An analysis of genetic variation in natural populations of
*Sticherus flabellatus* [R. Br. (St John)] using amplified
fragment length polymorphism (AFLP) markers

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Abstract

Amplified fragment length polymorphisms (AFLPs) were used to characterize the genetic diversity within and among natural populations of *Sticherus flabellatus*. Eight populations within the Sydney region of New South Wales, Australia were surveyed using 11 primer combinations. A total of 1108 reproducible bands were detected of which 469 (42%) were polymorphic. *F*$_{ST}$ estimates averaged over all polymorphic loci indicated that significant genomic differentiation occurs among populations (average = 0.783). Genetic diversity within populations was assessed according to average heterozygosity (*H*) and percentage polymorphic loci (*P*) per population. Within-population diversity ranged from *H* = 0.12 and *P* = 33.69 to *H* = 0.04 and *P* = 15.99. Analysis of genetic similarity among populations suggested that the eight populations studied fall into two groups of four populations, based on population size and the condition of the habitat. Phenetic analysis (*AMOVA*) indicated that genetic variation is greater among populations (74.34%) than within populations (25.66%). These findings suggest that the breeding system of *S. flabellatus* is predominantly inbreeding, with genetic diversity maintained by occasional outcrossing in larger populations. The results presented in this study could provide evidence to support the proposal to protect natural stands of *S. flabellatus*, which has implications for the Australian horticulture industry.

Keywords: breeding systems, conservation genetics, diversity, umbrella fern

Received 4 September 1999; revision received 22 November 1999; accepted 22 November 1999

Introduction

The preservation of genetic diversity both within and among natural populations is a fundamental goal of conservation biology (Hamrick *et al.* 1991). The amplified fragment length polymorphism technique (AFLP) (Vos *et al.* 1995) is a highly reliable and reproducible means of assessing genetic variation in natural populations. AFLPs also have the advantage of requiring no prior investment in terms of sequence analysis, primer synthesis or characterization of DNA probes (Vos *et al.* 1995).

*Sticherus flabellatus* (R. Br) St John, or umbrella fern belongs to the primitive fern family Gleicheniaceae (Harden 1990). *S. flabellatus* forms large colonies along creek banks and in moist gullies in tall eucalypt forests or rainforest edges along Australia’s eastern coast (Fairley & Moore 1989; Carolin & Tindale 1994). There is a great demand for *S. flabellatus* cut foliage by the floriculture industry, and currently all *S. flabellatus* sold is harvested from natural stands under licence. Although not considered vulnerable or threatened in the wild, *S. flabellatus* is to become a protected species due to its appeal and subsequent demand as a cut foliage product that could potentially cause excessive depletion of wild stock (National Parks and Wildlife Act Schedule 13 1974, New South Wales National Parks & Wildlife Service 1998).

Over 600 species of native plants are currently protected in New South Wales (NSW National Parks and Wildlife Service Schedule 13 1974). As with *S. flabellatus*, many of these are long-lived and slow growing, have breeding systems that are not fully understood, and have proven difficult to cultivate. *S. flabellatus* exhibits poor spore germination under controlled conditions, and once removed from the wild is extremely difficult to re-establish because of the disturbance to the rhizome.
To manage germplasm resources effectively, in terms of exploitation and conservation of biodiversity, a knowledge of the genetic diversity present in natural populations is required. The primary objectives of the present study were to detect and measure genetic variation in natural populations of *Stichurus flabellatus* in the Sydney region, and to examine the usefulness of AFLPs as genetic markers in this species.

**Materials and methods**

**Population sampling**

Within New South Wales, the majority of *Stichurus flabellatus* harvesting for the Sydney flower markets is centred within or near the Sydney region, an area of about 23 000 km² encompassing the catchment of the Nepean–Hawkesbury Rivers (Fairley & Moore 1989). Eight natural populations of *S. flabellatus* were selected within and on the periphery of the Sydney region: Hacking River (HR), Royal National Park; Fairy Bower Falls (FB), Morton National Park; Ku-ring-gai Creek (KC), Ku-ring-gai Wildflower Gardens; Gymea Bay (GB), Sutherland; North Watagan Mountains (WM), Watagan State Forest; Pearl Beach (PB), Crommelin Biological Research Station; Lane Cove (LC); Little Woombye Creek (GV), Grose Vale. Of these populations, the HR and FB sites were within National Parks (NSW National Parks and Wildlife Service) and the KC site was within Ku-ring-gai Wildflower Gardens (Ku-ring-gai Municipal Council). These populations existed within minimally disturbed habitats. The other populations existed within sites of varying degrees of protection; the WM site was within a state forest, the PB site was within a research station on the border of Brisbane Waters National Park, and the agricultural site of GV was protected by a conservation-orientated landowner. The GB and LC populations were in unprotected and isolated urban sites, currently managed by their respective councils. Of these eight populations, harvesting of *S. flabellatus* stems for cut foliage is known to have occurred at the WM site. The breeding system and the extent of vegetative reproduction in *S. flabellatus* is unknown. Therefore, in preliminary investigations a single patch within the KC population was sampled along a 16-m transect at 1 m intervals to identify the proximity of genets. This single AFLP reaction did not reveal any identical individuals (data not shown), and therefore a transect of approximately 100 m sampled at 10 m intervals was considered to allow sufficient distance between samples, consistency between the populations of varying size, and to be most accessible across the range of habitats. For each population, a total of 10 individuals were sampled and placed on ice in the field, and DNA was extracted upon return to the laboratory.

The populations of HR and KC were part of large *S. flabellatus* colonies that stretched long distances along river and creek banks. The transects were situated within 5 m of walking tracks that ran parallel to the waterways, but the populations were otherwise undisturbed. The populations of FB, PB and GV were also situated on creeks, but were part of smaller colonies. The PB population was within 4 m of a walking track and was otherwise undisturbed. The PB population lay within the landscaped grounds of the Research Station and the larger natural vegetation of the creek area had been cleared. The GV population was within an undisturbed gully that was surrounded by a dairy farm. This gully was approximately 10 m deep and up to 20 m wide and was unusual for the area because it consisted of natural vegetation. The GB population existed wholly within a residential area in a small depression that may have formerly been a natural creek line. The population was between the backyards of houses in a corridor of approximately 20 m across, and much of the natural vegetation had been cleared allowing the invasion of many exotic garden species. The WM population existed within a state forest where timber logging and bush-harvesting for many horticultural species occurs. The site sampled was 25 m from a track and has suffered minimal disturbance due to its inaccessible situation at the top of a steep gully. The LC population existed within the most modified urban environment. The site was situated between a major road and the rear of an industrial area, 10 km from the Sydney Central Business District. This site may have formerly been a natural creek bank and consisted of many fern species as well as native and exotic opportunistic species.

**DNA extraction**

DNA was extracted from fresh young tissue using a modified CTAB protocol (Doyle & Doyle 1988), with 3% w/v PVP (polyvinylpyrrolidone MW 40 000) added. Extracted DNA was quantified visually by agarose gel electrophoresis and ethidium bromide staining (Maniatis et al. 1982).

**AFLP protocol**

AFLP procedures were performed essentially as described by Vos et al. (1995) with minor modifications. The methylation sensitive restriction enzyme *PstI* (Grunbaum et al. 1981a,b) replaced EcoRI because it is known to preferentially cleave low-copy DNA in and adjacent to transcribed regions (i.e. genes) (see Burr et al. 1988; Roder et al. 1998). Eleven *PstI* + GAA: *MseI* + 3 primer combinations were used (Table 1).

Preamplification PCR reactions were performed with *PstI* + GA and *MseI* + O primers. The PCR reactions were

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performed in a Corbett PC-960C cooled thermal cycler using the following temperature cycle profile: a 1-min denaturation step at 94 °C, followed by 30 cycles of denaturation at 94 °C, annealing (see below) and extension at 72 °C. The annealing temperature in the first cycle of 65 °C was subsequently reduced by 1 °C over 10 cycles to 56 °C, and continued at 56 °C for the remaining 20 cycles. During the first 10 cycles the denaturation, annealing and extension steps were each for 1 min, with a reduction to 30 s each for the denaturation and annealing steps for the next 20 cycles.

Selective PCR was performed using a [\(^{32}P\)]-labelled EcoRI + GAA primer with each of the 11 unlabelled MseI + 3 primers. Selective amplification was performed using the cycle profile described for preamplification with an additional three cycles with 56 °C annealing temperature.

PCR products (3 μL) were electrophoresed on 5% denaturing polyacrylamide for 1.5 h on a BioRad 30 × 38 cm sequencing gel apparatus. Fingerprint patterns were visualized on Kodak PS autoradiography film, following an exposure of 18–36 h.

**Genetic analysis**

For each primer pair, the number of polymorphic and monomorphic bands was determined; however, bands that were monomorphic across all 80 individuals were excluded from analyses. Each locus was treated as a separate character and scored as either present (1) or absent (0) across all genotypes. The 0/1 data matrix was used to construct genetic similarity estimates representing all possible pairwise comparisons of individuals within and among populations using Jaccard’s similarity coefficient [\(G(ij) = a/(a + b + c)\), Jaccard (1908)]. Where \(G(ij)\) is the measure of genetic similarity between individuals \(i\) and \(j\), \(a\) is the number of polymorphic bands that are shared by \(i\) and \(j\), \(b\) is the number of bands present in \(i\) and absent in \(j\), and \(c\) is the number of bands present in \(j\) but absent in \(i\).

The Jaccard similarity matrix was generated using the program WinBoot ( Yap & Nelson 1996).

Descriptive statistics providing information on the genetic differentiation between the populations (\(F_{ST}\) values), average heterozygosities (\(H\)) and per cent polymorphic loci (\(P\)) for each population were estimated using the program FTPGA (Miller 1997a). \(F_{ST}\) values were estimated over all polymorphic loci and averaged over loci, and confidence intervals at the 99% confidence level were generated by boot-strapping at 1000 iterations. Average heterozygosities were calculated for each locus and averaged over loci according to the unbiased formula of Nei (1978). The percentage of polymorphic loci estimates was based on the percentage of loci not fixed for one allele.

The Jaccard genetic similarity matrix generated by WinBoot was used to perform multivariate genetic analyses using the program NTSYS-pc (Rohlf 1997). Cluster analysis was performed to construct a dendrogram using the unweighted pair-group method using the arithmetic means (UPGMA) procedure, and support for the clusters was evaluated by bootstrap analysis with 1000 iterations (Felsenstein 1985) using the program WinBoot ( Yap & Nelson 1995). The ordination technique, nonmetric multidimensional scaling (MDS) (Kruskal 1964), was used to reveal patterns of similarity and genetic variation within the genetic similarity matrix.

The assumption of Hardy–Weinberg equilibrium may not be justified in the characterization of genetic diversity using dominant markers such as AFLPs, and therefore a phenetic approach for assessing differentiation was used. Analysis of molecular variance (AMOVA) (Excoffier 1995), was used to statistically clarify patterns and degrees of similarity revealed by UPGMA and MDS. A pairwise Euclidean distance matrix was constructed using the program AMOVA–PREP (Miller 1997b), which prepares dominant marker data for Excoffier’s WINAMOVA. AMOVA performs a classical analysis of variance on the distance matrix and in this study partitioned the genetic variation between two hierarchical levels: among populations and among individuals. The significance of the AMOVA variance components and fixation indices was tested using non-parametric permutation procedures (Excoffier et al. 1992).

**Results**

**AFLP polymorphism**

The 11 primer combinations used generated a total of 1108 bands (mean ± SE, 100.73 ± 6.19 per primer combination),
of which 469 were polymorphic (mean ± SE, 42.64% ± 3.56 per primer combination) across 80 Sticherus flabellatus individuals representing eight populations of the Sydney region. Primer pairs varied in their degree of polymorphism (29–58%), and generally GC-rich Mse + 3 primers generated the greatest numbers of bands and revealed the highest levels of polymorphism (Table 2).

### Genetic diversity

The objective of this study was to characterize levels and patterns of genetic diversity within and among populations of S. flabellatus as reflected by average heterozygosity and percentage polymorphic loci. Each population consisted of 10 individuals.

<table>
<thead>
<tr>
<th>Population</th>
<th>Average heterozygosity</th>
<th>% Polymorphic loci</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>0.1175</td>
<td>33.69</td>
</tr>
<tr>
<td>FB</td>
<td>0.0395</td>
<td>15.99</td>
</tr>
<tr>
<td>GB</td>
<td>0.0669</td>
<td>24.73</td>
</tr>
<tr>
<td>WM</td>
<td>0.0492</td>
<td>17.27</td>
</tr>
<tr>
<td>KC</td>
<td>0.1064</td>
<td>35.18</td>
</tr>
<tr>
<td>PB</td>
<td>0.0619</td>
<td>17.70</td>
</tr>
<tr>
<td>LC</td>
<td>0.0666</td>
<td>20.68</td>
</tr>
<tr>
<td>GV</td>
<td>0.0770</td>
<td>24.73</td>
</tr>
</tbody>
</table>

Cluster analysis (Fig. 1) on all eight populations revealed two distinct clusters (Clusters 1 and 2) with 15% similarity. Furthermore, individuals within each population were clustered resulting in each population having its own specific node and demonstrated greater genetic variation between the populations rather than within. Clusters 1 and 2 were quite different in their patterns of genetic similarities among populations and between individuals within populations, with Cluster 1 exhibiting a much higher overall level of genetic diversity. The populations of Cluster 1 were 25% similar and individuals within the populations were from 40% to 51% similar. While the four populations of Cluster 2 were 67% similar and individuals within the populations were from 80% to 87% similar.

An exception to these eight population clusters were two outlying individuals from the GB population with 85% similarity to each other, and 67% similarity to the rest of Cluster 2. All individuals examined differed genotypically, with the two most similar individuals occurring in the GB population (94% similarity).

Ordination analysis complemented the cluster analysis.
by providing spatial representation of relative genetic similarities among individuals (Fig. 2). The three-dimensional MDS plot clearly differentiated the two main clusters revealed by uPGMA analysis, and the differences in genetic similarities within the clusters. Most of the variance was accounted for in the first dimension (85.22%), and a satisfactory minimum stress (stress 1 = 0.066) was achieved.

The apparent patterns revealed by clustering and ordination analyses were analysed by AMOVA to partition the variation between and within populations (Table 4). AMOVA attributed 74.34% of the genetic variation among populations and 25.66% within populations. Permutation-based significance tests of AMOVA variance components indicated that the among-populations classification was significantly better ($P < 0.05$) than random classification.

Table 4 AMOVA for 80 individuals of *Sticherus flabellatus* from eight populations of the Sydney region, using 469 AFLP markers. Statistics include sums of squared deviations (SSDs), mean squared deviations (MSDs), variance component estimates, the percentages of total variance (% of Total) contributed by each component, and the probability ($P$) of obtaining a more extreme component estimate by chance alone (estimated from 1000 sampling realizations).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>SSD</th>
<th>MSD</th>
<th>Variance component</th>
<th>% of total</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among populations</td>
<td>7</td>
<td>4602.09</td>
<td>657.44</td>
<td>63.55</td>
<td>74.34</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Within populations</td>
<td>72</td>
<td>1579.30</td>
<td>21.94</td>
<td>21.93</td>
<td>25.66</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>6181.39</td>
<td>679.38</td>
<td>85.49</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Genetic diversity

There is a substantial body of research on the genetic diversity of many natural populations of terrestrial and epiphytic fern species, based primarily on enzyme electrophoresis data. Although useful, enzyme techniques may underestimate true diversity because a limited number of loci, which do not represent a random sample of the entire genome (Gotelli 1981), are assessed (Korpelainen 1989). Molecular techniques such as AFLPs also have limitations, but are powerful in their ability to sample many loci randomly throughout the genome. There are very few reports detailing the use of PCR-based molecular techniques in fern species. Schneller et al. (1998) employed the RAPD technique (randomly amplified polymorphic DNA, Williams et al. 1990) to detect genetic variation in natural populations of the apomictic fern Dryopteris remota. The molecular technique exhibited a greater power for resolving genetic variation in this species, compared to previous research using allozymes that had revealed very little variation (Schneller et al. 1998; see also Bachmann 1994). As AFLPs generate a much greater number of markers per reaction (high multiplex ratio) compared to RAPDs (Breyne et al. 1997), the technique has the ability to provide substantial information for genetic studies of fern populations, as demonstrated by this study.

Heterozygosity values reported for outcrossing terrestrial fern species, based on enzyme electrophoresis data, range from 0.111 for Polypodiummunutum (Solts & Solts 1987b), 0.189 for Phegopteris (average of six species) (Hooper & Hauffler 1997), 0.206 for Bommeria hispida (Hauffler & Solts 1984), and 0.221 for Pellaea andromelidia (Gastony & Gottlieb 1985). These values are comparable to those reported by Hamrick et al. (1979) for populations of 100 outcrossing seed plant species. A much lower H value of 0.035 (range 0.000–0.077 for nine populations) has been reported for Hominostis pulmata (Ranker 1992), a species believed to have a variable mating system that is characterized by the ability to inbreed and colonize new habitats.

In the present study, the HR and KC populations had H values in the range of those obtained for outcrossing ferns and seed plants. Of the remaining S. flabellatus populations, four had H values in the intermediate range of 0.062–0.077, while two had H values in the lower range of 0.040–0.050. These intermediate and lower H values are similar to those reported for populations of H. pulmata (Ranker 1992). A study by Travis et al. (1996) reported heterozygosity detected by 220 AFLPs for critically endangered Astragalus crennphyphax var. crennphyphax populations. The values ranged from 0.037 to 0.134, comparable to the range detected for S. flabellatus in this study (see Table 3). Their study also detected a measurable level of separation over the entire genome as detected in this study.

In addition to high H values, the HR and KC populations also exhibited the highest P values (Table 3). Similar to the populations of FB, WM and PB with the lowest H values also exhibited the lowest P values. With the exception of the FB population, low P values in these populations reflected the fact that a high proportion of fixed loci (639 (58%) of the scored loci were fixed across every individual in every population). The four populations forming Cluster 2 (Fig. 1) all shared an additional 164 fixed loci that represented 35% of the 469 bands polymorphic across the remaining populations. These fixed loci, which may be either for advantageous or deleterious alleles (Ouborg & Van Treuren 1994), were not fixed in the FB population despite having the lowest H and P values. The combination of low H and P has been positively correlated with small population sizes (Ouborg & Van Treuren 1994).

Population structure

A high level of population fragmentation was detected in S. flabellatus by AFLP analysis, as indicated by high FST values (≥ 0.205) for 68% of the 469 polymorphic loci. This finding was supported by the AMOVA analysis that partitioned genetic variation between populations (74.34%). Enzyme studies on fern species have reported ST values ranging from 0.034 to 0.307 for mixed-mating Pteridium aquilinum populations (Korpelainen 1995), and from 0.109 to 0.203 for the inbreeding Ceratopteris thalictroides (Watan & Masuyama 1991), an average of 0.208 for the microsporad Dryopteris expansa (Solts & Solts 1987a), and an average of 0.264 for the outcrossing Chelanthcs gracilima (Solts et al. 1989). A much higher overall FST of 0.698 was reported for nine populations of H. pulmata (Ranker 1992). With the exception of the latter, the overall FST of 0.783 for the eight S. flabellatus populations is extremely high in comparison to those reported. The high FST reported for H. palustris, accompanied by the low range of H values, was described as typical for a species with a wide geographical distribution and high levels of inbreeding (Ranker 1992).

Multivariate genetic analyses revealed a division of the eight populations into two groups of four populations (Cluster 1 and Cluster 2, Fig. 1). This primary division is consistent with the two groups suggested by the average heterozygosity values (Table 3), with the exception of the FB population. The populations of the two clusters differed primarily in population size and in the condition of the habitat. The 'population size' is in reference to the overall size of the S. flabellatus colony from which the transect ‘population’ was sampled. Individuals sampled...
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from the HR, FB, KC and GV populations (Cluster 1, Fig. 1) represented subsets of much larger colonies that were distributed along creek banks. Large populations often exhibit greater genetic diversity compared to smaller populations, as was demonstrated for populations of Coreopsis integrifolia (Cosner & Crawford 1994). The populations of Cluster 1 were also within protected environments, where the habitat sampled had been stable for some time. In contrast, the populations of GB, WM, PB and LC were small (no greater than 120 m) and largely reproductively isolated, as a result of the variety of land uses that surrounded them. Larger and stable populations, such as those of Cluster 1, typically show higher heterozygosities and within-population genetic variation, while small populations such as those of Cluster 2 show lower variation regardless of their stability, primarily due to the effects of genetic drift (Loveless & Hamrick 1984). In stable habitats, higher heterozygosities have been favoured for increased competitive viability and the ability to adapt to changing environments and selection pressures (Lloyd 1974).

The partitioning of genetic diversity between populations rather than within populations has been documented in other small fragmented fern populations (Schneller & Holderegger 1996), as well as in rare angiosperms (see references in Cosner & Crawford 1994). This has been attributed to the homogenizing effects of restricted gene flow, genetic drift and inbreeding (Loveless & Hamrick 1984). The consequences of these effects are a reduced ability to withstand severe environmental perturbations and selection (Charlesworth et al. 1990) as well as inbreeding depression (see Lande & Schemske 1985; Schemske & Lande 1985; Lynch 1991). The loss of genetic variation within the smaller populations of S. flabellatus suggests that these populations have experienced drift and inbreeding through several generations (Van Treuren et al. 1993).

Breeding system

Plant breeding systems determine gene flow, the genetic structure of populations and the evolutionary potential of a species (Brown 1979; Loveless & Hamrick 1984; Korpelainen 1995). Information on the breeding system of S. flabellatus is not available. However, this species is a homosporous fern that reproduces via free-living bisexual gametophytes (Solits & Solits 1992). Early reports maintained that selling would be common in homosporous ferns (Klekowski & Baker 1966). However, enzyme electrophoretic data demonstrate that homosporous ferns possess various mating systems (see references in Masuyama & Watano 1990; reviewed by Solits & Solits 1990).

The range of \( H \) values obtained for S. flabellatus suggests that mating systems vary among the eight populations sampled (Lloyd 1974). Travis et al. (1996) reported that the similar range of \( H \) values obtained for A. cremnophylax was an indication of a mixed-mating system. Ranker (1992) also reported a range of \( H \) values for H. pulsatula that were similar to the S. flabellatus populations, particularly those populations forming Cluster 2 (Fig. 1). These findings, as well as the high degree of population differentiation in S. flabellatus, suggest that the most probable breeding system is one that is initially dominated by inbreeding, with diversity maintained in the larger populations through variable rates of outcrossing.

The genetic diversity of species with mixed-mating systems changes through time (Loveless & Hamrick 1984), with variations in the mating systems often resulting from selective pressures brought about by ecological conditions (Lloyd 1974). Occasional outcrossing in primarily inbreeding species may be attributable to the advantages of heterozygosity in adapting to changing environmental conditions and the production of viable progeny through the generation of a greater diversity of genotypes (Lande & Schemske 1985; Schemske & Lande 1985).

Population density

The prevailing population density is a function of the genetic mobility and breeding system of the species (Hamrick et al. 1991). Primarily inbreeding species typically display substantial variation in outcrossing rates (Schemske & Lande 1985), because they are particularly sensitive to changes in population density (Solits & Solits 1987a; Bawa & Ashton 1991). Spore migration is the only form of interpopulational gene flow in ferns, and the possibility and frequency of this is determined by the distance to the next spore source (Slatkin 1987). S. flabellatus typically forms patches of closely spaced fronds arising from creeping rhizomes, as observed in many ground-covering fern species, e.g. Runohora adiantiformis (Geldenhuys & van der Merwe 1988). The most important differences between the stable and disturbed populations sampled in this study was probably the distribution and density of fronds, and the proximity to a natural waterway. With the exception of the FB population, the large and stable populations of Cluster 1 (Fig. 1) consisted of several patches (HR: 4, KC: 5, GV: 5; FB: 2) of varying sizes (2 m to 30 m) and frond densities (12–59 stems per m\(^2\)), and were joined by sparsely distributed individuals. These populations were also on river and creek banks. The disturbed populations of Cluster 2 (Fig. 1) consisted of fewer patches (GB: 3, WM: 1, PB: 3, LC: 1), but each patch was large (10–120 m), distinct from one another (at least 3 m apart), and very dense in frond number (32–72 stems per m\(^2\)). Of these populations, only GB was situated on a natural creek. The presence of individuals between the patches in the stable populations would facilitate gene flow (Bawa & Ashton 1991), while small
populations that persist over several generations may lose genetic variation. This outcrossing effect was most evident in the larger colonies of HR, KC and GV. These findings also suggest that water may have an important role in *S. flabellatus* spore dispersal. The large colonies containing the HR and KC populations may have been promoted by the possibility of long-distance spore dispersal and migration (Silander 1985).

**Colonization**

The trend of large and stable populations having greater genetic diversity than small and disturbed populations does have exceptions. The FB and WM populations exhibited the lowest genetic diversity of the eight populations, but were grouped in separate clusters. The FB population was the least diverse, but was clustered with the most diverse populations of Cluster 1 and exhibited equivalent levels of within-population genetic similarity (Fig. 1). This population differed from the populations of Cluster 2 in that the low *P* value could not be related to a high incidence of fixed loci, indicating that the lack of diversity in this population was less influenced by genetic drift. This finding is consistent with habitat-determined selection pressures, because this population shared the habitat characteristics of the three other populations with which it clustered. The low genetic diversity of the FB population indicated a greater incidence of inbreeding that may be due to a recent rapid colonization event and founder effect. The founders of this population probably originated from a stable habitat, probably from another site within the National Park. 

Travis et al. (1996) attributed *H* levels of 0.04 (similar to those obtained for the FB and WM populations) in *A. crennophlyctis* to colonization events. The low *H* obtained at the time of sampling the FB population may be due to insufficient time passing to enable the population to evolve to equilibrium (Soltis & Soltis 1987c); i.e. colonization is in its early stages and full exploitation of the site has not occurred (Harper 1977).

The WM population clustered with the populations of Cluster 2 (Fig. 1), and it exhibited the lowest diversity of these populations. Unlike the other populations of Cluster 2, this was a large population that existed in a minimally disturbed habitat. In this case, however, the habitat was considered atypical for *S. flabellatus*, because it was situated near the top of a ridge, rather than a gully. The population was within a state forest at a site that is used for bush-harvesting of many horticultural species. *S. flabellatus* does not occur elsewhere in the local area, and the population studied has existed for approximately 10 years (R. Eagle, personal communication). The population appears to have arisen from a recent colonization event, and the lack of diversity may be due to a combination of founder effect, isolation (genetic drift) and subsequent forced inbreeding. Bush-harvesting of other species in the area may have led to the introduction of *S. flabellatus* spores from other harvesting sites. If the founding members of this population originated from such sites, they would be expected to have a reduced genetic representation of the original population. This is consistent with habitat-determined selection pressures, because the WM population was clustered with the other disturbed populations (Cluster 2, Fig. 1). Another important consideration is that bush-harvesting, although minimal, has occurred within the WM population. This practice would aid in maintaining a low level of genetic diversity because the reproductive potential of the population is reduced with the removal of spore-bearing fronds.

The GB population was also grouped with Cluster 2 and exhibited a low genetic diversity. This population could also have arisen as a result of a colonization event. There was evidence of migration with two outlying individuals belonging to the GB population (Fig. 1), suggesting the existence of other *S. flabellatus* populations in the local area. The higher diversity of this population compared to the FB and WM populations suggests either that more than one spore dispersal event has occurred to introduce new genotypes or that this population is older and has proceeded further towards equilibrium. Therefore, genetic variation within this population may increase in time (Schneller & Holderegger 1996), providing that the habitat allows for population increase. This latter factor is unlikely considering the altered state of the environment in which this population exists.

These three populations indicate that *S. flabellatus* can be an effective colonizer. This in itself provides additional evidence for a mixed-mating system because other colonizing ferns, such as *P. aquilinum* (Korpelainen 1995) and *H. palmata*, (Ranker 1992) also possess mixed-mating systems. From an evolutionary standpoint, this system is believed to be the optimal plant reproductive system (Klekowski 1969).

**Population conservation**

Genetic variation is the resource from which populations draw for short-term adaptation to environmental change and for longer-term evolutionary change (Frankel & Soule 1981). The aim of conservation, in addition to habitat preservation, is to maintain a species’ existing level of genetic variation (Simberloff 1988) in order to maximize its chances for persistence in the face of changing environments. The genetic information presented in this study may provide adequate evidence to support the protection of natural populations of *S. flabellatus*. In general, a loss of genetic diversity was apparent in the smaller populations compared to the larger populations, i.e. the
average coefficient of similarity increased within populations as the size of the population decreased (Dolan 1994).

It is often assumed that small populations that are isolated from one another are endangered (Barrett & Kohn 1991). This may be the case with one of the S. flabellatus populations studied. The LC population of Cluster 2 was the smallest population of the eight studied, and it existed within the most modified habitat. The greater level of genetic diversity of this population compared to the other three populations of Cluster 2 is evidence of a larger and more genetically diverse population in the recent past. Populations that have become small recently are considerably more diverse than those that have remained small for long periods (Barrett & Kohn 1991). Therefore, this site requires protection and regeneration if this S. flabellatus population is to persist.

Information on the genetic diversity within plant populations can be used in conservation management to determine which populations need protection and the effectiveness of existing reserves (Rieger & Sedgley 1998). In this study, the National Parks have proven to be successful at maintaining stable habitats such as the HR site, and in the case of the FB population its site will be protected for this population to reach its full potential. However, it has been demonstrated in this study that genetic diversity is not distributed evenly within S. flabellatus populations. Therefore, to retain existing diversity emphasis should be on conserving as many populations as possible. Genetic diversity may be partially restored to depleted populations through the introduction of individuals carrying novel genes (Butler et al. 1994). In doing so, care must be taken to avoid reductions in overall population fitness through the introduction of genotypes that have evolved under widely different selective regimes (Hamrick et al. 1991), i.e. outbreeding depression. Yet, it has been noted that populations with a long history of inbreeding may exhibit significant heterosis upon outcrossing (Schemske & Lande 1985).

This finding has implications for the horticultural industry which has a high demand for S. flabellatus fronds. Ferns are generally considered to be poor competitors, slow growing and show little resilience to defoliation (Grime 1985; Milton 1987; Geldenhuys & van der Merwe 1988). Milton & Moll (1988). A commercially feasible propagation protocol for S. flabellatus is currently in development, in an effort to phase out bush-harvesting and to meet the market demand. However, cultivation should be considered as assisting, not replacing, conservation in the wild (Leigh et al. 1984).

Conclusion

AFLP markers exhibited a high level of efficiency for detecting DNA polymorphism over a large number of randomly sampled loci, and proved to be very useful for detecting levels of genetic variation among natural populations of this species. AFLPs detected significant interpopulational differentiation, reflecting a predominantly inbreeding mating system and low levels of gene flow between the populations studied. Genetic variation within S. flabellatus populations was a function of population size and age, and the stability of the habitat. The higher levels of diversity in the larger populations will provide a greater capacity to withstand changing environments; however, the smaller populations may lack the potential to overcome the constraints of continuing habitat loss and fragmentation. The main factor contributing to the lower levels of genetic variation in small populations was genetic drift, in combination with founder effect and a higher incidence of inbreeding due to colonization events. These findings demonstrate the species’ inability to maintain genetic diversity in a disturbed environment; however, it is not yet known if a reduced genetic diversity affects long-term population viability. It has been observed in the largely asexual bracken fern that abundant phenotypic plasticity may allow species to be successful for long periods of time (Menges 1991). This emphasizes that once the biology of S. flabellatus is better understood, decisions about the future recovery and conservation of the populations can be made.

Acknowledgements

We would like to thank Dr Matthew Hayden for assistance with the AFLP procedure, Dr Peter Sharpe for access to laboratory facilities, and Dr Robert Parr for assistance with the statistical analysis. The following people are acknowledged for population sampling locations: Mr Richard Eagle and Mrs Katherine Eagle, Ms Sue Butler and Lane Cove Council, Mr Brian Mitchell and Ku-ring-gai Municipal Council, Dr Murray Henwood and Ms Janice Jacobs, Sutherland Shire Council, and the NSW National Parks and Wildlife Service.

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GENETIC VARIATION IN *STICHERUS FLABELLATUS* 581


The author’s research is concerned with the development of Australian native species for cultivation. The present work forms part of a larger project on the development of *Sticherus flabelatus* as a new cut flower foliage crop. The primary objective of this project is the cultivation of *S. flabelatus*, leading to the eventual phasing out of bush-picking. Currently, research is aimed at gaining an understanding of the interactions between populations and individuals (i.e. breeding system) of *S. flabelatus*, through the use of molecular markers.
