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Genetic variation and the mating system in the rare *Acacia sciophanes* compared with its common sister species *Acacia anfractuosa* (Mimosaceae)

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Abstract

Acacia sciophanes is an extremely rare and Critically Endangered species known from two small populations separated by less than 7 km. Specifically we aimed to investigate whether rarity in *A. sciophanes* is associated with decreased levels of genetic variation and increased levels of selfing by comparing patterns of genetic variation and mating system parameters with its widespread and common sister species *A. anfractuosa*. Fourteen polymorphic allozyme loci were used to assess genetic diversity with four of these used in the estimation of mating system parameters. At the species level *A. sciophanes* has lower allelic richness, polymorphism, observed heterozygosity and gene diversity than *A. anfractuosa* and significantly lower levels of gene diversity at the population level. Both species have a mixed mating system but the largest population of *A. sciophanes* has lower levels of outcrossing, higher correlated paternity and increased bi-parental inbreeding compared with two *A. anfractuosa* populations. However, both correlated paternity and bi-parental inbreeding appear to be at least partly influenced by population size regardless of the species. We suggest that *A. sciophanes* is likely to be an intrinsically rare species and that in particular the lower levels of genetic diversity and increased selfing are a feature of a species that has the ability to persist in a few localised small populations. Despite recent extensive habitat destruction our comparative study provided no clear evidence that such events have contributed to the lower genetic diversity and increased selfing in *A. sciophanes* and we believe its ability to exist in small populations may not only be an important factor in its survival as a rare species but also indicates that it may be less susceptible to the impacts of habitat loss and fragmentation. The key to this species conservation will be the maintenance of suitable habitat, particularly through improved fire regimes and control of invasive weeds, that will allow the two small populations to continue to persist in extremely restricted areas of remnant vegetation.

Introduction

Comparative studies of genetic variation and mating systems in pairs or groups of closely related rare and common species have proven valuable in developing an improved understanding of the genetic consequences of rarity (Karron 1991; Gitzendanner and Soltis 2000; Cole 2003). Findings from such studies may also have significant implications for the conservation and management of rare and threatened species with both the level and distribution of genetic variation, and the mating system informing on a range of possible management actions. These include germplasm collection and re-introduction strategies, *in situ* management priorities and actions involving population enhancement and gene flow augmentation. Assessing patterns of genetic and mating system variation in relation to rarity can also improve our understanding of the impact and

significance of recent reductions in population size and habitat fragmentation (Young et al. 1996).

Rarity in plants can be due to many factors including the evolutionary history of the species, habitat specificity and recent broad-scale habitat destruction or fragmentation (Fiedler and Ahouse 1992). Although rare species can exhibit different population sizes, structure, and geographical distributions (Rabinowitz 1981) they frequently have small disjunct populations and geographically restricted ranges. Thus theoretically, rare plants might be expected to show low levels of genetic variation both at the species and population levels due to selection under an narrow range of environmental conditions, and genetic drift and inbreeding in small isolated populations (Barrett and Kohn 1991; Ellestrand and Elam 1993). Low genetic variation within rare species may also be due to founder events associated with recent speciation (Gottleib 1981; Loveless and Hamrick 1988).

Broad comparisons between geographically restricted and widespread species have indicated that some trends are evident with geographically restricted species tending to have less genetic variability than widespread species (Hamrick and Godt 1989). Further studies comparing rare species and their common congeners show that rare species have significantly lower levels of genetic variation (Karron 1987; Gitzendanner and Soltis 2000; Cole 2003). In a comprehensive meta-analysis of isozyme variation in 247 plant species, summarised as 57 generic level comparisons of rare and common plants, Cole (2003) showed that at both species and population levels measures of percent polymorphic loci, number of alleles per locus, number of alleles per polymorphic locus, and expected heterozygosity were significantly less for rare species. More than 73% of the species and population level comparisons showed a reduction in measures of genetic variation. Although there are exceptions to this trend where comparable levels of genetic diversity have been found in rare species and their common close relatives (see Young and Brown 1996; Ge et al. 1999; Godt and Hamrick 1999; Coates et al. 2003.) the overall pattern indicates that the reduction in genetic variability in rare plant species appears to be more significant than previously thought (Cole 2003).

In addition to reduced levels of genetic variation it is also postulated that we might expect increased population differentiation because of reduced pollinator activity and increased selfing in the often-smaller populations of rare species (Cole 2003). However, this effect is likely to be confounded by the geographical distribution of the species with common species likely to have more widely separated populations distributed over a greater geographic range leading to overall reduced gene flow. It is perhaps not surprising therefore that Hamrick and Godt (1989) found no evidence for differences in genetic structure between geographically restricted and widespread species while Gitzendanner and Soltis (2000), and Cole (2003) found no difference in 22 and 38 rare and common congener comparisons, respectively. Although the effects of rarity on population differentiation are likely to be complex one useful approach is to investigate differentiation in rare and common species over the same geographic range. In one such study Young and Brown (1996) demonstrated three times the interpopulation genetic divergence in the rare *Daviesia suaveolens* compared with its widespread and common congener *D. mimosoides*.

The inconsistencies with many of these studies are not necessarily surprising, particularly with broad comparisons between rare and common species, because they will inevitably be confounded by differences in evolutionary histories and life-history traits. The key point advanced by Gitzendanner and Soltis (2000) is that by focussing on rare and common congeners one can more readily identify and understand historical and life-history causes of rarity and how they may affect patterns of genetic variation. In addition and of relevance in the development of conservation strategies is whether differences in levels of genetic diversity and mating system parameters are associated with human intervention, for example, rarity due to relatively recent broad scale habitat destruction and fragmentation. These kinds of studies may therefore also be useful in developing a better understanding of whether rarity and associated patterns of genetic variation are the result of anthropogenic or intrinsic causes (Fiedler and Ahouse 1992) or some combination of both.

Acacia sciophanes Maslin is an extremely rare species covering a geographic range of less than 7 km. It is currently ranked on IUCN criteria as Critically Endangered occurring in a heavily

fragmented landscape where much of the native vegetation has been cleared for agricultural production. It develops into a diffuse, openly branched, wispy shrub up to 2.3 m tall and is closely related to a more common species *Acacia anfractuosa* Maslin that occurs over a range of some 200 km. A Previous phylogenetic study based on cp DNA variation indicated that *A. sciophanes* and *A. anfractuosa* are sister species (Byrne et al. 2001). *Acacia sciophanes* is therefore typical of a significant component of the south-west flora of Western Australia where plant species often have narrow geographic ranges and adjacent allopatric or parapatric distributions in relation to close relatives (Hopper 1992). Like most other *Acacia* species *A. sciophanes* and *A. anfractuosa* are medium to long-lived woody shrubs and shed hard coated seeds that can remain viable for a considerable time (decades) in the soil. Native bees and wasps primarily pollinate both, and adult plants are killed by fire with populations re-generating from soil-stored seed. There are no obvious demographic reasons for the rarity of *A. sciophanes* with few differences in ecology and reproductive biology between *A. sciophanes* and *A. anfractuosa* (Buist et al. 2002). Indications are that *A. sciophanes* was probably geographically restricted and rare prior to land clearing for agriculture and that recent habitat disturbance and degradation are likely to have an ongoing impact on the two extant populations (Evans et al. 2000).

The aim of this study was to investigate whether rarity in *A. sciophanes*, due to intrinsic and or anthropogenic factors, is associated with decreased levels of genetic variation and increased levels of selfing by comparing patterns of genetic variation and mating system parameters with *A. anfractuosa*. Additional aims where to provide data that would assist in a better understanding of factors that may be contributing to rarity and important for the persistence of this species, and the development of more effective conservation actions that may enhance its recovery.

Materials and methods

Distribution, population sampling and site descriptions

Acacia sciophanes is extremely restricted occurring in only two populations at Wundowlin and Barbalin Rd (7 km further south) (Figure 1). Its closest relative *A. anfractuosa* has a geographic range of roughly 200 by 300 km. Their distributions do not overlap with *A. sciophanes* found some 20 km west of the nearest population of *A. anfractuosa*.

Both species are found on deep yellow sandy soils occurring in similar vegetation types consisting of floristically diverse low woodland and scrub heath communities that occur in the dry Mediterranean climate of this area. *A. sciophanes* can be defined as locally and geographically rare and a habitat generalist. It is possible that prior to land clearing *A. sciophanes* may have been locally common given that its habitat is not unusual. However, over the last 50 years the specific soil type has been largely cleared for agriculture resulting in a heavily fragmented landscape. The two small low density *A. sciophanes* populations, of 272 and 114 plants, occur partly in a modified environment of a rail and road reserve sandy soils but also in small relatively undisturbed vegetation remnants. The larger Wundowlin population is also found on a small 176 ha nature reserve. Six larger and generally higher density *A. anfractuosa* populations were included in this study from the more westerly part of its distribution. Although they do not cover the full *A. anfractuosa* distribution they do cover a range of population types from disturbed linear road reserve populations to a large Nature Reserve population. Details of populations sampled and sample sizes per population are given in Table 1. Seed was collected from multiple open pollinated pods per plant for isozyme analysis. Sampling was limited in the *A. sciophanes* Barbalin Rd population as the majority of plants did not produce any seed.

Isozyme electrophoresis

Seeds were germinated on moistened filter paper and seedlings with recently emerged radicles provided the best material for isozyme analysis. Sample sizes per locus are given in Table 2 for genetic diversity studies and 148, 155 and 151 seedling progeny were analysed for mating system studies in the *A. sciophanes* Wundowlin population and the *A. anfractuosa* Farina Rd and Koonadgin populations, respectively. Preparation of this material and the isozyme methods, using the Helena Laboratory cellulose acetate plate

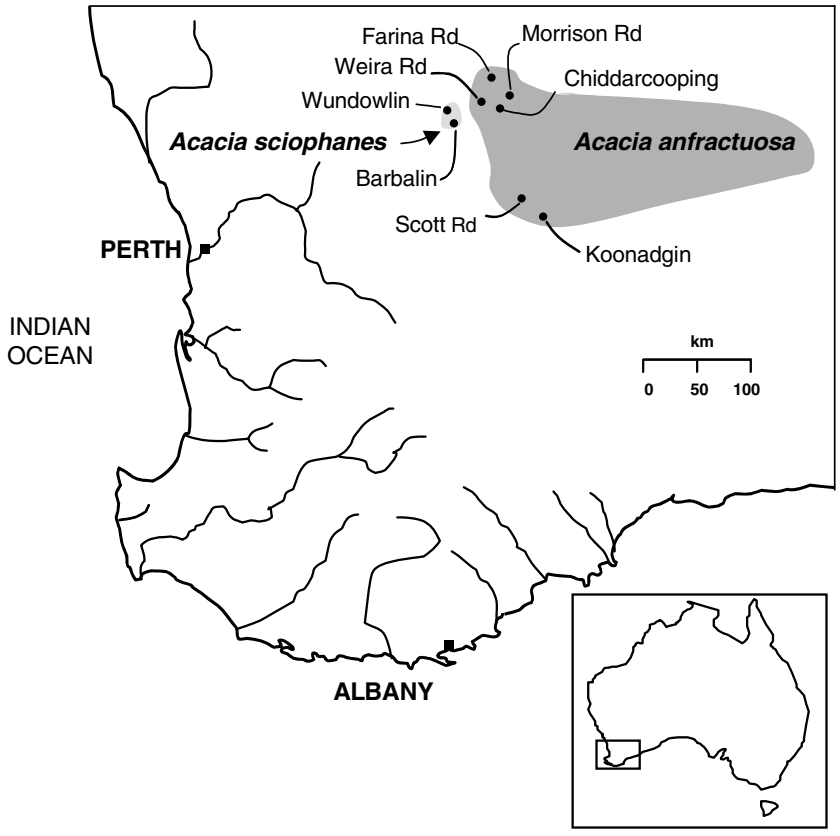

Figure 1. Distribution and populations sampled for *A. sciophanes* and *A. anfractuosa*.

Table 1. Population sizes, area occupied, plant density, disturbance regime and sample sizes for all populations of *Acacia sciophanes* and six populations of *Acacia anfractuosa*

Population/species	No. of mature plants	Area (ha)	Density (plants/m^2)	Disturbance regime	Total seed collected from (plants)
A. sciophanes					
Wundowlin	272	6.50	0.004	r, w	789 (16)
Barbalin Rd	114	1.50	0.008	g, w	128 (8)
A. anfractuosa					
Farina Rd	>1000	0.30	0.333	r	1117 (17)
Weira Rd	>1000	0.16	0.625	r	232 (13)
Morrison Rd	≈500	0.08	0.625	r	325 (11)
Chiddarcooping	>1000	0.4	0.250	r	767 (18)
Scott Rd	≈300	0.02	1.500	r	570 (20)
Koonadgin	≈400	25	0.002	r, w, g	1271 (15)

Disturbance: w, weed invasion; r, divided by road or firebreak which is actively maintained by grading; g, Disturbance in the form of clearing for gravel or sand extraction.

electrophoresis system, were described previously by Coates (1988).

Thirteen enzyme systems were assayed alcohol dehydrogenase (ADH, E.C. 1.1.1.1), esterase (EST, E.C. 3.1.1.-), glucose-6-phosphate dehydrogenase (G6PDH, E.C. 1.1.1.49) glucose-6-phosphate isomerase (GPI, E.C. E.C. 5.3.1.9), glutamate dehydrogenase (GDH, E.C. 1.4.1.2), isocitrate dehydrogenase (IDH, E.C. 1.1.1.41) leucine aminopeptidase (LAP, 3.4.17.1), malate

Table 2. Population sizes, genetic diversity statistics, fixation indices (F_{IS}) for all populations of *Acacia sciophanes* and six populations of *Acacia anfractuosa*

Population/species	Number of mature plants	*N*	*A* (SE)	*P* (SE)	H_e (SE)	H_o (SE)	F_{IS} (SE)
A. sciophanes							
Wundowlin	272	34.0	2.7 (0.2)	85.7	0.31 (0.05)	0.17 (0.04)	0.46 (0.08)
Barbalin Rd	114	21.5	2.4 (0.3)	78.6	0.28 (0.07)	0.19 (0.05)	0.26 (0.11)
Popn. mean			2.6 (0.15)	82.2 (3.6)	0.30 (0.02)	0.18 (0.01)	0.36 (0.10)
Species			2.9 (0.3)	85.7	0.31 (0.08)	0.18 (0.04)	
A. anfractuosa							
Farina Rd	>1000	26.7	2.9 (0.3)	85.7	0.38 (0.06)	0.21 (0.04)	0.34 (0.11)
Weira Rd	>1000	31.4	2.5 (0.3)	78.6	0.40 (0.07)	0.20 (0.04)	0.46 (0.09)
Morrison Rd	≈500	26.7	2.6 (0.3)	85.7	0.39 (0.07)	0.21 (0.05)	0.33 (0.11)
Chiddarcooping	>1000	30.7	2.8 (0.3)	85.7	0.38 (0.07)	0.17 (0.03)	0.41 (0.10)
Scott Rd	≈300	41.8	3.0 (0.3)	85.7	0.35 (0.06)	0.21 (0.04)	0.32 (0.09)
Koonadgin	≈400	28.3	2.5 (0.2)	85.7	0.38 (0.06)	0.19 (0.04)	0.44 (0.11)
Popn. mean			2.7 (0.1)	84.5 (1.2)	0.38 (0.01)	0.20 (0.01)	0.38 (0.03)
Species			3.5 (0.2)	100	0.40 (0.07)	0.20 (0.04)	

N, mean sample size per locus; *A*, mean number of alleles per locus; *P*, percentage of polymorphic loci; H_e, gene diversity; H_o, observed heterozygosity.

dehydrogenase (MDH, 1.1.1.37), malic enzyme (ME, E.C. 1.1.1.40), menadione reductase (MDR, E.C. 1.6.99.22), phosphoglucomutase (PGM, E.C. 2.7.5.1), phosphgluconate dehydrogenase (PGD, E.C. 1.1.1.44) shikimate dehydrogenase (SDH, E.C. 1.1.1.25). Fourteen zones of activity were scored and each zone was assumed to represent a single locus. All loci were polymorphic in at least one population. Their genetic interpretation was based on segregation patterns of progeny arrays from open pollinated families from the three populations used in mating system studies.

Genetic variation within and among populations, and gene flow

The average number of alleles per locus (*A*), percentage polymorphic loci (*P*), observed heterozygosity (H_o) and gene diversity (expected panmictic heterozygosity) (H_e) were estimated at the population level and species level using POPGENE (Yeh and Boyle 1997). Differences in genetic variation between the two species at the population level based on allelic richness, gene diversity and heterozygosity were tested using the FSTAT option for comparing these statistics among groups based on 1000 permutations.

Fixation indices (F_{IS}) (Wright 1978) were estimated to examine population deviation from random mating. The goodness of fit of observed genotype frequencies at each locus to those expected under Hardy–Weinberg equilibrium was tested by *G* test using POPGENE. The difference in F_{IS} between the two species was tested using FSTAT based on 1000 permutations.

Interpopulation divergence was estimated using Nei's (1978) unbiased genetic distance (*D*) and F_{ST} (Wright 1978). Nei's (1978) unbiased genetic distance (*D*) was calculated between all populations and then the mean *D* determined for populations of *A. anfractuosa*, and between populations of the two species. Separate estimates of F_{ST} were made for populations of *A. sciophanes* and *A. anfractuosa*. The indirect estimate of gene flow (*Nm*) was calculated over all populations and within the two species based on the relationship $F_{ST} = 1/[4Nm + 1]$ (Wright 1978). Nei's *D*, F_{ST}, and *Nm* were determined using POPGENE (Yeh and Boyle 1997).

Phylogenetic relationships among populations were investigated by restriction maximum likelihood method using gene frequency data. Robustness of the maximum likelihood tree was determined by constructing 500 bootstrap replicates of the gene frequency data for the maximum likelihood tree with SEQBOOT in PHYLIP. These bootstrapped data sets were then run through NEIGHBOR and CONTML, respectively, and

then a majority-rule consensus tree was generated in the PHYLIP program CONSENSE. PHYLIP 3.5 (Felsenstein 1995) was used for this analysis.

Bottleneck assessment

Understanding whether populations have recently experienced bottlenecks can be particularly useful for distinguishing between the genetic consequences of rarity caused by intrinsic factors such as evolutionary history and habitat specificity and anthropogenic causes of rarity associated with recent habitat destruction and fragmentation. In a population that has undergone a recent bottleneck allelic diversity is reduced faster than heterozygosity so that there is a transient deficiency in the number of alleles in that population that will be detectable for a short time of approximately 0.2–4.0 N_e (bottleneck effective size) generations (see Cornuet and Luikart 1996; Luikart and Cornuet 1998). Thus expected heterozygosity (H_e) becomes larger than the heterozygosity expected at mutation-drift equilibrium (H_{eq}). To evaluate whether any of the populations in this study have been through a recent bottleneck we carried out three different statistical tests: standardised differences test, sign test and Wilcoxon signed rank test using BOTTLENECK (Piry et al. 1999), based on the infinite allele model (IAM) and the stepwise mutation model (SMM) assumptions (see Cornuet and Luikart 1996). If H_e is significantly greater than H_{eq} according to these three tests then it is likely that the populations investigated have been through a significant recent bottleneck.

Mating system analysis

Two loci (*Lap-1*, *Pgi-1*) in the *A. sciophanes* Wundowlin population, three loci (*Adh-1*, *Lap-1*, *Pgi-1*) in the Farina Rd population of *A. anfractuosa* and four loci (*Adh-1*, *Lap-1*, *Pgi-2*, *Pgm-1*) in the Koonadgin population of *A. anfractuosa* were used to estimate mating system parameters. With the exception of Adh-1 (two alleles), three alleles were present for each locus in the three populations investigated. Mating system estimates were based on 10–11 families per population with a mean of 14.5–15.5 progeny per family.

Estimates based on different loci may be correlated if those loci show linkage disequilibrium. To test for associations between loci, Burrows composite measure of linkage disequilibria (Δ_{AB}) was calculated for all possible pairs of loci within populations with χ^2 tests for significance according to Weir (1996), using the computer package POPGENE (Yeh and Boyle 1997).

Maximum likelihood estimates of single locus (t_s) and multilocus (t_m) outcrossing rates (Ritland and Jain 1981) were based on the mixed-mating model of Brown and Allard (1970) with maternal genotypes inferred from progeny arrays (Ritland 2002). Correlation of outcrossed paternity (r_p) and the correlation of outcrossing among families (r_t) was estimated according to the correlated matings model (Ritland 1989). The inbreeding coefficient of maternal parents (F_m) was also calculated. All mating system parameters were estimated using the computer program MLTR version 2.4 (available from K. Ritland). Standard errors for the population estimates of t_s, t_m, r_p, and $t_m - t_s$ were based on 500 bootstraps with re-sampling among maternal plants (Ritland 2002).

Results

Genetic variation within populations

Two loci (*Gdh-1* and *Sdh-1*) were monomorphic in both populations of *A. sciophanes* and in five of the six populations of *A. anfractuosa*. Eight loci (*Adh-1*, *Est-1*, *Gpi-2*, *Lap-1*, *Mdh-2*, *Me-1*, *Pgm-1*, *Pgd-1*) had relatively uniform allele frequencies across all populations of both species. Four loci (*G6pdh-1*, *Idh-1*, *Mnr-1*, and *Pgi-1*) showed large allele frequency differences between the two species.

The population means and species values for allelic richness (A), percentage polymorphic loci (P), gene diversity (H_e) and heterozygosity (H_o) are presented in Table 2. At the population level there was significantly lower gene diversity ($P = 0.040$) in *A. sciophanes* compared with the *A. anfractuosa*, while there was non-significant but lower allelic richness ($P = 0.204$) and heterozygosity ($P = 0.270$), and no difference in polymorphism. At the species level, estimates for polymorphism, allelic richness, gene diversity and heterozygosity were all lower in *A. sciophanes*.

Population fixation indices (F_{IS}) were positive and significantly greater than zero in all populations of both species (Table 2). These results indicate a significant excess of homozygotes in the

seed progeny of both *A. sciophanes* and *A. anfractuosa*. This was reflected in Tests for Hardy–Weinberg equilibrium at individual loci, which showed that in the Wundowlin *A. sciophanes* population and four *A. anfractuosa* populations less than 50% of loci were in Hardy–Weinberg equilibrium. The exceptions were the Barbalin Rd *A. sciophanes* and Morrison Rd *A. anfractuosa* populations where 73 and 58% of loci were in Hardy–Weinberg Equilibrium. There were no significant differences ($P = 0.194$) in F_{IS} estimates between the two species.

Population and species differentiation

Divergence between *A. sciophanes* populations ($D = 0.039$, $F_{ST} = 0.055$) and *A. anfractuosa* populations ($D = 0.042$, $F_{ST} = 0.066$) was relatively low and was only slightly greater between *A. anfractuosa* populations even though they cover a far greater geographic range (Figure 1). Indirect gene flow estimates between populations within species indicated significant interpopulation genetic exchange was likely for both species, at least until the recent isolation of populations due to land clearing and habitat fragmentation. There was only a moderate level of differentiation evident between the two species based on the mean genetic distance between populations within the two species (Table 3).

Phylogenetic relationships shown by the maximum likelihood methods combined with geographical distribution gave no indication of any obvious phylogeographic pattern in *A. anfractuosa* (Figure 2). This is perhaps not surprising given the overall low level of differentiation seen among the populations of this species.

Bottleneck assessment

Under both IAM and SMM assumptions the standardised differences test, sign test and Wilcoxon signed rank test indicated no significant deviation from mutation-drift equilibrium with excess H_e and no evidence of recent bottlenecks for the *A. sciophanes* Barbalin Rd population or the *A. anfractuosa* Chiddarcooping and Farina Rd populations (Table 4). In the *A. sciophanes* Wundowlin Rd population only the standardised differences test showed a significant deviation from mutation-drift equilibrium with excess H_e under SMM assumptions. This seems likely to be a Type II error given the lack of support from the other tests under both IAM and SMM. In the other four *A. anfractuosa* populations we detected a significant deviation from mutation-drift equilibrium with excess H_e for at least two tests. The most compelling evidence for a recent bottleneck came from the Weira Rd data where all three tests indicated a significant H_e excess under IAM and two out three tests indicated a significant H_e excess under SMM. Less convincing evidence was available for Morrison Rd where two out of three tests indicated a significant H_e excess under IAM but none of the tests were significant under SMM. Similarly for Koonadgin all three tests indicated a significant H_e excess under IAM but none of the tests were significant under SMM. While for Scott Rd all three tests indicated a significant H_e excess under SMM but none of the tests were significant under IAM.

Table 3. Nei's unbiased genetic distance (D), F_{ST} and gene flow estimates (Nm) among populations of *Acacia sciophanes* and *Acacia anfractuosa*

Population group	D (SE)	F_{ST}	Nm
Acacia sciophanes	0.039	0.055	4.249
Acacia anfractuosa	0.042 (0.004)	0.066	3.505
A. sciophanes vs. *A. anfractuosa.*	0.116		

Mating system

The estimates of Burrow's composite linkage disequilibrium Δ_{AB} among the two loci (*Lap-1*, *Pgi-1*) in the *A. sciophanes* Wundowlin population, three loci (*Adh-1*, *Lap-1*, *Pgi-1*) in the Farina Rd population of *A. anfractuosa* and four loci (*Adh-1*, *Lap-1*, *Pgi-2*, *Pgm-1*) in the Koonadgin population of *A. anfractuosa* indicated no significant disequilibrium in any of the populations. Therefore all loci selected for estimating mating system parameters in each population were used.

Multilocus and mean single locus estimates of oucrossing rates for the Wundowlin population of *A. sciophanes* and the Farina Rd and Koonadgin populations of *A. anfractuosa* are given in Table 5. Both populations of *A. anfractuosa* had generally high outcrossing rates although only the multilocus estimate for the Farina Rd population was not significantly different from 1. The outcrossing rate in the Wundowlin *A. sciophanes* population

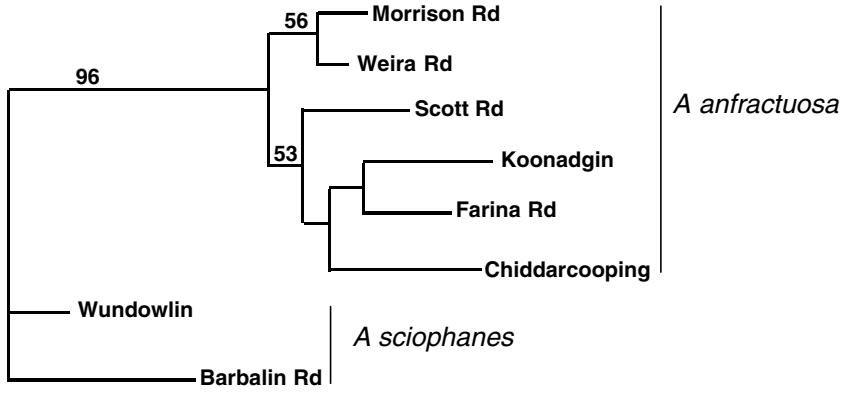

Figure 2. Phylogenetic relationships among populations of *A. sciophanes* and *A. anfractuosa* based on a continuous character maximum likelihood tree.

Table 4. Bottleneck assessment for all populations of *Acacia sciophanes* and six populations of *Acacia anfractuosa* based on three tests of heterozygosity (H_e) excess over heterozygosity expected at mutation-drift equilibrium (H_{eq}) under infinite allele model (IAM) and the stepwise mutation model (SMM) assumptions

Population/species	Probability of heterozygosity (H_e) excess over heterozygosity expected at mutation-drift equilibrium (H_{eq})					
	Sign test		Standardised differences test		Wilcoxon test	
	IAM	SMM	IAM	SMM	IAM	SMM
A. sciophanes						
Wundowlin	0.355	0.079	0.410	0.003*	0.850	0.093
Barbalin Rd	0.493	0.366	0.365	0.055	0.577	0.320
A. anfractuosa						
Farina Rd	0.221	0.399	0.050*	0.260	0.151	0.791
Weira Rd	0.006*	0.076	0.000*	0.035*	0.001*	0.009*
Morrison Rd	0.196	0.313	0.012*	0.275	0.011*	0.469
Chiddarcooping	0.450	0.081	0.100	0.094	0.151	0.301
Scott Rd	0.092	0.025*	0.165	0.015*	0.233	0.034*
Koonadgin	0.018*	0.350	0.009*	0.280	0.034*	0.423

*Significant difference between H_e and H_{eq} ($P < 0.05$).

($t_m = 0.61$) was noticeably lower than the outcrossing rates for the two *A. anfractuosa* populations ($t_m = 0.86$, 0.85). The single locus outcrossing estimates were significantly lower than the multi locus estimates in the Wundowlin *A. sciophanes* population ($t_m - t_s = 0.07$, SE 0.03) and the Koonadgin *A. anfractuosa* population ($t_m - t_s = 0.05$, SE 0.02) suggesting significant levels of bi-parental inbreeding.

The correlations of outcrossed paternity r_p (the probability that sibs shared the same father) were moderate and all were significantly greater than zero (Table 5). The Wundowlin *A. sciophanes* population had a noticeably higher correlated paternity ($r_p = 0.25$) than the two *A. anfractuosa* populations at Farina Rd ($r_p = 0.06$) and Koonadgin ($r_p = 0.15$). The average "paternal mating pool", the estimated number of sires per plant, was low in the *A. sciophanes* population (4 plants) but higher in both *A. anfractuosa* populations, particularly the Farina Rd population where there were estimated to be 4–5 times more "fathers" per family than in the *A. sciophanes* population. This was calculated as $1/r_p$ and is the number of sires that give rise to r_p assuming all sires have equal probabilities and consecutive matings are independent. The correlation of outcrossing among families r_t was low in all three populations and indicated low

Table 5. Mating system estimates for the largest population of *Acacia sciophanes* and two populations of *Acacia anfractuosa*

Population	Number of mature plants	F_m (SE)	t_m (SE)	t_s (SE)	$t_m - t_s$ (SE)	r_p (SE)	r_t (SE)	Paternal neighbourhood size ($1/r_p$)
Acacia sciophanes								
Wundowlin	272	−0.44 (0.28)	0.61 (0.13)*	0.54 (0.13)*	0.07 (0.03)	0.25 (0.08)*	0.15 (0.06)	4–5
Acacia anfractuosa								
Farina Rd	>1000	−0.49 (0.25)	0.89 (0.09)	0.89 (0.07)	0.01 (0.03)	0.06 (0.01)*	0.12 (0.08)	16–17
Koonadgin	≈400	−0.33 (0.27)	0.85 (0.04)*	0.81 (0.04)*	0.05 (0.02)	0.15 (0.05)*	0.08 (0.02)	6–7

*Significantly less than 1 for t_m and t_s, and significantly greater than zero for r_p ($P < 0.05$).
F_m, inbreeding coefficient of maternal parents; t_m, multilocus outcrossing rate; t_s, single locus outcrossing rate; r_p, multilocus correlation of outcrossed paternity; r_t, correlation of outcrossing among families.

levels of variation in outcrossing rates among families in both *A. sciophanes* and *A. anfractuosa*.

Fixation indices for the maternal plants in the three populations were negative and despite the relatively high standard errors (Table 5) were significantly lower than the positive population estimates based on seed progeny (Table 2).

Discussion

Genetic variation and population divergence

Our findings indicate that at both the population and species levels *A. sciophanes* has lower levels of genetic diversity than its widespread and common sister species *A. anfractuosa*. Given the restricted distribution of *A. sciophanes* and the highly fragmented landscape in which it now occurs there are a number of key causal factors that may explain the lower levels of genetic diversity and their likely association with rarity. These include intrinsic causes such as relatively recent speciation and associated founder events, directional selection associated with habitat specificity and persistence in small populations that have been subjected to significant climatic instability throughout the Pleistocene (see Hopper 1979). They may also include anthropogenic causes related to recent habitat destruction, degradation and fragmentation that have lead to reductions in population size and increased population isolation.

Previous phylogenetic studies based on cp DNA variation indicated that *A. sciophanes* and *A. anfractuosa* are sister species (Byrne et al. 2001). They do not share cp DNA haplotypes, are differentiated by five mutations and show 0.072% sequence divergence indicating that the divergence between these two species probably occurred relatively recently in the mid to late Pleistocene (Byrne et al. 2001). Lower levels of genetic variation in *A. sciophanes* could therefore be attributed to founder events associated with recent speciation (Gottleib 1981; Loveless and Hamrick 1988). However, we consider this unlikely to be a key causal factor recognising that the timescale indicated for the origin of *A. sciophanes* based on cp DNA divergence may be well beyond the timescale in which founder events following speciation would be expected to operate.

Habitat specificity also appears to be a less likely explanation for the low genetic variability in *A. sciophanes*. Both *A. sciophanes* and *A. anfractuosa* occupy extremely similar habitats being found on deep yellow sandy soils in floristically diverse low woodland and scrub heath communities. Similarly our findings do not provide any clear support for habitat loss and landscape fragmentation and associated recent reduction in population size as likely causes of the reduced genetic variation. It is not possible to determine population sizes prior to land clearing so it is difficult to estimate the magnitude of population size decline, if any. However, it is possible to assess whether a population has undergone a recent bottleneck by assessing whether expected heterozygosity (H_e) is larger than the heterozygosity expected at mutation-drift equilibrium (H_{eq}) (see

Cornuet and Luikart 1996; Luikart and Cornuet 1998). We found no clear evidence that either of the *A. sciophanes* populations had been through a recent bottleneck and had therefore been subjected to any significant recent reduction in numbers. In contrast in *A. anfractuosa* there was strong evidence for a recent bottleneck in the Weira Rd population and some indication that the Morrison Rd, Scott Rd and Koonadgin populations may also have been through recent bottlenecks. It is interesting to note that all of these populations are associated with road reserves and may have been subjected to significant disturbance associated with road and track maintenance over the last 50 years. These findings indicate that if anything recent habitat disturbance regimes seem to be influencing population genetic processes more in *A. anfractuosa* than in *A. sciophanes*.

We have excluded recent speciation, habitat specificity and recent habitat destruction and fragmentation as likely critical factors contributing to lower levels of genetic diversity in *A. sciophanes*. In addition there is little difference between *A. sciophanes* and *A. anfractuosa* in a range of reproductive and ecological attributes such as reproductive success, seed predation and dispersal, seed bank longevity and impact of edaphic factors on seedling growth (Buist et al. 2002). We therefore suggests that *A. sciophanes* is most likely an intrinsically rare species that has the reproductive and ecological capabilities to persist in this landscape as localised and relatively small populations and that this is the most likely explanation for its lower levels of genetic diversity compared with *A. anfractuosa*.

Similar patterns in rare and geographically restricted *Acacia* taxa, both in terms of high overall levels of genetic diversity and a reduction in genetic diversity in the rare species, have also been found in two other species complexes within the same geographic region; the *A. acuminata* complex, (Broadhurst and Coates 2002) and the *A. microbotrya* complex (Elliott et al. 2002). *Acacia* sp. 'Dandaragan' in the *A. microbotrya* complex occurs as a single large population over a range of topographies and soil types (Elliott et al. 2002). Like *A. sciophanes* it is extremely localised in distribution but unlike that species it persists as a very large population of some 25,000 reproductively mature individuals. This population is geographically close but allopatric to *A. microbotrya*. Phylogenetic analyses based on allozymes and morphological studies indicate that *A.* sp. ''Dandaragan'', although closely related to the two *A. microbotrya* variants, is a distinct species and has most probably recently diverged from *A. microbotrya* on the westerly margins of that species range (Elliot et al. 2002). In this case the lower levels of genetic variability were attributed to founder events associated with relatively recent divergence. In contrast reduced genetic variability in the rare and geographically restricted *A. oldfieldii* in the *A. acuminata* complex appears to be due to factors other than relatively recent divergence and speciation. Like *A. sciophanes* the level of genetic variation in *A. oldfieldii* is significantly lower than that observed in any of its common widespread sister taxa. Despite its clear morphological affinities with other members of the *A. acuminata* complex both cp DNA and allozyme studies indicate that *A. oldfieldii* is markedly divergent from its sister taxa suggesting extended isolation from the rest of the complex and that a late Tertiary separation is likely (Broadhurst and Coates 2002; Byrne et al. 2002). Thus like *A. sciophanes*, *A. oldfieldii* also appears to be an intrinsically rare species but the reduced levels of genetic variation are apparently associated with a much longer history of populations subjected to climatic fluctuations since the late Tertiary.

Our findings support the view that it is important to assess the genetic consequences of rarity in a phylogenetic context (Gitzendanner and Soltis 2000; Byrne et al. 2001). Despite lower levels of genetic diversity in *A. sciophanes* compared with its widespread closest relative these levels are amongst the highest recorded for *Acacia* species where population based allozyme variation has been investigated (Table 6). This includes comparisons with relatively widespread species such as *A. melanoxylon* and *A. burkittii*. Levels of genetic diversity in *A. sciophanes* are also high when compared to other long-lived woody shrub genera such as those summarised in Hamrick et al. (1992) and Young and Brown (1996). The high levels of genetic diversity in *A. sciophanes*, and to a lesser extent other rare *Acacia* species such as *A.* sp. Dandaragan and *A. oldfieldii*, compared with more distantly related widespread congeners brings into question the validity of such congeneric comparisons in large and phylogenetically diverse genera such as *Acacia*. For example, Maslin (2001)

Table 6. Estimates of allozyme variation (H_e, gene diversity) at the population level in rare/geographically restricted Australian *Acacia* taxa compared with common/widespread taxa

Species group	Rare/restricted	Common/widespread	Gene diversity (H_e)	Source
Western Australian *Acacia* species				
Anfractuosa	*A. sciophanes*		0.310 (0.080)	
		A. anfractuosa	0.380 (0.010)	
Microbotrya	*A.* sp. Dandaragan		0.109 (0.040)	1
		A. microbotrya	0.172 (0.013)	1
Acuminata	*A. oldfieldii*		0.168 (0.006)	2
		A. acuminata	0.238 (0.014)	2
		A. burkittii	0.287 (0.007)	2
		A. acuminata (narrow phyllode)	0.291 (0.020)	2
		A. acuminata (small seed)	0.245 (0.031)	2
Anomala	*A. anomala* (Chittering)		0.209 (0.027)	3
	A. anomala (Kalamunda)		0.079 (0.020)	3
Eastern Australian *Acacia* species				
		A. aulacocarpa	0.112 (0.033)	4
		A. auriculiformis	0.146 (0.020)	5
		A. crassicarpa	0.141 (0.010)	5
		A. mangium	0.017 (0.004)	6
		A. mearnsii	0.179 (0.006)	7
		A. melanoxylon	0.208 (0.009)	8

The Western Australian rare – common comparisons involve sister taxa.
1. Elliott et al. (2002); 2. Broadhurst and Coates (2002); 3. Coates (1988); 4. McGranahan et al. (1997); 5. Moran et al. (1989a); 6. Moran et al. (1989b); 7. Searle et al. (2000); 8. Playford et al. (1993).

documents 986 *Acacia* taxa in Australia in 10 different infrageneric groups. Gitzendanner and Soltis (2000) suggest that differences between congeneric species pairs are likely to be independent for each genus. The genetic diversity comparisons among *Acacia* species presented here suggest that some caution is needed in this approach and that in large genera comparisons are probably better carried out between species within the same section of the genus but not necessarily across more phylogenetically distant groups. Nonetheless, although this approach may be less appropriate for larger genera, such as *Acacia*, it should not detract from the value of making such comparisons in smaller less diverse genera. In those cases there are already a number of studies where valuable contributions have been made towards an improved understanding of patterns of genetic variation in rare plant species.

Mating system

Analysis of the mating system of both *A. sciophanes* and *A. anfractuosa* indicates a mixed mating system for both species with significant levels of selfing in *A. sciophanes* ($t_m = 0.61$) and low levels of selfing in *A. anfractuosa* ($t_m = 0.89$, 0.85). There is also evidence for bi-parental inbreeding in *A. sciophanes* and the smaller Koonadgin population of *A. anfractuosa*. These data contrast with the relatively few mating system studies on other *Acacia* species where very high outcrossing rates with insignificant levels of selfing have been found (Moran et al. 1989a; Casiva et al. 2004).

Generally high outcrossing rates in *Acacia* species are supported by comparative studies on seed set following selfing or outcrossing. These indicate that the majority of *Acacia* species investigated in those studies are either highly self incompatible or at least partially self incompatible (Bernhardt et al. 1984; Kenrick and Knox 1989; Morgan et al. 2002) while only one species was shown to be self compatible. Various phases of self incompatibility are postulated for *Acacia* species, both pre- and post-zygotic, with Kenrick et al. (1986) demonstrating pre-zygotic incompatibility in *A. retinoides*. In other cases post-zygotic effects appear to be prevalent. Post-zygotic mechanisms evidenced by higher abortion of immature seeds following selfing have been demonstrated in *A. baileyana* (Morgan et al. 2002). Such mechanisms are also likely to influence the outcomes of mating

system studies as a significant proportion of selfed seed in these species will be aborted before it could be assessed in a mating system analysis. It is therefore possible that the levels of selfing evident in *A. anfractuosa* and particularly *A. sciophanes* may not necessarily be unusual in *Acacia* populations. Rather the operation of post-zygotic lethals causing seed abortion may vary significantly between populations and species, but are less evident in these two species. Whether purging of lethals (Carr and Dudash 2003) is more likely in populations of *A. sciophanes* and *A. anfractuosa* is open to speculation. However, it is of interest to note that these *Acacia* species, like many other plant species in south-west Western Australia are likely to have undergone significant range expansion and contraction with past climatic events. Under these conditions frequent reduction in population size and increased inbreeding may have been conducive to purging of lethals from their populations.

In relation to *A. sciophanes* and its conservation, a key issue from this study is the significantly reduced outcrossing and increased correlated paternity compared with *A. anfractuosa*. Both measures indicate that in the smaller *A. sciophanes* population pollination events involve more selfing and bi-parental inbreeding, and each maternal plant is pollinated by fewer fathers. This trend is also evident in the smaller and lower density (Table 1) of the two *A. anfractuosa* populations although not as pronounced. Thus population size and plant density may be contributing to the mating system differences observed between these two species. In addition, we have also suggested that *A. sciophanes* is likely to be an intrinsically rare species so that an ability to tolerate increased selfing in small populations may not only be an important factor in its persistence but also suggests that it may be less susceptible to the impacts of habitat loss and fragmentation.

Pollination within *Acacia* is generally considered to involve a system of generalist entomophily (Bernhardt et al. 1984), as the flowering behaviour, floral presentation and polyad presentation of most species favour such a system. Pollen in *Acacia* is presented in the form of polyads (fused pollen grains) and all insects that forage for polyads will have immediate access to respective stigmas. Although we have not carried out any direct pollination studies in these two species the mating system studies suggest that insect pollinators spend more time on individual plants and move less frequently between different plants in the smaller *A. sciophanes* populations than the larger *A. anfractuosa* populations. Given this it is noteworthy that not only are the *A. sciophanes* populations smaller but the density of plants is noticeably lower (Table 1). In this situation insect pollinator behaviour is also likely to lead to fewer visits between plants and increased selfing.

Increased inbreeding due to increased selfing and bi-parental inbreeding, and the potential for increased inbreeding associated with increased correlated paternity are evident in *A. sciophanes* compared with *A. anfractuosa*. Although increased correlated paternity does not have an immediate effect in terms of inbreeding it will in subsequent generations given that over time fewer fathers have contributed to seed production. Nonetheless, it is not clear whether inbreeding depression is a consequence and whether there are any differences in levels of inbreeding depression between the two species. For example, there is no difference between the two species in terms of reproductive output measured as seeds produced per plant and proportion of inflorescences that produce fruit (Buist et al. 2002). Indeed studies on other rare *Acacia* species such as *A. cochlocarpa* and *A. aprica* indicate that reproductive output has not been significantly impaired in their small populations and that the greatest immediate threat to population persistence is the ability of seedlings to establish under regimes of severe competition from environmental weeds (Yates and Broadhurst 2002). Clearly there are effects, other than seed production, that could also be indicative of inbreeding depression in other parts of the life-cycle but these have not been investigated in these species. However, we do have strong evidence that inbred progeny are being eliminated as plants progress from seedling to reproductive maturity. Seedling fixation indices are high, ranging from 0.26 to 0.46 in *A. sciophanes* and 0.32 to 0.46 in *A. anfractuosa*. These values show that significant numbers of inbred seed are being produced in all populations of both species. In comparison, consistently lower and negative levels of gene fixation are evident in the maternal parents (Table 3) indicating that there is an excess of outcrossed (heterozygous) individuals in reproductively mature plants.

Conclusions and implications for conservation

In summary these findings demonstrate reduced genetic diversity and increased selfing in the rare *A. sciophanes* compared with its common close relative *A. anfractuosa*. We also found increased bi-parental inbreeding and increased correlated paternity in *A. sciophanes* although this appears to be partly influenced by population size regardless of the species. In contrast there is little difference between these two species in a range of reproductive and ecological attributes such as reproductive success, seed predation and dispersal, seed bank longevity and impact of edaphic factors on seedling growth (Buist et al. 2002). We suggest that *A. sciophanes* is likely to be an intrinsically rare species and that the low levels of genetic diversity and increased selfing are a feature of a species that has the ability to persist in a few localised small populations in the floristically diverse low woodland and scrub heath communities typical of this region. Despite recent extensive habitat destruction our comparative study provided no clear evidence that such events have contributed to lower genetic diversity and increased selfing in *A. sciophanes* and we believe that its ability to survive in small populations may not only be an important factor in its persistence as a rare species but also suggests that it may be less susceptible to the impacts of habitat loss and fragmentation. This of course depends critically on the maintenance of habitat that will allow the two small populations to reproduce and recruit in the relatively undisturbed but restricted areas of suitable habitat such as the 176 ha Nature Reserve that contains most of the larger population 1.

Although land clearing has largely ceased in this area habitat degradation associated with the impacts of inappropriate fire regimes, track or road maintenance and weed invasion are likely to have an ongoing impact on the *A. sciophanes* populations. The key to this species survival will be the amelioration of habitat disturbance and in particular strategies for implementing appropriate fire regimes and controlling invasive weeds. Although this species appears likely to be able to persist in relatively small populations any significant decline in numbers will also require management intervention such as population augmentation or translocation based on *ex situ* seed collections.

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