transcriptome analysis

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Usually woody plants are able to grow for many years through perennial cambium activity for development of the secondary vascular system. Specific molecular processes need to be evolved for directing secondary vascular development. Analysis of transcriptome in different vascular systems would help uncover the mechanisms underlying perennial woody plant growth. Genes and processes fundamental to vascular development across different vascular systems were identified. These genes are expressed mainly in vascular tissues, forming a complex of coexpression network with multiple functional connections. Near half of such networks have experimental descriptions, while more fundamental biological processes in controlling vascular system development remains to be elucidated. Transcriptome analyses reveal that approximately one third of the genes that are expressed in secondary vascular development in *Populus* occur alternative splicing. Characterization of the alternative splicing suggests that woody plants may have evolved some alternative splicing events which function particularly in regulating secondary vascular development. In summary, our studies identified a number of molecular networks which are specifically involved in the biological processes needed for perennial woody plant development. Characterization of such processes aids in advancing the understanding of woody plants.

T5-27: GWAS and Genetic Regulatory Networks

T5-27-01

A network-module of *LRR-RLK* genes quantitatively regulates root growth under iron-limited conditions in Arabidopsis

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Growth responses in plants are orchestrated by complex networks of genes that sense and transduce signals, and regulate specific developmental processes. While many genes have been identified that play a role in growth responses to the environment, very little is known about which genes and gene networks determine the extensive natural variation that can be observed for growth responses to the environment. For instance, the onset of root growth arrest upon iron (Fe) deficiency varies remarkably between different natural accessions of *Arabidopsis thaliana*. We therefore set out to identify networks of genes in which natural genetic variation affects the growth response to Fe deficiency. For this, we used a diverse set of 450 natural accessions of Arabidopsis that showed high variation in their primary root elongation when grown on Fe-deficient medium. We identified more than 20 statistically significant genomic loci that are associated with changes in root growth rate upon Fe deficiency. We used a whole-genome functional gene network model to identify genes in the immediate proximity of these associations that showed evidence for functional interaction. This analysis yielded several network modules of potentially interacting genes. Importantly, one of those modules, a cluster of 3 *Leucine-rich repeat receptor-like protein kinase* (*LRR-RLK*) genes and a protein kinase (*PK*) gene showed a strong signature of epistatic interactions. Each of the single mutant lines of these signaling genes displayed root growth rate reduction on Fe deficient media but not on full media, showing that this gene cluster is involved in growth regulation under Fe limited conditions. Due to their tissue specific expression pattern, we hypothesize that these four genes are coordinating growth responses in different tissues of the root. Consistently, we found that at least two of these LRR-RLKs physically interact at the protein level and we are currently investigating whether the combinatorial interaction of these 4 genes and their alleles are key determinants of the root growth response to Fe limited conditions.

T5-27-02

Global migration and adaptation in *Brachypodium* compilospecies

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To understand the genetic basis for wide adaptability, thousands of accessions were analyzed for genomic variation in ploidy and population structure. The allopolyploid *Brachypodium hybridum* is globally distributed with multiple introductions into Australia and N. America but little subsequent admixture. Diploid *B. stacei* is narrowly distributed in the Mediterranean, subsumed by *B. hybridum*. A core set of diverse diploid *B. distachyon* was selected balancing western and eastern Mediterranean regions of the dominant subspecies for detailed genomic and phenomic characterization. 1.5 M SNPs were identified among 75 accessions phenotyped with replication for early growth and reproductive traits under simulated seasonal and future climates conditions. Major QTL were identified flanking dozens of candidate genes for early vigour, flowering time in photothermal units and other agronomic traits.

T5-27-03

The added value of genome wide association mapping in the elucidation of quantitative trait regulation

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Over the last decade genome wide association (GWA) mapping has rapidly become a major tool for the identification of natural polymorphisms explaining observed population variation. Especially in *Arabidopsis* the field has rapidly developed and contributed to many aspects of modern biology. Here, we will highlight some of the advancements made and illustrate this with recent case studies. GWA mapping has been instrumental in identifying novel regulators of complex traits. As such, it has contributed to our further understanding of physiological traits, plant metabolism and adaptation and evolution. Now that many studies have appeared it is becoming increasingly clear that natural variation at specific loci can have strong pleiotropic effects on multiple traits. This is especially noticeable in the coregulation of plant metabolism, where often hotspots of trait associations are found. In addition, many examples of epistatic genetic regulation can be seen, further adding to the complexity of quantitative trait regulation. Nonetheless, GWA mapping has allowed the identification of many novel players in the pathway topology of many traits. Some of these show strong signatures of evolution, such as selective sweeps or balancing selection. This is often reflected in the geographical distribution of allelic variation and thus provides indications for ecological adaptation. We will provide examples to demonstrate the contribution that GWA mapping has made to our understanding of the evolution and regulation of quantitative trait variation.

T5-27-04

GWAS in *Arabidopsis thaliana* – Is work on a model organism obsolete?

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Understanding the causal relationship between genotype and phenotype is a major objective both in humans and plants. The main interest is in understanding trait architecture and identifying loci contributing to the respective traits. Genome-wide association mapping (GWAS) is one tool to elucidate these relationships and has been successfully used in many different species. However, most studies concentrate on marginal marker effects and ignore epistatic and gene-environment interactions. These interactions are problematic to account for, but are likely to make major contributions to many phenotypes. Understanding these interactions is crucial to conceive trait architecture and predict phenotypes. Here *Arabidopsis thaliana* serves as an excellent model for developing statistical frameworks to quantify these effects, as a plethora of both genetic and phenotypic data is publicly available. Data about new GWAS models, general considerations about GWAS and data quality will be presented.

T5-27-05

Genome wide association genetics, outlier analysis and RNAseq identify the genetic and genomic basis of drought tolerance in bioenergy *Populus nigra*

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More than 500 genotypes from a natural, *P. nigra* mapping population, drawn from across the species' western European range, were cultivated in the field under short rotation coppice (SRC) in Savigliano, Italy. The population was phenotyped for biomass yield and leaf size traits under irrigated and moderate drought treatments. Sequence capture genotyping by sequencing (GBS) targeting the gene space yielded 132,000 single nucleotide polymorphisms (SNPs) representing 29,400 genes. Genome wide association genetics (GWAS) identified candidate genes for biomass, leaf size and drought tolerance while genetic outlier analyses identified additional signatures of adaptive selection to important precipitation and temperature variables. A complementary glasshouse experiment applied a controlled drought to three morphologically and physiologically diverse genotypes from the natural population and these were subject to intensive phenotyping for leaf development and growth traits under control and moderate drought conditions. RNAseq was then employed to elucidate the transcriptomic response of these diverse genotypes to drought stress with over 2700 differentially expressed genes found to be involved in the drought response. When gene-phenotype networks were re-constructed for leaves of distinct developmental stages using the top 100 most differentially expressed genes, five key candidates were identified through gene ontology as being central to the drought response. These microtubule-related genes act as gene hubs in young leaves, playing a central role in the drought response. Subsequently, as leaves mature, these genes are reduced in their connectivity and are situated in the peripheral parts of the networks. Genes of interest overlapping between the GWAS, outlier and RNAseq analyses were identified and these are strong candidates for application in advanced molecular breeding or genome editing for the sustainable intensification of this important lignocellulosic crop.

T5-27-06

Evolutionary game theory reveals the origin of modularity

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Developmental modularity, i.e., coherent organization and function of developmentally related organs or traits, has been extensively studied in developmental and evolutionary biology. However, our understanding of how genes modulate developmental modularity has still been very limited. Here we describe and assess a conceptual model to map the genetic architecture of developmental modularity by integrating evolutionary game theory into a genetic mapping design. We view a developmental module as an ecological system in which the constituting components or traits compete for space and resources and cooperate with each other to govern the function and behavior of the module. We embedded mathematical aspects of competition and cooperation within systems mapping, a dynamic framework for genetic mapping, to test whether and how specific genes regulate the coordination of different but interconnected traits toward developmental modularity. The new model provides a powerful tool to study the genetic mechanisms underlying the origin, structure and function of developmental modularity and morphological integration.

T5-28: Genome duplication and variation

T5-28-01

Variations in gene copy numbers in crop wild relatives

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Gene copy number manipulation may be an under exploited mechanism for crop improvement. Analysis of copy number variation in crop wild relatives may reveal a source of useful variation for many important traits. Advances in genome sequencing and assembly are facilitating a growth in information on gene copy numbers and their variation in plants. Genome sequences for domesticated and wild rice species are now available for most species in the genus making this a good system to explore copy number variation in wild and domesticated plants. Rice grains are mostly starch and this abundant grain component is critical for many of the functional and nutritional attributes of rice. We have examined the genes for starch biosynthesis across the *Oryza* genus to identify any variation in copy number that might have a role in controlling rice starch properties. Most genes of starch metabolism show a high degree of conservation of genome location and copy number in rice and the wild relatives within the *Oryza* genus. A possible second pullulanase gene was detected in the published assembly of the *O. glaberrima* genome. A wild rice from Australia appears to have a second *GBSS* gene. Both the Australian and African taxa have novel starch properties including a high amylose content. The Australian taxa is descendant from a common ancestor of the Asian and African domesticated rice species. Changes in starch genes in the Asian and African wild populations may have been a key step in making the rice easier to cook and resulting in this A genome clade becoming attractive to domesticate.

T5-28-02

Gene expression patterns and RdDM-mediated epigenetic regulations of duplicated genes in *Brassica napus* subgenomes A and C

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Plant genome polyploidization and subsequent evolution is a crucial process for trait innovation and new species formation. Building of gene expression patterns of polyploidy duplicated genes is a first step in the process. Previous researches have indicated that these duplicated genes may inherit progenitors' expression patterns or form new patterns. In some polyploids, duplicated gene expression is biased toward one genome (genome dominance) while others are not. In all cases, mechanisms to form these expression patterns are poorly understood. In this study, we used a set of materials including the extracted AA subgenome (AeAe, n = 10) plants and its corresponding tetraploid *Brassica napus* (AnAnCnCn, n = 18) plus the tetraploid progenitors *B. rapa* (ArAr, n = 10) and *B. oleracea* (CoCo, n = 9) genomes to investigate duplicated gene expression patterns and further regulatory mechanisms of the patterns. Through analyses of whole genome re-sequencing and RNA-sequencing data, we found 80% of duplicated genes (in total about 23,000 gene pairs in leaves) inherited progenitors' gene expression patterns and 20% showed altered expression patterns. For the regulatory mechanisms of the altered patterns, we hypothesized that 24-nt siRNAs might be a kind of regulators because A and C have a big difference in transposable elements (TE) producing 24-nt siRNAs which mediate RNA-directed DNA methylation (RdDM) and thus affect nearby gene expression. To test this hypothesis, we performed small RNA sequencing and DNA methylation sequencing. By comparing to Ae, we found that 20% of the genes with expression alteration can be explained by RdDM in which this portion of TEs in the An genome could be targeted and methylated by 24-nt siRNAs from homologous TE copies on the Cn genome. For the inheritable gene expression patterns, we found that TEs which were asymmetrically inserted and then methylated by a small RNA-directed pathway, both already formed in each progenitor genome, have performed fixation on the inheritable patterns. The trends also exist in 15 Mya-triplicated subgenomic expression dominance. Our results revealed expression patterns of *B. napus* polyploidy duplicated genes and provided new insights into the regulatory mechanism of the expression patterns in polyploid evolution.

T5-28-03

Harnessing of untapped genomic variation in *Glycine* subgenus *soja*

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Soybean has undergone at least two rounds of genome duplication and produced mosaic of duplicated segments. The domesticated soybean (*Glycine max* (L.) Merr.) and its wild progenitor, *G. soja* exhibit substantial morphological difference in growth habits, seed-coat color, seed size and so on. To elucidate the genomic differences, seven representative *G. soja* accessions were *de novo* sequenced and compared with reference genome of *G. max*. We found uneven distribution of genetic diversity across 20 chromosomes. Inter-genomic comparisons identified 1,978 genes with copy number variation, 338 genes with *G. soja*-specific presence-absence variation, 2,094 genes affected by artificial selection during domestication for preferred agricultural traits. Comparison of candidate domestication-related genes with previous QTLs, as well as their homologs, we found some genes may contribute to variation of agronomic traits such as pathogen resistance, seed composition, flowering and maturity time, organ size and final biomass. Accordingly, our study of genome variation has yielded fundamental insights into the genes and biological mechanisms that underlie favorite trait for cultivar development.

T5-28-04

Structural and evolutive analysis of ancestral chromosomes fusion points within the hexaploid wheat genome

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The explosion of genomes sequencing has highlighted whole genome duplications (WGDs) as a major evolutionary driving force shared by multiples branches of the tree of life. However, the genome sizes and chromosome numbers have not increased exponentially over time because WGDs have been balanced by chromosome number reduction (CNR) and gene losses. In plants these two mechanisms have been found through genomes sequences comparisons and ancestral karyotype reconstruction. For monocots models have been proposed explaining CNR by nested chromosome fusions (NCFs). However, molecular mechanisms driving ancestral chromosome fusions that have led to the present day monocots karyotypes are still largely unknown. Based on this ascertainment, we aimed at characterizing the two chromosomes fusion points (FP) of wheat chromosome 1 that was formed by the nested fusion of two ancestral chromosomes. To obtain a high quality sequence of a FP we implemented a strategy based on the reconstruction of the ancestral grass karyotype, the definition of the synteny relationships between wheat and rice (as the modern representative of the ancestral 12 chromosomes grass karyotype) and the screening of wheat chromosome specific BAC libraries. We focused on the two FPs of chromosomal group 1. We investigated this region on chromosomes 1A, 1B, 1D obtaining two FP sequences (one on the short arm and one on the long arm) for each of them. The annotation of genes at these 6 loci allowed us to established orthology relations chromosome 5 and 10 of rice (images of ancestral chromosomes A5/A10) and homeology relations within the 3 subgenomes of wheat. The origin and the order of the genes neighboring the FP were confronted to models of chromosomes fusion of *Triticeae* described in the literature.

T5-28-05

Genome-wide identification, evolutionary analysis of Late Embryogenesis Abundant and Vicinal Oxygen Chelate family genes and their functions in drought stress tolerance and lipid metabolism in *Brassica napus* and *Arabidopsis*

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Background:
Drought stress is one of the most important abiotic stresses that determines crop yield, geographic distribution of plant species. *Brassica napus* serves as the third largest oil seed crops in the world. Many researches focus on the oil metabolism of *B. napus*. Late embryogenesis abundant (LEA) proteins are a diverse and large group of polypeptides that play important roles in desiccation and freezing tolerance in plants. The vicinal oxygen chelate (VOC) proteins are members of metalloenzyme superfamily that play roles in many biological reactions. Some members of *LEA* and *VOC* gene family have been systematically characterized in other important plants but not *B. napus*. Our study were to identify the members of these two gene families in *B. napus* genome and provided new insights in the evolutionary and functional analysis of *LEA*s and *VOC*s in *B.napus*.
Results:
A total of 108 *LEA* and 38 *VOC* genes were identified based on their conserved domains in *B. napus*. The results revealed that most of *BnVOC* and *BnLEA* genes have few introns, and all of *BnVOC*s contained the typical VOC structure of βαβββ modules. *BnVOC* and *BnLEA* genes are distributed unevenly across 19 chromosomes in *B. napus*, occurring as gene clusters in chromosomes A9, C2, C4, C5 and A6. The synteny of these genes is preserved. Synonymous (Ks) and nonsynonymous (Ka) values show most of

the *BnLEA* and *BnVOC* genes have Ka/Ks ratios less than 1, suggesting their conserve function and structure under evolutionary selective pressure. The expression of most *BnVOC*s and *BnLEA*s was increased in leaves and late stage seeds, further expression pattern results showed that seeds of *B. napus* with high oil content show higher expression levels of *BnVOC*s in drought stress. Different copies of *BnLEA* and *BnVOC* genes and the homologous *AtLEA* and *AtVOC* gene from *Arabidopsis* were cloned and then over-expressed in *Arabidopsis*, the analysis of transgenic seeds showed both the oil content and seed weight were increased 2%-5%, and the fatty acid composition were also affected. In drought stress, *Arabidopsis* T-DNA insert mutant *lea3* and RNA interference plants *atvoc-rnai* showed less drought resistance, exhibiting differences in many physiological parameters, including leaves temperature, chlorophyll content and so on .

Conclusion:

Whole-genome duplication and segmental duplication played a major role in the expansion of the *BnVOC* and *BnLEA* gene family in evolution, *BnVOC* gene family is a consequence of mesopolyploidy in *Brassica* evolution. Expression profiles and functional analysis suggested BnVOCs and BnLEAs might not only response to drought stress but also affect lipid metabolism. This present study gives a comprehensive analysis of the *LEA* and *VOC* gene family, provides new insights into the evolutionary pattern, and links their novel biological function of different aspects in *B. napus*.

T5-28-06

Analysis of Pinaceae gene family evolution suggests accelerated gene turnover in pine trees and lineage-specific amplifications of genetic networks

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Gene duplications and losses are major determinants of evolutionary change. Comparative genome-wide analyses of gene family evolution are often applied to infer patterns of gene turnover, i.e. gene gains and losses, across a range of species. Such investigations rely on gene annotations, which are often incomplete and partially inaccurate in draft genomes and transcriptomes. This issue is particularly relevant in species with large genomes (>10 Gb), including conifers. In this work we analyze gene family evolution in draft genomes of four conifers (loblolly and sugar pine, Norway spruce and Douglas-fir) using the likelihood framework implemented in the CAFE software, which allows estimating gene turnover rates and patterns in a species phylogeny. After grouping all annotated genes in orthogroups with OrthoFinder, we selected 16,118 gene families (~24,000-28,000 genes per species) occurring in at least two species for downstream analysis. The CAFE analyses indicated that gene turnover is at least twice as high in the two pines (~110 gene gains and/or losses per million years) compared to Norway spruce and Douglas-fir, a result that was robust to changes in the number of pine species used and the model of gene family evolution implemented in CAFE. We also identified 1,192 gene families with significant expansion or contraction changes, and a high proportion of gene duplications vs. gene losses in Douglas-fir. Overall, our results indicate that no less than 1/5 of all gene families experienced gains and/or losses during the evolution of these Pinaceae. However, we observed that up to ~50% of genes are much shorter than the longest gene in the same gene family, likely a result of pseudogenes missannotation. We applied a stringent length cutoff of <75% of the longest gene in the same gene family in order to remove putative pseudogenes from the 1,192 gene families with high gene turnover rates. Despite this conservative length threshold, we were able to identify many genetic pathways in the four Pinaceae with significant expansions in multiple gene families, indicating selective pressure toward retention of gene duplicates and amplifications of entire genetic networks.

T5-29: Genetics and Metablomics Studies of Higher Plants

T5-29-01

Biodiversity and regulation of wheat cuticle waxes in relation to drought

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Bread wheat, *Triticum aestivum*, represents about 30% of the world's cereal cultivation area and provides 20% of the calories for the human population. Wheat is cultivated on over 220 million hectares worldwide. Much of this land is often under the influence of limited water supply and heat that significantly impair the crop's yield. Understanding how a plant copes with these environmental challenges has a vital significance for the improvement of crop tolerance and yield. Plant cuticle is a continuous protective sheet that covers all aerial surfaces of the plant body and acts as an interface for the plant's interactions with surrounding environment. The main functions of the cuticle is to conserve water in internal tissues and protect the plant from harmful UV radiation. The goal of the current work is to get insights into cuticle structure, composition, and relatedness to drought tolerance in Australian wheat cultivars. Our analysis of wheat varieties with different levels of drought resistance and glaucousness show that considerable variation exists between genotypes in the composition and structure of the cuticle. In elite Australian wheat cultivars we identify β-diketones to be the main determinant of a glaucous phenotype; this determines the shape of wax crystals on the leaf surface and is an important contributor to efficient tissue water conservation. Our data reveals a complex relationship between the cuticle and drought tolerance, indicating that glaucousness and underlying β-diketones are not a single indicators of drought tolerance. Rather, cuticle thickness, specific cuticle wax composition (in particular the presence of significant contents of β-diketones, primary alcohols and alkanes, in concert with densities of stomata), all influence leaf water loss under the conditions of water deficit. These features of cuticle composition and deposition provide useful selection criteria for further exploration. Further, we demonstrate the drought-inducible expression of four wheat genes encoding *MYB* transcription factor (TF) genes. In wheat cells, the products of three genes (*TaMYB74, TaMYB31*, and *TaMYB24*) operate as the activators of cuticle biosynthetic genes. Functional MYB-responsive elements localized in the promoter region of the *SHN1* gene were specifically recognized by TaMYB74, but not by other MYB TFs. This data, integrated with other observations, allowed us to propose a scheme that links together drought, investigated TFs, downstream cuticle-related biosynthetic genes, and cuticle wax components. The overexpression of wheat homolog of the transcription factor WIN1/SHN1 (TaSHN1), which was previously shown to influence cuticle composition and drought tolerance in several plant species, influences both cutin and cuticle wax composition, plus the performance of selected wheat transgenic lines. Overall, our results extend the knowledge on cuticle biosynthesis regulation in wheat, and could potentially be used for engineering of cereal crops with enhanced tolerance and performance under drought.

T5-29-02

Insights into ROS sensing systems from algae to plants

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Reactive oxygen species (ROS), which are continually generated as a consequence of the normal metabolism in aerobic organisms, have recently emerged as important cellular signalling molecules controlling development, initiating cellular stress responses and cell death in a wide range of organisms. An important type of ROS is singlet oxygen ($^1O_2$), which is generated by energy transfer to molecular oxygen. What connects the primary chemical signals generated by $^1O_2$ (oxidized lipids and carotenoids) with the transcriptional responses at the level of nuclear gene expression is currently unknown. Searching for possible ROS sensor proteins with the combination of a ROS-inducible reporter in the unicellular green alga *Chlamydomonas* and reverse genetic analyses in *Arabidopsis*, we identified a small zinc finger protein, METHYLENE BLUE SENSITIVITY1 (MBS1) that is required for induction of $^1O_2$–dependent gene expression and accumulates in distinct granules in the cytosol upon oxidative stress. Using structural techniques in nuclear magnetic resonance (NMR), we firstly demonstrate that the solution structure of the MBS1 reveals the architecture of the single C_2H_2 zinc finger domain, which has emerged as a redox-sensing switch. Moreover, the conformational changes of MBS1 on the zinc binding site undergoes in response to $^1O_2$ exposure, but no significant conformational changes treated with another type of ROS, hydrogen peroxide (H_2O_2) *in vitro*, as shown by NMR and CD spectral. To gain a deeper understanding of the mechanisms involved in ROS sensing and, in particular, of the role of the novel protein MBS in $^1O_2$ sensing and signal transduction in *Arabidopsis thaliana*, point mutants have successfully been generated that eliminate the zinc binding sites in the protein and thus allowed us to directly test the hypothesis that the zinc finger acts as a ROS sensor *in vivo*. The outcome of these experiments was very clear: the zinc finger site of MBS1 is direct and specific $^1O_2$ signaling targets and required for acclimation of *Arabidopsis* to singlet oxygen. These findings provide new insights on a structural for the mechanism underlying the $^1O_2$ sensing of MBS1.

T5-29-03

Genetics and genomics of cotton fiber development

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Gossypium spp. produces two kinds of non-glandular trichomes. The first one is the hair trichome as same as in *Arabidopsis* and another is seed trichomes called fibers which are extremely elongated single cells and the largest source of natural textile grown throughout the world. Cotton fibers can be classified into two types: lint and fuzz. Lint initiates growth between anthesis and two days postanthesis (DPA) and can elongate 2.5-3.5 cm, whereas the fuzz which is approximately 0.5 cm in length initiates growth between 5 and 10 DPA. Based on map-based cloning strategy, for the first time, we isolated industrially important lint fiber production gene and fuzz development gene. We clarified that cotton has developed a unique, highly specialized transcriptional regulatory network responsible for fiber production, which is different from that in Arabidopsis leaf hair trichome development. The characterization of target genes and of the network that regulates fiber production has provided insights into the molecular mechanisms underlying cotton fiber development and underlying the increased lint yield produced by the induction of a greater number of epidermal cells to develop into lint fibers by genetic engineering.

T5-29-04

Duplication and neofunctionalisation of DODA was key to the emergence of betalains

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Betalains are plant pigments that produce a palette of pink, purple, yellow and orange colours. They are taxonomically restricted, being found only in the core Caryophyllales, whilst the rest of the flowering plants use anthocyanin compounds to produce a similar palette of colours. Intriguingly, anthocyanins and betalains have never been found together in the same plant. Betalains are derived from tyrosine and the biochemical pathway is very simple, requiring a minimum of two enzymes, DODA and CYP76AD, to produce the core betalain structures – betaxanthins (yellow-orange pigments) and betacyanins (pink-red pigments). The enzyme DODA, 4,5-DOPA-extradiol-dioxygenase, acts on L-dopa to produce betalamic acid, the principle chromophore of betalains. Betalains evolved once only and, just prior to their acquisition, DODA was duplicated. The ancestral DODA lineage (DODAβ) is found throughout the land plants whereas the derived lineage (DODAα) is only found in species with betalain pigmentation. Furthermore, in families that have reverted back to anthocyanin pigmentation, DODAα has been lost or down regulated. This suggests that duplication and subsequent neo-functionalisation of DODA was key to the emergence of betalains. We have isolated genes encoding DODAα and DODAβ from species representing different stages along the phylogeny and around the origin of betalains in order to test their capacity to produce betalain compounds. Only enzymes encoded by *DODAα* genes are able to produce a high level of betalains. Using phylogenies compiled from transcriptome data of 250 Caryophyllales species, we have determined the amino acid residues under positive selection and reconstructed the most likely ancestral DODA sequence preceding the duplication. We are functionally validating the role of these residues in betalain production and determining whether these residues are sufficient, alone or in combination, to convert the ancestral sequence into an enzyme that efficiently catalyses L-dopa. Ultimately, we will be able to determine the critical alterations that allowed an enzyme to specialize on a different substrate and assist in producing a novel class of plant pigments.

T5-29-05

Tracing recent domestication in endemic New Zealand plants.

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We are examining recent domestication by using genetic analyses of four endemic New Zealand plants that were brought into cultivation by Māori. New Zealand was one of the last places on earth to be discovered therefore cultivated endemic crops offer a unique window into the early stages of the domestication process. Prior to human arrival these species are thought to have been restricted to the northern North Island. Their occurrence in the south of New Zealand and the distant Chatham and Kermadec Island groups, is strongly associated with Māori archaeological sites, and is considered to result from translocations as part of their cultivation. Our research aims to determine the number of times each species was brought into cultivation and the way in which domestication has reduced genetic diversity in the cultivated populations. Another aim is to reveal prehistoric Māori settlement and trade routes. We have used a range of genetic techniques including whole chloroplast genome sequencing, HRM, Sanger sequencing, microsatellite genotyping and ddRAD.

T5-29-06

Application of optical topometry to the plant epidermis

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The plant epidermis regulates key physiological functions contributing to photosynthetic rate, plant productivity, and ecosystem stability. Yet, quantitative characterization of this interface between a plant and its aerial environment is laborious and destructive with current techniques, making large-scale characterization of epidermal cell parameters impractical. Here, I present the exploration of optical topometry (OT) for the analysis of plant organ surfaces. OT is a mature, confocal microscopy-based implementation of surface metrology that generates nanometer-scale digital characterizations of any surface. I report epidermal analyses in *Arabidopsis* (*Arabidopsis thaliana*) and other species as well as dried herbarium specimens and fossilized plants. We evaluate the technology's analytical potential for identifying an array of epidermal characters, including cell type distributions, variation in cell morphology and stomatal depth, differentiation of herbarium specimens, and real-time deformations in living tissue following detachment. As applied to plant material, OT is very fast and nondestructive, yielding richly mineable data sets describing living tissues and rendering a variety of their characteristics accessible for statistical, quantitative genetic, and structural analysis.

T5-30: Omics of Plant Stress Resistance

T5-30-01

Gene positioning by light

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Plant genomes are extremely sensitive to and can be developmentally reprogrammed by environmental light cues. Increasing evidence in yeast and mammalian models suggests that environmental cues can regulate gene activity by altering the spatial positioning of individual genes within the nucleus. However, if and how the spatial positioning of individual genes contributes to gene regulation in plants is still poorly understood. We have developed a method to label the positioning of individual genes by utilizing rolling-circle amplification of gene-specific circularizable oligonucleotides coupled with fluorescence *in situ* hybridization. Using this approach, we demonstrate that light triggers a rapid repositioning of the light-inducible *CAB1* locus in *Arabidopsis* from the nucleoplasm to the nuclear periphery during its transcriptional activation. *CAB1* repositioning is mediated by the red/far-red photoreceptors, the phytochromes, and is inhibited by the repressors of phytochrome signaling, including COP1, DET1, and PIFs. *CAB1* repositioning appears to be a separate regulatory step occurring prior to its full transcriptional activation. Moreover, the light inducible loci *RBCS*, *PC*, and *GUN5* undergo similar repositioning behavior upon their transcriptional activation. Our results support a novel gene regulatory mechanism in which phytochromes activate light-inducible loci by relocating them to the nuclear periphery. This study provides the initial evidence for the biological importance of gene positioning in plants and shows that plant genes can be spatially reorganized in response to environmental cues.

T5-30-02

Small RNAs contribute to plant resistance to *Phytophthora* pathogens

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A broad range of parasites rely on the functions of effector proteins to subvert host immune response and facilitate disease development. The notorious *Phytophthora* pathogens evolved effector proteins with RNA silencing suppression activity to promote infection in plant hosts. Small RNAs have been shown to regulate immunity, which requires transcriptional reprogramming. The *Phytophthora* effector PSR2 specifically affects the accumulation of phased small interfering RNAs (phasiRNAs), which are secondary siRNAs derived from coding or noncoding loci using microRNAs (miRNAs) as the triggers. *Arabidopsis thaliana* mutants defective in phasiRNA production are compromised in defense against *Phytophthora* infection, indicating that the phasiRNA pathway plays a positive role in plant immunity. Here, we report the identification of specific phasiRNAs that regulate immune responses and discuss the molecular mechanism by which PSR2 suppresses phasiRNA biogenesis and promotes infection.

T5-30-03

H3K9me2-mediated regulation of NB-LRR immune receptor expression control in *Arabidopsis thaliana*

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2. Nihon University

COPIA-R7, a transposable element (TE) inserted into the *Arabidopsis thaliana* NB-LRR-type immune receptor gene *RPP7*, has recruited the histone mark H3K9me2 to this locus. H3K9me2 is a suppressive epigenetic signal known to transcriptionally silence TEs in plants. However, at *COPIA-R7* this mark affects the choice between two alternative *RPP7* transcript polyadenylation sites and, thereby, influences the critical balance between *RPP7*-coding and non *RPP7*-coding RNAs. We further demonstrated H3K9me2 levels at *RPP7* to be controlled by EDM2, a novel type of nuclear localized PHD-finger protein. The module of 2 ½ atypical PHD fingers in EDM2 preferentially binds to certain combinations of R2, K4, R8 and K9 marks of histone H3. EDM2-mediated modulation of H3K9me2 levels at *RPP7* in response to defense induction dynamically adjusts the balance between coding and non-coding *RPP7* transcripts and is critical for immunity mediated by this immune receptor. As NB-LRR genes are often organized in clusters containing TEs, recruitment of TE-associated regulatory mechanisms to NB-LRR expression control is likely to be common. Consistent with this idea, we found EDM2-dependent H3K9me2 to control expression of several other TE-associated NB-LRR genes. We further identified additional components contributing to epigenetically-controlled alternative polyadenylation. New results on roles of EDM2 and other chromatin-associated regulators in defense-associated H3K9me2-mediated alternative polyadenylation will be presented at the meeting.

T5-30-04

Harness the power of big data – using gene modules to study plant nutrient uptake and utilization

Shisong Ma
University of Science and Technology of China

The advent of big data in biology offers excellent opportunities while poses daunting challenges for researchers to analyze the massive datasets to derive biological insights. We tackled such challenges by building an *Arabidopsis* gene network based on the graphical Gaussian model, using large-scale transcriptome data. The network contains 18,000+ genes that assemble into 600+ gene modules. These modules function in diverse cell organization, development, nutrients, metabolism, and stress response pathways. As examples, modules involved in the uptake and utilization of phosphate, nitrogen, and iron will be discussed in details, which contain key transcription factors, enzymes, and transporters as well as potential novel regulators for the related nutrient pathways. The network analysis was also extended to major crops and identified gene modules that determine agronomic traits. The modules revealed by our networks provide extensive resources for plant systems biology studies.

T5-30-05

Regulation and manipulation of communication signals in plant response to virus-vector mutulistic interactions

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Institute of Microbiology, Chinese Academy of Sciences

Vector-borne pathogens may alter promote the performance of their primary hosts in ways that influence the frequency and nature of interactions between hosts and vectors. The new concept in virology has been called ‘Perceptive insect behaviour’ and relies on a behavirour change induced by its transmitted viruses. This positive effect of a pathogen on its vector via their shared host plant is termed indirect mutualism. Previous work has reported a phytohormone Jasmonic acid (JA) -mediated terpene biosynthesis is suppressed in begomovirus-infected plants, leading to reduced resistance to whiteflies which transmit these viruses. Recently, we identified the proteins encoded by the monopartite or bipartite begomoviruses as the key genetic factor that suppresses JA-mediated terpene biosynthesis. Our new results suggested that plant innate immunity and other phytohormones also involve in the terpene-based odor resistance to whitefly. Meanwhile, geminiviruses have evolved sophisticated strategies to counter multiple-layer resistances against whitefly. Our results present a novel scenario of how a pathogen regulates host-derived olfactory cues for vector attraction via manipulation of plant host. The results will also help to address similar tripartite interaction systems in plants, animals or humans and will allow developing of innovative control methods through interference with vector transmission.

T5-30-06

Cross-kingdom RNAi and spray-induced gene silencing for plant disease control

Hailing Jin
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Small RNAs (sRNAs) are a class of short non-coding RNAs that mediate gene silencing in a sequence-specific manner. We have demonstrated that some sRNAs from eukaryotic pathogens, such as *Botrytis cinerea*, the fungal pathogen that causes grey mold disease on more than 1000 plant species, could be translocated into host plant cells and suppress host immunity genes for successful infection. This finding represented the first example of naturally occurring Cross-Kingdom RNAi in host-pathogen interactions. These sRNAs act as a new class of pathogen effectors that translocate into host cells to suppress host immunity. Recently we have found that transgenic plants expressing hairpin RNAs that targeting *Botrytis Dicer 1* and *Dicer 2* genes could effectively block the generation of fungal sRNA effectors and suppress grey mold disease. This strategy can be adapted to simultaneously control multiple fungal diseases. We show that *Arabidopsis* plants expressing hairpin RNAs that simultaneously target *DCL* genes of *B. cinerea* and *V. dahliae* show enhanced disease resistance to both pathogens. These results also suggest that sRNA trafficking is bi-directional and sRNAs could move from the host cells to the interacting pathogens. Most strikingly, we discovered that *Botrytis* could take up double-stranded RNAs and sRNAs from the environment. Applying sRNAs or dsRNAs that target *Botrytis Dicer* genes on the surface of fruits, vegetables and flowers significantly inhibits grey mold disease. Such pathogen gene-targeting RNAs represent a new generation of environmentally friendly fungicides.

T5-31: Genetically-Engineered Crops: Challenges and Potential

T5-31-01

Genetically-engineered crops: Current status and potential future developments

Peter Davies
Cornell University

Genetically engineered (GE) crops have now been grown for 22 years. They have received overwhelming adoption by farmers in countries where they are permitted. The number of countries where such crops are permitted (mainly the USA, Canada, Brazil and Argentina) is still limited, with further expansion hampered by outdated regulations originating for 30 years ago when the potential effects of genetic engineering were largely unexplored. Despite this there are numerous indications that farmers have taken events into their own hands and promulgated GE crops via “stealth seeds” despite official prohibitions. However the number of traits is still quite limited, being largely restricted to herbicide resistance, especially to glyphosate, and insect pest resistance through Bt. This has been an outstanding success in cotton growing in India with a 90%+ adoption rate, changing India from a cotton importer to a major exporter. Yet insect-resistant aubergine (*Solanum melongena*), which permits a massive reduction in the use of toxic insecticides, remains unutilized except in Bangladesh because of political opposition. Genetic engineering for disease resistance offers a major contribution, with the most notable success being in Hawaii where resistance to papaya ringspot has rescued the Hawaii papaya industry. Yet despite this success and demonstrated efficacy in Thai varieties in Thailand, the Thai government yielded to anti-GMO pressure to ban its adoption. Indeed the opposition to genetically engineered crops is solely political backed up by fear occasioned by the term GMO, coined by opponents, and false science. Consumers have seen little overt advantage to GE food products, as the increase in yields and decrease in pesticide usage is unseen by consumers. However should some food products collapse in availability (e.g., from citrus greening and various banana diseases) we may see more acceptance than at present. Other current and future potential advantages of GE crops include drought tolerance (especially in response to climate change), improved human-nutrient content (e.g., Golden Rice, though Golden Rice remains in limbo for various reasons 17 years after its initial development), improved plant-nutrient uptake, improved photosynthetic efficiency, and improved produce qualities for consumer acceptance (such as non-browning apples and potatoes). It will be up to the plant-science community to engage and inform the public at every opportunity about the productivity, environmental, and nutritional advantages of genetically-engineered crops in order to facilitate more widespread acceptance in future years.

T5-31-02

The development of disease resistance in tropical crops using genetic engineering

Frank Shotkoski
Cornell University

In many of the more developed countries, genetically engineered crops already contribute greatly to agricultural productivity and sustainability. Over the last few years, the largest growth in the adoption of genetically engineered crops has been in developing countries and this trend is expected to continue. The multi-national life sciences companies have been leading the way, but they are focusing primarily on a few crop/trait combinations that have high commercial value and occupy large international markets. The greatest success has been with insect resistant and herbicide tolerant maize, soybean, canola and cotton. Because of the costs and complexity of the issues related to crop biotechnology, many crops and traits of importance to subsistence and resource-poor farmers around the developing world have been overlooked. For example, there are few crops possessing disease resistance traits mitigated by genetic modification. Those that do exist, papaya ringspot resistant papaya and virus resistant squash, are resistant via viral coat mediated resistance. The development of fungal and bacterial disease resistant crops hasn’t been met with nearly the success compared to other traits, especially crops important to people in developing countries. More recently, crops genetically modified for fungi and bacteria resistance important to resource poor farmers in developing countries are in the pipeline for commercial release. I will present examples of several of these projects including Banana Xanthomonas wilt resistance in Uganda using the *pflp* and *hrap* genes, potato late blight resistance in Indonesia and Bangladesh using an R gene, *RB*, from a wild-type potato and attempts to generate fungal disease resistant banana using RNAi technology.

T5-31-03

A site-specific gene stacking system for GE crop

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A gene stacking system combining the use of the Cre-loxP, Bxb1-att and the CinH-RS2 site-specific systems is being deployed for engineering transgenic crops. In earlier work on the model plant tobacco, we have demonstrated Bxb1 integrase-directed site-specific gene stacking at a genomic target, followed by Cre-directed removal of unneeded DNA. To translate this technology to crop plants, target lines have been generated in both Japonica rice and Indica rice each with a single copy T-DNA insertion that harbors intact recombination sites in the genome. Gene integration into Japonica rice has been demonstrated with reporter genes and we are currently beginning the gene stack with a trait gene that reduces Cd accumulation in grain. Similar approaches in generating target lines are being taken in other important crops such as soybean and cotton. With this gene stacking system, adding new transgenes will not inflate the number of segregating loci when more traits are added; removal of marker genes and other nonessential DNA enables reuse selectable markers for subsequent transformation events as well as help ameliorate biosafety concerns of genetically engineered crops.

T5-31-04

CRISPR/Cas9 mediated base-editing efficiently creates non-GM herbicide resistant plants

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3. National Engineering Research Center for Veg

Introduction of a point mutation at a desired position remains a great challenge in plant genome engineering. Recently, an improved CRISPR/Cas9 system achieved precise base-editing in mammal cells and T0 rice plants, whether this new system would generate inheritable point mutations in plants needs urgent testing. In this report, we aimed to generate point mutations on ALS gene conferring herbicide resistance in *Arabidopsis* using this base-editing system and evaluate the editing efficiency. We showed that the base-editing efficiency was about 1.7% at T1 generation. Over 75% T2 seedlings of base-edited T1 lines, harboring known and novel herbicide resistant (HR) mutations at the desired position, survived herbicide treatment. Moreover, we identified six non-transgenic T2 HR plants with expected point mutations. These results demonstrated that the base-edited mutations are inheritable at high efficiency. Surprisingly, we discovered that the PAM regions could likely be a new base-editing region for this system, and revealed a second novel HR mutation on ALS gene. Collectively, this new base editing system is a powerful tool to generate known and novel inheritable mutations that bear great values and interests for basic research and crop improvement.

T5-33: Large-scale sequencing of biodiverse plants; beyond model organisms and crop species (two sessions)

T5-33-01

Mining biodiversity for optogenetics; mammalian neuroscience applications of algal proteins

Gane Ka-Shu Wong
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2. BGI-Shenzhen

1KP is an international multi-disciplinary consortium that has generated transcriptomics data across the Viridiplantae (green plants). We sequenced exemplars of all major lineages representing a billion years of evolution, including angiosperms, conifers, ferns, mosses, streptophyte and chlorophyte green algae. These efforts have increased plant gene numbers by about a factor of 50 relative to the public databases. One of our many goals was to demonstrate that biodiverse sequencing of species with no previously known utility can generate practical outcomes, at the level of individual molecules and complex pathways. In the former case, we discovered and quantitatively characterized many novel light-sensitive proteins that are used for optogenetics control of genetically defined neurons in conscious mammals. Some of these proteins have been licensed for use in clinical trials. Generalizing the concept, we found that billions of years of evolutionary “tinkering” has created a cornucopia of light-sensitive proteins that potentially allow us to control many other aspects of the cell, not just the electrical signals of the brain, but also the chemical signals that are ubiquitous in molecular cell biology.

T5-33-02

Elucidating the long evolutionary road to plant terrestrialization by comparative genomics

Shifeng Cheng[1], *Shanyun Xu*[1], *Yan Xu*[1], *Sijie Chen*[1], *Linzhou Li*[1], *Jing Qu*[1], *Chang Yue*[1], *Gane Ka-Shu Wong*[2], *Michael Melkonian*[3]
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3. Universität zu Köln, Germany

The origin and diversification of the green lineage (Viridiplantae), in particular the colonization of land, has profoundly changed our planet. However, the long complex stream of genetic innovations and morphological transitions from green algae to land plants is underexplored. I will present the recent progress from a large-scale comparative genomics and phylogenomics analysis incorporating 8 newly-sequenced algal genomes from 2 early diverging prasinophytes and 6 streptophyte algae. Using the new phylogenetic framework from the 1KP project, we determined the core nuclear and organellar genes that define the ancestor of the green lineage. Functional domains and metabolic pathways indicative of terrestrialization were investigated and categorized. In addition, we explored differential expression patterns and alternatively spliced forms in more than 30 transcriptomes from these 8 species. Together with other recent discoveries, it is becoming clear that many of the traits associated with land plants often have a molecular antecedent in the algae.

T5-33-03

Understanding the enigmatic dinoflagellates and their adaptive symbiotic systems with genomics

Senjie Lin
University of Connecticut

Dinoflagellata is a member of the alveolate phylum, a group of protists that also includes Perkinsozoa, Apicomplexa (malaria parasites) and Ciliophora. About 50% of the extant 4,000 species are photoautotrophic while the rest are heterotrophic; some species from both groups are mixotrophic. Dinoflagellates are widely distributed in aquatic ecosystems, important primary producers, a source of essential endosymbionts for coral reefs, and frequently responsible for harmful algal blooms and/or biotoxins in marine ecosystems. They exhibit unusual cytological and genetic characteristics, breaking nearly all of the major rules in biology textbooks, and epitomize the most complex plastid evolution known to science. Although the molecular mechanisms behind these enigmatic characteristics remain elusive, novel insights have begun to emerge from genome and transcriptome analyses. Recent advances will be presented to address what makes these organisms so ecologically successful.

T5-33-04

Deciphering nitrogen-fixing root nodule symbioses Part I – Phylogeny to synthetic biology

Pierre-Marc Delaux
Université de Toulouse

Many of the plant traits that are important for agriculture are quantitative traits that are complex and under the influence of many genetic and environmental factors. This is particularly true for root-nodule symbiosis (RNS), a specific association between a few plant lineages and nitrogen-fixing bacteria. The plant benefits from this association by gaining access to the pool of atmospheric nitrogen, providing an essential nutrient for plant growth. With the rise of synthetic biology, we can envisage engineering desirable traits, such as RNS, in cereal crops for increasing sustainability and productivity in agriculture. However, a comprehensive understanding of the molecular basis of this trait is required before such an undertaking can proceed. I will discuss recent findings supporting the use of comparative phylogenomics to identify genetic innovations behind key plant traits, and how this fits into a global collaborative effort to engineer RNS into cereal crops.

T5-33-05

Algal ancestor of land plants was preadapted for symbiosis: Recent progress and updates

Pierre-Marc Delaux
Université de Toulouse

Physiological studies and fossil records strongly indicate that the ability of the first land plants to form symbiotic associations with beneficial fungi was one of the critical innovations that permitted them to colonize land. In angiosperms, genes required for the perception and transduction of diffusible fungal signals for root colonization and for nutrient exchange have been characterized. However, the origin of these genes and potential correlation with land colonization remain elusive. A comprehensive phylogenetic analysis of 259 transcriptomes generated by the 1KP initiative, as well as 10 green algal and basal land plant genomes, coupled with the characterization of the evolutionary path leading to the appearance of a key regulator (a calcium- and calmodulin-dependent protein kinase) showed that the symbiotic signaling pathway predated the first land plants. In contrast, the downstream genes required for root colonization and their expression patterns appeared subsequent to the colonization of land. Hence we concluded that the most recent common ancestor of extant land plants and green algae was preadapted for symbiotic associations. Subsequent improvement of this precursor stage in early land plants through rounds of gene duplication led to the acquisition of additional pathways and the ability to form a fully functional arbuscular mycorrhizal symbiosis. Here, I will present recent advances made to validate these evolutionary hypotheses.

T5-33-06

A genomic approach to understand the molecular basis of plant-cyanobacterium symbiosis

Fay-Wei Li
Boyce Thompson Institute & Cornell University

Plant symbiosis with nitrogen-fixing cyanobacteria is a unique form of mutualistic association that has independently evolved in diverse yet isolated lineages-from a few species of bryophytes, ferns, and cycads, to a small genus of flowering plants. Compared with other nitrogen-fixing microbes, cyanobacteria are much less dependent on the plant host, and therefore can be an ideal partner for engineering symbiotic nitrogen fixation into crop plants. However, our current understanding of plant-cyanobacterium symbiosis is rudimentary, in large part because of the lack of genomic and genetic resources for their plant hosts. Through a global collaborative effort, we have generated genomic and transcriptomic data for several plant species capable of cyanobacterial symbiosis-*Azolla* (a fern), *Anthoceros* (a hornwort), *Blasia* (a liverwort), and *Gunnera* (a flowering plant). I will highlight our key findings and discuss future directions.

T5-33-07

Discovery and design of hyperstable bioactive cysteine-rich peptides from medicinal plants

James Pingkwan Tam
Nanyang Technological University

Our interest in orally-active peptides has led to a systematic profiling and characterization of the disulfide-rich family of cysteine-rich peptides in medicinal plants and functional foods. Of particular interest are peptides with 2 to 6 kDa molecular weight and 3 to 6 disulfides as framework crossbraces. These highly disulfide-constrained small proteins are super-compact, hyperstable to heat, chemical and enzymatic degradation, i.e. they are an underexploited class of bioactive compounds for the design of orally-active therapeutics. The most well-known are defensins, whose antimicrobial activities contribute to the host defense mechanism. However, profiling of over 1,000 plant species and >100 families show that defensins, which are not constitutively expressed, are not the most common cysteine-rich peptides in medicinal plants. Two themes are emerging. First, each family with a distinctive cysteine motif has a distinctive scaffold with highly evolvable and hypervariable non-cysteine residues. This feature has been exploited for drug design by grafting a metabolically-labile bioactive peptide to a cysteine-rich scaffold, rendering it metabolically-stable and orally-active. Second, molecular diversity is produced from a single gene by two methods, fuzzy bioprocessing from the precursors and post-translational modification, a theme commonly observed in secondary metabolites.

T5-33-08

Deciphering nitrogen-fixing root nodule symbioses Part II - Comparative phylogenomics

Maximilian Griesmann
Ludwig-Maximilians-Universität München

Of all the essential plant nutrients, nitrogen is required in the largest quantity and is frequently the limiting factor in crop productivity. Although plants are surrounded by atmospheric nitrogen, they are unable to use it. Some flowering plant lineages escaped the requirement for fixed nitrogen in soil by forming root nodule symbioses (RNS) with nitrogen-fixing bacteria. This capability is restricted to four closely related orders: Fabales, Fagales, Cucurbitales and Rosales. As a result of this relationship, there is a hypothesis that RNS evolved many times, independently, from a predisposition event that occurred before the divergence of these orders. However, the molecular mechanisms that allowed RNS to evolve remain largely unknown. Through a global collaborative effort, we have sequenced the genomes and transcriptomes of 13 species that together cover the breadth of symbiotic states in the Fabales, Fagales, Cucurbitales and Rosales orders. I will present the key findings from our comparative phylogenomics analysis of this dataset.

T5-33-09

Supporting large-scale genomic studies through open platforms at China National GeneBank

Xin Liu

BGI-Shenzhen & China National GeneBank

China National GeneBank (CNGB) held its opening ceremonies in Shenzhen late in 2016, after five years of construction. The organization was launched in 2011 by the Chinese government as the nation's first genomics repository. With funding from the central and the local government, CNGB was constructed and is operated by BGI. It is focused on establishing open platforms for global scientific research. CNGB has already established a biorepository with the capacity for storing dozens of millions of samples, a database with 20 Pb (petabase = 10^{15} base) of storage capacity, and a living biobank. It houses a sequencing platform with a throughput of 10 Tb per day (terabase = 10^{12} base). Several large-scale genomics studies are already launched, e.g. the avian 10K genomes http://b10k.genomics.cn. Progress towards this and other large-scale genome projects will be discussed. We are actively seeking global collaborations to utilize CNGB's open research platform.

T5-33-10

Was there a Chlamydiae contribution to primary endocytobiosis and the origin of Plantae?

Michael Melkonian

Universität zu Köln

Plantae (Viridiplantae, Rhodoplantae, Glaucoplantae) genomes contain many essential genes that are homologous to genes found in Chlamydiae, a group of intracellular bacteria that function as energy parasites in many eukaryotes, including even humans. However, no extant Plantae are known to harbor intracellular Chlamydiae. Over the last ten years, a hypothesis has been developed that these chlamydial genes are the relics of an ancient endosymbiosis of Chlamydiae that occurred in the ancestor of the Plantae at the time of their acquisition of cyanobacteria, and that these chlamydial genes were essential for the establishment of the primary endosymbiosis and consequently of the origin of plastids in Plantae. This hypothesis has been controversial because the taxon sampling on which the original arguments were based was limited. In the analysis to be presented, we considered 79 chlamydial genes from 362 taxa of bacteria and eukaryotes, many identified from novel transcriptomes and genomes sequenced for this study. These results were subjected to phylogenetic analyses, yielding novel insights into the origin and function of chlamydial genes in Plantae.

T5-33-11

Discovering the molecular basis of C_4 photosynthesis through wide sampling of natural diversity

Steven Kelly

University of Oxford

Much of biology is associated with the evolution of convergent traits. For example, the most productive crop plants (e.g. sugar cane) use C_4 photosynthesis-a more efficient form of photosynthesis than the C_3 form that is seen in 85% of plants (e.g. rice, wheat, soybean). C_4 has evolved independently from C_3 in more than 60 lineages. Although insight into the molecular basis of this trait can be gained by studying model organisms, elucidating the pathway to a level necessary to re-engineer crop plants has been challenging. By comparing the transcriptomes from plants representing 18 independent origins of C_4 photosynthesis, we quantified the extent to which this convergent trait employs identical molecular mechanisms. Nearly all of the established biochemical changes that characterize C_4 species were recovered by an analysis of these data. In addition, we expanded the existing paradigm by four metabolic pathways not previously associated with C_4 photosynthesis. These results show that comparative transcriptomics of non-model species can provide detailed insights into the molecular basis of complex traits.

T5-33-12

Network approaches for plant phylogenomic synteny analysis: Challenges and prospects

M. Eric Schranz

Wageningen University and Research

Network analysis approaches have been widely applied across disciplines. In biology, it is now frequently adopted to organize protein-protein interactions, organize pathways and/or to interpret gene co-expression patterns. In contrast, comparative genomics is still largely reliant on pairwise comparison and linear visualization methods. We present the challenges and prospects for establishing a generalized phylogenomic synteny network approach that is required to interpret the wealth of new and emerging genomics data. This will be illustrated by an analysis of synteny across 101 completed plant genomes. A broad approach like this holds great promise for understanding the evolutionary history of genes and genomes across phylogenetic groups with long divergence times. Additional details are available online at the preprint server http://www.biorxiv.org/content/early/2017/01/17/100990.

THEME VI: PLANTS & SOCIETY

T6-01: Latin American botanical research and the journal impact factor (two sessions)

T6-01-01

Meeting the challenges of impact factors

Tod Stuessy

Ohio State University

Impact factors were designed to aid decisions on which biological and medical journals should be included in *Current Contents*, a popular summary of the major journal literature during the 1960s and 1970s. As time progressed, university administrators realized that these impact factors could be used to evaluate the quality of scientific work published by staff members within their institutions. Because of the difficulty of evaluation of published research in many diverse fields, it is understandable that decision-makers would turn to quantitative and qualitative assessments of productivity. On the one hand, encouraging staff researchers to publish in international and more visible journals is laudable, and this can work to the benefit of the individual as well as the institution. On the other hand, this search for higher impact can sometimes interfere with appreciation and encouragement of research that an individual is best suited to produce. A further problem arises when institutions set impact goals that are also tied directly to salary or other aspects of career development, which can lead to negativism. It is important to realize that the bibliographic monopoly of developed countries is now virtually gone, and if a researcher has Internet connection, then most of the world's literature is at his/her disposal. However, to be able to use the international literature, one must be reasonably fluent in the English language. Although this may seem overly restrictive, it is a natural trend to have one primary scientific language, which initially started with Latin, then French, German, and now English. Accepting this reality, young scientists must become immersed in English to a level where the international journals can be read and understood. In addition to this needed linguistic preparation, the researcher must develop self-confidence to address international topics, instead of only regional ones. Pursuing research topics that are limited geographically in the home country may eclipse the likelihood of addressing questions of broader interest. Sometimes the financial resources are so limited, or so directed to national initiatives, that travel to other countries is difficult. In these cases, search for international funding may be the only avenue for successful global competition. The young researcher must seek out training in laboratories in developed countries, obtain mentoring from international researchers, and select globally significant research topics. Such adjustments in career development will naturally result in publication in higher impact journals, hence relieving pressure from the institution. Achieving this in developing countries is much more challenging than in developed countries, but it can be accomplished with effort.

T6-01-02

Journal Impact Factor and evaluation of researchers in Latin America

Alina Freire-Fierro
IKIAM University and Asociación Latinoamericana de Botánica ALB

Although originally created to evaluate journals, the Journal Impact Factor has nowadays become one of the main factors to evaluate productivity and impact of scientific researchers worldwide. These bibliometrics, though understandable applied to journals published in English, are completely unfair to non-English journals. This situation is worse to Latin America, where the tradition of research/publishing high quality papers, even in Spanish, is relatively new. Additionally, and while for other sciences the impact of journals can be measured in a time span of 2, 3, 20 years, in botanical research many articles/works published more than 200 years ago are still being cited. Adding to these problems, the evaluation of impact factor of journals is made primarily in English language publications, as it has been reported for example, that out of 100 Panamian publications, none had been included in the evaluation of the recently modified science index, the Science Expanded Index (SCI-Expanded). While even in English-speaking countries, many institutions have agreed not to use the Journal Impact Factor as a measurement for a researcher's productivity or impact (e.g. signage of the DORA agreement in the US) or have used other methodologies for this evaluation (e.g. the Research Excellency Framework REF2014 in the UK); in Latin America, many governments/institutions are still using it. The JIF is being used for example to categorize Mexican researchers in the National System of Researchers (SNI), or by Colombian Colciencias, and by the Ecuadorian National Registry of Researchers (RNI). In Perú for example, JIF and H indices count up to 35% of the total score for a researcher's categorization in the National Directorate of Researchers and Innovators (DINA). This unfair evaluation from Latin American governments and institutions towards a researcher's impact really hurt scientific development in the region. Since very good and experienced Latin American researchers want to keep their high academic status, they will continue to publish in English, giving therefore a positive feedback for journals published in English in detriment of journals published in Spanish. Although just as recent as of December 2015, Clarivec Analytics, the current owner of former Thompson Reuters' Institute for Scientific Information (ISI), creator of the IJF, has developed a new index that does not consider the Journal Impact Factor, the Emerging Sources Citation Index. Still though, one of the requirements for journals to be evaluated by this Index is that it has to have the cited literature in English. Taking into consideration the stated above, I consider and encourage to the botanical community and to scientific organizations to sign the DORA agreement, and to follow the methodologies used by REF2014, but modified to reflect Latin America's scientific research reality. Additionally, and as a scientific community, we consider that it is our duty to request the Latin American governments to stop using the JIF as one of the most important elements in their evaluations of researchers in the region.

T6-01-03

The Impact Factor and the Mexican System of Researchers (SNI-CONACyT): Stuk in the "carrot and stick" traditional approach.

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In Mexico, the National System of Researchers (SNI-CONACyT) evaluates the performance of botanists in Area II (Biology and Chemistry) and awards four types of nominations (Candidate, rank I, II, and III), with $ 600 to $ 1200 USD per month (accounting for 25-50% of total revenues). The problems include: 1) In the referees predominate in researchers in the area of chemistry, who ascend more quickly with their publications in journals of greater Impact Factor (IF); 2) the evaluation, supposedly, takes into account many other criteria, however in practice they require you to publish at least one paper per year in JCR journals with rigorous IF (> 0.5). Implications of this "Carrot and stick" approach: 1) Many traditional botanists abandon discipline in pursuit of a more profitable trade, while students prefer to study molecular biology than field botany, since there is almost no support for field research projects. 2) It is more likely that a stressed and sedentary national researcher will first acquire one of the Non-Transmisible Diseases (NCDs) before reaching “the carrot” (a promotion to rank II or III); either that or they first reach their retirement age without receiving their corresponding recognition and encouragement. PROPOSALS: The evaluation of the quality of botanical research can be considerably improved through a better diversity of indicators: 1) INTERNATIONAL PLANT NAME INDEX (IPNI): Consider the number of new species described as author or co-author, or whether the researcher is an authority in a particular plant group, which could be easily generated by IPNI. 2) INDEX HERBARIORUM (IH): Give special value to outstanding or emblematic botanical collectors for each herbarium, this data are available in this data base. 3) GENE & DATA BANKS. Contemplate the number of plant accessions or DNA sequences, recorded in gene banks, databases, botanical or public gardens. 4) Taxonomic keys to identify species in a given group should be specially valued as a tool for curating collections of herbariums, museums and databases and for the formation of new young taxonomists. 5) Geographical and social coverage of research should also be considered. 6) The botanical monographs represent the works of a lifetime of a researcher, must be valued for the quality of their content in a very special way, years of experience in the subject, geographical coverage, scope, degree of complexity, research risks, or rarity of organisms under study. 7) The floristic checklists of a great mountain or of a floristic region with hundreds or thousands of species should also be considered as monographs. 8) Generate algorithms (search engines) to count the number of times that a species or species author has been historically cited in the publications. 9) Consider metrics, additional to the IF: the SNIP, Source Normalized Impact per Paper; the RESEARCH GATE SCORE and total readings that an author has; and the GOOGLE SCHOLAR that provides the h index. 10) Provide greater financial and logistical support to botanical and related journals to improve their capacity for review, editing and dissemination.

T6-01-04

Latin American botanical research and the journal impact factor

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An analysis of the low impact factor of Latin American botanical research is presented as well as future perspectives for Latin American botanical journals are described. It is widely known that the best scientific works carried out in biological sciences, including botany, are published in journals outside the Region and furthermore, published in English. Accordingly, in recent years there has been a growing effort to change to English language the published articles in local journals as means of increasing its level of circulation. However, this process goes to the detriment of the botanical scientific knowledge in the original countries of publication where Spanish or Portuguese language is spoken. In general, there is little information on the impact factor for Latin American journals and, in particular, on journals associated to botany, which are very scarce in the Region. Four Latin American countries have more than one journal registred in the Science Citation Index: Argentina, Brazil, Chile and Mexico, whose average impact factor is less than 1; the remaining countries in the Region do not have botanical journals in this Index. It is clear that the impact factor of Latin American journals is low, however, this factor sometimes takes a second place considering that these journals constitute one of the few if not the only mean of spreading the botanical knowledge in their own countries. Although this last argument is valid, the situation is critical for the botanical research carried out in Latin America and it raises some questions. Should the language of publication be changed to English, considering this language as the main way to spread scientific knowledge, or should be published bilingual journals (Spanish/English or Portuguese/English)? or, alternatively, should be considered more important the dissemination of knowledge in the countries of origin than spreading the knowledge globally? On the other hand, frequently there is a lack of recognition of articles written in Spanish since researchers in other non-Spanish speaking countries do not take the time to read them. Additionally, botanical articles on Latin American topics written in English reach higher degrees of impact and international knowledge. Considering the above, the translation to English language would be a requirement if we really want to improve the impact and dissemination of research published in Latin American journals. However, as stated before, these journals are the main source for in-country botanical education for many students and represent their first contact with research. It is important to note that although the impact factor is an unfair measure for a number of countries, including Latin American ones, it is an universally established scientific measurement that we must consider. Finally, some potential actions to increase the international dissemination of Latin American journals could include their bilingual publication in electronic format as well as the physical document. Another step could be the distribution of Latin American journals by international companies responsible for spreading scientific knowledge, therefore allowing wide availability of articles in full text.

T6-01-05

Citing nomenclatural authors makes a difference: Quantifying effects of alternative citation practices in taxonomy, exemplified by a bibliometric analysis of the literature on cotoneaster

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Background: An important reason for the crisis in taxonomy lies in the rather modest performance of taxonomic publications when evaluated using current bibliometric tools. Recently, proposals for alternative citation practices in taxonomy with a focus on new approaches on citing nomenclatural authors have been put forward. However, no quantitative data was available on the potential bibliometric effects of such alternative practices. Purpose: The present study was performed in order to quantify bibliometric effects of alternative practices of citation using a representative corpus of taxonomic literature on the genus Cotoneaster (Rosaceae) as a sample. The main question was: What difference would it make if taxonomic publications additionally included all nomenclatural authors in their end references section? Furthermore, we attempted to analyse the basic characteristics of a representative corpus of taxonomic literature and develop qualifiers that might help to reassess various practices of citation, and various practices of bibliometric evaluation. Methods: As it is often difficult to delimit taxonomic literature from literature belonging to other disciplines, pragmatic criteria for doing so were developed. The taxonomic literature making up the sample corpus, pertinent to the genus Cotoneaster, was retrieved as comprehensively as possible. Citation relationships between publications in the corpus were extracted and stored in a database, using a subset of citing publications. Citations were classified, with a particular focus on nomenclatural citations attached to taxon names (Linnaean style references), as opposed to end references. Data analysis was performed using standard software. Results: The taxonomic literature on Cotoneaster comprised some 450 publications. From a subset of 120 citing publications, some 3,600 citation relationships to other publications were recorded. Linnaean style references (ca. 2,400) outnumbered the end references (ca. 900) greatly. The median age of the cited publications was approximately 70 years; almost 90 percent of cited publications were older than 10 years. Linnaean style references tended to be older than end references. Lists of the most-cited publications differed widely depending on whether they were based on Linnaean style references or end references. Conclusions: Using the corpus of the taxonomic literature on Cotoneaster as a sample, bibliometric effects of including all nomenclatural authors in the end references sections of publications could be simulated for the first time. It would make large differences in several bibliometric parameters if Linnaean style references were treated in this manner. Implications of our results for taxonomy as a discipline are discussed with specific regard to current usage of bibliometric parameters, such as impact factors, for research evaluation by academic administrations.

T6-01-06

The discreet charm of publishing an Argentinean botanical journal in the Journal Impact Factor Era

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The *Boletín de la Sociedad Argentina de Botánica* has published 51 volumes since 1945, in all disciplines of Plant Biology and related groups. From the year 2000, it has published regularly at the rate of one volume per year and from 2013 it has published four trimestral issues. In 2010, the journal was included in the Science Citation Index Expanded without requiring use of English.

Why do we publish a scientific journal? 1. To validate and legitimize the ownership of the contributions; the fact that an investigation appears in a prestigious journal, through the filter of a pertinent evaluation, guarantees the intellectual quality of the work. 2. To serve as a way of preserving the heritage of science. 3. To professionally promote the researcher by making his work public. Why do researchers publish their work in journals? 1. To report the results of their investigations. 2. To contribute to the advancement of knowledge. 3. To be properly evaluated, obtain grants and have prestige, in a system where they are evaluated by their publications. 4. To make their work known before other researchers. The publication itself is, then, at the base of the scientific scheme. The JIF has become a chief and widely used tool to rank journals, although there are other systems (SJR, Scopus, Latindex, Scielo, *etc.*). It is not only important to publish, but where to publish. The journal in which a paper is published has become more important to researchers and administrative evaluators than the research itself. Journals, indirectly, endorse the contents of the articles, contents that the administrative evaluators do not care to read. Journals of the Northern Hemisphere have the best rankings whereas Latin American journals, when included, barely reach less than 1. This system indirectly defines what is valid science and it discriminates through low rankings for many science journals. According to Scimago Journal Rank, from ca. 91,000 papers in Plant Science from 2013 to 2015, the USA and the UK published ca. 20,000 each, the Netherlands ca. 14,000, Germany ca. 6,000, and Latin America ca. 4,360 papers (less than 5%; Brazil 2,400, Mexico ca. 600, Chile and Argentina ca. 500 each.) The scientific production of some developing countries is smaller than that of a single USA university department. The publication of a scientific journal is a complex task, more in our countries where they are generally edited by non-profit societies with altruistic goals and meager resources. National universities and research councils fund our research and sustain our jobs, but force us to publish our work in Northern Hemisphere journals, as evaluation committees do not consider papers published in journals from our region. Open access Latin American botanical journals with the best possible quality, both in form and content, should be considered a priority and the work published in them acceptable by administrative evaluators. Our journals are part of our intellectual and cultural heritage and represent us in the community of nations. It may be like swimming against the current, but this is discrimination that must not be left unopposed.

T6-01-07

Measuring the impact of scientific research in Colombia: Towards the globalization of its science

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Research in Colombia has grown exponentially, despite maintaining a low expenditure in Science, Technology and Innovation (ST&I: 0.4% of GDP). Colciencias, the government agency that supports scientific research, keeps a national register of 10,042 researchers working in Colombian institutions, 2,324 of them in natural sciences, mainly in 17 of the 289 universities in the country. Also, it recognizes 4,638 research groups, 855 of which are in natural sciences. In line with the policy of research internationalization, more than a decade ago Colciencias established a registration and evaluation system of both groups and researchers, implemented on the platform ScienTI (with CVLac and GroupLac profiles). Initially, a National System of Indexation and Homologation of Specialized Publications in ST&I (Publindex) had been created. Publindex contains 562 Colombian journals, but only 75 are listed in Thomson Reuters impact factor (JCR) and SCImago Journal Rank (SJR), which means most of the Colombian journals and publications are not even considered for Impact Factor (IF). Moreover, of all those journals, only 14 are in biological sciences, and just a few are in botany (i.e. Caldasia). The WoS reports yearly 8,000 scientific documents, including 6,700 journal articles, from Colombian institutions, being second in South America after Brazil. However, only 600 of them are in the biological sciences, and roughly 50% in botany. After a comprehensive evaluation on the impact of Colombian research, led by the National Observatory of Science and Technology and Colciencias, three conclusions arose: 1) Colombian researchers publish mainly in journals of low impact; 2) Colombian journals have low presence in international indexes; and 3) Colombian science has low impact in the international scientific community. After comparing various metrics, in 2016 Colciencias decided to start classifying publications using the impact factor provided by JCR and SJR, instead of Publindex, with various consequences under hot debate: 1) The system assumes that the main research paradigms in the country are similar to those of interest by the high IF journals, but this is not always the case. 2) Assessment of both researchers and research groups depends on where they publish. Since publishing in high IF journals is expensive, main universities may take advantage against regional universities. 3) Accreditation of graduate programs may be affected by the impacts of the new ranking system. 4) Funding opportunities may remain restricted to the highest ranked researchers and groups, leaving out possibilities for many regional groups and researchers to emerge. 5) Pay scales may depend on the ranking of researchers, based on Colciencias lists, possibly affecting researchers from certain areas or subdisciplines for which impact factors are usually low (e.g. ethnobotany). 6) There might be a loss of social diffusion of scientific knowledge, since most of the high IF journals are in English, and many Colombians (and especially in the regions) do not understand the language. Despite the critiques and problems of this system, the country might see an improvement of the research impact in the scientific international community in the near future.

T6-01-08

Please count taxonomic citations as regular citations: An appeal

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Since their invention by Vasily Nalimov (1969), various citation indexes (CI) became an extremely important tools to measure scientific productivity and success. There is however, one important gap in CI calculations in the field of biological taxonomy: taxonomical citations (which supply scientific names) does not count and therefore the citation level of all taxonomic works is severely underestimated. We appeal to all CI database holders and to all scientific community -- please include taxonomic citations!

T6-01-09

Where do public interest and academic goals converge? Critical shortfalls of narrow endemic plants conservation in Chile

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Chilean flora is composed by around 4,300 species, from which a 45% is endemic. This is one of the highest rates of endemism in the Andean region. Central Chile is globally known as the "Central Chilean biodiversity hotspot". Just a low percentage of the flora has been assessed regarding its conservation status. The taxonomic identity of some groups is still uncertain, and the distributional information is very sparse. These shortfalls are currently known as the Linnean and Wallacean shortfall within the framework of conservation biogeography. These shortfalls are getting more critical nowadays, in the fate of climate change driving distributional changes and high environmental risks, like the unexpected wildfires that dramatically affected almost all central Chile this last summer. An increasing interest within the botanical scientific community towards narrow endemic and threatened species is expected. This is but not the case. A revision of articles published in principal botanical journals, show a minor presence of Chilean plants, and from these, a much more restricted percentage of narrow endemic and threatened ones. Also, botanical journals are not interested in publishing distributional information, which is crucial for the IUCN classification and for taking real actions towards conservation. Some exceptions have been published by *Phytotaxa*. The Chilean highest impact botanical journal, *Gayana Botanica*, is not accepting reports regarding distributional notes anymore. These notes are being still published by Museum low impact journals like the *Boletin del Museo Nacional de HIstoria Natural* or electronic journals like *Chloris chilensis*. The impact of these journals is certainly minor, and these articles are not recognized by the Science Commission Fund (Fondecyt-Conicyt) for projects funding. The disincentive for such publications is evident. We also checked if the Management Plans of the National Park Service (CONAF) do have complete species' lists. The results are dramatic: only half of the protected areas has useful botanical information. And from this half, only another half shows a taxonomic and distributional coherence with the control database. Our results show a high dissociation between the information gaps that need to be filled for taking actions in botanical conservation, and the incentives for research and publication following international "excellence" standards. A profound reflection within the botanical scientific community is needed for building the bridges between academic goals and the real society needs regarding the benefits of botanical conservation and the ecosystem services associated. Not mentioning the important challenges that are related nowadays to climate change and the unexpected impacts like the wildfires that affected central Chiles this last summer and certainly affected important populations of narrow endemic species, that are today, more threated than ever. We emphasize the importance of low impact journals for the continue updating of the distributional knowledge of endangered species, and the need of a long term botanical information program, independent of academic high impact publication needs.

T6-01-10

Neotropical Biodiversity: An open access journal initiative from Ecuador

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Latin America is the most diverse region on Earth. This vast biodiversity is still poorly known at all levels: from it basic description to the interactions at the ecosystem level and its conservation status. A collective effort to facilitate original research and communication is greatly needed in the region. At the same time, publishing in mainstream journals are challenging for Latin American researches. One of the reasons is the regional scope of studies focus in the area. Also the language barrier makes it difficult for researchers to publish in high impact journals. With the aim to expand publication and dissemination of scientific work from Ecuador and Latin America, the Ecuadorian Government and its National Secretariat for Higher Education, Science, Technology and Innovation (SENESCYT in Spanish) launched in 2015 the Neotropical Biodiversity Journal. Neotropical Biodiversity is an open-access and peer-reviewed journal that publishes original research articles on the biodiversity within the Neotropics. The journal publishes research at all scales of biodiversity: population, species, and ecosystem level. It is general in scope and the main criteria for acceptance are originality, scientific rigor and appeal to an international readership. SENESCYT covers all the fees of articles published in the journal, which is distributed through Taylor & Francis, a well-recognized scientific publisher. This eliminates any financial barriers that scientists may encounter when accessing or publishing research and warranties that all papers are widely available to the global scientific community. Even though this endeavor counts with financial support, several challenges are faced in launching a new journal. One of the challenges is making the journal visible to the scientific community. Another issue is convincing scientists to collaborate with the journal as reviewers or authors. Assembling a committed editorial board was difficult, but now the journal counts with 32 associate editors from 11 countries. Also even though Neotropical Biodiversity is free and open access, as a new journal, it is not indexed and has no impact factor. Impact factor measure the yearly average number of citations to papers published in the journal. Impact factor has been criticized as a valid measure of scientific quality; however, it is still used as a measure of a researcher merit in Latin American countries. As a result, researches might not be inclined to contribute their best research in a recently launched journal. The philosophy behind the creation of Neotropical Biodiversity is that scientific information should be available to any interested person and the only criterion to publish research should be quality and no economic factors. The challenges are still current but our expectation is that the journal will become one of the most influential venues to publish research from Latin America.

T6-01-11

How the journal impact factor affects the publication of agriculture and natural sciences research in Argentine journals

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AgriScientia is a periodical publication in the agricultural and natural sciences including Botany in Argentina. It began in 1980 as Revista de Ciencias Agropecuarias and in 1991 changed its name to the current one. Initially its scope was to channel the results of research by the faculty of the academic unit (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba) and later it accepted papers submitted by other nationals and foreign researchers. About two decades ago the National Research Council (CONICET) created a journal advisory body (CAICYT) to set the editorial standards of argentine scientific publications. CAICYT helped AgriScientia with the indexing in Scielo and Scopus among other indexing systems as well as implementing the open journal system. The irony behind this praiseworthy action is that the evaluation committees from CONICET are still using the Journal Impact Factor (JIF) as a measurement for researcher productivity, to allocate project funds, to establish promotions, to award scholarships, *etc*. Therefore, the low JIF of national journals prevent the consideration of the contribution of those papers to enhance the local knowledge. AgriScientia has a low JIF and consequently it is not receiving many contributions from the CONICET scientific community. This has a negative impact on the relevance of published articles and therefore it becomes a vicious circle because the journal is not cited. To attract more contributions, AgriScientia accepts papers written in Spanish, Portuguese and English over a wide range of subjects in agriculture and natural sciences by doing so it became one of the top journals in the country. Unless some changes are introduced in the current evaluation system based on the JIF, most of the scientific journals of Latin America are subject to be ignored or not properly considered. Fortunately, there are some international actions such as the DORA agreement which can reverse the current unfair system for scientific publications in Latin America.

T6-02: Interpretation, interrelationship and representation of plants as symbols in different cultural expressions

T6-02-01

The long journey of South American sacred plants

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South America’s ethnic diversity rivals its biological diversity, from the Apurina to the Quichua and from its astonishing tropical forests to its surprising arid environments. Once, this subcontinent was exclusively inhabited by hundreds of diverse indigenous people that made use of its natural resources. Among them, many vascular plants with different psychoactive compounds were prepared by shamans in rituals for divination, prophecy, cure, and ecstasy. The shaman, after consuming them, perceived and expressed the voice of a deity. Thus, these plants were considered to possess divine qualities. The usage, the effects, and the peculiarities of some of these emblematic species used by indigenous people is here discussed, together with the current decontextualized practice of these sacred plants. Ayahuasca has a long history of entheogenic practices among the peoples of Western Amazonia, where it has been revered for millennia. It is basically prepared with *Banisteriopsis caapi* (Griseb.) C. V. Morton (Malpighiaceae) mixed with additional plant species. It has provided humankind with a powerful medicine of healing and transformation, purging of body, mind, and spirit. Ayahuasca healing remains a prominent and accepted shamanic practice in indigenous and mestizo communities throughout tropical South America. Tobacco (*Nicotiana tabacum* L., Solanaceae) was traditionally used by indigenous peoples for religious purposes. It is a spontaneous hybrid native to the Andes of Peru and Ecuador found only in cultivation. It has stimulatory effects of the sympathetic and parasympathetic systems. Shamans consumed it in different ways to see inside the patient and diagnose his illness. Adopted by the Spaniards, tobacco had a rapid global spread and became an industrial product, away from the earlier ritual use. *Echinopsis pachanoi* (Britton and Rose) Friedrich and Rowley (Cactaceae), the San Pedro cactus, is a spineless columnar cactus that generally grows over 1,800 m a.s.l. in Andean warm and temperate regions. It is one of the oldest ceremonially consumed plants with archaeological records from 1,300 years B.C. This intoxicating and hallucinogenic drink is ingested by shamans and healers for divination purposes, diagnosis of diseases, and witchcraft. As Angel's trumpet or floripondio, several tree species of the genus *Brugmansia* Pers. (Solanaceae) are recognized. They are grown as ornamental and medicinal and are used in divination practices, initiation rituals and magic in the Andes and the Amazon for its hallucinogenic and psychoactive properties. There are records of its use in ceramics from 600 B.C. The coca (*Erythroxylum coca* Lam., Erythroxylaceae) is a native shrub to the Andean region. Its leaves have been widely used by native people for thousands of years in their religious cosmology. Chewing coca leaves is common in indigenous communities to reduce pain and as a stimulant against altitude sickness and to overcome fatigue, hunger, and thirst. Its leaves are important in Earth offerings and are also read in a form of divination in shamanic rituals. In the late 19th century, its psychoactive alkaloid (cocaine) was popularized for local anesthesia and was included into different products, such as prescription drugs, medicine, and soda drinks.

T6-02-02

Interpretation, interrelationship and representation of plants as symbols in different cultural expressions

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All cultures have some myth or tales related with nature and humans. In the context of the social imagination, mankind has taken the plants as symbols and endowed them with an extraordinary and powerful personality. The ancestral memory, the racial memory, as well, as the cultural memory of society, plays a fundamental role in the perception of plants and in the creation of myths about them. Ancestral myths evolve, regress and get new perception through time and come up to our days with the same power they have born. This field of work is very exciting because there are several connections between plants and cultures, such as this example among others. In Greeks and Roman mythology the oaks trees (*Quercus* spp.) are associate with their highest god, Zeus or Jupiter which is the divinity of the sky, the rain, and the thunder. Two different species from different families such the rushes (*Juncus* sp., Juncaceae) and the fennel (*Foeniculum vulgare*, Apiaceae), are the plants that allowed Prometeo to stole the fire from the month Olympus and give it to humans. The bay, (*Laurus nobilis* L, Lauraceae) is the transformation of the dryad Daphne consequently became the symbol of victory and merit. Several genera, species or other taxonomic range were given by botanists in honor to mythological characters such as *Achillea* for Achilles, hero of Troy and *Eragrostis* for Eros, god of love. Also, there are botanical terms related to mythology such as hermaphrodite, which is a flower having both male and female reproductive organs which derived from Hermaphroditus the bisexual child of Aphrodite and Hermes. Also, there are several mythological gods or goddesses associated with nature, trees, gardens and orchards such as Flora goddess of wild plants and Ceres goddess of agriculture. From all of those examples, the myth of Narcissus and Hyacinthus, as well as, the metamorphosis of mythological characters in plants such Philemon and Baucis (transformed on oak and linden trees) are the ones that offers the major impact in human philosophy. Plants names are sometimes in relation with human feelings of joy or sadness such the legend of the sad tree *Nyctanthes arbor-tristis* L. which means "sad tree" which is related with ancient stories in Hindu Mythology. From ancient times till the present plants have relations with different religions cult, such the rose, as attribute of Venus (Aphrodite), symbol of love that was taken by Christianity as a symbol of the Virgin Mary. The Egyptian considered that from the flower of *Nymphaea lotus* L. the God Sun Re or Ra was born. The sacred flower of India (*Nelumbo nucifera* Gaertn.) is the seat of Lakshmi the goddess of luck and good fortune. Also, the lotus flower is the base from where is standing up Guan Yin or Kwuan the goddess of fertility in Taoist myth.

T6-02-03

The myth and uses of three Mexican plants by the Maya and Aztecs

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Mexico has a great variety of native species used for nutritional and medicinal purposes which were also part of the ceremonial rites of Maya and Aztec communities. The traditional uses before the arrival of the Spanish conquerors to the New World were compiled in the Badianus Manuscript or "Little Book of the Medicinal Herbs of the Indians" (1552). In those times the botanical name and chemical composition of the plants were unknown but the knowledge of the Tlazolteolt (Goddess of Medicine) has being related with the uses of the species in current times by several scholars. In this presentation three species were selected to review the uses and relationships with ancestral mythology. One of the species of major uses in current time is maize (*Zea maíz* L.) which was considered in the myth as the plant from which the Maya race originates. According to "*Popol Whu"* (Popol-Vuh the sacred book and the myth of pre-colonial America), the goddess Tepeu and Gucumatz created the first man and first woman from cornmeal dough using yellow and white maize. Kukulcan or Quetzalcoatl the feathered snake is the major figure of Nahua mythology and represents the force of life and, in the form of a black ant, stole maize from the red ants for the benefit of mankind. Xipe-totec, the god of fertility and regeneration, was associated with the crop of maize and several sacrifices were done in his honor to assure a good harvest. The origin of the chocolate started as a fermented beverage used in ceremonial rites. It was prepared by mixing roasted cacao seeds (*Theobroma cacao* L.) with seeds of *Bixa orellana* L., chili pepper (*Capsicum* spp.) and vanilla (*Vainilla planifolia* Andrews). In Nahuatl, the language of Aztecs, the plant was called "kakaw" and chocolate derived from "xocolatl" which meant bitter water. In present time, chocolate has an important international market in the candy and cooking industries. The myth of the pulque or aguamiel which is the alcoholic beverage made from fermented sap of the “manguey cactus” which is not a real cactus, instead is a variety of mezcal (*Agave americana* L.) started when Quetzalcoatl ascended to haven to request the assistance of the goodness Mayahuel to prepare a placebo to bring happiness to humans. In the earth Quetzalcoatl and Mayahuel fell in love and became trees with the branches interconnected. Tzitzimitl, the grandmother of Mayahuel destroyed the tree but, from the debris the manguey cactus was born. This agave plant was used to obtain fibers for clothing and bags and is the raw material for mescal a distilled spirit of Mesoamerica, similar to tequila the traditional alcoholic drink of Mexico.

T6-02-04

Key plants preserve elements of culture: A study over distance and time of fresh crops in Puerto Rican markets in Hartford, CT (U.S.A.) — “A Moveable Feast”

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People retain culinary customs when they migrate. We tested this commitment via study of Puerto Rican fresh produce markets in the continental U.S.A. (Hartford, CT) over time, 18 years, and space, by comparisons of the Hartford markets with the markets in Puerto Rico. A survey of Puerto Rican markets in Hartford in 1993-94 was repeated in 2009-10. A comparative study was made at three major open-air markets in Puerto Rico in 2009. Surveys recorded fresh crops, and consultations with vendors and interviews with Puerto Rican residents in Hartford provided context for the use of the plants in cooking. Altogether, 84 plant crops (64 species; 32 families) were recorded for seven categories. The largest category was ‘viandas’ (fresh, starchy “root crops” and immature fruits), followed by ‘saborizantes’ (flavorings). More than 80% of the crops were still present in the second Hartford survey nearly 20 years later. Approximately 90% of the Hartford 1993-94 crops and 75% of the Hartford 2009-10 crops were shared with markets in Puerto Rico. Our results suggest two new concepts. The persistence of these largely tropical foods in a temperate market far removed from insular, and tropical, Puerto Rican markets shows the importance of basic foods as an element of cultural identification. We recognize this stability despite significant time and geographic separation as an example of ‘Culinary Cultural Conservation’. Secondly, analysis of these fresh produce markets leads to the conclusion that viandas are the most prominent in diversity, persistence over time and distance, volume, and in terms of consumers’ “willingness to pay” (willingness to pay more to forego cheaper local substitute foods). Accordingly, we consider the viandas a good example of a ‘Cultural Keystone Food Group’, extending the previous concept of ‘Cultural Keystone Species’ to a group. We posit that the viandas are an example of a culturally elemental food group preference of sufficient strength that migrants bring this preference with them wherever they travel, and that the analysis of this Cultural Keystone Food Group can be used for a type of “rapid assessment” of the degree of culinary conservation in different migrant Puerto Rican communities. Furthermore, our results suggest that the crops that compose a Cultural Keystone Food Group, such as viandas, do not have to be native to a particular area, such as Puerto Rico, in order for them to become iconic of the cultural cuisine, and that several crops once associated with the slave trade to Caribbean islands are now celebrated components of Puerto Rican cuisine.

T6-02-05

The double life of a holy tree: The quishuar in Catholic temples and Andean homesteads

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In many cultures, trees have importance, both, material and spiritual, which represents a potential for their survival. Several ethnographic studies from India, the Philippines, Madagascar and Papua New Guinea, offer accounts of trees being perceived as having a material life and existence that transcends human life: “trees never die; they just morph into a different form”. This cultural assertion holds a potential that could prevent the extinction of many arboreal species. The quishuar o kishwar (*Buddleja incana*), from the *Scrophulariaceae* family, is a tree native of the Andes region shared by Bolivia, Colombia, Ecuador, and Peru. It grows at an altitude between 3,000 and 3,500 meters above the sea level. For centuries, Andean people have considered it a sacred tree. At the time of the Spanish conquest, trees had a symbolic and economic importance. During the Inca Empire, trees represented the origin of human life and embodied grandparents and ancestors. The quishuar in particular had multiple uses: as firewood, to manufacture tools, in buildings and to carve out *keros* (sacred vases). Also, this tree was perceived as tree with magical powers because of their capacity to link the underworld with the world of humans. Throughout centuries, quishuar has kept its sacred character. In Ecuador, in two catholic temples built during the 17th century, expose the body of Jesus Christ crucified, sculpted from quishuar, continues being worshiped. This paper analyzes the intertwined condition of the quishuar: its religious symbolism associated with the body of Christ and its magical character as caretaker of the underworld. Based on the analysis of colonial Latin American Indian chronicles and ethnographic work, this paper aims to articulate the current symbolic and religious importance of the quishuar. I suggest that the (*Buddleja incana*), thanks to its usefulness in Andean homesteads continues being perceived as a magical entity and, thanks its homology with the human body, has become part of the Christ crucified during worship services in Catholic temples.

T6-02-06

Cultural variation in the depiction of plants in Indian literature

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‘Culture and ‘Literature’ are closely associated terms as literature is the written portrayal of any culture. In recent times, one of the most important issues in the world in all its diverse forms is the issue of culture. Culture in the traditional sense is considered to be an assemblage of beliefs, practices, traditions, rituals, myths and art of a particular society. Logically, all facets of our life in some or the other way, are confronted with the effects of culture which intern gets manifested in to a local literature at temporal and spatial scales. Since ancient times, man has had an intimate contact with various environmental assets in general and plant resources in particular. The mankind depends on plants not only to fulfill their basic needs and necessities but also for the associated cultural values at personal and social level. In rites and rituals, ceremonies and sacrifices observed across all religions and customs, plants have played a significant role in various forms. Globally it has been observed that while accepting variety of transformations, interestingly literary writers have incorporated the changing phases in plant science in their writings and have reinforced the notion that 'culture' is not a static phenomenon. Depiction of plants and plant parts in Indian literature is also found to be obedient in following this rule. In the present study, a modest attempt to comprehend how culture contributes to the depiction of plants in diverse ways in literature is made. The manner in which plants are depicted in literature bring out the concepts, ideas, thoughts, beliefs, and superstitions of a particular group of society. It is interesting to unravel the threads of good or bad bioclimatic, biogeographic and social conditions as expressed in the respective literature. This study presents examples with reference to plants from literature written in different languages in India, folk literature and also Indian literature in English and argues that references to the plants is the representation of varied cultural and behavioural patterns with customs and traditions in India. It also highlights how value attached to a particular plant differs spatially in different traditions of Indian context as well as the western context. This exercise concludes that it is advisable and would be an enriching experience if the people from literature and plant sciences jointly analyse how various properties and features of plants are reflected in literature. This can provide significant insights about plants to a person from literature and make the reading of literature an enriching and rewarding experience for the person having science background under a variety of cultural settings.

T6-04: Prescriptions for overcoming plant blindness

T6-04-02

Understanding core concepts about photosynthesis - a case study of an experiment-based teaching strategy

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From a human perspective, photosynthesis is the most important process on earth. During photosynthesis, plants are able to use the solar energy and raw materials-carbon dioxide and water-to build glucose and all organic compounds necessary for survival. All organisms depend on photosynthesis-the main pathway by which carbon and energy enter the web of life. Moreover, photosynthesis releases oxygen (O_2) in the air we breathe, and it is this oxygen that plays such an important role in cellular respiration and the accompanying synthesis of ATP. Thus, without photosynthesis, plants and animals, including humans, would suffocate and starve. Core concepts about photosynthesis are important for students to understand, but they are difficult and there are many misconceptions, not only for middle/high school students, but also for college/university students majoring biology. An experiment-based teaching strategy was used in the present study to improve students' biological literacy about photosynthesis. The results presented are based on a series of efficient and reliable inquiry-based photosynthesis experiments including: (1) Oxygen release from submerged pondweeds (*Elodea*, *Hydrilla*, *Spirogyra*). (2) Microscopic observation of stomata. (3) Paper chromatography of photosynthetic pigments. (4) Investigation of environmental factors affecting photosynthetic rate.

T6-04-03

Planting Science online mentoring program opens students' eyes to plants and plant scientists

Catrina Adams

Botanical Society of America

In this presentation, we share how the Botanical Society of America and 17 other scientific society partners fight plant blindness by bringing plants and plant scientists into secondary school classrooms around the world. Through PlantingScience.org, small teams of students design and execute plant science projects in their classroom with online support from a volunteer scientist mentor. The award-winning program has reached over 24,000 students from 41 U.S. states and five countries. More than 1,000 mentors have registered to participate from 39 countries. As Wandersee and Cleary wrote in 2006, *"The presence of a plant mentor earlier in one's life (someone who helped the mentee observe, plant, grow, and tend living plants) is a key predictor of that person's awareness, appreciation, and understanding of plants throughout the lifespan."*. PlantingScience makes it easy for practicing plant scientists to develop relationships with secondary school teachers and their students with a very small weekly time commitment, regardless of how physically close they are to a school. We will share and discuss examples of interactions that demonstrate how scientist mentors provide students with diverse role models of who scientists are, how they think about problems, how science works, and how they value plants. PlantingScience also provides an opportunity to increase secondary students' exposure to plant biology, and to bridge the gap between secondary and post-secondary education. Most U.S. high school biology teachers have never taken a botany course, and many secondary school teachers feel underprepared to teach plant science to their students. Without support from the plant biology community, biology teachers may frequently choose to use animal examples over plant examples to teach concepts relevant to all life, thus reinforcing plant blindness in their biology students. Through the National Science Foundation-funded Digging Deeper program, PlantingScience brings early career plant scientists together with high school biology teachers for extended, blended professional development. Teachers and scientists work together in-person during a week-long workshop and continue their interactions online as students work through a photosynthesis and cellular respiration investigation theme using the PlantingScience platform. Such partnership programs are mutually beneficial, as many graduate students and early career biologists are underprepared to teach using active learning principles; they are also unfamiliar with secondary school curricula and benefit from relationships with secondary teachers. We share data and examples highlighting benefits of this program for students, teachers, and early career scientist mentors, and lessons learned for facilitating such online partnerships.

T6-04-04

To raise plant appreciation to beta-level through integrative and literary approaches to plants as covered in literary works, cultural context, and economic uses

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Getting to know plants is to love plants. The very first attraction to a plant is often induced by its beauty and fragrance, whereas the alpha-level of knowledge comes with the association of the plant with its names. Labels of plants in gardens and color photos and descriptions in pictorial guide books and websites could serve as vehicles to deliver such information, which would enhance amateurs and college graduates, including those with trainings in life sciences, to reach this level. However, to bring them a step further to the beta-level for ingrained admiration and respect of plants, we find it effective to introduce them, in vivid and subtle messages and stories, to the relevant literary works, cultural backgrounds, and economic uses of the plants. Below are a few examples of our approaches presented in a weekly column on a local newspaper. To demonstrate the importance of scientific names, we cite an essay written by the famous Taiwan writer Lung Ying-tai (龍應台) who found the city flower of Hong Kong, Yangzijing (洋紫荊), was actually Yanzijing (艷紫荊) of Taiwan. Some bloggers then mocked at Hong Kong people for this stupid mistake, unearthed by a non-scientist. The truth is both vernacular names refer to the same plant Bauhinia blakeana, which is called by a third vernacular name, Honghua Yangtijia (紅花羊蹄甲), in mainland China. If botanists from the three places meet, it would be very confusing if they do not use scientific names. To introduce lichens to the laymen, we describe the news of the cosmetic disaster of reflective white patches on the face of Peng Liyuan, wife of the current Chinese leader Xi Jinping, during their visit to London in 2015. Such patches are compared with those on the smiling faces of buddha sculptures in Angkor, Cambodia. Further elaboration is then added on lichen botany and their impacts on cultural relicts. Through an announcement of a wedding banquet, we review Chinese greetings, particularly the reference to conjoined images, and elaborate with succinct details on inosculation and the botany of conjoined trees as well as their differences from hugging trees which have no fusion of plant tissues and from stranglers of the genus Ficus. A higher level of knowledge of plants would enhance deeper appreciation of plants as found in relevant literary works, cultural context and wisdom in the economic uses of plants, which would, in turn, enhance higher expectations for conservation of plants and their sustainable uses.

T6-04-05

Overcoming plant blindness with active learning

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Learning to identify plants is extraordinarily difficult. This difficulty leads to what has been called plant blindness “the inability to see or notice the plants in one’s own environment.” Yet, despite this difficulty, many botanists can quickly and easily make species identifications at a glance, sometimes out of the window of a moving vehicle. They do this without recourse to the use of traditional characters or keys. This talk will present a method for teaching this type of instant plant recognition and overcoming plant blindness. Plant identification is difficult because of variability in the plants’ appearance, the difficult of bringing them into the classroom, and because it is often difficult to take students to the field, especially on urban campuses. To solve these problems, we developed and tested a cognitive psychology-based computer program to teach plant identification. The program incorporates presentation of plant images in an active-learning format that was developed to stimulate expert-level visual recognition. *Visual Learning – Plant Identification* (*VL-PI*), and its sister program *VL-Agricultural Plants of the Riverina* (*VL-APR*), is a cross-platform visual learning application that has been experimentally shown to improve plant recognition. In a controlled experimental test against traditional study methods use of the program resulted in up to a 25% improvement in exam scores. The software demonstrates how the use of interleaved examples, spaced repetition, and retrieval practice (SPRINT) can be used to train identification of complex and highly variable objects. The software is open source, and easily customizable. A brief four-minute introduction can be found at https://youtu.be/lfoeldV-_94. A longer introduction, including instructions on how to add your own images is here: https://youtu.be/qMq0n8q8B8Q. The source code and executables can be found here: https://github.com/Jasig/ImageQuiz/releases.

T6-04-06

Biodiversity description simulation: a tool to stimulate interest in plant taxonomy during class

Rafael Medina
Augustana College

Many future biologists complete their college education with just minimal training on plant biology, and the lack of time and resources make impossible a deeper immersion in plant taxonomy practice and the description of biodiversity during an introductory course. If the students are never exposed to this field, it becomes harder for them to gain the appreciation or pursue any interest on a field that would be so needful in the next generation of biologists. The activity proposed here is designed to stimulate the interest on taxonomy with a game in which groups of students simulate to compete to be the first to describe and publish a new plant species. This activity has been tested successfully with classes of about 40 students in a time frame of 75 minutes, which makes it suitable to fit in a single class or lab session. The students are divided in a variable number of groups with three different roles: explorers, editors and curators. Each group receives a different briefing depending on their role: to identify a plant and send a manuscript with its description; to review submitted manuscripts and publish them when they comply with the code of nomenclature; and to accession and database plant specimens in a herbarium. The skills needed to participate in the game can be adjusted to the desired competencies of an introductory botany course. The debriefing discussion may include topics such as the challenges of biodiversity description, the peer-reviewed publication process and the importance of scientific collections.

T6-05: Botanical contributions from the Biodiversity Heritage Library

T6-05-01

The Biodiversity Heritage Library: Empowering discovery through free access to biodiversity knowledge

Martin R. Kalfatovic
1. Biodiversity Heritage Library
2. Smithsonian Libraries

The Biodiversity Heritage Library (BHL) is an international consortium working together to make biodiversity literature openly available to the world as part of a global biodiversity community. Through its extensive network of Members and Affiliates, over 50 million pages of biodiversity literature are now available through the BHL portal. Eight Global BHL Nodes work with institutions in their regions of the world to build capacity for digitization and to promote open access to the biodiversity literature. The BHL was created in 2006 as a direct response to the needs of the taxonomic community for access to early literature. Designed with meeting these needs, the BHL was grant-funded and quickly proved its value to its target users. The original BHL organizational model, based on US and UK partners, provided a template for, first, BHL Europe, and then a series of global nodes, most recently, BHL Africa, BHL Singapore, and BHL Mexico. As the BHL moved from project to a cornerstone of biodiversity infrastructure, sustainability, appropriate expansion, collaboration with national and pan-national digital libraries (Europeana, the Digital Public Library of America) became more important. With significant contributions from many of the major botanical garden libraries and related collections throughout the world, the BHL is an important tool in global botanical research.

T6-05-02

Building botanical literature collections for global use: Highlights from the Biodiversity Heritage Library

Caroyln Sheffield
Biodiversity Heritage Library/Smithsonian Libraries

As an open access digital library, the Biodiversity Heritage Library (BHL) provides extensive and broad coverage of biodiversity literature. Drawn from the collections of libraries from around the world, botanical literature composes both a significant portion of this corpus and a foundational aspect of the consortium. Formed in 2006, four of the original ten founding institutions were the libraries of botanical gardens and herbaria. New partnerships continue to be formed and today there are nine participating institutions making major contributions to the botanical collections including the Chinese Academy of Sciences, Royal Botanic Gardens, Kew, and Singapore Botanic Gardens, among others. From their collections and those of other natural history libraries, botanical literature remains a strong and growing portion of the online corpus. Over the years, special projects have also provided new opportunities to further grow the botanical coverage in BHL. Partners have collaborated across geographic boundaries to build an extensive collection of Seed and Nursery Catalogs. Other projects have focused on scientists' field notes. Thousands of pages of unique, archival materials documenting explorations, discoveries, and botanizing efforts from around the world are now freely available on the BHL website. These, along with the core book and journal collections, enable researchers to investigate and increase understanding of plant life more efficiently than ever before. BHL users have shared some examples of how they're asking big questions in new ways, thanks to the open access literature now available online. Examples include mining BHL metadata to identify agricultural resources and coverage; researching the phytogeography of species; and studying early land plants to inform conservation efforts. This presentation will highlight the breadth of botanical content within the BHL corpus; showcase some of BHL's projects to analyze and build collections of botanical literature within the digital library; and feature some of the real world use cases for BHL's botanical content.

T6-05-03

Real use cases for semantic information in BHL

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The Biodiversity Heritage Library (BHL) wants to encourage the reuse of its content by creating an accessible, computational, and servable data store for the purpose of data mining and semantic operations. The Mining Biodiversity project explores the transformation of the BHL into a next-generation social digital library resource to facilitate the study and discussion (via social media integration) of legacy science documents on biodiversity by a worldwide community and to raise awareness of the changes in biodiversity over time in the general public. The resulting digital resource would provide fully interlinked and indexed access to the full content of OCR text library documents, via semantically enhanced and interactive browsing and searching capabilities, allowing users to precisely locate the information of interest to them in an easy and efficient manner. Learning from the experiences of colleagues involved in efforts to enhance the accessibility of biodiversity data through well-defined semantics-rich representations, it was determined that our focus should start first by addressing real questions that BHL users had. However, initial consultations with potential users of this semantically-enhanced information, suggested that the search functionality we had planned would not be fully-seized by taxonomists in their daily use of data. Nevertheless, further consultation with BHL users from other areas allowed our team to identify a valuable set of real use cases for Semantic Information that could be supported. An example search interface implementation will be shown. This talk will demonstrate current real information needs that interoperable semantics for biodiversity data and knowledge discovery can support in BHL. It will also highlight areas challenged by the lack of machine-interpretable semantics that could help addressing and suggest potential solutions. Finally, switching from supporting use cases using text mining for semantic searching over the BHL document collections to support knowledge curation, we will mention two examples of implementation in follow-up projects, one for Philippine biodiversity in general and another one for dipterocarps.

T6-05-04

The development and progress of BHL China

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We have some development and progress as for the project of BHL China. As BHL has been blocked from China, we have downloaded more than 120,000 volume books from BHL website and put them in the website of BHL China. We also have developed new automatic camera program based on gphoto2 environment (IA scanning machine) to make the scanning machine working again. More support for other biodiversity informatics projects: ABCDNet (Asia Biodiversity Conservation and Database Network), the plant list in Aisa, NSII (National Specimen Information Infrastructure). We also visited some herbaria and libraries in four Asian countries, such as Thailand, Vietnam, Indonesia and Philippines. Some libraries were very interested in BHL project. We hope there are more participation and cooperation from these countries.

T6-05-05

The challenges of a botanical library in the tropics and the need for restoration and digitisation of materials

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The financial stringencies faced by libraries is a major challenge worldwide as the economy slows down and budget cuts becomes a regular exercise in many private and public organisations. The Library of Botany and Horticulture in the Singapore Botanic Gardens (SBG Library) has amassed a large collection of printed scientific materials and an extensive portfolio of serials in areas of tropical botany, taxonomic, horticulture, conservation, ecology, ethnobotany, landscaping and design. Modest budget allocation which has not seen much increase over the years pose challenges to the library to respond to new opportunities as offered by the digital provision of content and to increase acquisition. Another, is how the executive board of its parent organisation views the library as a unit that is "good to have" but need not be extensively staffed and budgeted. The library is expected to sustain existing types and level of services with limited qualified staff to meet the goals of the whole organisation. There is shortage of skilled and trained librarians for employing as such librarians are attracted to and hired by larger libraries that are able to manage and meet the staff's career advancement expectations and training needs as compared to the gardens where the bulk of the budget has always gone into the greening projects and maintenance, horticulture and research staffing, exhibitions and events, equipment purchases, and field trips. The SBG library is also facing constraints in physical space for expansion of its collection and have resorted to using compactors to overcome this issue. With the advent of the internet and availability of e-journals and e-books, the physical use of the library by staff has also seen a dramatic reduction since the late 1990s. Ability of staff to access and obtain information via internet search engines and to use more e-journals and e-books rather than resorting to searches from hardcopy books has meant forgoing the library and the need of librarians except in cases when the access to the needed information is not available freely or not lodged in any electronic form. This has led to an increase in demand by the research staff for the library, with its limited budget, to subscribe to major electronic journals and e-books. However, these e-publishers' policies on pricing and accessibility are another big challenge as this inhibit take-up by this small library. Collections of rare, scholarly printed works and botanical artworks are seeing various stages of deterioration due to poor storage facilities in the past. The environment, poor handling and storage of these collection overtime are unconducive to keeping the literature collections in good state perpetually. The rather exorbitant cost of restoration and digitisation and lack of professional restorers are issues presently faced when the library wishes to engage in preserving increasing number of deteriorating collections. The library will need to review its management and acquisition of information resources and staffing and to take advantage of digital information format and find strategies to keep cost down and still meet demands of the organisation .

T6-05-06

A checklist of the orders and families of medicinal plants in the Philippines

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A checklist of the orders and families of Philippine medicinal plants is prepared to provide an up-to-date reference for students, teachers and researchers alike. The accepted names, families and orders in this checklist follow the recent classification of angiosperms largely based on DNA sequence data by the Angiosperm Phylogeny Group (APG) IV. Available and comprehensive references on Philippine medicinal plants were used to come up with the checklist. This was complemented and updated by online resources retrieved from reputable search engines with a focus on ethnobotanical researches conducted on some indigenous peoples in the country. The authors likewise included an alphabetical listing of older and conserved family names. Results indicated a total of 35 orders out of the world total of 45 (77%) and 120 families of the world's 413 (29%) which were classified according to the APG groupings (Magnoliids, Asterids Commelinids, Fabids, Malvids, Lamiids and Campanulids) and further organized into subclasses and classes of the presently used Cronquist system (Magnoliidae, Liliidae, Commelinidae, Rosidae and Asteridae) for convenience and practical use. Moreover, the list recorded more or less 900 medicinal plant species and it also included the common disease each species is utilized for. The checklist does not only prove the rich medicinal flora of our country but as well as the extensive knowledge of our local "herbolarios". Thus, it is imperative for the proper documentation and promotion of these valuable resources through modern platforms to maximize its potential in contributing to our country's health sector.

T6-06: Early land plants: from early adopters to transformative models for citizen science engagement connecting natural history collections to biodiversity research and education

T6-06-01

A three-tier citizen science monitoring scheme for the distribution of plants, bryophytes and lichens

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FLORON, Dutch Botanical Research Foundation

Biodiversity surveys are often carried out to answer specific questions. To overcome the problem of shifting data demands due to changes in endangered species lists and environmental laws and regulations, monitoring all taxa of a certain species group can be an alternative. In this paper the authors describe how a citizen science approach in the Netherlands succeeded in monitoring all wild vascular plants, bryophytes and lichens since 1989. Two different methods are used to monitor common taxa and rare taxa. The third tier assures sufficient spatial coverage. All three methods use data from previous years to calculate when a grid cell or taxon needs to be resurveyed. The resulting data set was used to create live distribution maps. Occupancy modelling was used to calculate national and regional annual species trends. Trends and distribution maps are used as material for National Red Lists and the evaluation of nature management targets. Results over the past ten years showed that the method is robust enough as a long-term monitoring project. The principle of using existing data to assign monitoring tasks is efficient, but it provides also a transparent way of communicating with volunteers participating in citizen science, who will find their work more meaningful.

T6-06-02

Crowd sourced science: A transformative model of engagement connecting natural history collections to biodiversity education and conservation

Matthew Von Konrat[1], *Brian Carstensen*[2], *Laura Trouille*[2], *Dara Arabsheibani*[3], *Audrey Aronowsky*[9], *Laura Briscoe*[4], *Mike Bryson*[5], *Tom Campbell*[3], *Ben Carter*[7], *Steve Cohen*[5], *Charlie Delavoi*[10], *Eve Gaus*[1], *Juan Larraín*[6], *Beth Sanzenbacher*[8], *Caitlin Vaughn*[1], *Zak Zillen*[3], *Taylor Walker*[11]
1. The Field Museum
2. Adler Planetarium
3. Northeastern Illinois University
4. New York Botanical Garden
5. Roosevelt University
6. Pontificia Universidad Católica de Va lparaíso, Campus Curauma
7. San Jose State University
8. Bernard Zell Anshe Emet Day School
9. University of Chicago
10. University of Connecticut
11. Hollins University

Alarmingly, the world's biodiversity is diminishing rapidly and undergoing an extinction crisis. Biological collections of museums and academic institutions, documenting the fossilized and living members of the world's ecosystems and their changes over time, are uniquely poised to inform the stewardship of life on Earth. The National Science Foundation program Advancing Digitization of Biodiversity Collections (ADBC) is enhancing and expanding the national resource of digital data documenting biological and paleontological collections at a rapid pace. We provide a collaborative model founded on these digitization efforts that work towards addressing the critical urgency of the loss of taxonomic expertise and the rapid decline of biodiversity. Scientists and educators are partnering with leaders in online Citizen Science, Zooniverse (see www.zooniverse.org), and have a coordinated network including students and professionals at universities and partnering high schools and middle schools to accelerate the pace of scientific discovery. The project has the specific goal of engaging a broader audience, especially students and citizen scientists, to partner with our efforts in recording critical data sets from digitally rendered images. A web-based tool has been developed (see http://microplants.fieldmuseum.org) demonstrating a model approach to aid in relieving some of the taxonomic impediment, accelerating biodiversity documentation and discovery. To date, over 8,000 participants have generated over 90,000 data points that are being utilized by researchers to help with species delimitation and aiding in uncovering cryptic species. The project focuses on a mega-diverse liverwort genus occurring in Australasia, the Malay Archipelago, and southern South America-representing several global biodiversity 'hotspots'-including the Polynesia-Micronesia hotspot, which is considered the epicentre of the current global extinction crisis by Conservation International. Only scant data exist for liverworts (living descendants of the earliest land plants) compared to many animal and seed plant groups of the region. Data derived directly from the participation of Citizen Scientists, particularly cryptic complexes of species, has a significant impact on our understanding of species distribution patterns and direct implications for conservation of these organisms in the region.

T6-06-03

Bryophytes in Australian ecosystems-opportunities to educate the wider community

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4. Australian National Botanic Gardens, Canberra
5. Friends of the Box-Ironbark Forests, Castlemaine

Australian vegetation is very different from that of the northern hemisphere, featuring plant families and genera, such as Proteaceae, *Eucalyptus* and *Acacia,* which have spectacular, eye catching and colourful flowers; little wonder that minimal attention has been paid to early land plants, in particular bryophytes (mosses, liverworts and hornworts). However, bryophytes play a vital role in ecosystems, from minimising soil erosion in arid regions to humidifying the upper canopies of rainforests. Bryophytes have an intrinsic beauty and once observed, it is easy to generate interest in these exquisite miniatures of the plant world. In Australia, bryologists are few and far between, but in recent years there have been some outstanding initiatives to educate the wider community in the importance of these easily unnoticed plants. Technological opportunities to promote bryophytes are not extensive. The online Atlas of Living Australia has proved to be an immensely useful tool to check distribution of individual species, as well as list species recorded for given locations. The Australian National Botanic Gardens in Canberra has developed web pages designed and written to educate and generate interest, rather than distance the wider community from the scientific aspects of bryology. Other, more traditional methods may be successful in raising the awareness of these plants with the public. A living bryophyte display at the entrance to the Australian National Botanic Gardens' Visitors Centre is ideally placed to capture the interest of visitors. Engagement of the art community has also been an excellent opportunity to showcase bryophytes, including exhibitions resulting from *Artists in Residence* programs or exhibitions of scientific botanical illustrations of bryophytes. The publication of Read and Slattery's *Mosses of Dry Forests in South-eastern Australia,* a community project with the Friends of the Box-Ironbark Forests of northern Victoria, promoted as a '*guide for students and absolute beginners-technically accurate, but free of technical language*' has been a great success. Many Australian herbaria have set up volunteer programs, involving anyone from university students to retirees, to work on bryophyte collections. Inviting local professional and amateur botanists to participate in bryological field trips was a success in a recent bryophyte workshop. Teaching programs prepared for schools and universities developed some years ago are still successfully incorporated in educational programs today. Bryophytes have also featured in tourist magazines in tropical northern Queensland and have captured the interest of locals and also international visitors. Australian institutions may not have the capacity to support high-tech infrastructure to generate citizen science on-line, but the enthusiasm and active participation by Australian bryologists in using a wide range of opportunities to promote bryophytes can go a long way to educate and generate interest in these less-well known, albeit spectacularly beautiful and environmentally essential, group of plants.

T6-06-04

Bryophytes: Easily overlooked but unique plants for public awareness of natural beauty and biodiversity conservation

Shan Li, *Qin Zuo, Lihui Mao, Li Zhang*

Fairy Lake Botanical Garden, Shenzhen & Chinese Academy of Sciences

Bryophytes, including liverworts and the more familiar mosses, together form the second largest group of land plants after flowering plants. Some bryophyte species are quite conspicuous and common even in urban areas. Unfortunately, they are poorly known by the public generally due to their miniature size and perceived limited influence to human's daily life. On the other hand, there are many unique features of bryophytes that make them appealing to attract the attention of the general public especially the students. The low commercial value of bryophytes provides distinct opportunities for educators and scientists to be free from the commercialization and focus purely on the natural beauty through their exquisite and varied morphological features. For the audience, the pure interest helps to maintain a long-term focus on bryophytes with further study and consideration of the related environmental topics, namely ecological system and biodiversity conservation *etc.* In Fairy Lake Botanical Garden, we have carried out diverse methods to introduce general knowledge of bryophytes to the public in the past decade. These include: publication of popular science books; booklets and a field guide; exhibitions and lectures; and training volunteers as environmental tutors of both regular courses and irregular environmental education activities. We also teach how to look for, observe and identify common local bryophytes, and identify the current threats they face, such as habitat loss. We found that the above multi-pronged approaches have been very successful. People of diverse backgrounds and ages show continued interest towards bryophytes beyond their initial exposure. And 35 volunteers who passed the training exam became the core members in the relevant environmental education projects of the botanical garden. Presently, Fairy Lake is the only botanical garden in China to provide such courses on bryophytes. In the near future, a moss nursery will be established, and this will provide an easier access for the public and students to familiarize themselves with bryophytes. Novel innovations are being considered, for example, to organize art exhibitions and handicraft workshops on bryophytes, to collaborate with schools introducing bryophytes into classroom curricula, and to develop an interactive App about bryophytes and bryological research.

T6-07: Interpretation of plants to the public

T6-07-01

'Searching for Green Treasure in NBG '- an interesting game to learn from plants

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Botanic gardens are important centres for education, they offer a unique window to the wonders of plant kingdom, and they are uniquely placed to teach the public about the importance of plants in our lifes and in the global ecosystem. Nanjing Botanic Garden (NBG), the first national botanic garden in China, puts emphasis on environmental education since the late 1990s. The environmental education programme of NBG have been carried out in various ways. Scientific guides, printed materials, plant lables, exhibitions, lectures, training courses have been provided; various activities including summer camp and interactive game have been organized. A pant-based game called 'Searching for Green Treasure in NBG', mainly designed for young students is introduced in details in this paper. The game was first held in NBG in 2006, and approximately 3,000 visitors, including adults, took part in the searching activity within 2 months. After that, the game has been redesigned especially for pupils and junior high school students. Involved plants and places (specialized gardens) varied according to weather, students' age and activity duration. With the combination of plant-serching game, team work and competition, the game has become one of the most popular activities ever held in NBG for students, it not only has been interesting, but also has aroused participant's curiosity and encouraged them to observe and discover the uniqueness, beauty and secret of plants, to cultivate a love and respect for them and to create a sense of biodiversity protection.

T6-07-02

Ethnobotanical study of domesticated plants in Ile-Ife, Ilesa and Osogbo areas of Osun state, Nigeria

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Ethnobotanical study of domesticated plants in Ile-Ife, Ilesa and Osogbo areas of Osun state was carried out to explore and document the extent of general and medicinal use of the plants; and to also provide ways of conserving various plant species that are useful to man. Questionnaires were structured in such a way that adequate information revealing the familiarity of people with the plants was collected in addition to the usage of their various parts. The returned questionnaires were analysed using IBM SPSS Statistics 21 analytical method. All the domesticated plants are of economic importance and are very useful to people where they are found. *Mangifera indica*, *Newbouldia laevis*, *Spondia mombin*, *Gliricidia sepium*, *Ficus* spp., *Blighia sapida* and *Argyreia speciosa* are used to provide shade apart from their other economic values. The fruits of *Mangifera indica*, *Spondia mombin*, *Annona muricata*, *Terminalia catappa*, *Artocarpus communis*, *Blighia sapida*, *Chrysophyllum albidum* and *Elaies guineensis* are edible. *Polyalthia longifolia*, *Newbouldia laevis* and *Dracaena arborea* are used for landmarks and as boundary. *Gliricidia sepium* was observed to be the most diverse of all the plants in consideration and the most commonly used in various environments. *Calotropis procera* was largely found to be a wild species as it was encountered in various locations where it was not planted, but was not uprooted because of its importance in milk brewing. *Elaeis guineensis* is known to be vast in its importance and its value cannot be over emphasized, since all its parts are useful economically. Ethnobotanical study shows that plants are highly valuable and useful for various purposes such as landmarks, shed, traditional purposes and several medicinal values for treatment of several ailments like fever, cough.

T6-07-03

The Interpretation of volunteer educators: A link between researchers and the public in the botanic garden

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Volunteers are essential but overlooked human resource in Chinese botanic gardens for a long time. By the chance of the Environmental Education project, we recruited and trained the first two batches of volunteers, who were ordinary people with limited academic background, as the environmental educators. After a set of training courses and examination, the volunteer educators mainly undertook the informal environmental education in Fairy Lake Botanical Garden. Moreover, these volunteers helped to transfer the scientific knowledge about plants into an easily accessible way to the public and find out the fascinating topics from the perspective of the public. However, there are limited researches about the volunteer team in the botanic garden. To assess the effect of the interpretation of the volunteer educators and improve their performance in the interpretation, we set a series of survey focusing on the volunteer characteristics, motivations, goals, achievements, demands, self-feedbacks and feedbacks from the audience. These surveys enabled us to understand more about our volunteers and gave us suggestions to improve the interpretation given by our volunteers.

T6-07-04

Cultural significance of plants in traditional Chinese gardens

Jinyong Chen

The Museum of Chinese Gardens and Landscape Architecture

Plant is one of the important elements in the traditional Chinese gardens. Plants are integrated with surrounded landscape, not only improve beauty of the scenery, but also give people strong impression by the cultural connotation. From the very beginning, Chinese people live on plants for food, medicine, shelter, and gradually appreciate them. In the Book of Songs published between 1100 B.C. and 600 B.C., about 138 species of plant were mentioned in the 311 songs. For example, ‘the young man presents peony flowers to the lady when he made date with her’, showing his love. During that time people planted willows surrounding the house as a garden, outside the garden were mulberries and inside the garden was *Pteroceltis tatarinowii*. In Chinese gardens, plants are appreciated not only by the natural beauty, but also the artistry and culture, which deeply rooted in the 3,000 years history. In the Analects of Confucius, Zaiwo stated that the national tree of Xia Dynasty (2205-1782 B.C.) was pine, that of Yin Dynasty (1600-1046 B.C.) was cypress, and Zhou Dynasty (1100 -256 B.C.), chestnut tree. Similarly, Sun Jue in Guweishu (published between Ming and Qing dynasty) stated that pines were planted in the mausoleum of king, cypresses were planted in the duke’s mausoleum, chestnut tree were around the official’s tomb, *Sophora japonica* around the scholar’s, and common people have no tomb but only willows. This defined the hierarchy of different trees, which affected the planting in the traditional gardens. In addition, plants were personalized in Chinese culture. Pine tree, bamboo and Mei flower were recognized as ‘Three Friends in the Winter’ since they were prosperous in the winter. This beautiful plant landscape was commonly applied in Chinese gardens. Mei, orchids, bamboo and chrysanthemum were praised as ‘Four Gentlemen’. The combination of these species was designed with rocks in the classic gardens, forming an elegant cultural landscape. For the royal gardens such as Summer Palace, the Temple of Heaven and Chengde Summer Resort, pine and cypress were extensively planted, not only because the emperor recognized their importance, but also they symbolized eternity due to their evergreen and longevity. In the Summer Palace, different plants have a certain cultural connotation. Cypresses were planted in two rows inside the east gate, standing for the civil and military ministers. Moving forward, near the palace was *Sophora japonica* ‘Pendula’ representing the emperor by its dragon-like branches. Tree peony, the imperial flowers were planted on the both sides of the palace. *Firmiana simplex* was planted in the empress’ yard, meaning phoenix (symbolizing Queen) inside. Magnolia, crabapple and peony were planted in the Summer Palace as well because they symbolized noble and wealthy in Chinese culture. For the private gardens (especially scholar’s garden), the design concept usually comes from paintings and poems. For example, Yuanxiang Hall in the Humble

Administrator's Garden originated from an essay Ode to the Lotus Flower, indicating the fragrance of lotus flower in the garden can be smelled from far away. At the same time, plants were bestowed good wishes by their appearance. *Osmanthus fragrans* and *Magnolia denudata* were commonly planted in the courtyard, implying 'gold and jade fill the hall' for they have golden yellow and pure white flowers. Similarly, varieties of yellow flowers and white flowers were planted in the Yuyin Cottage Garden in Guangzhou, which had same meaning. Loquat was frequently planted in the private gardens such as the Humble Administrator's Garden in Suzhou. Its fruits look like gold hanging on the tree. The orange tree has same indication, which is recognized as auspicious in southern China gardens. In the Buddhist temple, plants related to Buddhism are usually planted, such as *Ficus religiosa*, *Saraca dives*, S*horea robusta*, *Corypha umbraculifea*. Moreover, 'Five Trees and Six Flowers' are commonly planted in Dai temple of Xishuangbanna. In northern China, Ginkgo, horse chestnut, bamboo are commonly planted in the temple garden since they are mentioned in the Buddhist legend. In the Taoist temple garden, plants are somewhat different. Peach trees are commonly planted since peach symbolizes longevity. A famous poet Liu Yuxi in Tang Dynasty described thousands of peach trees in Xuandu Temple. Pine, cypress, lotus and *Camellia japonica etc.* plants are related to the Taoism and are commonly used in the temple gardens. Actually plant culture can be appreciated everywhere in the traditional Chinese gardens. For the pavement, some special pattern such as Meikaiwufu with five petals of Mei flower, means five blessing. The paintings or decoration with plants on the architecture (house, pavilion, corridor *etc.*) are also common. For instance, bottle gourd (Hulu in Chinese) has a special meaning of good fortune. Pomegranate and grape with many seeds indicate successive progeny and prosperous family. All these plant culture will add more interest to the traditional gardens.

T6-07-05

Plants and their symbols: Conservation of cultural knowledge of the Mamanwa tribe of Malimono, Surigao del Norte

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Tribal communities have a closer relationship with the natural environment due to their long-term use of their natural resources. The documentation of their indigenous knowledge regarding the uses of plants is important in this critically degrading environment. This paper presents the ethnobotanical study, more specifically the plants considered as symbolic and those plants used in rituals by a Mamanwa community in Barangay Binocaran, Malimono, Surigao del Norte. An open-ended interview with the use of semi-structured questionaire were done on ten (10) respondents with an age range of 50-78 years to ensure the validity of the information gathered. The respondents provided information on 15 plant species that are used in ritual ceremonies and nine (9) symbolic plant species. This study also identifies the conservation efforts of the tribe towards their cultural knowledge and plant resources.

T6-07-06

The role of botanical garden in science education for young people

Jianjiang Chen

Beijing teaching Botanical Garden

A botanical garden plays an important role in scientific research and public education. It opens to the public and serves as base for species protection. It also makes great function in environmental science and life education for young people. In the past, botanical garden was just a public garden of touring and leisure for primary or secondary school students, and was research site just for the professional plant researchers. However in nowadays, the botanical gardens are trying their best to abstract the public attention to environmental protection through proper ways and means. They want to let the public to understand more about the truth of biodiversity crisis, and aware the importance of the rescue plants protection. Although after several years developing, the environmental education in botanical garden in China is still in the initial stage. There are lots of problems such as single method and form for interpretation, low degree of public participation, the lack of adequate maintenance of educational facilities, lack of a continuous system of environmental education activities, less publications, *etc.* Beijing teaching botanical garden is a special garden, because it was built just for biological and environmental education for the young people aged 6-16. In more than sixty years' development, the garden now makes many efforts in the area of explore experimental activities and gains experience in making full use of botanical garden for student education. The teachers make a theme for the activities all the year after discussion, then design and implement teaching activities. The experiment and activities include but not just "The Climate Change", "Plant Physiology", "Relationship between Soil and Plants". Each lesson lasts about 90 mins. Parts of the lesson held in the garden, and another part in the lab. Those research activities abstract children who living in the city almost all the year to be closer to nature. In Beijing teaching botanical garden they measure the temperature, humidity and other concerns about the causes of climate change and greenhouse effect and coping strategies. With a hand-held magnifier, boys and girls research on plants, insects, soil and water, thinking of social value, ecological value and make their choice. The special experience which cannot be provided by public school will give them chance to thinking about the occurrence of things around life. We have insisted the activities more than 3 years, seven thousand children came and jointed the activities. Multiple use of botanical garden, let the garden to be a platform of environmental and ecological diversity of education for young people will be a good idea and wise choice.

T6-08: Transgene flow in plants: its possible social and environmental impacts

T6-08-01

Transgene flow from herbicide-resistant rice (*Oryza sativa*) and its possible social and environmental impacts

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Rice is an important world crop providing staple food for nearly one half of the world population. The management of weeds infesting rice fields is challenging. It is even more difficult to control conspecific weedy rice (*O. sativa* f. *spontanea*) that can frequently acquire genes/traits from cultivated and wild rice through gene flow and introgression. Such a process can result in adaptive evolution of weedy rice in diverse rice ecosystems worldwide. The potential commercialization of genetically engineered (GE) herbicide-resistant rice varieties has stimulated great concerns over its possible social and environmental impacts. The commercial cultivation of the non-transgenic rice (Clearfield®) that is resistant to imidazolinone herbicides has already caused more serious weedy rice problems in many rice planting regions of the world, due to crop-weed gene flow, and eventually affected farmers' incomes. Hypothetically, the commercial cultivation of GE herbicide-resistant rice (e.g., glyphosate resistant) will most likely cause the similar problems. It is found that overexpression of endogenous *epsps* (5-enolpyruvoylshikimate-3-phosphate synthase) gene from plants, such as cultivated rice and *Arabidopsis* species, can also increase the resistance of the GE plants to the glyphosate herbicide. Overexpression of endogenous *epsps* gene can provide an alternative method of genetic engineering to produce glyphosate resistant crops. Recent studies demonstrated that overexpression of the endogenous *epsps* gene isolated from cultivated rice has substantially increased the resistance of GE rice plants to the glyphosate herbicides. Unexpectedly, the *epsps* transgene has significantly increased the photosynthesis ratio, tryptophan concentration, and fitness (survival and fecundity) of crop-weed rice hybrid descendants derived from crosses between glyphosate-resistant GE rice and weedy rice accessions, without the application of any glyphosate. Similar results were also obtained in crop-wild rice hybrid descendants. The enhanced survival and fecundity of crop-weed/wild hybrids and their descendants resulted from transgene flow between glyphosate-resistant GE rice and weedy/wild rice may pose great challenges for weedy rice control and environmental biosafety issues, in addition to social consequences related to farmers' incomes, because the problem of transgene flow from GE rice to its coexisting weedy rice or nearby wild rice populations is not avoidable. This might be particularly true for glyphosate-resistant GE rice containing the overexpressed *epsps* transgene that may transfer to wild relatives of rice. This is because the *epsps* transgene may enhance fitness of crop-wild hybrids even without exposure to herbicides. Importantly, EPSP synthase that determines the survival, growth, and development of plants is a key enzyme in the shikimate pathway that is found in all plant and many microorganism species. The glyphosate herbicide can competitively inhibit EPSP synthase, causing weakness and even death of plants at a certain dosage. Therefore, before the application of such a widely used herbicide in GE rice, the social and environmental impacts caused by transgene flow should be thoroughly evaluated.

T6-08-02

Potential social and environmental impacts of plant transgene flow: A 30 year overview

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Concern about unintended transgene flow from engineered plants dates back to the 1980's. At that time, a single concern was voiced, the evolution of more difficult weeds via crop-to-weed hybridization. The initial general view was that relevant gene flow rates were too low to be of much consequence. Relevant data were scarce. Now, over 30 years later, the situation has evolved. Natural rates of gene flow as well as the fitness effects of transgenes in the field have been quantified. Both were sometimes found to occur at significant magnitude to drive introgression. Descriptive and experimental field studies involving deregulated transgenes in field conditions have often yielded surprising results. Novel transgenic traits, from pharmaceutical-production to seed-sterility to gene drive have stimulated the identification of novel potential impacts, both detrimental and beneficial. Furthermore, about two dozen reports of spontaneous transgene flow into unintended populations are informative, illustrating impacts to date. Collectively, the foregoing accumulated data suggest that unintended transgene flow can have idiosyncratic impacts that depend on the recipient plant, the recipient population, the recipient ecosystem, and the values of the human system that interacts with those plants. Like natural gene flow, transgene flow has had and is anticipated to have infrequent significant effects. Whether the rare negative effects are realized will depend on mindful decision-making prior to environmental release. Continued research on case studies, such as those discussed in other presentations in this Symposium, will help guide that decision-making.

T6-08-03

Environmental impact of insect-resistance transgenes flow into rice wild relatives

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Gene flow from genetically engineered (GE) crops allows novel traits to spread to their sexually compatible wild relatives. Ecological impact caused by transgene flow into wild relatives is largely determined by the fitness effect brought by a transgene. Traits such as resistance to insects may enhance the fitness those weed and wild relatives, but few studies have tested for these effects under natural field conditions. In this study, we established multiple-generation hybrid progeny system between GE rice with insect-resistance transgenes (*Bt*, *CpTI*, and *Bt*/*CpTI*) and its wild relatives including weedy rice (*Oryza sativa* f. *spontanea*) and common wild rice (*O. rufipogon*), to investigate the long-term fitness effects of different transgenes on weedy rice and common wild rice populations in common-garden experiments with different insect environments and planting modes, from 2005 to 2013 in Fuzhou, China. Results from rice crop-weed hybrid descendants (F1-F7) demonstrated that *CpTI* alone did not significantly affect fecundity, although it reduced insect damage. In contrast, under certain conditions with high insect pressure, *Bt*/*CpTI* conferred significantly reduced less insect damage and increased fecundity in transgenic crop-weed hybrid lineages from F2-F7 relative to non-transgenic controls consistently. A positive correlation between insect index and fecundity change was detected, stressing the important role of ambient insect pressures in assessing fitness effects caused by *Bt*/*CpTI* transgenes. However, the increased density of transgenic plants in different cultivation plots could inhibit the insect occurrence and limit the fitness benefit brought by *Bt*/*CpTI* transgene. With the possible commercial cultivation of insect-resistant GE rice in future, significantly reduced ambient insect pressure and limited fitness advantage of insect-resistance transgene by extensive presence of GE rice plants in paddy field, will likely result in minor ecological risk by crop-weed transgene flow. Results from rice crop-wild hybrid progeny (F1-F2) demonstrated no significant differences in insect damages were observed between the wild rice parent and GE hybrid descendants under high insect pressure condition. The wild parent showed significantly greater relative survival-regeneration ratios than its hybrid descendants under different insect pressures. However, more seeds were produced in transgenic hybrid descendants than their non-transgenic counterparts under high-insect pressure. Given that the introduction of *Bt* and *Bt*/*CpTI* transgenes did not provide greater insect resistance to crop–wild hybrid descendants than their wild parent, we predict that transgene flow from GE insect-resistant rice to wild rice populations may not cause considerable ecological risks.

T6-08-04

Use of case studies of past transgene flow experiences to inform future releases

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Past experiences with gene flow from transgenic crops should be used to direct research, deployment, and regulation of transgenic crops. The frequent gene flow occurrences provide examples of the avenues by which both pollen and seed move in the environment. Studies often report on the occurrence of cross pollination but not on retention of the transgene in subsequent generations nor the consequences of gene flow. Most often the fact that the transgenic species will hybridize is not new information but rather confirmation of a fact that is already known. It is more important to assess the impact that a trait might have in the environment. Gene flow via seed movement which is a more frequent occurrence than via pollen is rarely studied in relationship to gene flow. Seed movement can move a trait globally which can easily impact global markets if a trait is not approved by the importing country. The use of case studies of gene flow from transgenic crops such as alfalfa (*Medicago sativa*), creeping bentgrass (*Agrostis stolonifera*), rice (*Oryza sativa*), and wheat (*Triticum aestivum*) which have different levels of cross pollination, growth habits and markets encompass a variety of issues and impacts which are crucial to consider when releasing other transgenic crops or traits.

T6-08-05

Evolutionary dynamics of weedy rice (*Oryza sativa*) and implications for adaptation through transgene escape

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Weedy crop relatives pose unique challenges to the commercialization of GE crop varieties. Crop-weed interfertility facilitates transgene flow and can promote introgression of GE traits such as herbicide resistance. In the case of rice, GE crop varieties have existed for well over a decade but have never been commercialized, in part because of concerns over rapid transgene escape into weedy rice populations. Weedy rice is a major agricultural weed in rice fields worldwide and can reduce harvests by >80% if left unchecked. Gauging the risks of transgene flow into weedy rice requires an understanding the weed's origins, population structure, and levels of gene flow with cultivated and wild rice. This presentation describes recent insights into the evolutionary origins and genetic mechanisms of adaptation in US and Asian weedy rice strains. Using a combination of genome-wide SNP analyses, comparative QTL mapping, and genome scans for selection, we find evidence for the following: 1) Weedy rice has evolved multiple times independently through "de-domestication" from different cultivated rice ancestors. 2) Weed evolution has likely occurred both early and late in the history of rice cultivation. 3) Relatively few genetic changes are required for the emergence of weedy traits, and these can evolve independently through more than one genetic mechanism. 4) Despite a high selfing rate, weedy rice can rapidly evolve through adaptive introgression from both cultivated and wild *Oryza* populations. These findings suggest that adaptive GE traits such as herbicide resistance could spread rapidly into weedy rice, and that weed populations could serve as a genetic bridge for transgene movement into wild *Oryza* species. Given the major economic impacts of weedy rice on global rice production, caution is warranted in GE rice commercialization.

T6-08-06

Metabolic redesign of tocopherol biosynthetic pathway: Potential application for human health and abiotic stress alleviation in plants

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Oxidative stress is one of the major manifestations of unfavourable environmental conditions faced by plants as also of several different diseases, including cancers, in humans. α-tocopherol, the biologically most active form of vitamin E, is a major antioxidant that bulwarks the cells. It constitutes a small fraction of the total tocopherol pool in most oilseed crops. We generated transgenic (TR) *Brassica juncea* plants with ~6 fold higher α-tocopherol levels by overexpressing γ-tocopherol methyl transferase. To better understand the roles of different tocopherol forms in plants we compared the performance of TR plants under conditions of abiotic stresses induced by salinity, heavy metal and drought. This resulted in an increase in total tocopherol levels in both the wild type (WT) and TR plants. Seed germination and leaf disc assay showed that TR *B. juncea* had enhanced tolerance to these stress and that induced by high temperature and methyl viologen. Damage caused by the induced stress was lower in TR plants compared to WT plants as assessed by their higher relative water content, lower MDA and electrolyte leakage. Lesser superoxide and H_2O_2 accumulation was observed in TR seedlings exposed to these stress. The levels of antioxidant enzymes and molecules were higher in TR plants when compared to WT plants under similar stress. Analysis of chlorophyll a fluorescence kinetics showed that there were differential effects of the stress on different sites of the photosynthetic machinery. These effects were found to be alleviated in TR plants. We further checked the efficacy of feeding α-tocopherol enriched seeds in securing the antioxidant defense in mice. We found significant increase in the content of various phase I and phase II enzymes with a corresponding decrease in peroxidative damage. Also, the transgenic seeds were found to have chemopreventive effects against DMBA-induced skin papillomagenesis in mice model. Our results highlight the potential of increased α-tocopherol in transgenic *B. juncea* in health of humans and agricultural crop plants.

T6-09: Turning toxins to treasure using plants

T6-09-01

Carbofuran degradation using iron oxide nanoparticles derived from the bark extract of *Hevea brasiliensis* Muell. Arg.

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Hevea brasiliensis is the primary source of natural rubber. Millions of tons of bark are discarded during tapping process for natural rubber, and it is a renewable resource. So usage of rubber tree bark for chemical synthetic process is a sustainable practice. In contrary, pesticide degradation from the agriculture water release and products is a serious health issue need to be resolved. It is well known that iron oxide nanoparticles are used in environmental remediation. Functionalized iron oxide nanoparticles have more reactivity compare with non functionalized particles, and increase efficiency of reactions for which nanoparticles are applied. Carbamate pesticide carbofuran is degraded by iron oxide nanoparticles derived from *Hevea brasiliensis* bark extract in the present study. Synthesis of nanoparticles was carried out by mixing water extract of bark and ferric chloride solution. Surface plasmon analysis of isolated particles confirmed presence of iron oxide in the reaction mixture. SEM-EDS analysis indicated nanoscale size and the presence of iron in the particles. The iron oxide was confirmed as magnetite with help of powder X-Ray diffraction and ESR analysis. The capping agents in nanoparticles were detected using FTIR. Phytochemicals responsible for the formation and capping of nanoparticles were identified with help of a comparative study of bark extract before and after the reaction via GC- MS. The synthesized nanopartices were utilized for the degradation of carbofuran. It is found that mixing of nanoparticles lead non photo catalytic degradation of carbofuran. Optical absorption studies and nuclear magnetic resonance studies were carried out to describe the reactions involved in the breakdown of carbofuran. In summary, we established methodology for the synthesis of nanoparticles from bark extract of *Hevea brasiliensis*. We also identified phytochemical responsivble for nanoparticle formation, physico-chemical properties of the nanoparticles, and action of the nanoparticles that lead decomposition of carbofuran.

T6-09-02

Morphophysiological, ultrastructural and element uptake in response to cadmium in 2 *Calophyllum brasiliense* Cambess. (*Calophyllaceae* J. *Agardh*).

Pedro Antonio Mangabeira
Santa Cruz State University

Cadmium (Cd) is a metal known for its genotoxicity and cytotoxicity, much concerned for its potential environmental and human-health impacts. This study evaluates the toxic effect of Cd in *Calophyllum brasiliense* plants. The plants were cultivated for thirty days in full nutrient solution in order to adapt, and after that be dayperiod, for fifteen days in nutrient solution without Cd or with 4, 8, 16 and 32 μmol Cd L^{-1}. After this period under Cd exposure, anatomical analysis of the leaf showed no significant effects of Cd on epidermal thickness in abaxial and adaxial sides, mesophyll, palisade and spongy parenchyma. Contrastingly, changes were noticed in the ultrastructural level in the leaf mesophyll cells as rupture of the membrane of chloroplasts and disorganization of the thylakoid membranes, in starch grains and in mitochondria with rupture of the membrane and invagination of the nuclear membrane. Electrodense materials into cells of the cortex and vascular bundle was also observed. In the cells of the root system, the observed ultrastructural changes were disruption of the cell wall and electrodense material deposition in the cortex cells and vascular region. Cd accumulated in roots with low translocation into shoot. Cd toxicity also affected the photosynthetic activity, inducing stomatal closure and photosynthetic assimilation reduction and the instantaneous carboxylation efficiency, drastically reducing the leaf transpiration. The nutrient content in the stem and root was variable, according to Cd increase in nutrient solution. Based on the experimental evidence, it can be concluded that *C. brasiliense* has potential to bioconcentrate high Cd levels in the root system.

T6-09-03

Cadmium-induced tolerance mechanisms in seedlings of *Avicennia schaueriana* Stapf & Leechman ex Moldenke (Acanthaceae)

Pedro Antonio Mangabeira
Santa Cruz State University

Cadmium (Cd) is a toxic metal with several folds of increase in the environment in recent decades. The response of tropical woody species in contaminated environment is rather scanty. It is therefore proposed to investigate the mechanisms that regulate the uptake and tolerance of Cd in the mangroves. The main objective of this study was to identify possible Cd-tolerance mechanisms of *Avicennia schaueriana* (Acanthaceae) seedlings. Seedlings were collected and placed in pots with nutrient solution for sixty days. After this period, the following concentration of Cd were added to the treatments: control (without Cd), 16,32 and 64 mg L^{-1}Cd in the form of CdCl2.5/2 H_2O, for five days. At the end of the exposure period, no anatomical changes were observed on the leaf blade of *A. schaueriana*. The increasing doses of Cd in the nutrient solution did not significantly alter the thickness of the abaxial and adaxial surfaces, the palisade and spongy parenchyma thickness, and the total mesophyll thickness. Polarized light microscopy revealed an increase of calcium oxalate crystals in the leaf mesophyll in the dose of 64 mg L^{-1} Cd and this is considered one of the tolerance mechanisms of the plant to the metal. At the ultrastructural level, the leaf mesophyll cells showed an increase of starch grains and electron-dense material deposited in the cells of the cortex and vascular bundle at 64 mg L^{-1} Cd. An accumulation of Cd was also observed in the root system, with low translocation to the aerial part. The regression equations showed that Cd affect the levels of macro- and micronutrients in different ways. The present study demonstrates for the first time the adaptive mechanism whereby *A. schaueriana* seedlings tolerates high levels of Cd by eliminating part of the absorbed Cd through the precipitation of calcium in the salt glands and calcium oxalate deposits.

T6-09-04

Plants for prevention of pollution and resource recovery

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Phytodiversity is being used as raw material for environmental decontamination and this field has grown phenomenally in recent years. On the other hand the volume of toxic and contaminated substrates (water, soil and air) are increasing due to anthropogenic and technogenic sources of organics and inorganics. The contemporary world is facing pollution. Sound policies and affordable technologies are required for combating environmental pollution (domestic and industrial). There is increasing demand for food, fodder, fuel, fertilizer and fiber. Cost of phytomass is gradually increasing due to land cost and water scarcity. Therefore, there is a need to address the toxic waste management and curb pollution due to industrial and domestic waste. This presentation will focus recovery of a wide variety of metals from environmental waste which otherwise is extremely toxic. Plant diversity is useful for Prevention of pollution and resource recovery (=phytotechnologies). Plants being photoautotrophs, can meet this demand by providing renewable resources and substitutes for non-regenerable resources. There is a need to bridge the research gap at the interface between pollution mitigation and boosting the biobased economy aiming at United Nations notified 17 sustainable goals. Pollution abatement and the pressing need to create viable value chains from toxic substrates are gaining considerable importance in developing economies. Thus, the efficient recovery and processing of the resources for recycling are significantly gaining importance globally. Awareness and opportunities to obtain valuable resources from process and bleed streams are of paramount importance in recent times. This would attain not only creating revenues but also achieving environmental sustainability. In this presentation various examples of plants and their societal benefits including their role in turning toxins into treasure shall be covered citing examples of plants and their societal benefits. Specific examples taken from a) Mangroves - acts as biofilters of toxic waste and mangrove wood and plants products are invaluable economic products b) Medicinal plants - e.g. Selenium bioconcetration in *Opuntia-ficus indica* c) Air Pollution tolerant plants – Large number of plants with high air pollution tolerance index are appropriate for social, urban forestry e) Ornamentals - Some cut flowers such as marigold (Tagetes spp.) and water lily handle a variety of toxic substances and generate revenue f) Plants for ecocatalysts and green chemistry - Metallophytes serve as invaluable plant resources for Green Chemistry. Recently metal accumulators, viz. *Noccaea caerulescens* and *Anthyllis vulneraria* are used as starting raw materials to prepare novel poly–metallic catalysts g) Phycoremediation and value additions including biodiesel h) Bioeconomy and sustainable environment - promising bioeconomic potential also exists for metallophytes for production of Biofortified nutraceuticals for human health, animal feed/poultry feed.

T6-09-05

The potential of *Etlingera philippinensis* (Ridl.) R. M. Sm., a Philippine endemic wild ginger against *Phytophthora infestans* (Mont.) *de Bary*: prospect as fungicide

Florfe Acma, *Girlie Guillen*

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Etlingera philippinensis of the Zingiberaceae family, is a Philippine endemic wild ginger which grows abundantly in certain areas of the Philippines and with no known use for the local communities. Some species of *Etlingera* were reported to possess antimicrobial and insecticidal activities. Hence, this species was screened against *Phytophthora infestans*, the fungal pathogen of potato and tomato late blight to evaluate its antifungal activity. This study was conducted to isolate *P. infestans* from infected tomato leaves and to test the antifungal efficacy of the ethanolic extracts of *E. philippinensis* leaves and rhizomes against *P. infestans* using poisoned food method. *P. infestans* was isolated from infected tomato leaves and were inoculated into the potato dextrose agar following the tissue culture method. The antifungal assay using poisoned food method showed that both the leaves and rhizome ethanolic extracts of *E. philippinensis* have antifungal activity against *P. infestans*. On the 3rd day up to 9th day of incubation, the leaf extracts with concentrationof 10 mg/mL showed a low level of efficacy compared to the leaf ethanolic extracts 1.0 mg/mL and 0.10 mg/mL. However, on the 12th day of incubation, the ethanolic extracts of the leaf with the concentration 10 mg/mL showed the greatest antifungal activity among the leaf ethanolic extracts. On the other hand, all tested concentrations of the rhizome ethanolic extracts showed greater antifungal activity compared to the activity of the leaf ethanolic extracts. Mycelial inhibition on the 12th day of incubation at 0.10 mg/mL concentration of the rhizome ethanolic extract is comparable with inhibition of the commercial fungicide. It is concluded that both the rhizome and the leaf ethanolic extracts of *E. philippinensis* exhibit antifungal activity against *P. infestans*. Further, it was found out that higher concentration of the leaf extracts exhibited higher antifungal activity while the rhizome extracts exhibited higher antifungal activity at lower concentrations. The study reveals that *E. philippinensis* has a great potential as a fungicide.

T6-09-06

Impact of environmental variables on plant communities of the Thandiani sub forests division of the Western Himalayas of Pakistan

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In the summers of 2012 and 2013, we surveyed the vegetation of Thandiani in the Western Himalayas of Pakistan. We analyzed how the plant associations differ and develop foreseeable relationships with its respective environmental gradients. We take evidence from links between species and environment and changes in the community structure in fifty analysed stations with 1,500 m^2

plots. Preliminary results showed that the family Pinaceae was the most abundant family with 1892.4 F.I.V, followed by Rosaceae with 14.78.2. Out of 97 plant families, Rosaceae with 20 species was the most dominant family, followed by Asteraceae and Ranunculaceae with 14 and 12 plant species respectively. CANACO and GEO database methods demonstrated strong correlations among species distributions and environmental variables i.e., elevation, topography and edaphic gradients. Our findings show an increas in diversity index and species richness from lower elevation (1,290 m a.s.l.) to higher altitude (2,626 ma.s.l.). It was concluded that soil composition, aspect and altitude were the main factors affecting the vegetational composition of Thandiani sub forests division. The low P value ($P \leq 0.002$) showed that the variation in the vegetation Composition in the study area was highly significant in terms of test statistics. The habitats of the five association types overlapped broadly but still make strong correlation with environmental variables.

T6-10: Capturing biodiversity for food security

T6-10-01

Application of genomics to enhanced utilization of cereal diversity

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Genomics may greatly enhance utilization of plant genetic resources in support of food security. Analysis of wild and domesticated genetic resources may identify new sources of genetic variation for breeding. Genomics of cereal (especially rice and wheat) genetic resources are key to global food security. Whole genome sequencing and transcriptome sequencing can contribute to discovery of valuable new genes and alleles. Sequencing of the genomes of wild rice (*Oryza*) populations has revealed new ancestral populations that widen the effective gene pool of rice for breeding. These newly identified genetic resources will provide a new source of diversity for use in breeding for disease resistant and tolerant to climate change in rice. Analysis of the transcriptome of developing seeds of diverse wheat germplasm has revealed genetic diversity that explains differences in carbon assimilation, flour yield in milling and bread quality on baking. Together these gene discoveries offer a significant opportunity to accelerate the rate of genetic gain in wheat breeding.

T6-10-02

Genetic dissection of starch biosynthesis diversity for grain quality improvement in rice

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Rice is one of the most important foods in the oriental world, and more peoples prefer to consume rice with good sensory and health characteristics. Starch is the major component of rice endosperm, and the composition and structure of starch play a dominant role in the quality of the rice grains, as well as their industry application. In the world, especially in China, there are abundant rice germplasms, including lots of local landraces, and the grain quality variation occurred widely among these landraces. Thus, it is very important to mine novel alleles of starch quality-related genes for rice quality improvement. In our studies, we focused on the starch-synthesis related genes (SSRGs), and carried out to mine novel alleles for quality improvement of both *japonica* and *indica* rice cultivars. By using the approaches of re-sequencing and genic molecular markers, the allelic variation of SSRGs among lots of rice germplasms were analyzed and several new alleles were mined. The results showed that there was different combination of these alleles in current cultivars. In order to investigate of the effects of these genes and their alleles on grain quality in rice, a lot of near isogenic lines (NILs) and transgenic lines were developed, and their backgrounds were nearly the same to the receptors. Through determining and comparison of the physiochemical properties of rice grains and the fine structure of starches between the NILs/transgenic lines and their parents, the results showed that there has a certain impact of the allelic variations on grain quality. The resulted and related materials resulted from this study will be very useful not only to rice breeding on quality improvement, but also to understand the genetic diversity of grain quality in rice.

T6-10-03

Large-scale plant genomics studies and CNGB's effort in food nutrition development

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Plants play a crucial role in ecosystems, development of medicine and particularly as the fundamental resources in nutrition and food productions. Understanding plant genetics and biodiversity is a key to develop new and improved varieties with integrated efforts of germplasm resources collection and storage, genotypic and phenotypic data analysis, transformation and agricultural application. In the past one and half decade, series of innovative technologies (e.g., Next-Generation Sequencing technologies) have been developed and widely applied in sequencing plants at various levels (e.g., genomes and multi-omics data), which has tremendously accelerated and reshaped the researches in biodiversity and practices in food development. With such a remarkable explosion in sequencing technology and analytical tool development, multitudes of fundamental questions in plant genetics, populations and biodiversity were unprecedentedly asked and addressed at genome-wide scale (e.g., the 1KP project: https://sites.google.com/a/ualberta.ca/onekp/). The China National GeneBank (CNGB), with its digitization platform (e.g., BGISEQ-500) and supercomputing platform, is aiming to decode all life forms on this planet especially for plant resources. Furthermore, CNGB is dedicated to developing improved crop varieties, through gene editing and bio-synthesis platforms, and aims to establish a 'super crop' centre focusing in food and nutrition. We call for global collaborations from various research and application areas in food and nutrition with united efforts, and CNGB will provide an important vehicle to the gateway to the new era of global food security.

T6-10-04

Conservation of *Oxalis tuberosa*, "oca", in Ecuador: An iconic Andean tuber

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Yachay Tech (School of Biological Sciences and Engineering)

Oxalis tuberosa, “oca”, is an ideal model to understand how the interaction between environment and humans has influenced the origin and maintenance of the diversity of varieties among Andean tuber species. However, in recent decades, cultural and market changes have caused certain varieties of “oca” to disappear in some areas of Ecuador, which can potentially lead to genetic erosion of the species and jeopardize the long-term food sovereignty of the country. During the last two years, Yachay Tech and Yachay Botanic Garden have intensively and extensively collected varieties of “oca” throughout the high Andes of Ecuador. Collections were obtained directly from the farmers themselves (indigenous people and mestizo peasants). Each visit was complemented by a semi-open interview on the agricultural practices related to this tuber. In the near future, we will conduct genetic analyses to measure the genetic diversity and understand the phylogenetic history of the “oca” varieties in Ecuador. Such analyses probably will also include the “oca” collections maintained by Ecuador′s Instituto Nacional de Investigaciones Agropecuarias (INIAP), mostly collected a few decades ago. The results of this project can help to design better strategies and management practices for the conservation of Ecuador′s native plant genetic resources.

T6-10-05

Diversity of edible aquatic plants from Thailand

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Aquatic plants are diverse in Thailand, over 400 species occurred with several taxa are edible popularly consumed or sale in the local markets of the region. This is one of key biological asset from the wetland habitats, play important roles of ecosystem services. This study observed edible aquatic plants utilized in Thailand, at least sixty-sixth species including marginal plants thirty-eight species, emerged plant seventeen species, submerged plant two species and floating plant four species. Moreover, five species of ferns are also found. They are consumed in many recipe types including for vegetable, cereal or starch and cooked.

T6-10-06

Utilizing pulses diversity for ensuring food and nutritional security in Asia and Africa

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Pulses because of their higher nutrient contents play an important role in providing food and nutrition security in developing countries. Breeding efforts for enhancing crop productivity could not meet the desired targets that are critical to feed the vastly growing global population. This has been partly attributed to low utilization of genetic diversity from genebanks to breeding programs. With an objective to understand the genome architecture and apply genome diversity in breeding, draft genomes of chickpea and pigeonpea were assembled. Subsequently, large scale re-sequencing efforts have been initiated in these crops for identifying new sources of genetic variation and allelic variants of candidate gene (s) associated with beneficial traits. In each of these pulse crops, more than 500 lines including reference set, elite lines, parents of several mapping populations have been re-sequenced. Millions of variants have been identified by aligning the re-sequencing data to their respective draft genomes. Detailed analyses provided comprehensive data on diversity features, gene loss, domestication and selection sweep. Multi-location phenotyping data for high priority traits for breeding along with SNPs was used for genome-wide association studies and several marker trait associations have been established. To accelerate molecular breeding efforts further, “The 3000 Chickpea Genome Sequencing Initiative” has been initaited. In summary, significant advances have been made in the area of assessing and utilizing sequence diversity in breeding programs for improving crop productivity.

T6-11: Exploration of new ornamental plants

T6-11-01

The genetic diversity and pedigree analysis of *Bougainvilleas*

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Bougainvillea is a genus in the family Nyctaginaceae with 10 to 18 species native to South American countries such as Argentina, Bolivia, Brazil, Ecuador, Paraguay and Peru. Over 300 cultivars have been developed as the result of natural selection and breeding so far. *Bougainvilleas* have become one of the most common ornamental plant groups in the tropical and subtropical regions worldwide for their variegated foliage and especially showy bracts appearing in various shades of many colours except the blue. *Bougainvillea* cultivars are drought-tolerant, warm-requiring, easy to care and long lasting blooms with strong colours, and are widely cultivated for striking soft landscape. They were selected as official flower of several cities and counties in a number of countries and regions as well as the national flower of Zambia. In order to figure out the genetic relationship among the abundant cultivars and their wild relatives, over 60 SSR molecular markers have been developed based on the transcriptomic sequencing of *Bougainvillea arborea* and the validation with more than 500 samples of cultivars, which further provides resolutions for the precise cultivar identification and the germplasm renovation. This study will mainly present the genetic diversity and pedigree analysis of *Bougainvillea* cultivars and the taxonomy and phylogeny of their wild relatives.

T6-11-02

Hoya, the unknown country of beauty

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Hoya is a large genus within the milkweeked subfamily Asolepiandiodeae, the dogbane family Apocynaceae. It consists of over 300 species widely distributed from the tropical and subtropical Asia to Oceania. Similar with the well-known epiphytic plants, most of the *Hoya* species also share this special habit. Morphologically, the genus exhibits diverse inflorescence (e.g., umbel, pesudumbel or umbel-like inflorescence), and also is characterized by the highly diversity in color, morphology and the size of single flower, which making it to be an economically and ornamentally important plant group. The Roy Garden, located in Guangxi Province, China, has been endeavoring to collect wild resources of *Hoya* species since 2003, and now over 90% of the *Hoya* species are cultivated there. Based on these obtained materials.The Roy Garden has cultivated *Hoya* plants for more than 10 years, and also summarized the experience of the daily cultivation and conservation about *Hoya* plants, while collecting the wild *Hoya* genus resources. Now, in the Roy Garden, the cultivation and conservation of *Hoya* plants can be controlled with the lighting, temperature, humidity, substrate, fertilizers, moisture and some other conditions. Over 200 species of *Hoya* plants had bloomed in the Roy Garden, every year, from March to October, the Roy Garden looks like an ocean of "umbel" flowers. The Roy Garden has gradually increased for the application of the *Hoya* plants since year 2016, especially in landscape and gardening development. On the basis of exchanging communication and coorperation with public *Hoya* fans in China for several years and in connection with the *Hoya* plants with high potential in landscape and gardening using. The Roy Garden selected about 200 species of *Hoya* plants, and assess them with the plant characteristics, growth patterns, the flower characteristics and fragrance, ease of cultivation and conservation and some other horticultural indicators. Finally, we screened about 40 species of *Hoya* plants from them as the key targets for landscape and gardening using. Now in China, at least 3 botanical gardens have began to cultivated *Hoya* plants as a pilot, the special "Umbel" flower attracted large number of tourists' attention. And the same time, as a new element of family greening, the Roy Garden hope the *Hoya* plants can entered more and more household gradually. From 2014, in order to improve the adaptability of *Hoya* plants cultivate in the subtropical and even temperate environment. The Roy Garden began to develop the new *Hoya* varieties and a new *Hoya* variety named *Hoya henschkeliana* "Roy" independently by using interspecific hybridization. It will be published in 2017. The Roy Garden hopes to make more contribution to the global gardening work, and want to show the world an unknown country of beauty.

T6-11-03

New ornamental magnolias from controlled breeding

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Controlled breeding has significantly accelerated the speciation and plant evolution. The diversity of *Magnolia* taxa in today's ornamental market and gardens has brought us much more complication in the field of plant sciences, especially delineation of a species. Although "cultivar" has been widely applied for cultivated plants, the bridge between natural species and cultivated plant taxa has not fully constructed. From the oldest hybrid *M.* x *soulangiana* (*M. denudata* x *M. liliiflora*) by Mr. Etienne Soulange-Bodin in 1820, sterile little girl cultivars from *M. liliiflora* and *M. stellata* by Dr. Francis De Vos in 1956, to a unnamed cross between *M. sieboldii* and *M. insignis* by Mr. Kevin Parris in 2014, we had reviewed their morphological characteristics, phonological data, cytological variations, molecular evidences, and adaptation. Ornamental important *Magnolia* taxa have been greatly advanced under controlled hybridization and many outstanding ornamental cultivars from diploid to decaploid had been bred and available on the market. From these various hybrid cultivars, natural species delineation, such as cross-incompatibility (reproductive barriers), should be challenged. Results from our recent intra- and inter-section cross-hybrids of Section *Michelia* provided support to adapt "cultivar" instead of "hybrid species". Further studies should be placed all puzzles from natural species to cultivars, morphological traits to molecular relationships, by botanists, taxonomists, and horticulturists as one big picture.

T6-11-04

Plant hunters enriched the ornamental plant world

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Plant hunter is a keen person with botanical knowledge and brave mind for achievement. In today's 369,000 flowering plant species known to science, half of them are discovered by plant hunters. In the ornamental plant world, plant hunters almost provided all original species and majority of ornamental plants in gardens, landscapes, parks, greenhouses, and nursery trade nowadays are directly or indirectly from "golden age" for plant exploration and collection in late 19th and early 20th century. From Joseph Banks (1743-1820), who turned plant hunting into a profession and the first promoter of plant expeditions, to Robert Fortune (1812-1880), Ernest Henry "Chinese" Wilson (1876-1930), Frank N. Meyer (1875-1918, losing his life during the collecting trip), Frank Kingdon-Ward (1885-1958), Zhan Wang (1911-2000), Roy Lancaster (1937-), and Daniel J. Hinkley (1954-), the passion for collecting plants had motivated these greatest plant hunters. Their journey of plant collecting trips, especially difficulties and achievement, had been reviewed. Some milestone plants from these plant hunters were examined and their performance in our gardens were summarized. Their plant collection does not only enrich our gardens, but also contain critical genetic resources for our future plant breeding. New plants are new blood for the ornamental industry. We should foster more plant hunters and fully support and manage to fund plant exploration and collecting. There are still 15-30% new flowering plants to be discovered and shared with gardeners. But the most important task for plant hunters is to rescue and conserve endangered and threatened plants under mismanaged and expropriated habitats.

T6-11-05

Morphological characterization of cultivated and wild *Lupi-*

nus (Fabaceae, Faboideae) to be introduced as ornamental and garden plants in Argentina

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Flower plants have been used from antiquity for decoration and grown only for display purposes. Although most species of *Lupinus* qualified as ornamental plants for its colorful papilionoid flowers, the erect racemes and the unique palmately compound leaves, few of almost 600 species are commercialized as garden plants. The most popular of them is the perennial *L. polyphyllus* Lindl., and its hybrids known as "Russell lupins" developed in England by George Russell around 1,911. Other perennial species such as *L. arboreus* Sims (yellow bush lupin), *L. albifrons* Benth. (white-leaf bush lupine); *L. nootkatensis* Donn ex Sims (nootka lupin) and the annuals *L. texensis* Hook. (Texas bluebonnet) and *L. hartwegii* Lindl. (sunrise lupin) are available in the international market, but cultivation are almost restricted to cold areas mainly in Europe and North America. In Argentina, *L. polyphyllus, L. arboreus* and the Russell lupins were introduced in the mountainous region of Patagonia and now are growing wild, mainly in the island of Tierra del Fuego, where the later is the icon flower of Ushuaia city. With the aim of making lupins more popular as garden plants in Argentina, cultivated as well as native species were evaluated by its ornamental and reproductive features as was done for wild species of *Viola* sp. A practical garden guide for lupin cultivation, with the approaches of xerophytic gardening is under preparation. For this presentation, the habitat, habit, plant and inflorescence architecture, flower color and garden uses were analyzed in cultivated lupins (*Lupinus albus* L., *L. angustifolius* L., *L. luteus* L., *L mutabilis* Sweet), in two introduced species (*L. arboreus* Sims., *L. polyphyllus* Lindley) and four wild species natives to Argentina (*L. albescens* Hook & Arnott, *L. gibertianus* CPSm., *L. magnistipulatus* Planchuelo & Dunn and *L. honoratus* CPSm). Recommendation for seed collection of wild species is described and symbols were designed to illustrate plant characteristics, uses and care. As a result of this study, the species analyzed will be presented as ornamental plants in alphabetical order by scientific name, in a booklet with a short description of characters, photographs, ornamental features, habit, uses as garden plant and other possible uses, along with natural origin and distribution. Places and conditions which are needed to be grown, and recommendations for plant care are listed.

T6-11-06

The conservation and utilization of begonias, action by Chinese botanical gardens

Wenguang Wang, *Shouzhou Zhang*

Fairylake Botanical Garden, Shenzhen & CAS

Begonias are well known ornamentals which are cultivated for thir spectacular blooms and range of leaf shapes and coloration. Presently around 1,800 wild species of Begonia are recognized, making this genus one of the largest among the flowering plants. The species occur in subtropical and tropical regions, with the greatest diversity in America and Asia (>750 species each), while being relatively poor in Africa (160 species) and absent in Australia. China has the largest number of Begonia species in Asian, nearly 200 species. Most Begonias have a single living habitat, and some species distributed only in few sites. Artificial acquisition and environment destruction result in some of them endangered. Living collection in botanical garden is significant and pressing. Fairylake Botanical Garden, Shenzhen & CAS have collected more than 250 wild species and 170 cultivated varieties. In this presentation, we'll introduce the adaptability of cultivation conditions incl. using Begonias in landscape, and review the begonias conservation in China. The morphological and genetic diversity of *Begonia edulis* Lévl. (B. Section. Platycentrum), a wide spreading species in China, will also presented.

T6-12: Challenges of botanic gardens for a sustainable future

T6-12-01

Crabaple, the city flower of Baoji

Jian Quan, *Ling Guo*

Beijing botanical garden

Crabapple is confirmed as the city flower of Baoji (in Shaanxi province, China) in 2009. "Crabapple" in Chinese is pronounced as "Hai Tang", and "Hai Tang" also stands for many plants in Chinese, such as crabapple (*Malus*), Japanese quince (*Chaenomeles*) or begonia. Since Shaanxi province is the distribution center of the Genus *Malus* in China, then crabapple is the best symbol plant for Baoji. It was called "Xi Fu" (west prefecture) from the north of Wei River to the west of Jing River in Guanzhong area, including Baoji city, in ancientChina. That was why peoples generally recognized "Xi Fu Hai Tang" was determined as the city flower of Baoji. According to Flora of China (English Edition), the scientific name of "Xi Fu Hai Tang", is *Malus* × *micromalus* Makino. But the description in Flora of China (English Edition), was different from the original publication by Makino. It is provide some possibility of advanced discussion. "Xi Fu Hai Tang" is considered as *Malus spectabilis* 'Riversii' in scientific name according to the literature research in this paper. Crabapples with highly ornamental characteristics, looking forward in the future, more cultivars are appearing and could be the symbol of many cities.

T6-12-02

Impacts of climate change on indigenous communities: The role of botanic gardens in biocultural conservation

Christopher Dunn

Cornell University

Global climate change is having a significant, and negative, impact on the biological diversity and, thus, on the integrity of natural systems. What is less well understood, yet just as critical, are the impacts of climate change and of changes in natural systems on indigenous peoples. In other words, as biological diversity is eroding, so too is the cultural and linguistic diversity of the world. In fact, of the approximately 7,000 extant languages still spoken, fully 50% are at risk of extinction, with the vast majority in the tropics and subtropics. This rate of extinction of languages (and thereby of human cultural diversity) is considerably higher than most estimates of extinction risks to plants and animals. In addition, traditional ecological knowledge and livelihoods are being lost. As an example of the latter, many tropical (and other) indigenous cultures rely on phenological or "ecological calendars" to determine appropriate timing of planting, hunting, harvesting, among other necessary activities. Climate change, and consequent impacts on natural systems and resources, is completely disrupting wellbeing of tropical communities. Thus, it is not enough to consider just the effects of environmental change on plant life within the current context of the global conservation initiatives, such as the Convention on Biological Diversity (e.g., Article 8j), the Global Strategy for Plant Conservation (Target 13), and the Aichi Targets (Target 18). Botanic gardens are uniquely positioned to actively engage in understanding the broader impacts of environmental change to biocultural diversity to achieve biological, cultural, and economic resilience. Examples of how botanic gardens in several parts of the world are defining key ways to better understand tropical and cultural conservation will be presented.

T6-12-03

Gardens are inspirational for conservation bloom

Rajesh Rajaselvam

Dalhousie University

Admiring nature's beauty won't be enough to conserve it. Valuing the value of nature should be expressed by allowing nature to run the show. Since the clock of extinction is ticking fast, the message on conservation to educate the younger generation should be faster. Admiration comes through experiencing something good, thus gardens are inspirational for practical education in botany, landscaping and plant and nature conservation. Not only did the school outreach activities on conservation boost, but also the enthusiasm, enrollment, and the success rate increased in undergraduate plant related courses, labs and field activities in Nova Scotia with the active involvement of botanical gardens. My review concluded that garden visits, hands on field activities and commitment of resource personals are found out to be the key in motivation for plant conservation in young generations. Additional garden related activities have been scheduled to confirm this success.

T6-12-04

The introduction, selection and landscape application of native plants in Labagoumen Nature Reserve in Beijing of China

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The introduction and application of native plants from the Labagoumen nature reserve, which is one of the most abundant plant species areas and has the unique geographical environment and the advantage of plant resources in Beijing, were carried out in order to enrich the diversity of native plants and to create a landscape scene with rich geographical characteristics of Beijing. According to the investigation, there are 668 species, belonging to 367 genera and 102 families in Labagoumen nature reserve which accounts for 1.4%, 53.2%, and 38.7% respectively for vascular plants in Beijing. Many of them have high ornamental value, which are good materials for landscaping and greening, including *Caragana pekinensis, Rosa bella, Hydrangea bretschneideri* and so on. 123 taxa of local plants in Labagoumen were introduced, including 15 woody stocks, 58 perennial roots and seeds for 50 plant other species. 50 introduced plants were observed and evaluated basis on growth, ornamental characteristics, heat resistance, drought resistance, light resistance and shading resistance. 30 species of native plants were selected based on comprehensive evaluation, including *Elsholtzia stauntonii, Deutzia grandiflora, Clematis heracleifolia, Clematis tangutica, Dianthus superbus, Hemerocallis lilioasphodelus, Clematis hexapetala, Scutellaria baicalensis, Dracocephalum rupestre, Platycodon grandiflorus, Dictamnus dasycarpus, Paeonia obovata, Scabiosa tschiliensis, Pedicularis resupinala, Geum chiloense, Adenophora hunanensis, Veronicastrum sibiricum, Phlomis umbrosa, Actinidia arguta, Trollius chinensis, Aquilegia yabeana, Lythrum salicaria, Campanula puncatata* and so on. These selected native plants have good ornamental value and strong adaptability to Beijing environment, which can be applicable for Beijing Landscape greening.

T6-12-05

Population ecology of *Pterocarpus angolensis* DC. in the Lowveld National Botanical Gardens, Mpumalanga Province, South Africa

Peter Tshisikhawe

University of Venda

Pterocarpus angolensis DC is a tree species belonging to Fabaceae family. It is one of the tree species that is utilized for various purposes around the world. In South Africa it is mainly used for its medicinal bark and its good quality wood in furniture production. The great demand of the species has resulted in its high level of harvesting wherever it grows. The overexploitation is therefore threatening its population viability outside protected area. The current studies aimed at understanding the status of a population within the protected pristine area of the Lowveld National Botanical Gardens. Data on population parameters was collected from twenty-six line transects of 100 m x 10 m in sizes. Parameters sampled were plant height, crown health estimates, stem circumferences and evidence of debarking. Results showed a healthy population that displayed an inverse J-shaped curve as analysed from some of the parameters sampled. The population in the Lowveld National Botanical should be monitored in order to keep its good health status. Factors that may have negative impact on the population were also identified.

T6-12-06

Ethnobotany and conservation of Okomu Forest Reserve, Edo State, Nigeria

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An ethnobotanical survey of useful plants was conducted in Okomu Forest Reserve, with a view to documenting indigenous knowledge of medicinal plants used by the inhabitants of the communities and enclaves around the reserve. The information were gathered with the use of structurally designed questionnaires. A total of 90 angiosperm species belonging to 45 families were identified to be useful in the management of various ailments within the study area. Euphorbiaceae and the Legumes constituted the highest species occurrence while the largest number of plant families (22) had only one species each, being represented. Further findings showed that the leaves and stem barks are the most useful parts of the plants while the flower and pith were the least useful. In general, the trees were the most useful of all plant habits followed by the herbs and the shrubs. While this work reflects the biodiversity richness of the study area and its environs, it also suggests the enforcement of conservation strategies as a measure to mitigate species loss.

T6-13: Citizen science and conservation: inspirational projects to get the common public into investigating and conserving regional biodiversity

T6-13-01

The Inheritance and Innovation of Chinese Landscape Architecture—On the Past and Present Life of Shenzhen Fairylake Botanical Garden

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Over three decades, Shenzhen, having made great achievements in the construction and development of landscape architecture, also created numerous Number One in China in the field of landscape architecture. Led by Professor Sun Xiaoxiang and Academician Meng Zhaozhen from Beijing Forestry University, a group of experts and scholars have practiced the uniquely glamorous Shenzhen Experience with their actions and wisdoms. Since the early 1980s, from site selection to the whole practicing of planning and design as well as the highlighting of the post effect, Fairylake Botanical Garden mainly underwent start-up stage, stable stage developing stage and mature stage, got gradually matured and perfected after years of development and has been enjoying a growing popularity. The start-up, construction and development of Fairylake Botanical Garden, lasting for as long as over 30 years, cannot do without the protracted and unremitting efforts of the landscape architecture practitioners of generations and shows the excellent practice in inheritance and innovation of Chinese landscape architecture.

T6-13-02

The Million Orchid Project: Using citizen science to restore orchids within a large urban area

Carl Lewis, *Jason Downing*
Fairchild Tropical Botanic Garden

Southeastern Florida (USA) historically supported more than 30 species of native orchids (Orchidaceae). Beginning more than a century ago, the region developed into the Miami Metropolitan Area, which now includes more than 6 million people. As a result of habitat loss and overharvesting, many of the native orchid species are now extinct in the region, and nearly all are reduced to fewer than 100 plants. The Million Orchid Project, established in 2013, is a citizen science program that aims to propagate and restore orchids to the entire Miami Metropolitan Area. The project is a massive experiment, conducted by thousands of citizen scientists, to determine whether densely populated urban areas can support the recovery of rare orchids. Specific research includes (1) experiments on propagation and planting techniques, (2) studies of the genetic diversity of wild and reintroduced orchids, (3) monitoring of survivorship and growth rates in a variety of different urban habitats, and (4) isolation and characterization of fungal endophytes. Volunteers, teachers, and schoolchildren are involved in all parts of the project. More than 100 schools now have orchid propagation supplies and equipment within their classrooms. A specialized mobile micropropagation laboratory, built within the frame of a decommissioned school bus, visits schools throughout the region to give students hands-on experience with sterile orchid culture. To date, eight native orchid species have been propagated from seed, and five have been introduced into urban landscapes. Citizen science data have helped us select optimal growth media, transplanting protocols, and outplanting sites for for each orchid species. As a result, we have seen survivorship improve as the project progresses. Orchids have been succeeding in neighborhoods that were previously thought to be too urbanized. Our results suggest that an engaged community can help restore rare orchids, especially within densely populated urban areas.

T6-13-03

Citizens are supporting the *ex-situ* cultivation of botanic gardens: A community approach in Germany

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The ambitions to fight against the global loss of plant biodiversity are high (Secretariat of the Convention on Biological Diversity, 2010). But when it comes to specific provisions, often the societal acceptance is little and the lack of money is high. To protect local biodiversity the cultivation of wild plants in ex-situ collections of botanic gardens is an established tool, jet. For preserving the whole range of local/regional plant biodiversity in accessions, preferably under near natural conditions, the need of space and money becomes a big problem. Particular when it comes to handle more than one population per species, or even several species with many populations. To overcome the mentioned problems of acceptance, space and money the Science Shop of Potsdam (Germany) initiated and mediated between local urban gardening communities, local authorities and the botanic garden of Potsdam an appropriate approach to protect local plant-diversity. Here we will present the experiences of the initiation stage and future steps to evaluate the process.

T6-13-04

Promoting indigenous plants in a pilot project to support more efficient use of water, natural resources and regional biodiversity in the state of Sinaloa, Mexico

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This project aims are to strengthen the interaction between the

University of Sinaloa and the community of the State of Sinaloa, Mexico, through the development of a Green Campus where plants are used to promote diverse methods for the protection of the environment and water resources. In Sinaloa droughts are recurrent and cause depletion of water resources both in surface water and in aquifers. The rational use of water and the sustainable management of natural resources is a social problem that must be addressed in an integrated manner including public policy, infrastructure and reducing consumption by the productive and domestic sectors. In 2013 to 2015 a project was implemented to promote these objectives through a cooperative approach between researchers, students, external consultants and the community as part of the Drought Prevention and Mitigation Program (PMPMS in Spanish) to support the efficient use of water and natural resources and promote biodiversity in the State of Sinaloa. In this context plants can contribute to mitigating droughts as they play a beneficial role by protecting the soil to avoid erosion, filtering contamination and conserving water resources. To demonstrate this fundamental role of plants, a proposal for a "Green University City" was planned for the improvement, adaptation and modernization of university grounds to promote a culture of environmental care and the generation and application of knowledge on relevant issues related to sustainable development. The project includes research into the adaptation of plants to drought conditions, water consumption and their impact on the total water balance, to establish which plants are most suitable for the local climatic conditions. In the university campus the project will include changes to the landscape by making more efficient use of irrigation water and promoting the knowledge of native plants for their conservation. It is proposed that a major part of the green spaces of the campus will be turned into a succulent garden by planting native species of *Opuntia* commonly known as nopales used in many traditional Mexican dishes; *Mammillaria*; *Cephalocereus*; *Cylindropuntia*; *Echinocactus*; *Sclerocactus* and *Selenicereus* between other representatives of the Cactaceae family. Also, species of Agave which is one the plants from which the traditional drink tequila is made, and species of Yucca, will be cultivated in different locations of the campus. The trees to be incorporated include: *Ceiba pentandra* (L.) Gaertn. and Cordia boissieri A.DC. Also patches of native grasses and herbs will be considered as part of the landscape design. Overall it is expected that plants will contribute to conserving at least 30% of the precipitation that at present is lost as surface water flow which in turn causes flooding and limits the recharge of aquifers. Thus it will be demonstrated to the community that plants can have a beneficial use in managing water resources. In turn it is hoped that this example will motivate the community to adopt a more sustainable approach to the selection of plants so that native plants are preferred over introduced species.

T6-13-05

Plant symbols in Turkey and importance of plant symbolizing for nature conservation

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From past to today, plants are used as a symbol for cities. They appear in flags, coins, stamp *etc.* With regard to Turks rose and tulip are abundant during all periods. Tulip usages and senses in Ottoman Empire's and Turkish cultures are very common. Anatolian Seljuki Turks used motifs of rose, rose products in their literary works. Sultan Mehmet II, the conqueror of Istanbul in 1453, once depicted in miniature smelling roses. At the present time Isparta Province is center of rose cultivation in Turkey, and it is called city of rose. The tulip is used as an icon and the festival is organized in Istanbul. In Turkey wild plants are also used as a symbol from the stand point of conservation. During the "Important Plant Areas" project along BTC pipeline in Turkey, 10 plants (*Cousinia sivasica*, *Cyclamen pseudibericum*, *Helleborus vesicarius*, *Hyacinthus orientalis* subsp. *chionophilus*, *Fritillaria alburyana*, *Iris iberica* subsp. *elegantissima*, *Lathyrus karsianus*, *Lilium kesselringianum*, *Orchis anatolica*, *Tchihatchewia isatidea*) were chosen as a symbol of each city. Plants were rare, endemic or economic. Also one of the municipalities in Istanbul chose "*Crocus olivieri* subsp. *istanbulensis*" as their logo. Based on these examples symbolizing flowers/plants make awareness of local people for protection the habits and also plants. Ultimately plants as a symbol are good source to understand culture of nations, history of cities and good instrument for nature conservation.

T6-13-06

Plant species composition and bioculture of urban greenspace in Kunming city, Southwest China

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Plant species composition in urban greenspace reflects the natural environment, human management and cultural connotation of the city. But many studies do not discuss the cultural connotation of urban plants. The plant species composition and bioculture were studied based on the field data collected in 22 greenspaces in Kunming City China. A total of 143 families, 448 genera and 716 species of vascular plants was recorded, of which, 649 species were in the campus and 374 species in the parks, indicating the higher species diversity in the campus. In teams of growth form, the herbs, trees, shrubs and vines respectively accorded for 46.51%, 27.79%, 22.49% and 3.21%. The proportion of trees in the parks (33.69%) was higher than that in the campus (28.35%). In terms of species origin, the proportion of alien plants in the total species, campus species and park species were 39.25%, 38.52% and 41.44% respectively. Too many tropical plants introduced caused annually a large number of plant frost damage and management input. The plant composition in the urban greenspace embedded the distinct traditional morality and real aesthetic. Most plants played the roles of afforestation and beautification, reflecting the season changes of colorful flowers and leaves. More local bioculture was showed in the aged greenspaces, compared the dull afforestation and visual sense in the new ones. The key taxa of plant system evolution, state or local protection plants and local endemic species were highlighted in the plant configuration of campus green, promoting the culture service functions for teaching and scientific research.

T6-14: Botanical Gardens and the Exploration of Salt Tolerant Plants

T6-14-01

Finding climate change ornamentals for Galveston Island, Texas, and the strategies needed to deal with a salt challenged environment

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Stephen F. Austin State University

In 2008, Galveston Island, Texas, USA, was affected by Hurricane Ike which inundated most of the island with sea water. The storm killed fifty-five thousand large live oaks, *Quercus virginiana*, many of which were over one hundred years old. With funding by the Moody Foundation, a long term platform for salinity research and discovering ornamentals adapted to the island was initiated in early 2016. The full sun one ha plot is located on the west side of Moody Gardens, a nationally known resort on the island, and elevation varies from 1 to 1.5 m above sea level. Soil samples taken across the plot indicate a soil with high SAR at the one meter depth and varying degrees of high salinity depending on proximity to the bay. The research plots are also subject to intense coastal winds for much of the year. This project has three goals. 1) Develop a long term evaluation program for woody ornamental trees and shrubs. A survey of plants in the evaluation program and their performance will be presented. 2) Studying treatments that affect soil amelioration and plant establishment in the project area. In this first study, a randomized block design was utilized with three species, eight treatments, six replications and 2 plants per replication, for a total of 288 plants in the study. The three woody plants tested include *Quercus virginiana*, *Hibiscus hamabo*, and *Taxodium* X 'T406', the latter a hybrid of bald cypress and Montezuma cypress introduced by the Nanjing Botanical Garden. Plant performance and soil analysis change over time will be presented. 3) assess and characterize microbial communities in the salt affected soil on Galveston Island and the opportunities for mycorrhizal colonization of select species to enhance their salt tolerance and performance.

T6-14-02

Analysis of the environmental characteristics and salt-tolerant plant screening involving the issues of coastal area afforestation in Jiangsu Province

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Jiangsu province is located in the eastern part of China with 954 km of coastline along the Pacific Ocean. With 670,000 hectares of costal land equivalent to 6.5% of the entire area of the province, this land area is almost 25% of the coastal land of China. Silting is enlarging the area at an increasing rate of 1,300 hectares per year. Jiangsu province includes the transition zone of the more temperate north and the south subtropics. Its mild climate and abundant rainfall (900-1000 mm annually), is conducive to the growth of a wide diversity of plants. The utilization of coastal land resources and the restoration of vegetation have drawn increased attention by local governments. However, vegetation restoration and establishing wind-break forests on the bare coastal land are often associated with difficult environmental factors, including soil salinity, nutrient shortage, wind strike and water logging. Plant growth, especially tree growth, is especially vulnerable and high mortalities are common. For many years, the author has engaged in a variety of studies that focus on the introduction and screening of salt-tolerant plant materials. Communication with national and international scholars interested in this common topic is critical to sharing and developing new ideas, finding new plants and discovering the best strategies for restoring vegetation on the salty coastal lands in China and other countries facing similar issues.

T6-14-03

The contribution of economic plant exploration studies of Nanjing Botanical Garden in the past 50 years

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The results of researches on introduction, cultivation and breeding of economic plants were briefly reported. Species of *Dioscorea, Castanea, Taxodium distichum, T. mucronatum, Cynodon dactylon, Vaccinium, Rubus* (*black berries*)*, Lycoris, Stevia rebaudiana, Cistus ladaniferus, Olea europaea, Coreopsis drummondii, Chimonanthus pracox* and *etc.* were included. Research achievement made obvious benefits to national economy and ecological conditions. In the meantime theoretical study on plant introduction and breeding, conservation of biodiversity and environment protection were conducted also.

T6-14-04

The salt tolerance assessment, improvement and utilization of native turfgrass in China

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Saline soil is a type of soil widely distributed on the world, accounting for 25% of the total land area. It mainly distributed in the inland arid and semi-arid areas and coastal areas. Along with the development of the industrial construction and ecological construction in saline land, it has brought about a large-scale landscape engineering and ecological restoration project, which required a lot of salinity-tolerant green plant cultivars. In addition, the application of saline reclaimed water and snow melt salt in winter also increased the demand of salinity-tolerant greening plants. Bermudagrass (*Cynodon* spp.) is a kind of salinity-tolerant grass, which has a great potential for application in greening and ecological restoration on saline land. In this research, the regulation mechanism of sodium and potassium ion and the establishment and maintenance technique of salinity-tolerant bermudagrass on heavy saline soil was studied based on the evaluation of salinity tolerance of bermudagrass resources, and then a 'saline soil turf' model was proposed and demonstrated. We preliminary confirmed the genetic variation of salinity tolerance in Chinese bermudagrass resources, and their regulation mechanism of sodium and potassium. Bermudagrass could reduce Na^+ accumulation and maintain K^+ stability in leaves under salinity stress by restricting Na^+ into bundle in roots, selective transport K^+ over Na^+ from root to leaf, and Na^+-selective secretion via leaf salt glands, at the same time maintaining appropriate stomatal conductance to maintain photosynthetic efficiency. We also found salinity stress increased salt gland density, the appropriate increase of K concentration in rhizosphere was beneficial to the competitive absorption of K^+ over Na^+, and the different ion regulation ways were interaction each other. By using cDNA-AFLP differential expression technique, some gene fragments which may be related to ion regulation were obtained. A simplified turf establishment and maintenance in saline soil was proposed based on the model of 'Saline soil-Salinity tolerance turfgrass cultivar-Saline water irrigation' and demonstrated in Jiangsu Province, Shandong Province, and other heavy saline soil including the ecological reconstruction of islands in Chinese South Sea. This research provided a good basis for further mining the salinitytolerant gene, continuingly breeding salinity-tolerant cultivars and demonstration in heavy saline soil in bermudagrass.

T6-14-05

Research on the establishment of coastal shelter forest systems with the function of anti soil erosion and wind breaking

Haibo Hu

Nanjing Forestry University

The suitable trees, allocation patterns, silviculture and management techniques of anti-erosion and anti-fouling forests were studied in the sandy, muddy and rocky coastal areas. Nineteen species of anti-erosion trees had been selected, such as *Fraxinus chinensis, Robinia pseudoacacia, Ascendens mucronatum, Bruguiera gymnorhiza, Kandelia candel* and so on. At the same time, we established the database of south China coastal salt tolerant plants, including two hundreds and forty-six kinds of salt-tolerant plants. We used the silviculture means of lifting isolation layer, cavernous membrane of replacing soil, base plate of replacing soil and the ecological floating bed purifying aquaculture water technology to carry out shelter forest establishment. The soil physical and chemical properties and erosion resistance of coastal shelter forest were researched. In sandy coast, the reunite degree of soil is higher for the stands of *Suliu plant, Fraxinus americana* and *Populus L.*, and the disperse rates were lowest, the resistance to erosion was stronger. The main factor influencing the soil anti-erosion index was the number of fine roots diameter class of trees 0 to 1mm and 1-2 mm of in the rocky coast, and soil anti-erosion index of six forest types were higher than non-forest lands, and the anti-scouring index of 0 to 20 cm soil were higher than that of 20 to 40 cm. On the ditch slopes of the sandy coast, the anti-erosion ability increased 33.2% on average. By means of Land sat TM, World View and Quick bird satellite images and topographic map data, for monitoring the coastal landscape and vegetation dynamics, we develop and apply of the coastal shelter forest vegetation and landform change detection of remote sensing image processing software applications. By the method of layered extraction, site classification and area changes of the muddy coastal mangrove were researched. Using SPOT 5 remote sensing data, we monitored the vegetation and landscape change in sandy and rocky coast of Daishan island. What's more, we accessed a computer software copyright registration "coastal shelter forest vegetation and landform change monitoring and remote sensing image processing software applications (2012SR038689)".

T6-14-06

Interactive effects of sulphur and humic acid on soil properties, and growth, green yield and some chemical constituents of *Pisum sativum* plants grown on newly-reclaimed salt-affected soils

M.M. Rady[1], A. Osman[1], H. Mohamed[2], R.M. Nassar[3], M. Abd El-Hady[4], ***Magdi T. Abdelhamid***[4]

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2. Ain Shams University
3. Cairo University
4. National Research Centre

About 900,000 ha of Egypt's agricultural lands are suffering from salinity build-up problem. Therefore, two field experiments were conducted on newly-reclaimed moderate saline sandy clay loam soil at the Experimental farm of Faculty of Agriculture, Fayoum University, Fayoum, Egypt during the two successive growing seasons, to examine the positive effects of applied elemental sulphur (S) at the rates of 0, 120, 240, 360 and 480 kg ha^{-1} and humic acid (HA) at the rates of 0.0, 0.5, 1.0, 1.5 and 2.0 g L^{-1} and their combinations on pea (*Pisum sativum* L.) growth, yield of green pods, green seeds and their quality parameters as well as some soil chemical properties after harvesting pea plants. The obtained data showed a significant positive response for the applied treatments of the S and HA as either solely or in combination with a superiority towards the highest levels of combined ones. Also, the obtained results indicate that the greatest values of green seed starch, total soluble sugars and protein contents are more attributed to the positive effect of nutrients. The obtained results reveal that organic humic acid at the rate of 1.5 g L^{-1} and S at rate of 360 kg ha^{-1} should be used to face a great problem of either soil salinity or excessive $CaCO_3$ content. These beneficial effects are more attributed to the achieved soil amelioration in the values of ECe, pH and ESP as well as released nutrients from either native source or applied organic manure, which supported the aforementioned pea traits. So, it could be recommended that organic (humic acid) and elemental sulphur could be used to alleviate the adverse effects of soil salinity.

T6-15: Species richness of the pan-tropical genus Begonia and its conservation challenges at Internet Age

T6-15-01

A better strategy for conservation of Chinese Begonia

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Begonia is one of the most diverse plant taxa and the sixth largest angiosperm genera consisting of over 1,800 accepted species names. The species number of this genus sharply increased particularly in the last 20 years, and only in China it increased from 80 to 200 species during this period and is close to that of Brazil with the richest *Begonia* species in the world. Based on recent field survey, the number of *Begonia* species in China is predicted to be between 250 and 300. Most of *Chinese Begonias* are narrowly distributed, and the wild populations are facing shrinking due to unique, isolated and fragmented habitats subject to negative influence of climate change and human's activity. In addition, the exploitation of wild *Begonia*s with ornamental and medicinal values has been accelerated by fast developing internet. More and more species should be categorized into rare and endangered class and therefore need immediate protection. Ex situ conservation of *Chinese Begonias* started in 1996, and so far nearly over half of the total species have been introduced by several major botanical gardens belonging to Chinese Academy of Sciences. However, only fewer research institutions, limited funds and human resources have been involved in *Begonia* conservation, and no project has ever been conducted on reintroduction. Therefore, more systematic work and a better conservation strategy remain to be developed. A deep taxonomical study is needed to understand the real situation of *Chinese Begonia* germplasm. A better conservation strategy is proposed for *Chinese Begonias* depending on a solid taxonomical foundation, understanding population diversity, and integrating multiple approaches of protection, such as in situ, quasi situ, near situ and ex situ conservation, seed bank, plant reintroduction, legislation and public education. Although the current protected areas (any types nationally and regionally) provide harbors for the majority of wild *Begonia* species, the species or population targeted conservation zone or micro-reserve should be established for either endangered species or unique populations which are distributed outside the current protected areas. On the other hand, the national and regional level law on plant protection should be extended to prevention of illegal collection and transaction of wild plants particularly the species with unique habitats and small populations.

T6-15-02

Begonia placentas response to the global cooling events via chloroplast genomics

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Ice ages are among the most important climatological events in the history of the earth. The multiple global cooling events have been well documented from earth science. During the process, the biotas experience multiple gradual and sudden changes, such as extinction, rapid radiation and evolutionary shift of growth habit. However, it is unknown which definite biotas throughout the world provide clear evidence of the progress of glaciations and what adaptive characters of these biotas are affected by glaciations. Here we show that *Begonia* placentas responses to the global cooling events in polyphyletic, polychromic and polytopic ways via ecotypes. We sampled 110 species in *Begonia* worldwide with diverse placentation and ecotypes and built up the stable phylogenetic tree based on the chloroplast genome. We observe the placental development in ovary of four species from the different linages and confirm the evolutionary direction of placentae from parietal to axile placentation. We also observed systematically the spatial change of placentation respectively in 9 positions in the ovary from 56 species via paraffin section and in three positions from 67 species via cutting of fresh ovary. Here we show that the derived state (axile placentation with single segment in geophytes and therophytes adapted to cold and dry climates) tends to occur at the base of the main clades in the phylogenetic tree. The comparison of development, phylogeny and ecotype shows that *Begonia* has experienced multiple influences from climate freezing, as indicated by the basal groups in the phylogenetic tree with derived characters and cooling-adpative ecotypes during the glacial periods and the main linages with the rapid radiation of species during the interglacial. The evolution of *Begonia* placenta not only responses to global cooling events but also indicated the occurrence time of some tectonic events in global history. It seems that the taxonomic ranks in *Begonia* were gradually formed under the influence of global cooling. The above case seems also happen outside the genus *Begonia* even across the whole biotas, justifying a broader survey of the evolution of entire biotas within the context of global climate change.

T6-15-03

Diversity of the genus *Begonia* L. in Laos

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Indo-Burma region is one of the biodiversity hotspots where more than 7,000 endemic plants live. Laos is located at the centre of this biogeographic region bordering to China, Cambodia, Myanmar, Thailand and Vietnam, thus grasping plant diversity in Laos is crucial to understand how such a huge endemism was developed. However, many endemic species are endangered by recent human activities in Laos. Some projects were urgently launched on the flora of Laos, such as those by Royal Botanical Gardens Edinburgh (RBGE), Muséum National d'Histoire Naturelle (MNHN), Kunming Institute of Botany (KIB) of the Chinese Academy of Sciences (CAS), but much more efforts are required to clarify the whole picture. *Begonia* L. (Begoniaceae) is widely distributed in the tropical and subtropical regions of Africa, America and Asia, and consists of ca. 1,820 species worldwide. Laos is a part of Indo-China, where very few documentation on Begoniaceae has been published. As there is no botanist focuses on *Begonia* of Laos, an obvious gap in Indo-China, while the nearby regions with richness of *Begonia*, such as Yunnan of China (about 100 species), Vietnam (about 80 species) and Thailand (about 70 species). The most account of Lao *Begonia* was published after 2007, including 13 species or 17 taxa. Recently, some new species has been described from this country such as *Begonia afromigrata* J.J.de Wilde, *B. lamxayiana* Souvann. and *B. viscosa* Aver. & H.Q.Nguyen. To put forward the inventory on Lao plants, especially on the genus *Begonia*. We carried out a joint field collection and the examination of the available specimens from Laos in 2016 to explore its potential diversity of *Begonia.* We try to submit the current state of *Begonia* in Laos in Congress via the collaborative study with many botanist in the other countries to fill the gap.

T6-15-04

The diversity and conservation of *Begonia* in continental SE Asia

Ruth Kiew

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The greatest obstacle to studying any aspect of *Begonia* diversity and conservation in SE Asia is the taxonomic impediment. Among continental SE Asian countries, only Peninsular Malaysia has a monograph for *Begonia*s. From existing specimens in herbarium collections and the rate of published new species and records, it is clear that existing checklists for Myanmar, Thailand and Vietnam include less than half the countries' species. The flora of Laos and Cambodia are woefully incomplete. Habitat diversity in all these countries emphasize the species richness of *Begonia* on karst limestone, particular in northern Vietnam, which is a biodiversity hotspot for *Begonia*. The major problem confronting *Begonia* conservation is the extremely high level of endemism (over 80%) with many species known from a single locality, and secondly the taxonomic impediment. At present, more than half the species fall within the IUCN Data Deficient Category. Major threats throughout the region are deforestation and logging, quarrying of karst limestone, and the construction of hydroelectric dams. Concerted action is required to conserve *Begonias* by (a) revising *Begonia* floras on a national basis, (b) assessing conservation status of species, and (c) because *Begonias* are frequently indicators of biodiverse habitats, identifying and designating areas as Important Plant Areas to form the basis of a network of legally Totally Protected Areas.

T6-15-05

Reconstructing phylogenetic relationship of *Begonia* sect. *Coelocentrum* (Begoniaceae) using targeted enrichment of nuclear genes

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With estimated more than 1,800 species, *Begonia* L. (Begoniaceae) is one of the largest genera of vascular plants, distributed throughout tropical and subtropical Asia, Africa and America. *Begonia* sect. *Coelocentrum* was first established by Edgar Irmscher in 1939 on the basis of the parietal placentation and geographical distribution. Section *Coelocentrum* comprised ca. 65 species with a variety of leaf shape, texture and variegation and is usually confined to the cave-like microhabitats of karst areas in southern China and northern Vietnam with most species highly localized. A recent molecular phylogenetic study based on DNA sequences of nrITS and chloroplast rpL16 intron indicated that a majority of Sino-Vietnamese limestone *Begonia* (SVLB), including sect. *Coelocentrum* and five species of sect. *Diploclinium*, *Leprosae*, and *Petermannia*, form a strongly supported monophyletic group but deeper nodes are very poorly resolved, which suggests a rapid species radiation across the Sino-Vietnamese limestone karsts in SVLB clade. To construct well-supported molecular phylogeny of sect. *Coelocentrum*, we employed a novel target enrichment method using a set of custom capture probes designed across four *Begonia* transcriptomes, coupled with next generation sequencing (NGS) to gather phylogenetic information from thousands of nuclear loci. We sequenced over 90% of *Begonia* sect. *Coelocentrum* species and used bioinformatic and phylogenetic programs to produce a phylogenetic tree to 1) test the current phylogenetic hypothesis; 2) revise the current taxonomy, and 3) understand the evolutionary history in this group. These data will provide important information for further studies investigating evolutionary mechanisms and plant speciation in limestone flora.

T6-15-06

Vietnam-the history museum of the evolutional platform for *Begonia*-needs to be explored and conserved with priority

Hieu Nguyen
Center for Plant Conservation Vietnam

Begonia L. is comprised of more than 1,800 species, most of which are local endemics with very restricted distribution in tropical and subtropical regions of the world. Inventory of the genus species composition and diversity in local floras, particularly in Indochina remains very far from acceptable completion. When over 175 species are reported from China, with 143 local endemic (82%), very few species known originate from countries of eastern Indochina. Thus, only 36 species of the genus till now have been tentatively reported from Vietnam. Relatively more extensive attempts were undertaken in studies of the genus in Vietnam. Since 1919, French plant taxonomist – François Gagnepain (1866-1952) was the pioneer in exploring *Begonias* in Indochina and a few *Vietnamese Begonias* documented. The first checklist reports 41 native species and 6 cultivated by Vietnamese plant taxonomist, Pham Hoang Ho (1991,1999). Over the last 10 years, 29 species have recently been added by other plant taxonomist as Ruth Kiew, Ching-I Peng, Shui-Yu Min, Nguyen Quang Hieu, Mark Tebbit, Leonid Averyanov, *etc.* Many of them were found in North Vietnam. However, many are isolated in ancient mountain formations, particularly karstic rocky remnant limestone massifs well presented in Vietnam and in Laos undoubtedly provide home to numerous still undescribed as local endemic species. Our explorations during the last few years confirm this supposition and truly outline areas of eastern Indochina as the history museum of the evolutional platform of *Begonia*-needed to be explored and conserved with priority. Our model conservation chooses the right place within local village and engages local community to plant and manage the collections.

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