

Low RAPD polymorphism in *Archangiopteris itoi*, a rare and endemic fern in Taiwan

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Abstract. *Archangiopteris itoi*, an endemic fern in Taiwan, is under threat due to human disturbance. Based on fossil evidence the origin of *Archangiopteris* can be dated to the Middle Jurassic Age. Field survey indicated that only eighteen individuals were left in the wild. The population at Lienhwachi, on which the species was named, is now extinct. The genetic variation of the population remaining at Wulai was assessed by RAPD fingerprinting. Of 40 oligo primers, only one revealed polymorphism. Nevertheless, this primer enabled identification of six haplotypes. The low variability within the population is ascribed to the small population size, which was constrained largely due to habitat disturbance. In the small population, genetic drift will have greater effects leading to loss of variation at most loci. In this study, RAPD data indicated that some variation existed within the population, thus suggesting that materials were available for re-establishment of the populations.

Keywords: *Archangiopteris itoi*; Conservation biology; DNA fingerprinting; Ferns; Genetic variation; RAPD; Taiwan.

Introduction

Archangiopteris Christ and Gies. is one of the eusporangiate ferns recognized as an ancient group of pteridophytes. The genus is characterized by huge plants, pulvini of fronds, and fleshy stipules as in other marattialean ferns (Copeland, 1947). Eleven taxa of *Archangiopteris* are distributed in southeast China, north Vietnam, and Taiwan (Ching, 1958; Hsieh, 1970), with high levels of endemism in local floras. Based on the fossil record, the genus has been traced to the Middle Jurassic period (Hill and Camus, 1986). Phylogenetically, this genus is closely related to *Protomarattia* Hayata, distributed with a limited range in north Vietnam, and *Angiopteris* Hoffm., another marattialean genus endemic to Southeast Asia (Hayata, 1919; Chang, 1975). Extant species of these genera, *Angiopteris* s. l. (Hill and Camus, 1986), represent relics with a long evolutionary history through several glaciation and vicariance events.

Archangiopteris itoi Shieh, a species endemic to Taiwan, was first described by Shieh (1970), based on the specimens collected from Lienhwachi in central Taiwan. The species is distinct from *A. henryi* Christ & Gies. var. *somai* (Hayata) Tagawa, another indigenous taxon on the island, by differentiation of recurring veinlets between each pair of true veins and larger fronds (DeVol, 1976).

Recurrent veinlets have been used for distinguishing *Angiopteris* from most *Archangiopteris*, except for *A. itoi* and *A. cardieri* C. Chr. & Tard., a species endemic to north Vietnam. Pinnae with sori close to the margins also indicate a close relationship between these two species. This phylogeny reflects a close geographical history among Taiwan, northern Vietnam, and southern China (Chiang, 1997).

As a relic taxon, *Archangiopteris* provides valuable information for unveiling the evolutionary history of eusporangiate ferns. However, not much attention has been paid to this interesting fern until recently (Wen, 1993a,b). The floristic inventory of Wen indicated that *A. itoi* is under threat of extinction. The "type" population, on which the species was based, has been completely destroyed when a trail for lumbering was constructed a couple of years ago. Fortunately, another remaining population was recently discovered at Wulai in northern Taiwan (Figure 1) (Wen, 1993b). However, the population size is very small, with only 18 individuals. All plants were found at the same site in an original broadleaf forest on government land.

For species with limited population size and number, randomly amplified polymorphic DNA (RAPD) fingerprinting provides an ideal tool for detecting existing variation within and between constituting individuals (Hadrys et al., 1992; Lynch and Milligan, 1994; Rieseberg and Swensen, 1996). In our study, after screening with 40 oligo primers, very low genetic polymorphism was detected in the only extant population of *A. itoi*.

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Materials and Methods

Young leaves of the remaining 18 individuals were sampled from the population at Wulai (Figure 1) and stored on silica gel for rapid drying. All individuals were found at the same site of a 100 m² area in the broadleaf forest at Wulai.

Total DNA was extracted following CTAB procedures (Murray and Thompson, 1980; Doyle and Doyle, 1987). PCR amplifications were carried out in a thermal cycler (Perkin Elmer 480) using the following temperature protocol: an initial 3 min denaturation at 94°C, followed by 35 cycles of 15s at 94°C, 20s at 36°C and 60s at 72°C, and a 10-min

final extension at 72°C. Reactions were carried out in 25 µl volumes with 0.5 ng of template DNA, 2.5 µl of 10× reaction buffer, 2.5 µl of MgCl₂ buffer, 2.5 µl of dNTP (8 mM), 0.1 µM primer, and 0.7 unit of *Taq* polymerase (Promega). Forty 10-base oligonucleotide primers (Nos. 21-60) obtained from the UBC Series one (a gift from Dr. John Carlson, University of British Columbia) were screened. Amplified products were electrophoresed on 2% NuSieve 3:1 agarose gels (FMC BioProducts) in 1× TBE buffer, stained with ethidium bromide, and photographed with Polaroid type 667 film.

Results and Discussion

After surveying with 40 oligo primers, 37 gave positive amplifications and all yielded monomorphic banding patterns, except for primer no. 57 (5'-TTCCCCGAGG-3'). The polymorphism with primer 57 was observed with four DNA fragments: 650, 700, 750, and 800 bp. Six genotypes were identifiable according to the fragment patterns (Table 1, Figure 2). Types A, C and D, with 5 (27.7%), 6 (33.3%), and 4 (22.2%) individuals respectively, predominated. Three types (B, E, F) were rarer, with only one individual each.

In this study, most of the RAPD loci appeared to be monomorphic among plants of *A. itoi*, although polymorphism was detected with primer 57. Dominance at RAPD loci might also mask some rare allelic variants, but overall, little genetic variation has been observed in this species. The low level of genetic variation is usually highly correlated with limited population sizes, in which the stochastic processes of genetic drift might have led the genetic composition toward allele fixation at most loci with high probability (Hartl and Clark, 1997). Obviously, genetic variation is difficult to preserve within a small population size of no more than 20 effective individuals, unless some genetic agents, such as self-incompatibility, have evolved to preclude inbreeding. However, no such mechanism has been reported in the Marattiaceae or in related families. Low genetic variation has been detected in many endangered species of both animals (e.g., turtles, Gray, 1995; seals, Kretzmann et al., 1997) and plants (e.g., *Limonium cavanillesii*, Palacios and Gonzales-Candelas, 1997). The bottleneck effect and apomixis have been found responsible for the loss of genetic variability in many cases (Palacios and Gonzales-Candelas, 1997; Koch et al., 1998).

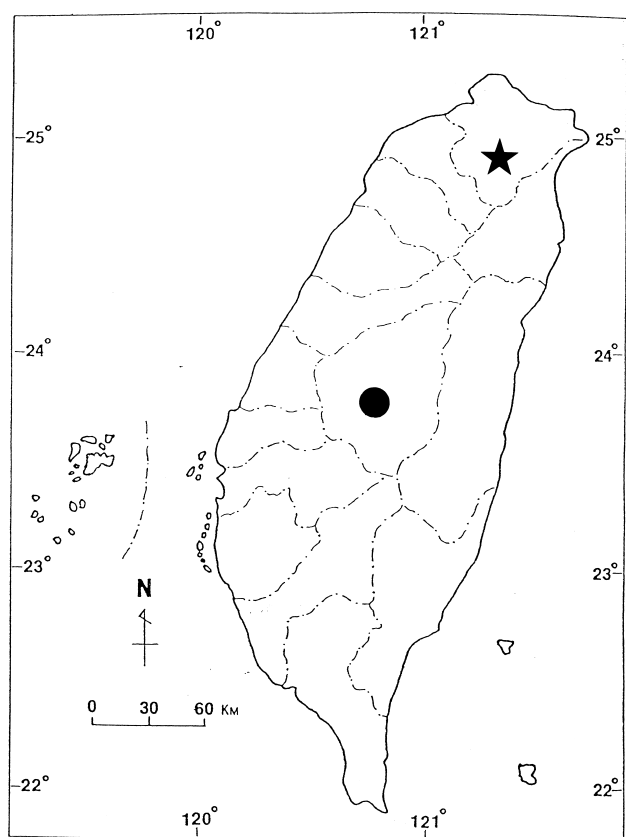


Figure 1. Distribution of *Archangiopteris itoi* in Taiwan with an extinct population at Lienhwachi (●) and an extant population at Wulai (★).

Table 1. Distribution of polymorphic DNA bands amplified by primer 57.

DNA fragments (bp)	Individuals																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
650	0	0	0	1	1	0	0	1	1	1	1	1	1	1	1	0	1	1
700	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
750	1	1	0	1	1	1	1	0	1	1	1	0	0	1	1	1	0	1
800	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0
Haplotypes ^a	A	A	B	C	C	A	A	D	E	C	C	D	D	C	C	A	D	F

^aThe haplotypes (A-F) in the eighteen individuals (1-18) of *Archangiopteris itoi*. 0: absent, 1: present.

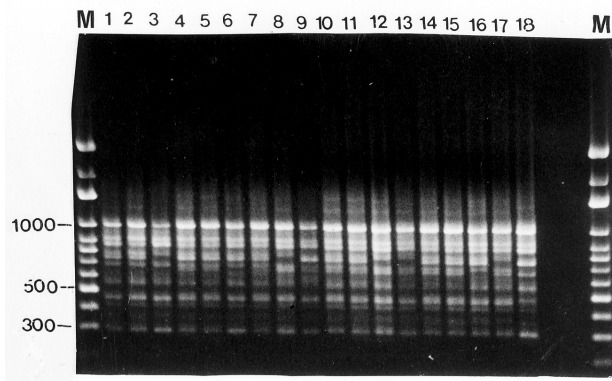


Figure 2. RAPD fragments amplified from individuals (1-18) of *A. itoi* by the primer 57.

Although no direct evidence has been obtained to suggest that the removal of the population at Lienhwachi had caused the loss of variation, theoretically, highly isolated small populations should maintain higher overall genetic diversity than a single larger population, even if homogeneity was dominant in one of the populations (cf. Hartl and Clark, 1997). It should be noted that the loss of genetic elements is forever and irreversible (Meffe and Carroll, 1997). Nevertheless, as it is generally known, mutation is the eventual source of genetic variation and is occurring continually. This process is very slow. When threatened by extinction, an entire population can hardly have a chance to recover in a short span from the sudden diminishment in genetic diversity caused by drift (Meffe and Carroll, 1997). In this study, the limited population size, the small number of remaining populations, and the low level of residual genetic variation indicate that *A. itoi* is faced with a critical need for conservation. A species like *A. itoi*, an endemic and relic taxon with conservation values at high taxonomic levels, and with small populations under threat, should be given a high priority by conservationists (cf. Given, 1994).

Although several rare species like *A. itoi* may maintain low genetic diversity, we disagree that most taxa of the Marattiales have remained inactive evolutionarily due to their ancient history (cf. Ching, 1958; Wen, 1993a). For instance, populations of *Angiopteris lygodifolia* Rosenst., one of the most common ferns on this island, are not only "healthy" ecologically, but also heterogeneous genetically at most loci according to a preliminary RAPD survey (data not shown). Extinction is certainly a natural process, like speciation, for most extant species or populations, but extinction should not necessarily be the fate of *A. itoi*. In this investigation, both gametophytes and young sporophytes were available, growing at the same site as their parents, indicating the "health" of the plants. However, the population size of *A. itoi* was constrained by the range of suitable habitats. In the last two decades, most original broadleaf forests at low elevations have been completely removed by commercial development. Habitat fragmentation definitely has made many lowland species rare or extinct (cf. Meffe and Carroll, 1997). The extinc-

tion of *Schizaea dichotoma* (L.) Sm. (Schizaeaceae) and the rarity of *Hemionitis arifolia* (Burm.) Moore (Adiantaceae) in Taiwan are other examples to be noted.

Efforts have been made to produce plants of *A. itoi* via tissue culture, using spores (C. R. Wen, personal communication), but without success. The absence of symbiotic fungi, namely mycorrhiza, may be one of the major factors causing the difficulties in culture (Zhao and Du, 1997). The pulvini have been thought to have potential for vegetative propagation (late Prof. Kramer, personal communication). Unfortunately, cultures of pulvinus have not shown any progress so far. Nevertheless, RAPD data from our study indicate that some variation still exists within the single remaining population of *A. itoi*; thus, this population could provide materials for re-establishing other populations.

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台灣的稀有及特有蕨類植物：伊藤氏原始觀音座蓮蕨之低的 RAPD 多型性

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伊藤氏原始觀音座蓮蕨為台灣的特有蕨類植物，因人為干擾處於極度瀕危，原始觀音座蓮蕨屬的起源依化石證據可追溯到中諸羅紀，野外的觀察指出目前在野外，僅 18 個植株存活於烏來的單一族群中。而當時本種發表所根據的模式蓮華池族群則已完全消失；本研究利用 RAPD 指紋技術偵測個體間的遺傳變異，經 40 個引子的複製，只有一個引子顯現出個體間的多型性，並鑑定出 6 個不同的基因型。族群內低的遺傳變異度可能歸因於過小的族群，而族群大小則受到棲地破壞的影響及限制，在一個小族群內，遺傳漂變有較大的效應使大部分的基因座失去遺傳變異。在本研究中，RAPD 資料雖然顯現極低的遺傳變異，但一方面也指出重建族群所需遺傳物質的可得。

關鍵詞：伊藤氏原始觀音座蓮蕨；保育生物學；蕨類；DNA 指紋；遺傳變異；隨機複製多型性 DNA 片段；台灣。