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出席『21世紀蕨類植物研討會』報告

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內容摘要: 「21 世紀蕨類植物研討會」於 2004 年在英國愛堡舉行，共分系統分類與大演化 15 篇、生態與植物誌 10 篇、保育 7 篇、種化與小演化 8 篇、基因組 3 篇、發育與構造 6 篇等，另有海報 23 篇。本人代表我國發表的文章爲「利用托葉繁殖進行台灣原始觀音座蓮與伊藤氏原始關音座蓮的區外保育」，由於試驗完整，並具原創性，與會學者均表達高度的興趣與肯定，也讓國際學者瞭解台灣在保育研究的努立。

本文電子檔已上傳至出國報告資訊網

目錄

一、前言

台灣原始觀音座蓮與伊藤氏原始關音座蓮為台灣兩種固有且瀕臨絕滅的蕨類植物，目前只知分別在烏來地區與中部蓮華池有少數分布，其中前者約僅一千株，後者更少於一百株，其保育實刻不容緩。

此兩種植物在以往之研究，除生育地有零星之調查，其生殖行為並無完整之報告。本人以多年之孢子繁殖經驗，嘗試進行其孢子繁殖。歷經多年之實驗，雖可使台灣原始觀音座蓮的孢子發芽，但後續之配子体生長卻極為遲緩，在歷經 2.5 年之培養，孢子体繁殖成功的比率僅約 1%；而對伊藤式原始觀音座蓮則更無法使其孢子發芽。欲突破此種低繁殖率甚或零繁殖率，本實驗嘗試以孢子体之托葉進行繁殖，並獲得極佳之效果。此結果不僅有助於此二稀有種之保育，也可供世界其它地區本屬稀有種之保育參考。

二、行程

日期	行程地點	工作內容
93 年 7 月 9 日~10 日	台北→愛丁堡	行程
93 年 7 月 11 日	愛丁堡	植物園參訪
93 年 7 月 12 日~16 日	愛丁堡	研討會。
93 年 7 月 17 日~18 日	愛丁堡	植物園參訪
93 年 7 月 19 日~21 日	愛丁堡	標本館參訪。
92 年 7 月 21 日~22 日	愛丁堡→台北	返程。

三、會議內容

五天之研討會共分「系統分類與大演化 Systematics and macroevolution」、「生態與植物誌 Ecological and floristic studies」、「保育 Conservation」、「種化與小演化 Speciation and microevolution」、「基因組 Whole genomics」、發育與構造 Development and structure」等幾個主題：

「系統分類」的研究佔最多數：

Kathleen M. Pryer 以三種葉綠体 DNA(*rbcL*, *atpB*, *rps4*)及一種核 DNA(18S rDNA)序列，分析 62 群植物後，結果顯示除石松門(Lycophytes)外，其餘可歸為一單系門(Monilophytes)。紫萁科(Osmundaceae)為薄囊蕨的姐妹群；雙扇蕨科(Dipteridaceae)、Matoniaceae、裏白科(Glechiniaceae)、膜蕨科(Hymenophyllaceae)等則形成一複雜之起源群系；莎草蕨(schizaeoid)則為薄囊蕨中心群的姐妹群。

Raymond Cranfill 以 *rbcL*, *rps4* 分析 55 屬 97 種真蕨類，可重新定義出 sinopteroids, vittarioids(包含鐵線蕨 *Adiantum*), taenitoids, pteridoids(包含 *Cosentinia*), acrostichoids (包含水蕨 *Ceratopteris*), cryptogrammoids (包含鳳了蕨 *Coniogramme*)等六大系；其間關係為(cryptogrammoids (acrostichoids (taenitoids, pteridoids)))((sinopteridoids)(vittarioids)) 形態特徵如孢子囊群的分佈、假孢膜的有無、葉子的質地、脈型等，無法做為以上六大自然群的分類依據；而許多生態條件如水生、紅樹林、附生、乾旱、溫帶、亞熱帶、熱帶、極地等，

則更能反應此六自然群系之環境適應情況。

Tom A. Ranker 以二個 cpDNA 基因分析 73 種禾葉蕨之親源關係，結果顯示 *Adenophorus, Ceradenia, Calymmodon, Cochlidium,, Enterosora, Mepomene* 為單群系；其它如 *Ctenopteris, Grammitis, Lellingeria, Micropolypodium, Prosaptia, Terpsichore* 等則為多起源群系。葉身分裂程度、根莖的鱗片、腺狀附絲等形態為趨同或平行演化的特徵；以往被忽視的特徵如根的排列與鱗片的亮澤等，更能反應自然群系之分類。

Chile Tsutumi and Masahiro Kato 野外觀察發現附生蕨類植物依孢子發芽與孢子体生長的地點，可區分為攀緣(climber)、半附生(secondary hemiepiphyte)及絕對附生(obligate epiphyte)三種生活型。攀緣植物指孢子自地上萌芽，而後孢子体爬附於樹上，唯其根部終生與土壤接觸，如藤蕨、蘿蔓藤蕨；半附生型類似攀緣型，但在孢子体階段，其下半段可能脫離土壤(人為砍除或土壤乾燥而枯萎)，但上半部則繼續生存，如同絕對型之附生植物，如條蕨與腎蕨。以 *rbcL, accD, atpB* 基因序列分析廣義的骨碎補科(Davalliaceae)及水龍骨科(Polypodiaceae)不同生活型的種類，發現絕對附生型的種類緣起自半附生型，而與攀緣型之親源關係較為疏遠。

「生態與植物誌」一節中，大部份為地區植物誌之調查，且都集中於熱帶美洲與熱帶亞洲進行，如巴西、哥斯達黎加、安地斯山、馬來群島、菲律賓等地，顯示此等地區之調查仍有待加強。除植物誌外，其它為競爭、虫食、及物候等之探討。

「保育」一節與植物誌之研究相反，多為歐洲學者所發表，此外僅墨西哥及台灣各一篇研究，顯示保育與經濟發展之相關性。蕨類之保育分由現地與區外進行，包括孢子的貯存、配子体與孢子体的繁殖、組織培養等。本人代表台灣以「托葉繁殖進行台灣原始關音座蓮與伊藤氏原始觀音座蓮的保育」為文發表(附錄一)，獲得極多之迴響，亦顯示我國在保育的努力。

「種化與小演化」之研究，都以染色体之觀察，配合 DNA 及同功酵素之研究，探討種的來源與親源。多篇研究均以鐵角蕨屬為對象，多倍体化(包括同源與異源)為其種化之主要原因。一般多倍化後之種，可行自交，因此其孢子飛散後形成之單一配子体可成功產生孢子体，而有助於種的擴散。

「基因組」的研究中，C-value 被使用於探討基因組的演化。目前發現即使在同一系群(clade)中，其差異也很大，例如在石松們(Lycophytes)之差異可達 75 倍(*Selaginella kraussiana* 為 0.16，*Isoetes lacustris* 為 11.97)；而在 Monilophytes 中更達 95 倍(*Azolla microphylla* 為 0.77，*Psilotum nudum* 為 72.67)。以系群平均值而言，Osmundaceae 及 Psilotaceae 屬“極大型”，其值超過 35；水生蕨類則為“極小型”，其值小於 1.4。顯示基因組之演化可能朝增加與減小兩個不同方向進行，唯蕨類植物此方面研究正在起步，需有更多的數據才能獲得較具体的結論。

「發育與結構」之研究，結合基因、生理、與解剖，探討苔蘚單倍体之配子体與蕨類植物二倍体之孢子体之分生組織起源，獲得二者非同源之結論。此外對蕨類配子体分生組織之發育也有更進一步的研究，就水龍骨科而言，一般心藏型配子体的分生細胞發現最後發育為邊緣的分生組織；而帶狀型的配子体，其頂端分生細胞最後則轉為中肋分生組織，負責中肋與藏卵器的形成。孢子体具走莖之種類在產生新芽後，其走莖可能形成離層斷裂，而與母株脫離，狀似由孢子發芽而形成之新植株，以往對野外蕨類的生殖可能高估了其孢子與配子体繁殖的速率。

四、會議心得

1. 維持全球生物多樣性應：

   (1) 瞭解並記錄植物多樣性

   (2) 保育植物多樣性

   (3) 永續利用植物多樣性

   (4) 促進對植物多樣性的認知

   (5) 建立植物多樣性的保育能力

2. 蕨類植物是一群古老的陸生植物，它們是全球環境變遷下的生存者，但並不意味往後也能永遠生存。在維護世界生物多樣性的同時，蕨類植物多樣性是否得以確保，完全取決於我們。

3. 蕨類植物系統分類研究之危機：

   在分子生物日益進步的今天，傳統形態的分類研究卻急速減少，在可預見的將來，這已成為植物鑑定、植物誌、系統分類等科學的隱憂，而蕨類植物之研究學者更為稀少，其危機也更大。這些現象主要源於下列原因：

   (1) 蕨類植物在各大學與研究機關之研究漸少。

   (2) 政府經費的短缺與不確定。

   (3) 熱帶生態系的複雜增加了研究的困難。

   (4) 就業市場的壓力迫使學生不易投入此領域。

   (5) 標本館之不易維持使得無可替代的標本無法完整的被保存。

五、建議事項

(一)生物多樣性的保育與永續利用已成為全球的重視議題，相關的政策擬定與研究之進行刻不容緩。

(二)系統分類學之研究為生物多樣性保育與利用之基礎，標本館的經營管理更為系統分類學的基礎，政策與經費應有更明確的支持。

(三)植物園為生物多樣性保育之搖籃，應該受到更多之重視與支持。舉辦本次研討會之愛丁堡植物園即受到英國皇家政府的長期支持，其聲譽也因此受到全世界之肯定。該園四合一之策略，使各不同類型植物獲得最妥善之保育及研究，值得台灣植物園系統之學習。

六、附錄、論文發表全文

CONSERVATION OF ENDANGERED FERNS, *ARCHANGIOPTERIS SOMAI* AND *A. ITOI* (MARATTIACEAE: PTERIDOPHYTA), BY PROPAGATION FROM THEIR STIPULES

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Archangiopteris somai Hayata and *A. itoi* Shieh are two endemic ferns of Taiwan and are categorized into endangered and critically endangered species, respectively. Five fresh stipules were stripped from each of 10 sporophytes of *A. somai* and *A. itoi*, growing in Wu-lai, northern Taiwan, and totally 50 stipules were used in each species. These stipules were rinsed 3 minutes with clean water and put on the medium (4:1, soil: peat moss) under room temperature and fluorescent light (12 hours/day). After one-year culture, plantlets were produced from 40% and 90 % of stipules of *A. somai* and *A. itoi*, respectively. The average sprouting rate and time needed to sprout of these two species were not significant differences among stipules of various sizes of stem. The stipule sizes were not significantly different between the sprouting and non-sprouting stipules. Those average time needed to sprout were very low (*A. somai*) or not (*A. itoi*) related to their stipule sizes. The growth of the stripped mother plants is not affected by removing those stipules comparing to the last year and to the control plants. The simple practical way of stipule propagation of these two species efficiently provides a selection for horticulture, *ex situ* conservation, and *in situ* restoration.

Key words: *Archangiopteris itoi*, *Archangiopteris somai*, conservation, Marattiaceae, endangered plants, stipule propagation, Pteridophyte, reproduction.

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Introduction

Archangiopteris Christ and Gies. is recognized as one of the ancient lineages of pteridophytes. Eleven species of *Archangiopteris* are distributed in southeast China, northern Vietnam, and Taiwan (Ching, 1958). Most taxa are endemic in the above areas. The origin of *Archangiopteris* can be traced back to the Middle Jurassic period, based on fossil records (Hill & Camus, 1986). This genus is phylogenetically closely related to *Angiopteris* Hoffm., another marattialean genus endemic to Southeast Asia, and *Protomarattia* Hayata, distributed in a limited range of northern Vietnam (Hayata, 1919; Chang, 1975). Extant species of these genera represent relics of an ancient lineage that evolved through several glaciation and vicariance events. As a relic taxon, *Archangiopteris* provides valuable information for unveiling the evolutionary history of eusporangiate ferns. However, not much attention has been paid to them until recently (Hsu et al., 2000; Chiang et al., 2002). Even more, many of them are at rare or endangered status in terms of the conservation criteria.

Two *Archangiopteris*, *A. somai* Hayata and *A. itoi* Shieh, have been documented in Taiwan (DeVol & Shieh, 1994). Both species are endemic and categorized into endangered and critically endangered species, respectively (Kuo, 1997; Moore, 2001). Only two populations are known: one in Wulai and another one in Lienhwachi, in northern and central Taiwan, respectively. However, the population of *A. itoi* in Lienhwachi has never been found since the species was first published. The population size and number of both species are strictly limited. Individuals are estimated less than 1,000 for *A. somai* and less than 100 for *A. itoi* (personal investigation). The gametophyte growth is very slow for *A. somai*, and only about 1% of them produced sporophytes after 2.5-year gametophyte-cultures (Chiou and Huang, unpublished). For *A. itoi*, no any spore germination or gametophyte culture has been documented. Tissue cultures of sporophytes of these 2 species have not been reported. In the field, the young sporophytes are very rare. All these cause a severe conservation issue. To conserve these 2 endangered ferns, we then tried to propagate them by using their stipules, as some horticulturists used in other Marattiaceae (Hoshizaki and Moran, 2001; Jones, 1987). The sprouting rate and time needed to sprout were observed. The influence of stem sizes and stipule sizes on the sprouting is analyzed. Morphology of young fronds was studied to assist further investigation in the field. Results provide a practicable method for their horticulture and conservation.

Materials and methods

Because of such a rare status of *Archangiopteris somai* and *A. itoi*, any resource used for experiment and propagation is needed to be very careful. We first tried to stripped above-soil stipules but found they were such strictly tightened on their stems. To avoid damaging the "mother plants", underground stipules, which were relatively loosely tightened, were stripped from their sporophytes of the population in Wulai. Five stipules from one sporophyte and 10 sporophytes of each species were sampled. Fresh stipules were sealed in plastic bags right after being stripped to avoid over evaporation. These stipules were rinsed 3 minutes with clean water and lay on and half covered by the medium (4:1, soil: peat moss) in plastic boxes as soon as they were brought to the laboratory. All cultures were maintained under white fluorescent illumination at about 24 μ mole $m^{-2}s^{-1}$, 12 h/d and the temperature was between 20 and 28℃.

To understand if sprouting was affected by the status of the mother plant and stipule, diameter of the stem and stipule size (width x length) were measured (Table 1). Stipule sprouting rates were counted every month for 1 year. The plants of which stipules were stripped were monitored through out the experiment to see if such treatments would damage their growth.

Results

The sizes of stem and stipules of *Archangiopteris itoi* were significant larger than which of *A. somai*. Stipules began to sprout plantlets at 3 and 4 months after being cultured for *A. itoi* and *A. somai*, respectively. After 7 months of culture, the sprouting rate of *A. itoi* stipules reached to 90 % and did not increase anymore thereafter during one-year culture. On the other hand, the sprouting rate of *A. somai* continuously increased little by little and reached to 40 % after one-year culture (Fig. 1). The stiplues of *A. itoi* not only had a significantly higher sprouting rate but also needed a shorter time to sprout plantlets than that of *A. somai* (Table 1).

The sprouting rates of stipules being stripped from different stem sizes did not show significant difference for both *A. somai* and *A. itoi*, although there was a trend of a higher sprouting rate of stipules being stripped from the smaller stem of *A. somai*. Moreover, the average time needed to sprout of various stem sizes of both species were not significantly different although the stipules of the smaller stems of *A. somai* tended to sprout earlier (Table 2).

Whether the stipule size affecting plantlet emergence is also inspected. Results show that stipule sizes were not significantly different between the sprouting and non-sprouting ones in both species (Table 3). There is a weak negative correlation between the stipule size and time needed to sprout of *A. somai*, whereas which of *A. itoi* is not significant (Fig. 2).

Seventy five percent of the first emerged fronds from *A. somai* stipules were single frond, whereas 93 % of them were with 1 to 3 pairs of pinnate in *A. itoi* (Table 4). All plants of which stipules were stripped for this study were no obvious damage during the next year. The growth and other phonological characteristics such as the number of new frond emergence and season of spore production were similar to which of the year before stripping their stipules and similar to which their stipules were not stripped (data not shown).

Discussion

Expansion of stipule buds of *Danaea wendlandii*, a species of another genus of Marattiaceae, is rarely seen in the field (Sharpe & Jernstedt 1991). The same situation occurred in these two studied species. Stipules have been used to artificially produce offspring of this family. Stipules cut from living fronds were suggested in some marattialean species (Jones, 1987). In *A. somai* and *A. itoi*, however, only 2~5 fronds were produced in one sporophyte each year. Any living frond cutting may seriously damage the plant unexpectedly. In this study, we used underground stipules which did not bear any living frond and relatively easy to be stripped. Results show that those underground stipules are capable of sprouting and producing plantlets well, neither damaging living fronds nor affecting phenological characteristics of their mother plants in the subsequent year.

The fact that no significant difference of sprouting rates and time needed to sprout among stipules being stripped from different stem sizes suggests that the underground stipules of different sizes of stem have the same capability of producing plantlets, at least in the stem size range we used. However, due to population limitation and conservation concern, only 10 plants of each species were used in this study. More duplicates may lead different results, especially in *A. somai* of which average sprouting rate of stipules from 3-cm stem was about 2 times of other treatment and time needed for sprouting was one month shorter too. Thus stipules of 3-cm stem of *A. somai* are still suggested to be selected first for artificial propagation if materials are limited. On the other hand, the sprouting rates of *A. itoi* were high up to 90% and the time needed to sprout was no more than 7 months. It suggests that the selection of stem size is not so relevant in this species.

Similar to the stem size, the stipule size is not very either related to the sprouting rate or time needed to sprout for these 2 ferns. Thus the consideration of stipule sizes is not so important in terms of their vegetative propagation.

Spores are usually to be used for fern propagation naturally and artificially. When the spore source is limited, vegetative propagation is used. Budding of fronds is the most common way of vegetative propagation in ferns. Tissue culture is also applied in artificial vegetative propagation of ferns. For *A. somai*, gametophytes are able to be produced successfully from spores but grow very slowly. During 2.5 year only about 1% offspring sporophytes occurred in our multi-spore cultures (unpublished). For *A. itoi*, no report regarding to its spore germination has been found, and we are not able to make them germinated either. Tissue culture of these 2 species was tried but not successful, mainly due to strictly insufficient materials both in the wild and in the greenhouses or gardens (Gen Chang, personal communication). Stipule culture provides a feasible method to increase plantlets efficiently for materials of the tissue culture and for conservation of both *in situ* and *ex situ*, e.g., in botanical garden (Ranker, 1994). For some other rare Marattiaceae species, this method may also facilitate their propagation and conservation.

Another advantage of the stipule culture is to have plants matured earlier, comparing to the spore culture. The first frond of sporophytes reproduced from gametophytes is usually single frond. Plantlets reproduced from stipule cultures had a quarter of 1~2 pairs of pinnate and 93 % of them with 1~3 pairs of pinnate for *A. somai* and *A. itoi*, respectively. In other words, those plantlets with various pairs of pinnate will grow faster and achieve mature stage earlier than those only with single frond. This condition is favourable to the subsequent horticulture, *ex situ* conservation and *in situ* restoration.

Disadvantage of vegetative cultures is the arrest of their gene diversity. The genetic diversities of these two ferns are unexpected high, from the evidence of the *ATP*B-*RBC*L intergenic spacer of chloroplast DNA (Chiang et al., 2002). Thus plantlets produced from stipules of different plants are able to promote the maintenance of their various genetic diversities.

A limitation of stipule cultures has to be addressed, however. Only 2~5 fronds were produced one year in each sporophyte for both of these species (unpublished). It means only 2~5 stipules will be added to an individual plant each year. Besides, how long of those stipules could survive is not clear. Obviously number of stipules stripped needs to be very careful and cannot be cut unlimitedly.

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Table 1. Sizes of stem and stipules, sprouting rates and time to sprout of *Archangiopteris somai* and *A. itoi*.

	A. somai	*A. itoi*	t-test[1)]
Diameter of stem (cm)	4.1±0.9	8.6±1.9	**
Stipule size			
Width (cm)	1.7±0.3	2.7±0.6	**
Length (cm)	2.3±0.4	3.4±0.5	**
Width x Length	3.9±1.3	9.2±3.0	**
Sprouting rate (%)	40.2±21.1	90.0±4.1	**
Sprouting time (mo)	7.2±2.4	4.6±1.1	**

[1)]: Significant differences (P value < 0.01) appear in all compared items of these 2 ferns.

Table 2. The average sprouting rates and time needed to sprout of stipules from various stem sizes of *Archangiopteris somai* and *A. itoi*.

A. somai

Diameter of stem (cm)	3 (N=3)	4 (N=2)	5 (N=5)
Sprouting rate (%)[1]	60	30	32
	(40-80)[5]	(0-60)	(0-60)
Time needed to sprout (mo)[2]	6.1	7.0	8.4
	(4-12)	(6-8)	(6-11)

A. itoi

Diameter of stem (cm)	6 (N=1)	7 (N=1)	8 (N=4)	9 (N=2)	10 (N=1)	13 (N=1)
Sprouting rate (%)[3]	100	60	95	90	80	100
Time needed to sprout (mo)[4]	3.8	5.3	4.6	4.6	4.8	5.2

[1,2,3,4]: No significant difference of the same row in t-test.

[5]: Number in the parenthesis shows the range.

Table 3. Stipule size of various sprouting status of *Archangiopteris somai* and *A. itoi*.

Species	1. A. itoi			2. A. somai			
Sprouting status[1)]	a	b	t-test	a	b	c	t-test
Stipule size (cm^2)	9.3±3.0	7.6±1.7	ns	3.5±1.1	4.4±1.4	3.7±1.5	ns
Stipule number	45	5		20	24	6	

[1)]: a = sprouting stipules, b = non-sprouting stipules which died after 1 year culture, c = non-sprouting stipules which were alive after 1 year culture.

Table 4. Types of the first frond emerged from stipules of *Archangiopteris somai* and *A. itoi*.

%	*A. somai*	*A. itoi*
Single frond	75	7
Frond with one-pair pinnate	20	51
Frond with two-pair pinnate	5	31
Frond with three-pair pinnate	0	11

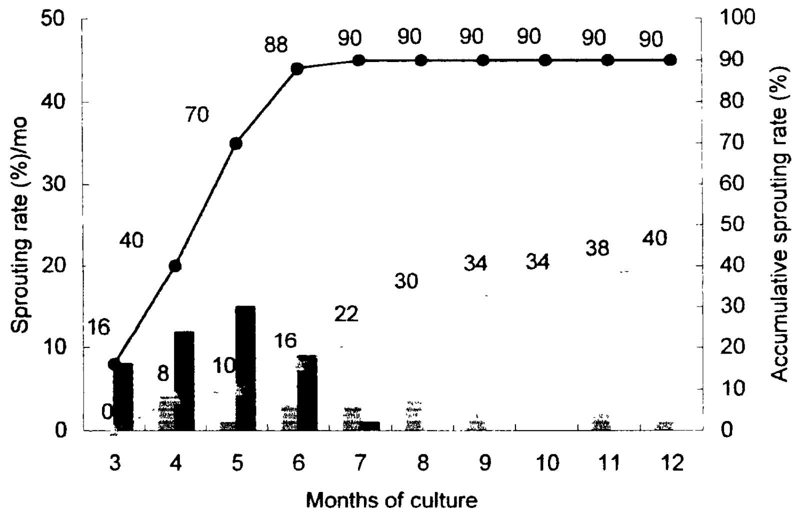
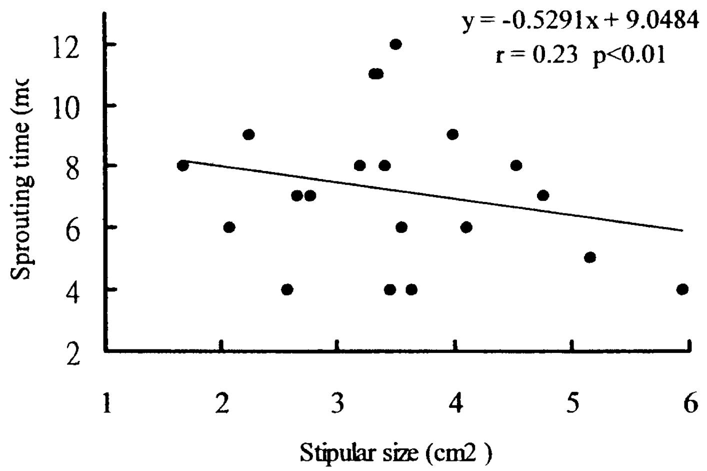


Fig. 1. Sprouting rates (left axis, column) per month and accumulative sprouting rates (right axis, curve) in one-year culture of stipules of *Archangiopteris somai* (gray) and *A. itoi* (black).



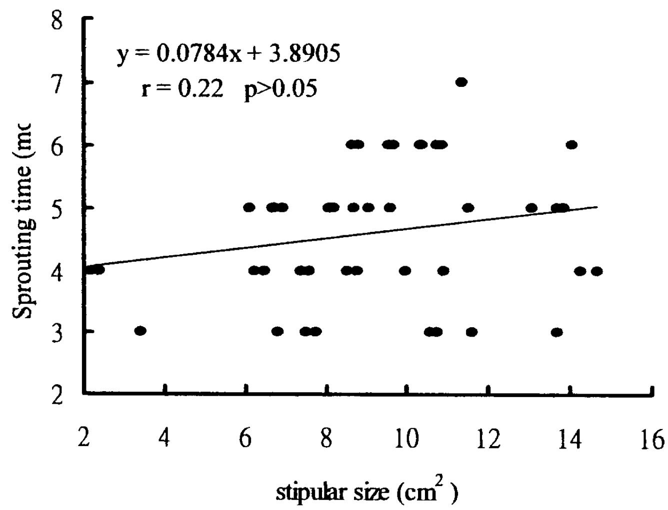


Fig. 2. Correlation between stipule size (width x length) and time needed for plantlet emergence in *Archangiopteris somai* (up) and *A. itoi* (low).