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Conservation genetics and ecology of *Angiopteris chauliodonta* Copel. (Marattiaceae), a critically endangered fern from Pitcairn Island, South Central Pacific Ocean

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Abstract

Angiopteris chauliodonta, endemic to remote Pitcairn Island, was until recently thought to occur in only two small populations. Survey work carried out on the island in 1997 increased the number of populations to six, but as the total number of plants found was 774 (of which only 147 were mature adults) the species should be regarded as critically endangered. The species occurred in native fern-rich *Homalium taypau* and *Metrosideros collina* forest that was in many areas heavily invaded by *Syzygium jambos* and *Lantana camara*. RAPD analysis identified related populations but there was no correlation between genetic and geographical distance. The highest levels of genetic diversity was partitioned within populations ($H_S = 0.154$; $D_{ST} = 0.116$), although the larger populations were not necessarily the most diverse. Threats are primarily due to forest clearance, invasive species and erosion. Conservation management for this species will be through reinforcement of existing populations to maximise their genetic diversity and translocation of new populations to suitable habitats.
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1. Introduction

Island biota and ecosystems are highly vulnerable to disturbances associated with human activities, the most serious of which are the introduction and release of grazing mammals and alien plants (Given, 1981; Mueller-Dombois and Loope, 1990; Paulay, 1994; Wright and Lees, 1996). Currently one in three of all known threatened plant species are island endemics (Whittaker, 1998). The reason for the susceptibility of island populations to extinction is controversial, but once the taxon's numbers have been depleted, the *coup de grâce* is usually delivered by stochastic factors, whether anthropogenic, demographic, environmental, catastrophic or genetic (Rosenzweig, 1995; Frankham, 1997). Four genetic factors that can contribute to higher extinction rates in island populations are: inbreeding depression, loss of genetic variation, accumulations of mildly deleterious mutations and genetic adaptations to island environments (Frankham, 1997). Thus genetic studies of species which occur in fragmented island populations are likely to benefit conservation management programmes.

The ultimate goal of conservation biology is to maintain the evolutionary potential of species by maintaining natural levels of diversity, as this genetic diversity is essential for species and populations to respond to long and short term environmental change and thus overcome stochastic factors which could otherwise result in extinction (Frankel and Soulé, 1981; Lande and Barrowclough, 1987; Rossetto et al., 1995). In addition, genetic diversity has been shown to be positively and significantly correlated with population fitness (Reed and Frankham, 2003). The use of molecular analysis as an integral component in the conservation of rare and endangered species is still in its infancy, but is becoming more widely used as the techniques become more

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accessible and less expensive. Knowledge of the genetic structure and relationships within and between populations leads to more appropriate population management from the beginning of conservation efforts, when options may be the most flexible (Haig, 1998). In addition, genetic surveys are a more accurate means of inferring demographic fragmentation, and the resulting level of risk of local extinction (Templeton et al., 1990). This information can also be used to select appropriate genotypes to maximise genetic diversity when formulating long term conservation strategies (Haig, 1998; Rossetto et al., 1995). The primary conservation goal should be to establish self-sustaining populations whenever possible, if necessary using ecological manipulations to minimise inbreeding and maximise genetic variability within populations (Holsinger and Gottlieb, 1991; Lande, 1988). Genetically effective management varies between species, and so for any plan to be drawn up there must also be a detailed knowledge of the species' natural history (e.g. ecology, breeding system, life-history), and in the case of pteridophytes spore dispersal may allow much more interpopulation gene flow than in most seed plants (Soltis and Soltis, 1990). It has been suggested that a recovery or management plan drawn up without considering this information may be subject to error (Hamrick et al., 1991).

A commonly used PCR technique for the detection of genetic variability is Randomly Amplified Polymorphic DNA (RAPD) (Williams et al., 1991). The RAPD technique is particularly useful for population studies (Parker et al., 1998; Williams et al., 1991), as it surveys the entire genome, rather then selected fragments, as with minisatellites and allozyme markers. RAPDs therefore provide unbiased estimates of genetic and clonal identity, making them useful in the development of breeding programmes and recovery strategies (Stewart and Porter, 1995). RAPD technology is also thought to be more liable to detect variation in inbred species (Williams et al., 1993, in Dowe et al., 1997), and consistently find more polymorphism than AFLP or ISSR (Lu et al., 1996; Parker et al., 1998). It has major advantages in molecular ecology because of its wide applications and the fact that it requires the least in technology, labour and cost, without the necessity of radioactivity and requiring only small amounts of DNA (Hadrys et al., 1992; Dawson et al., 1993; Fischer et al., 2000).

Angiopteris Hoffmann (Marattiaceae) is a widespread genus of giant ferns with bipinnate fronds up to 4 m in length which arise from a rosette. The genus occurs from Madagascar across South East Asia and Polynesia, and north to Japan. The taxonomy of the genus is debated with some authorities retaining only one species, *A. evecta*, in the genus while others allowing up to 200 (Verdoorn, 1938; Copeland, 1947). *Angiopteris* is distinguished from the morphologically similar genus

Marattia as having rounded sporangia arranged in two rows (normally of 5–7 sporangia), while in *Marattia* the sporangia are fused into a single linear synangium (Copeland, 1947). *Angiopteris* is distinguished from *Archangeopteris* by being bipinnate and large in size (Copeland, 1947).

Little is known about the breeding biology of *Angiopteris* species, or about the life cycle of individuals within the genus. In addition no literature was found relating to the population dynamics of species within the genus or in closely related genera. *Angiopteris* species, however, reproduce both sexually by spores and vegetatively by means of the large fleshy globular rhizomes found at the base of the frond (known as 'stipules'). The gametophytes are large and long lived; they are thought to be obligate mycorrhizal (Hepden, 1960), although not necessarily to a specific mycorrhiza (Cooper, 1976), but were successfully germinated on sterilised compost in Trinity College Dublin.

Angiopteris chauliodonta Copel., endemic to Pitcairn Island, was first described by Copeland in 1938 based on collections made by during the Mangarevan expedition in 1934. The fronds are bipinnate, with the pinnules slightly toothed and the sori formed by two rows of sporangia. The rachis is densely covered in scales when young. Closely related species include *A. longifolia* Grev. & Hook. from the Cook, Austral and Society Islands, from which it differs in having broad, sterile apices of the pinnules, with sharp, narrow, incurved teeth at their bases (Copeland, 1938). Pitcairn material was assigned by Brown and Brown (1931) to *A. longifolia*, and the type specimen for *A. longifolia* is also attributed to Pitcairn (Copeland, 1938). *A. longifolia* is, however, confined to the Society Islands, the Cook Islands and Rapa, and the species was probably described from Tahitian material, mistakenly labelled as having been collected from Pitcairn (Copeland, 1938; the type specimen is labelled as collected in 1830, but as it was published by Hooker in 1830, it was probably actually collected in 1825 on Captain Beechey's voyage to the Pacific). The local name on Pitcairn is nehe, and the plant is used for bedding and as an ornamental.

Angiopteris chauliodonta is a biogeographically significant component of the genus as it represents the most south-easterly species in the genus. In addition it is almost certainly one of the rarest *Angiopteris* species both in terms of its distribution on only a single island and its reduced population size.

The aim of this study was to gather information on the ecology and population structure of *Angiopteris chauliodonta* in order to understand the reasons for the species' limited distribution and population size on Pitcairn Island, and to use this information to develop scientifically sound conservation management for the species.

2. Methods

2.1. Population survey

A population survey was carried out during a 3 month period of fieldwork in 1997. Sites surveyed were those where the species had been previously recorded, or those where the islanders said they had previously seen it. The name of each site and the numbers of mature, fertile and juvenile individuals in each of eight size classes were recorded. Size classes were assigned based on the length of the longest frond (<0.1, <0.5, <1, <1.5, <2, <3, <4, >4 m). The position of each population was mapped using GPS.

2.2. Ecological observations

The ecology of *A. chauliodonta* was recorded by means of field observation and quadrat data. The latter formed part of a larger vegetation survey of the island (Kingston and Waldren, 2003), which recorded species associations, altitude, aspect, and slope. Photosynthetically active radiation (PAR; the component of solar radiation used in photosynthesis, between 400 and 700 nm) and red to far-red ratio (R:FR; the ratio of red (660 nm) to far-red (730 nm) light penetrating the canopy) were recorded from the centre of the quadrat using vertically positioned sensors (Skye Instruments). In addition a hemispherical photograph, using a vertically levelled fisheye lens, was taken for canopy analyses and to measure the Leaf Area Index (LAI). Canopy analyses used the Hemiphot computer package (ter Steege, 1994). A soil sample was also taken from the centre point of the quadrat, and was analysed using standard protocols (Grimshaw, 1989) for percentage Loss on Ignition (LOI; an estimate of the organic matter content estimated by causing the ashing of the organic material in high temperatures) and pH (using a combination electrode in a slurry of 1:2 soil to distilled water).

Nomenclature follows Kingston (2001), which is modified from Florence et al. (1995) to reflect the more recent collections made in 1997 and revised synonymy for some of the taxa found in the region.

2.3. RAPD analysis

2.3.1. Plant material collection

Tissue samples for DNA analysis were collected at the same time as the population survey and placed in zip-lock bags in the field and rapidly dried in silica gel, as recommended by Chase and Hills (1991). Where possible six collections were made from each of the populations of *A. chauliodonta*, with care being taken not to sample from obvious clones. In two of the smallest populations, Faute Valley and Garnets Ridge, only three collections were made as plants were thought to be clones. Samples collected were of frond material, and were collected from young, actively growing shoots, where available.

2.3.2. DNA extraction

DNA was extracted using a standard protocol modified from Hillis and Moritz (1990). A dried sample of 0.1–0.3 g was ground with liquid nitrogen and extracted using 2% CTAB extraction buffer [150 mM Tris[hydroxymethyl]aminomethane hydrochloride (Tris-HCl) (pH 7.5), 1M NaCl, 15 mM Ethylenediaminetetra-acetic acid (EDTA) (pH 8.0) and 1% Hexadecyltrimethyl-ammonium bromide (CTAB)]. One 24:1 chloroform: isoamyl alcohol deproteinisation step was carried out. Following centrifugation, ice-cold isopropanol was added to precipitate the DNA. Following precipitation the DNA pellet was cleaned with ethanol and suspended in 100μl of TE buffer [10 mM Tris-HCl (pH 7.5), 1 mM EDTA (pH 8.0)]. Determination of the concentration and purity of the DNA was by agarose gel electrophoresis and the samples were stored in a freezer at -20 °C.

2.3.3. RAPD reaction

RAPD-PCR reactions were made to a total reaction volume of 50 μl, containing PCR Buffer $\times 10$ [10 mM Tris–HCl (pH 9.0), 50 mM KCl], 3 mM $MgCl_2$, 200 μM each of dATP, dCTP, dGTP and dTTP, 2 units of Taq polymerase, 0.5 μM primer (see below), and 1 μl of total DNA (the mass of DNA in 1 μl varied slightly). Reactions were carried out using a Perkin-Elmer 480 Thermal Cycler (40 cycles of 94 °C for 1 minute, 36 °C for 2 minutes and 72 °C for 2 min, followed by 1 cycle of 72 °C for 5 min). Negative controls and replicates were included in each thermo-cycler run to check for contamination and ensure reproducibility.

PCR amplification products were visualised by electrophoresis on a 2% agarose gel stained with ethidium bromide, and using a 1 Kb DNA ladder (GibcoBRL) standard.

Primers from Operon Technologies 10-mer kits A, B and C were screened and five from kit A that showed clear polymorphic banding were selected (Table 1).

Table 1
Summary of the data obtained for the five primers used with *Angiopteris chauliodonta*

Primer	Sequence	Fragment size range (bp)	No. loci
OPA 02	TGCCGAGCTG	280–1630	11
OPA 03	AGTCAGCCAC	300–1190	8
OPA 04	AATCGGGCTG	260–860	11
OPA 09	GGGTAACGCC	290–1900	11
OPA 10	GTGATCGCAG	290–1170	12
Totals			53

Presence of bands was scored by eye and only unequivocal bands were scored, with weak and spurious bands not being included. Unfortunately PCR amplification was unsuccesful for several individuals, or the bands were too weak for scoring. In total 53 reliable bands were found, and scored for 25 individuals.

2.3.4. Data analysis

Genetic distance was calculated using Nei's (1978) unbiased genetic distance, which allows for small sample size (Nei, 1987), and plotted using UPGMA cluster analyses. UPGMA was used over other methods (e.g. Neighbour-joining, Dollo parsimony) as it more accurately resolved the relationships between individuals. This has also been noted by other authors (Stewart and Porter, 1995; Ayres and Ryan, 1997; Dowe et al., 1997), who recommend it for use with studies of rare and endangered species.

To complement the cluster analyses, an ordination of the data was carried out using the technique of Nonmetric Multidimensional Scaling (NMDS) using the Sørensen coefficient as the distance measure, and a Monte Carlo test of 100 runs to test for robustness. Multivariate analyses in general allow a derivation of the natural groupings among individuals, and NMDS in particular complements the cluster analysis by allowing an assessment of the robustness of the groups obtained

(Ayres and Ryan, 1997). In addition to the graphical analysis, Nei's (1973) gene diversity statistics were calculated for an assessment of within and between population diversity. A Mantel test was used to test for correlation between geographic and genetic distance (Sokal and Rohlf, 1995).

NMDS and Mantel tests were calculated using PC-ORD ver. 3.2. Nei's (1978) genetic distance, Nei's (1973) gene diversity statistics and UPGMA dendrograms were calculated using POPGENE ver.1.31 (Yeh and Boyle, 1997).

3. Results

3.1. Population survey

Angiopteris chauliodonta, previously recorded from only two sites, was represented by six populations, the location and distribution of which are mapped in Fig. 1, with population demographic information in Table 2.

Fertile plants account for only 19.0% of the total of 774 plants recorded on the island, although one population, High Point 1, had 41.9% fertile (or mature adult) plants. Plants <1 m in size account for 63.8% of the total. Plants <2 m were never fertile, but only 31% of plants >2 m in size were sterile.
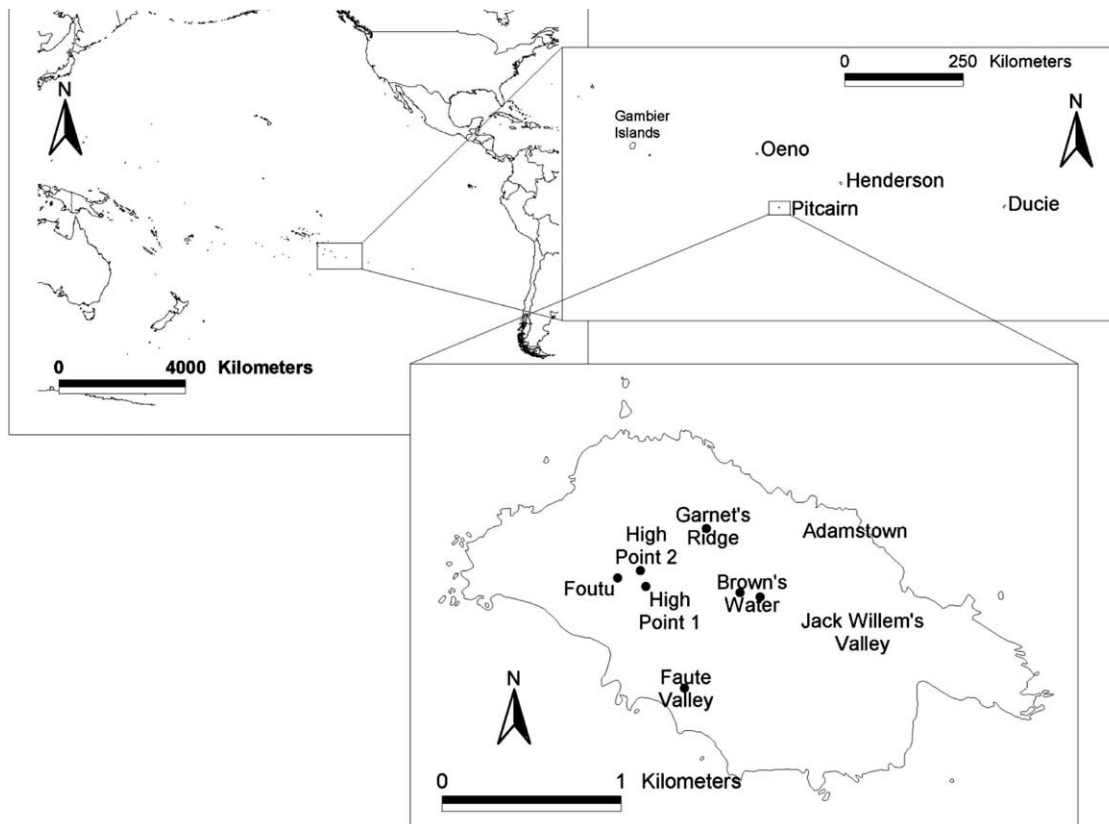

Fig. 1. The location of Pitcairn Island, with close up map of the island to show the location of the six *Angiopteris chauliodonta* populations. Place-names listed are those mentioned in the text.

The Faute Valley population contained no fertile individuals, and Garnets Ridge only had one small fertile individual. The Foutu population had 80% fertile individuals, but this was combined with virtually no evidence of juvenile individuals. The populations at High Point have the most balanced demographic structure, with few large individuals, but with individuals in all size classes, both fertile, sterile and juvenile. The Brown's Water population had a comparatively large number (455) of small, sterile and juvenile individuals, with only 12 fertile plants. However, there was evidence that many plants have become reduced in size, with many having leaf bases from much larger fronds persisting, suggesting that some individuals were at least larger and perhaps fertile in the past.

3.2. Ecological observations

Angiopteris chauliodonta occurs in sheltered gullies in a range of forest habitats, but predominantly in high altitude cloud and damp forest that is now heavily invaded by *Syzygium jambos* and *Lantana camara*. In all habitats the associated species are mainly native pteridophytes such as *Arachnoides aristata*, *Loxoscaphe gibberosum*, *Cyathea medullaris*, *Lastreopsis pacifica*, *Phymatosorus commutatus*, *Pneumatopteris costata*, *Psilotum nudum*, *Trichomanes enderlichianum*, and less commonly with *Nephrolepis cordifolia*, *N. hirsutula*, *Trichomanes tahitense*, *Davallia solida*, *Diplazium harpeodes* and *Christella parasitica*. Where the canopy is not dominated by *Syzygium jambos*, canopy-forming trees of *Homalium taypau*, *Metrosideros collina* and *Glochidion pitcairnense* occur.

The ecology of the populations vary depending on the vegetation community in which they occur (Table 3). This variation relates primarily to the canopy forming tree species, notably in the light environment, but also in the soil characteristics. Where the populations occurred in *S. jambos* forest a low R:FR ratio was consistently recorded (0.34–0.72), but the R:FR ratio was

Table 2
Population statistics and demography for all *Angiopteris chauliodonta* populations from Pitcairn Island. Sterile and fertile individuals are divided into size classes

Location	Sterile (m)						Fertile (m)				Totals		
	<0.1	<0.5	<1	<1.5	<2	<3	<2	<3	<4	>4	Overall	Fertile	% Fertile
Faute Valley	1	1	2	1	2	2					9	0	0
Garnets Ridge		5			7		1				13	1	7.69
Foutu		2	3				5	5	5	5	25	20	80.0
High Point 1	19	41	21	6	11		49	19	3		170	71	41.5
High Point 2	12	25	13	4	6		30	12	1		102	43	41.2
Brown's Water	111	152	86	56	24	14		4	4	4	455	12	2.64
Total	143	226	125	67	50	16	85	40	13	9	774	147	19.0
% Total	18.4	29.2	16.1	8.7	6.5	2.1	11.0	5.2	1.7	1.2			

Table 3
Summary ecological information for the six *Angiopteris chauliodonta* populations. Italics indicate that the values shown are for the vegetation community in which the population occurs, rather than for the location of the individual population

	Faute Valley	Garnet's Ridge	Foutu	High Point 1	High Point 2	Brown's Water
Vegetation community	*Metrosideros collina* forest	Eroding slopes	*Metrosideros collina* forest	*Syzygium jambos* forest	*Homalium taypau* forest	*Syzygium jambos* forest
Slope (degrees)	40	40	*20–40*	42	*20–50*	30–78
Aspect (degrees)	200	182	300	66	90	85
Red:Far Red ratio	0.89	1.16	*0.50–0.88*	0.72	*0.77–0.90*	0.34–0.57
Photosynthtically Active Radiation ($\mu mol\ m^{-2}\ s^{-1}$)	270	1290	*17–500*	15	*32-100*	7.4-12.6
Leaf Area Index	0.39	0.26	*0.2-1.0*	0.48	*0.1-0.6*	0.6-0.8
% Organic content	22.7	13.0	*15.9–29.9*	22.0	*10.1–25.1*	21.4–34.2
Soil pH	7.7	5.3	*6.6*	6.0	*6.6*	5.8-6.3
% cover of invasive species in community	26.8%	25%	*15%*	22.9%	*33.4%*	68.9%
Threats[a]	I, P	C, I, P, Er	I, P	I	I	I, Er, Ex
Altitude (m)	110	240	*300–310*	250–300	*280–300*	160–200
Local topography	Steep sided gully	Slope	Slope	Depression	Depression	Steep gully

[a] C = clearance of native forest; I = spread of invasive species; P = very small population size; Er = erosion; Ex = exploitation.
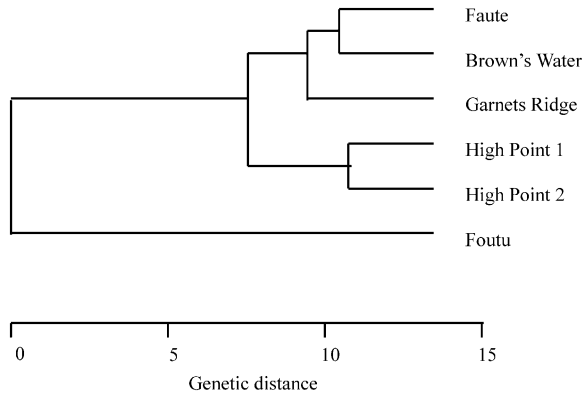
Fig. 2. UPGMA dendrogram based on Nei's (1978) genetic distance between populations (drawn from data on lower diagonal of Table 3).

higher when associated with native tree species such as *H. taypau* (0.77–0.90 in High Point) or *M. collina* (0.50–0.88) in Faute Valley and Foutu). In fact the highest recorded R:FR in any forest type on the island was in *H. taypau* forest (0.90). The same pattern was found for PAR (7.4–15.0 µmol m^{-2} s^{-1} in the *S. jambos* forest, but 570.0 µmol m^{-2} s^{-1} in the native forests) and the reverse pattern for LAI (0.48–0.8 in the *S. jambos* forest, but only 0.2–0.6 in the native forests).

The Garnets Ridge population differed from the others as it occurred on an actively eroding slope, on the edge of a *Metrosideros collina* and *Cyathea medullaris* forest. The light environment here showed very high levels of PAR (1290 µmol m^{-2} s^{-1}) and R:FR (1.16) due to the fact that the population is no longer protected by a forest canopy.

In all of the forest types *A. chauliodonta* sites had a high LOI (10.1–34.2%), but pH, aspect and slope varied widely. All sites occurred at altitudes of above 110 m, and up to just below the highest point on the island at 320 m. No measurements of site humidity or rainfall were made, but most populations occurred in areas of cloud forest and especially in gullies and on damp soils or spring lines, and as such were likely to be areas of high humidity.

Most of the mature plants had released their spores during the field visit in 1997, but no information on maturation, fruiting or spore release could be gathered during the limited observation period.

3.3. RAPD analysis

Nei's (1978) genetic distance was calculated between each population (Table 4) and used to plot a UPGMA dendrogram (Fig. 2). This shows that High Point populations 1 and 2 are the most closely related, with the lowest genetic distance (0.0494), while the Foutu and the Faute Valley populations are the most distant (0.3377). The distinctness of the Foutu population is because this population contains the only exclusive band in the data set, and also is represented by only four individuals. The Mantel test showed that while there is a slight positive association between geographic and genetic distance (standardised Mantel statistic $r = 0.030$) this was not significant ($P = 0.459$; based on a Monte Carlo randomisation of 1000 runs). Thus the similarity between the two most geographically distant populations at Faute Valley and Garnets Ridge (0.0616) may be an artifact of low sample size, as the Faute and Garnets Ridge populations are only represented by two and three individuals respectively. A low sample number would mean that only the most common bands are probably represented by these populations.

In order graphically to represent the relationships within the populations from Pitcairn an NMDS analysis was carried out, using two unrotated dimensions to ensure the lowest stress levels (26.48). The analysis was robust with P values of <0.01 for both dimensions for 100 runs using a Monte Carlo test (Table 5). The resulting graph (Fig. 3) shows a similar pattern to the UPGMA dendrogram, the Foutu population ordinating with high values for both dimension 1 and dimension 2, and the other populations ordinating with low dimension 1 and dimension 2 values. There is considerable overlap between individuals and the populations are not distinctly separated.

Analysis of the RAPD loci data showed low levels of diversity within the individual populations on Pitcairn

Table 4
Geographic distance (in metres; above diagonal) and Nei's unbiased measure of genetic distance (Nei, 1978; below diagonal) between populations of *Angiopteris chauliodonta* from Pitcairn Island. Mantel statistic (r) = 0.03, $P = 0.459$

	Faute	Foutu	High Point 1	High Point 2	Brown's Water	Garnets Ridge
Faute	****	811	676	784	703	1027
Foutu	0.3377	****	189	162	838	635
High Point 1	0.1016	0.2169	****	108	649	527
High Point 2	0.1289	0.2170	0.0494	****	703	487
Brown's Water	0.0692	0.2793	0.0781	0.0839	****	487
Garnets Ridge	0.0616	0.2903	0.1414	0.1753	0.0800	****

Island, but the overall observed diversity (H_T) was comparable to populations of other pteridophyte species (Soltis and Soltis, 1990). In the Pitcairn samples, the highest levels of observed diversity (H_T) were found in the High Point 1 and 2 populations, and the lowest in the Faute Valley population. Total observed diversity (H_T) was divided into within population diversity (H_S) and between population diversity (D_{ST}), with H_S (0.154)

being higher than D_{ST} (0.116) (Table 6). There was no significant correlation between sample size and total observed diversity (H_T; $P > 0.05$).

4. Discussion

4.1. Population structure

Previous botanical surveys on Pitcairn had found only two populations of *Angiopteris chauliodonta* on Pitcairn, one from Garnets Ridge, and one from the hills above Adamstown (probably the Brown's Water population; St. John, 1987; Waldren et al., 1995). Our 1997 survey increased the number of populations to six, with reasonable certainty that no further large populations will be found, as the island was thoroughly searched.

No individuals had identical RAPD banding patterns, suggesting no clones were sampled, something that can be a problem when sampling from vegetatively

Table 5
Stress, significance (*P*) values and R^2 correlation scores for NMDS of 2 dimensions for RAPD data for *Angiopteris chauliodonta* populations

	Stress	*P*	R^2
Dimension 1	26.48	0.010	48.3
Dimension 2	15.75	0.010	42.6

P = proportion of randomised Monte Carlo test runs with stress < observed stress (i.e. *P* = (1 + no. permutations < observed) / (1 + no. permutations). R^2 = correlations between ordination distances and distances in the original n-dimensional space.

Fig. 3. NMDS ordination diagram for *Angiopteris chauliodonta* calculated for 2 dimensions (R^2-Dimension 1 = 48.3; Dimension 2 = 42.6). Key: Faute ■, Foutu ◆, High Point 1 ■, High Point 2 ▲, Brown's Water ■, Garnets Ridge ▲.

Table 6
Summary population diversity statistics for *Angiopteris chauliodonta*, calculated using Nei's (1973, 1987) gene diversity statistics

Location		Faute	Foutu	High Point 1	High Point 2	Brown's Water	Garnets Ridge	*All*
Sample size		2	4	6	5	5	3	*25*
Total observed diversity	H_T	0.088	0.122	0.207	0.190	0.138	0.180	*0.270*
Within-pop diversity	H_S							*0.154*
Between-pop diversity	D_{ST}							*0.116*
Gene diversity between pops	G_{ST}							*0.429*
Gene diversity within pops	$1-G_{ST}$							*0.571*

reproducing species. In addition, being able to distinguish all individuals suggests that adequate numbers of primers were analysed, thereby increasing the validity of the results.

While the smaller populations did not necessarily have less variation, the high levels of genetic diversity in the two High Point populations may be explained by their large size, high number of fertile individuals and spread of individuals in all size classes. In addition their proximity to each other increases the effective population size and the chances of gene flow between the populations. The lowest levels of diversity in Faute Valley could be explained by the population's relative remoteness (>675 m to nearest populations at Brown's Water and High Point) and very small size (only nine individuals). High levels of diversity in the Garnets Ridge population, even though the population is small, may relate to its proximity to the Brown's Water and High Point populations. This is backed up by the NMDS analysis of the populations, in that the Garnets Ridge population overlaps with the High Point and Brown's Water populations on the resulting ordination. The levels of genetic variation suggest that the Garnets Ridge population may be a newly expanding population, but considering its location in an area threatened by severe erosion it is more likely that it is a remnant of a once larger population.

The Brown's Water population is the largest on the island, but one that is at risk from substrate erosion and road widening. There is evidence that the population once contained larger plants, as older stem bases on many individuals suggest leaves formed previously were larger than those present at the time of our survey. The apparent reduction in the size of the population has also been noted by islanders who used to collect fronds from this site (B. Christian, pers. comm.). While this population still shows the highest levels of regeneration of any population on the island, many of the young plants are along an unstable and steep stream bank and are therefore unlikely to persist to maturity.

The Foutu population is the most distinct population. This population occurs close to the High Point populations, but is separated from them by a major ridge and dense cloud forest. This dense vegetation surrounding the population may reduce gene flow from this population to the others. The genetic distance between the Foutu and the Faute populations was 0.3377 (Table 6), and while this is substantially higher than other genetic distances in the populations (0.0494–0.2903), it is lower than genetic distances between populations in other species. For example in *Hordeum spontaneum* populations from Israel, genetic distances between populations of up to 0.393 have been recorded using the same methods employed here (Dawson et al., 1993).

Within-population diversity for the Pitcairn populations was 57.1% of the total diversity found, which is comparable to values found in the rare and endangered fern *Dryopteris cristata* (Landergott et al., 2001), but considerably higher than values found in the more widespread fern *Sticherus flabellatus* (25.66%; Keiper and McConchie, 2000). Soltis and Soltis (1990) suggest that for pteridophytes inbreeding populations distribute most of their variation between populations, which implies that *Angiopteris chauliodonta* may be an outbreeding species. The relatively large amount of between-population genetic variation also suggests that the populations of *A. chauliodonta* are not showing high levels of fragmentation as is common in other rare plant species (Schneller and Holderegger, 1996). Realistically, it may be artificial to consider the species to be divided into separate and distinct populations when the total area covered by the species, and the distance between the populations, is so small.

4.2. Population ecology

Angiopteris chauliodonta is not closely associated with any distinct vegetation community or habitat type, but rather it occurs in a range of high altitude and humid forest types. The primary differences in ecological factors observed between populations was in the light environment and soil characteristics, which are both closely associated with the variations in canopy-forming species. This supports the theory that the species was more widespread across the island in the past, but has declined due to various environmental threats (see below). The ability of the species to occur in various habitats suggests that if the populations are maintained in the short term, they will be able to cope with any long-term environmental changes that might occur.

There is no significant correlation between the ecology and genetic structure of the populations, but the high altitude populations show a significantly higher level of fertility ($P = 0.05$). These populations tend to have suffered less from disturbance and this may explain their improved fertility.

4.3. Threats

In recent conservation and threat assessments of the entire flora of Pitcairn Island, *Angiopteris chauliodonta* was found to be one of the most threatened species on the island, second only to *Coprosma benefica*, another endemic angiosperm species which is reduced to just 12 individuals. *A. chauliodonta* was assigned the IUCN category of 'critically endangered' (based on criteria A1(c-e), B1, B2(a-e), and C2 from IUCN, 2000) due to the species narrow geographic range, small fertile population and suspected reduction in population size (Kingston and Waldren, 2002).

A decline in *A. chauliodonta* on Pitcairn has been reported by several of the islanders and is due to several

factors; forest clearance, exploitation, invasive species, substrate erosion and the reduced reproductive potential resulting from these environmental threats (Table 3). In the past the species covered larger areas, and fronds were cut for their scent and as bedding (B. Christian, pers comm.). More recently plants have also been collected as ornamentals (C. Warren, pers comm.). Invasion of the taxon's natural habitat by both *L. camara* (High Point, Foutu, Garnets Ridge, Faute Valley) and the spread of *S. jambos* trees (Brown's Water, High Point) probably account for most of the reduction in populations. *L. camara* spreads in canopy openings preventing regeneration of other species, and while *S. jambos* provides dark and humid conditions, it also causes erosion by eliminating the ground flora that binds the soil (especially problematic at Brown's Water). It has been documented that slow growing pteridophytes are badly affected by invasive species while more 'weedy' pteridophytes may actually benefit (Given, 1993). Natural erosion, exacerbated by anthropogenic activities, threatens the Garnets Ridge population, which is precariously located on the edge of a large area of bare ground known as the Hollow. The Faute Valley population, even though located in a remote valley, is probably the population most at risk in the long term, as the population is small, with a low genetic variation and no fertile individuals.

4.4. Conservation actions

A. chauliodonta requires both in and ex-situ conservation actions. In-situ conservation could be carried out in protected areas suggested for the island (Kingston, 2001). These reserves have been selected to maintain some of the best habitats containing native vegetation, but in areas which are rarely visited or utilised by the islanders, thus avoiding land-use conflicts. One of the potential reserve areas houses the three largest and most genetically diverse populations, at Foutu and High Point, ensuring that a viable level of genetic diversity is maintained. Ex-situ conservation would be in the recently constructed island nursery where stock plants could be grown for reinforcement of existing populations, for ornamentals, and for translocating into areas that would be cleared of invasive species. There is much scope for using this architectural species in amenity plantings around the settlement, ensuring its survival on Pitcairn. In addition to the participation of one of the islanders at a propagation course in Kew, a recently established long-term conservation project aims to provide the islanders with the expertise needed to maintain the nursery and facilitate species recovery programmes.

In 1997 a trial translocation of 40 stipules from Brown's Water, High Point and Foutu was planted into Jack Willems Valley. The site was chosen as it had appropriate protected and inaccessible habitat, but was visible from a road. Monitoring in 2003 showed a 65% survival rate, with a mean frond number of 3.26, and a mean size of 21.7cm. The islanders had expressed an interest in having the species more widespread as an ornamental, and their pleasure at seeing the 'new' population. The success of this trial suggests that a low maintenance, low technology and locally acceptable solution may be feasible. Growth trials from spores have been relatively unsuccessful, as while gametophytes have formed, production of sporophytes has not been achieved.

A vegetation survey of the island (Kingston and Waldren, 2003) has identified several other locations that would be suitable for translocation of populations have been identified. A nursery stock of plants will be grown from stipules collected as appropriate from all populations, and by removing small and young plants which are vulnerable from the Brown's Water population. The lack of clear population differentiation means that conservation management programmes should aim to maintain this high genetic variability and minimise inbreeding by mixing individuals from the most diverse populations (i.e. High Point and Garnets Ridge) with those from the less diverse populations (i.e. Faute Valley and Foutu). As no populations are genetically isolated, recovery programmes could consist of populations with individuals from several populations. This mixing would utilise the high genetic variability of the larger populations to recover those with lower genetic variability. Mixed population translocation programmes with monitoring for outbreeding depression, are thought to be the most successful in the long term (Templeton et al. 1990).

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