

Genetic divergence and the mating system in the endangered and geographically restricted species, *Lambertia orbifolia* Gardner (Proteaceae)

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Population genetic structure and the mating system were investigated in the endangered plant *Lambertia orbifolia*. This species is geographically restricted with two disjunct groups of populations. Twelve out of 19 allozyme loci were polymorphic and four were suitable for mating system studies. Levels of genetic variation within populations were comparable to other long-lived woody shrub endemics. Genetic divergence between population groups was very high ($D = 0.252$) and the F_{ST} over all populations was 0.441. Gene flow estimates within population groups were low even though the maximum geographical distance between any pair of populations is 15 km and could be attributed to the localized movement of bird pollinators. Mating system studies on four populations showed consistently low levels of outcrossing, compared with other Proteaceae. Correlations of outcrossed paternity were moderately high and all were significantly greater than zero. Values ranged from $r_p = 0.33$, in the two largest and very dense populations, to the highest value of $r_p = 0.54$ in a smaller low-density population. The current population genetic structure in *L. orbifolia* is probably the result of local extinction of intervening populations because of Pleistocene climatic change and increased aridity, and extended isolation of the two remnants. It is proposed that the phylogenetically distinct Narrikup population group be recognized as a separate conservation unit and be given high priority for conservation action.

Keywords: conservation units, endangered species, gene flow, genetic structure, habitat fragmentation, mating system.

Introduction

Naturally fragmented and disjunct population systems are a feature of the rich and diverse flora in south-west Australia. A significant proportion of the plant species is likely to be relictual and probably had wider, more continuous distributions during favourable climatic regimes up to the early Pleistocene. Increased aridity and climatic instability during the Pleistocene are considered to be major factors in the localized extinction and fragmentation of many plant species in this region (Hopper *et al.*, 1996).

The level of geographical separation between population groups within these fragmented species suggests that recent gene flow, either by long-distance seed dispersal or pollen movement, has been limited or

absent for a long period. Reduction or absence of gene flow, and fluctuations in population size caused by climatic instability, are likely to lead to significant interpopulation divergence. Studies to date on rare and geographically restricted species from south-west Australia support this view. Significant genetic divergence among populations associated with clear geographical separation is a feature of a range of species in a number of flowering plant families such as Mimosaceae (Coates, 1988), Myrtaceae (Moran & Hopper, 1983, 1987; Sampson *et al.*, 1988), Proteaceae (Coates & Sokolowski, 1992) and Stylidiaceae (Coates, 1992).

A major confounding factor in assessing the evolutionary patterns observed in the south-west flora are the broad-scale effects of recent widespread land clearing over the last 150 years and the associated extensive habitat fragmentation. These events also have very significant implications for the conservation of this flora. Plant species in south-west Australia are now affected by

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rapid and recent habitat fragmentation superimposed upon a system of historically fragmented and isolated populations (see Hopper *et al.*, 1996).

The genetic consequences of recent habitat fragmentation generally relate to the effects of reduced population size and increased geographical isolation among populations (Barrett & Kohn, 1991; Young *et al.*, 1997). In species with a continuous metapopulation structure, fragmentation is predicted to lead to a loss of genetic variation and increased divergence among populations. These effects result from increased drift, increased inbreeding, reduced gene flow between populations and local population extinction (Young *et al.*, 1997). Limited studies to date support the prediction that habitat fragmentation and associated reduction in remnant size result in loss of genetic variation (see Prober & Brown, 1994). The population genetic consequences of recent habitat fragmentation on already fragmented population systems are likely to be similar, although in those species with historically small isolated populations, loss of genetic variation and increased inbreeding may be more severe. Importantly, Young *et al.* (1997) point out that the effects of fragmentation have only been studied in a few plant species covering a limited range of ecological, reproductive and demographic traits and more studies are needed before more specific generalizations can be developed.

Population fragmentation, whether historical or resulting from recent habitat disturbance, might also be expected to affect the mating system, particularly in small isolated populations (Sampson *et al.*, 1996; Young *et al.*, 1997). There is only limited evidence to date to indicate a relationship between increased inbreeding and fragmentation (Young *et al.*, 1997). However, Sampson *et al.* (1996) have shown that, in a number of species where fragmentation is also associated with habitat disturbance, lower levels of outcrossing are a feature of

highly disturbed populations. In these cases habitat disturbance was linked to loss of pollinators, change in pollinator behaviour and the introduction of new pollinators.

Lambertia orbifolia is an endangered large woody shrub known from only seven populations that have a significant disjunct distribution. Like many Proteaceae, flowers are protandrous and geitogamous pollination is likely to be common. It is bird-pollinated with bird movement relatively restricted within populations (Whitaker & Collins, 1997). Recent habitat fragmentation has affected all populations of *L. orbifolia* both in terms of reduction in size and increased isolation. The current population structure could be caused by recent fragmentation of a more continuous system of populations but appears more likely to be the result of historical fragmentation associated with Pleistocene climate change.

The aim of this study was to investigate the population genetic structure and the mating system in relation to both historical patterns and recent effects of fragmentation.

Materials and methods

Distribution, population sampling and site descriptions

Lambertia orbifolia has a disjunct distribution, with populations occurring in two discrete areas approximately 200 km apart in south-west Western Australia (Fig. 1). Five populations are located in the Scott River Plains area east of Augusta and two populations are found 30 km north of Albany near Narrikup.

The five Scott River Plains populations are found on sandy soils over ironstones in winter-wet depressions and seasonally inundated wetlands. These areas

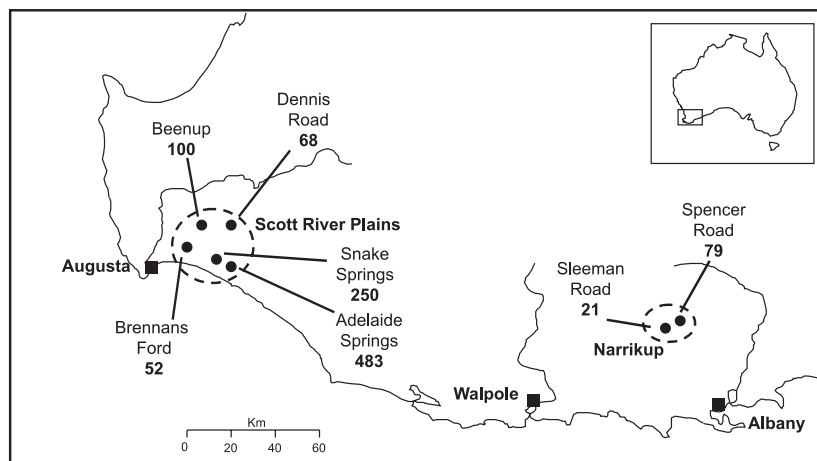


Fig. 1 Distribution and number of mature plants for all populations of *Lambertia orbifolia*.

comprise floristically diverse low woodland and scrub heath communities. The coastal sites at Adelaide Springs and Snake Springs comprise relatively large, dense populations of large shrubs to small trees up to 5 m in height. In the three smaller inland populations at Brennans Ford, Dennis Rd and Beenup, *L. orbifolia* occurs as clumps of several plants or as dispersed individuals. The two smallest populations at Narrikup are found on sandy loams over laterite in open, low forest with a species composition different from that found in the Scott River Plains area. Plant density in both Narrikup populations is similar to the large coastal Scott River Plains populations. Population sizes are shown in Fig. 1.

The level of habitat disturbance resulting from land clearing differs markedly between populations. In the populations at Adelaide Springs, Snake Springs and Brennans Ford, vegetation disturbance is limited to the population periphery and adjacent areas. At Beenup both past grazing and disease caused by the root rot fungus *Phytophthora cinnamomi* has resulted in some habitat degradation, particularly in adjacent areas. Road verge populations at Dennis Rd, Spencer Rd and Sleeman Rd are characterized by significant habitat degradation associated with weed invasion and loss of the associated flora. The Spencer Rd population is also infected with *Phytophthora* root rot and a canker fungus, and has shown significant decline from 139 plants at the time of sampling in 1994–95 to 79 plants in 1998.

Seed was collected from at least 10 plants per population in conjunction with larger seed collections for *ex situ* germ plasm storage. Follicles contain one to two seeds each and were collected from inflorescences scattered over the plant. Follicles become ripe within 8–9 months (Sage, 1994) and then dehisce. Seed samples therefore generally represent the products of fertilization for the year of sampling.

Isozyme electrophoresis

Up to 25 seeds per plant were germinated on moistened filter paper. The number of seeds used per population was dependent upon sample size requirements for mating system studies. Seedlings with recently emerged radicals, minus the bulk of the large endosperm, provided the best material. Preparation of this material and the isozyme methods, using the Helena Laboratory cellulose acetate plate electrophoresis system, have been described previously by Coates (1988).

Eleven enzyme systems were assayed: aspartate aminotransferase (AAT, EC 2.6.1.1), alcohol dehydrogenase (ADH, EC 1.1.1.1), esterase (EST, EC 3.1.1.-), glucose phosphate isomerase (GPI, EC 5.3.1.9), glutamate dehydrogenase (GDH, EC 1.4.1.2), leucine aminopepti-

dase (LAP, EC 3.4.11.1), malate dehydrogenase (MDH, EC 1.1.1.37), malic enzyme (ME, EC 1.1.1.40), menadi-one reductase (MDR, EC 1.6.99.22), phosphoglucosomutase (PGM, EC 5.4.2.2) and shikimate dehydrogenase (SDH, EC 1.1.1.25). Nineteen zones of activity were scored and each zone was assumed to represent a single locus. Sixteen polymorphic loci were detected. Their genetic interpretation was based on segregation patterns of progeny arrays from open-pollinated families from the four populations used in mating system studies. The locus *Me-1* was only resolved in the Narrikup populations and therefore not used in the population genetic studies but was used in the mating system analysis of the Spencer Rd population.

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. Differences in genetic variation between the Scott River Plains and Narrikup populations were determined using a nested ANOVA. 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.

Interpopulation divergence was estimated using Nei (1978) unbiased genetic distance (D) and F_{ST} (Wright, 1978). Nei (1978) unbiased genetic distance (D) was calculated between all populations and then the mean D determined for populations within the Scott River Plains area, the Narrikup area and for all populations. It was also used to construct a UPGMA phenogram. Separate estimates of F_{ST} were made for the Scott River Plains populations, Narrikup populations and for all populations. The indirect estimate of gene flow (Nm) was calculated over all populations and within the two population groups based on the relationship $F_{ST} = 1/[4Nm + 1]$ (Wright, 1978).

Single-locus diversity measures, F_{IS} , Nei's D , Hardy–Weinberg test, F_{ST} , Nm and the UPGMA phenogram were determined using the computer program POPGENE (Yeh & Boyle, 1997). Phylogenetic relationships among populations were also investigated by the restriction maximum likelihood method using gene frequency data. Multiple datasets (500) were generated by bootstrapping, a phylogeny determined for each dataset by the restriction maximum likelihood method and a consensus tree generated. The computer programs SEGBOOT, CONTML and CONSENSE in PHYLIP 3.5 (Felsenstein, 1995) were used for this analysis.

Mating system analysis

Four loci were used to estimate mating system parameters in four populations: Adelaide Springs, Snake Springs and Beenup from the Scott River Plains area and Spencer Rd from the Narrikup area. Three loci (*Est-1*, *Est-3* and *Sdh-2*) were used to estimate mating system parameters in the Scott River Plains area whereas *Est-1*, *Est-3* and *Me-1* were used to estimate mating system parameters in the Narrikup population. Estimates were based on 13–18 families per population with a mean of 11.5–20.5 progeny per family.

Mating system estimates based on different loci may be correlated if those loci show linkage disequilibrium. To test for associations between loci, Burrows's 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 (1990), using the computer package POPGENE (Yeh & Boyle, 1997).

Maximum likelihood estimates of single-locus (t_s) and multilocus (t_m) outcrossing rates (Ritland & Jain, 1981) were based on the mixed-mating model of Brown & Allard (1970) with maternal genotypes inferred from progeny arrays. Correlation of outcrossed paternity (r_p) was estimated according to the sibling-pair 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 0.9 (available from K. Ritland). Standard errors for the population estimates of t_s , t_m , r_p , F and $t_m - t_s$ were based on 500 bootstraps with resampling among maternal plants (see Sun & Ritland, 1998).

Results

Genetic variation within populations

Three loci (*Aat-2*, *Mdh-1* and *Pgi-1*) were monomorphic across all populations. Five loci (*Pgm-1*, *Pgm-2*, *Aat-1*, *Est-2* and *Mnr-3*) were monomorphic in six out of seven populations, with a rare allele found at each locus in the remaining population. There was also a fixed difference between the two population groups, Scott River Plains and Narrikup, at the *Mnr-2* locus. The other 10 loci were polymorphic in most populations. Six loci (*Adh-1*, *Est-1*, *Gdh-1*, *Lap-1*, *Sdh-1* and *Sdh-2*) had relatively uniform allele frequencies across all populations. Three loci (*Est-3*, *Mnr-1* and *Mdh-1*) showed significant allele frequency differences between the Narrikup and Scott River Plains populations (Fig. 2), whereas at the *Aat-3* locus, a unique allele was found at a frequency of 0.20 in the Spencer Rd population.

The population means for allelic richness (A), percentage polymorphic loci (P), gene diversity (H_e) and heterozygosity (H_o) are presented in Table 1. Polymorphism, allelic richness, gene diversity and heterozygosity were all moderately lower in the Scott River Plains populations than in the Narrikup populations, although the differences were not significant. The mean for all population estimates, $P = 41.1$, $A = 1.5$, $H_e = 0.11$, $H_o = 0.06$, is relatively low for animal-pollinated outcrossing species (see Hamrick & Godt, 1989).

Population fixation indices (F_{IS}) were positive in all populations and significantly greater than zero in the Snake Springs, Brennans Ford, Dennis Rd and Spencer Rd populations. These results indicate a general excess of homozygotes in the seed progeny.

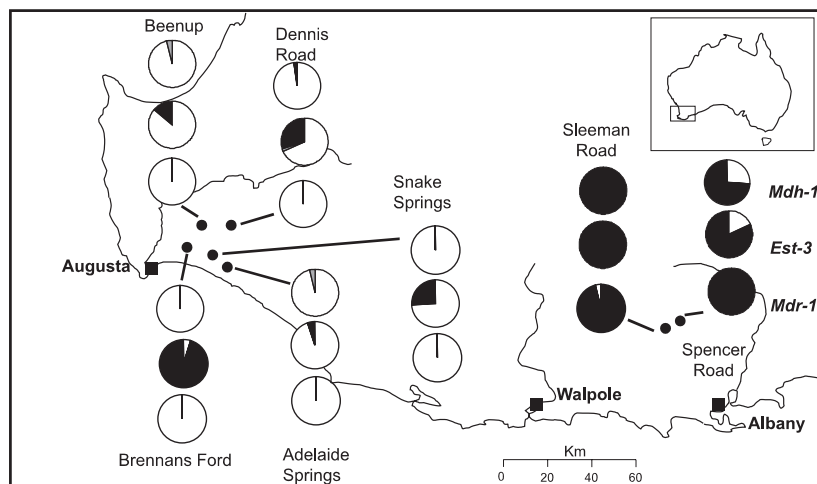


Fig. 2 Allele frequencies for the loci *Mdh-1*, *Est-3* and *Mnr-1* in all populations of *Lambertia orbifolia*.

Table 1 Population sizes, genetic diversity statistics†, fixation indices (F_{IS}) for all populations of *Lambertia orbifolia*

Population	Number of mature plants	A (SE)	P (SE)	H_e (SE)	H_o (SE)	F_{IS} (SE)
<i>Scott River Plains</i>						
Adelaide Springs	483	1.4 (0.1)	36.8	0.08 (0.04)	0.06 (0.03)	0.24 (0.15)
Snake Springs	250	1.4 (0.1)	36.8	0.10 (0.04)	0.05 (0.03)	0.31 (0.12)
Brennans Ford	52	1.4 (0.1)	31.6	0.08 (0.04)	0.04 (0.02)	0.29 (0.14)
Beenup	≈100	1.7 (0.2)	52.6	0.11 (0.04)	0.07 (0.03)	0.26 (0.16)
Dennis Rd	68	1.4 (0.1)	35.0	0.13 (0.05)	0.06 (0.02)	0.47 (0.11)
Mean		1.5 (0.1)	38.5 (3.6)	0.10 (0.02)	0.06 (0.01)	0.31 (0.03)
<i>Narrikup</i>						
Spencer Rd	79	1.6 (0.2)	47.4	0.16 (0.05)	0.08 (0.03)	0.37 (0.09)
Sleeman Rd	21	1.6 (0.1)	47.4	0.10 (0.04)	0.07 (0.03)	0.29 (0.15)
Mean		1.6 (0.1)	47.4	0.13 (0.03)	0.08 (0.01)	0.33 (0.04)
Mean all populations		1.5 (0.05)	41.1 (3.0)	0.11 (0.01)	0.06 (0.01)	0.32 (0.03)

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

Population differentiation

Divergence between the Scott River Plains and Narrikup populations, measured by F_{ST} and Nei's unbiased genetic distance (D), was extremely high (Table 2). The average F_{ST} among all populations was 0.441 and the mean D was 0.142. The genetic distance between the Narrikup and Scott River Plains populations was $D = 0.252$. The level of divergence, measured by D , among populations within both areas was low. However, the F_{ST} values were relatively high suggesting that significant genetic differentiation is present even among geographically close populations.

Gene flow estimates within population groups were low even though the maximum geographical distance between any pair of populations is 15 km in the Scott River Plains area. The overall estimated level of gene flow among all populations was extremely low ($Nm = 0.317$) and is indicative of long-term historical isolation.

The analysis of phylogenetic relationships among all populations, based on UPGMA or maximum likelihood methods, gave identical tree topologies (Fig. 3). The

Narrikup and Scott River Plains populations were clearly separate lineages. Coastal populations in the Scott River Plains area (Adelaide Springs, Snake Springs) grouped together and were separated from the more inland populations at Beenup and Dennis Rd.

Mating system

The estimates of Burrows's composite linkage disequilibrium Δ_{AB} among the four loci (*Est-1*, *Est-3*, *Sdh-2* and *Me-1*) indicated no significant disequilibrium in any of the populations. Therefore all four loci were used in estimating mating system parameters.

Multilocus and mean single-locus estimates of outcrossing rates for four populations (Adelaide Springs, Snake Springs, Beenup and Spencer Rd) are given in Table 3. All four populations had outcrossing rates significantly less than one. The outcrossing rate in the small Narrikup population (Spencer Rd, $t_m = 0.41$) was significantly lower than the mean outcrossing rate for the larger Scott River Plains populations ($t_m = 0.61$). There was no relationship between population size and outcrossing rate within the Scott River Plains populations. Although there was no significant difference between the mean single-locus and multilocus outcrossing estimates the single-locus estimates were moderately lower in all populations. Low levels of biparental inbreeding may be contributing to this trend.

The correlations of outcrossed paternity r_p (the probability that sibs shared the same father) were moderately high and all were significantly greater than zero (Table 3). Values ranged from $r_p = 0.33$, in the two largest and very dense populations at Adelaide Springs and Snake Springs, to the highest value of $r_p = 0.54$ in the smaller, low-density population at

Table 2 Nei's unbiased genetic distance (D), F_{ST} and gene flow estimates (Nm) within and between the two population groups in *Lambertia orbifolia*

Population group	D (SE)	F_{ST} (SE)	Nm
Scott River Plains	0.042 (0.009)	0.238	0.801
Narrikup	0.049	0.143	1.494
All	0.142 (0.024)	0.441	0.317
Narrikup vs. Scott River Plains	0.252		

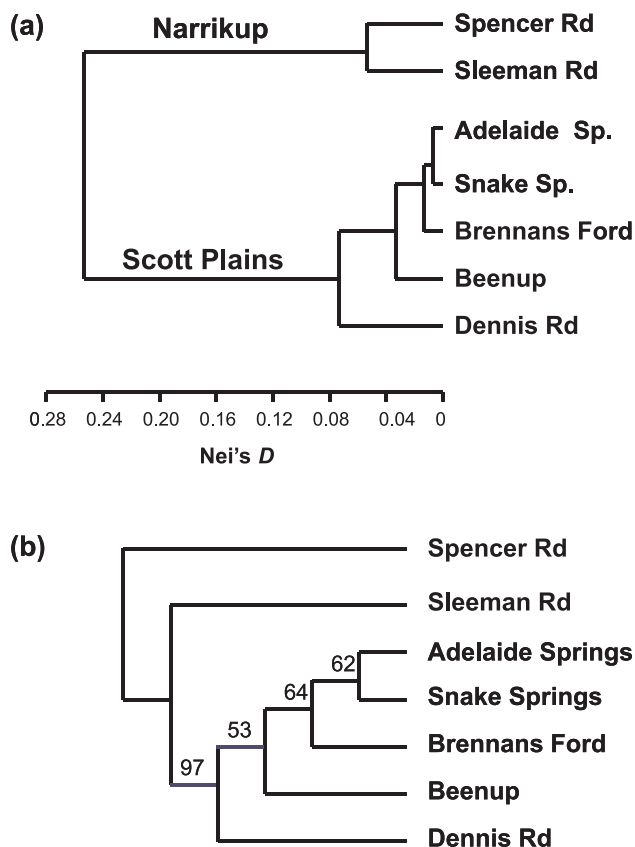


Fig. 3 (a) UPGMA clustering based on Nei's unbiased genetic distance. (b) Restriction Maximum Likelihood tree based on allele frequency. Bootstrap percentages are shown for each branch.

Beenup. The smallest relatively high-density road verge population at Spencer Rd had a correlated paternity ($r_p = 0.35$) similar to the two largest populations. The 'paternal mating pool', the estimated number of sires per plant, was low in all populations, ranging from three to four plants in the larger more dense populations to one to two plants in the Beenup population. These low overall values probably reflect the size of the plants and the limited movement of the bird pollinators between plants. These data indicate that the size of the paternal mating pool is associated with population density.

The average fixation index for the maternal plants ($F_m = 0.09$, Table 3) is significantly lower than the average population estimate based on seed progeny ($F_{IS} = 0.32$, Table 1). This indicates selection favouring heterozygous individuals as plants mature from seedling to adult.

Discussion

Genetic structure and population divergence

The high levels of genetic divergence and population phylogenetic analyses indicate that the two disjunct population groups at Scott River Plains and Narrikup have been historically isolated for an extended period of time. There is little evidence to suggest, from these data, that the current distribution of populations is the result of recent habitat fragmentation. In addition, large areas of undisturbed vegetation still occur between the two population groups and, despite extensive surveys, no further populations have been located. The possibility

Table 3 Population size and mating system estimates† for four populations of *Lambertia orbifolia*: (d) indicates dense populations where individual plants were usually separated by 1–3 m, (s) indicates plants were sparsely distributed, usually separated by 3 m or more

Population	Number of mature plants	F_m (SE)	t_m (SE)	t_s (SE)	$t_m - t_s$ (SE)	r_p (SE)	Paternal neighbourhood size ($1/r_p$)
<i>Scott River Plains</i>							
Adelaide Springs (d)	483	0.28 (0.16)	0.53 (0.08)*	0.46 (0.08)*	0.07 (0.03)	0.33 (0.06)*	3–4
Snake Springs (d)	250	0.01 (0.03)	0.72 (0.10)*	0.63 (0.09)*	0.09 (0.04)	0.33 (0.08)*	3–4
Beenup (s)	100	0.04 (0.08)	0.57 (0.11)*	0.55 (0.11)*	0.02 (0.02)	0.54 (0.18)*	1–2
Mean		0.11 (0.09)	0.61 (0.06)*	0.55 (0.05)*	0.06 (0.02)	0.40 (0.07)*	
<i>Narrikup</i>							
Spencer Road (d)	56	0.03 (0.08)	0.41 (0.08)*	0.39 (0.08)*	0.02 (0.02)	0.35 (0.07)*	2–3

† F_m , inbreeding coefficient of maternal parents; t_m , multilocus outcrossing rate; t_s , single-locus outcrossing rate; r_p , correlation of outcrossed paternity.

*Significantly less than 1 for t_m and t_s , and significantly greater than zero for r_p ($P < 0.05$).

that the two road verge populations are the result of recent introductions from the Scott River Plains area can also be discounted, given the level of the genetic differences between the population groups.

The long-term effects of Pleistocene climatic change and increased aridity on the south-west Australian flora suggest that the current population genetic structure in *L. orbifolia* is the result of local extinction of intervening populations, and extended isolation of the two remnants. Local extinction and range contraction, resulting from climate change, are particularly evident in a number of large forest *Eucalyptus* species that occur in areas between the two *L. orbifolia* population groups (Wardell-Johnson & Coates, 1996).

All populations of *L. orbifolia* appear to have been affected to some extent by recent habitat fragmentation. This has resulted in a reduction and ongoing decline in population size, particularly in the road verge populations at Dennis Rd, Spencer Rd and Sleeman Rd. From theoretical predictions and a number of empirical studies the smaller *L. orbifolia* populations might be expected to show reduced levels of polymorphism and allelic richness (Barrett & Kohn, 1991; Young *et al.*, 1997). However, comparisons between populations showed no association of decreased population size with a reduction in any measure of genetic variation (Table 2). The smallest isolated populations at Narrikup had marginally higher levels of allelic richness and polymorphism than the larger Scott River Plains populations. It is possible that the initial reduction in population size has not been great enough to cause a detectable loss in genetic variation. The Narrikup populations may also have been much larger until relatively recently. Land clearing has been more widespread and ongoing adjacent to the Narrikup populations than in the Scott River Plains area. It is also likely that insufficient time has elapsed for drift and inbreeding, because of small population size, to have any major effect, particularly as the life cycle of these plants is probably 25 years or longer.

The level of genetic diversity observed in *L. orbifolia* ($H_e = 0.11$), although low when compared with other long-lived, woody angiosperms (Hamrick *et al.*, 1992), is similar to or moderately higher than other members of the Proteaceae. Allozyme studies on species from a number of genera within the Proteaceae such as *Banksia* (Coates & Sokolowski, 1992; Carthew, 1993; Sampson *et al.*, 1994), *Grevillea* (Ayre *et al.*, 1994) and *Persoonia* (Krauss, 1997) have revealed relatively low levels of genetic variability within populations. Krauss (1997) suggested that the low levels of genetic variation in *Persoonia mollis*, which is killed and regenerates from seed after fire, resulted from localized extinction and founder events caused by frequent fires in a fire-prone

habitat. He also proposed that other Proteaceae and non-Proteaceae with similar life histories may be affected in the same way. Although Krauss (1997) points out that further studies are required, limited investigations to date do not support his proposal; for example, studies on other genera with analogous life histories that occur in fire-prone habitats, such as *Acacia* (Mimosaceae), *Daviesia* (Papilionaceae) and *Eremaea* (Myrtaceae), indicate relatively high levels of genetic diversity (Coates, 1988; Coates & Hnatiuk, 1989; Young & Brown, 1998).

The evolutionary history of the populations provides the most probable explanation for the low levels of genetic variation found in *L. orbifolia*. Fluctuations in population size and local extinctions, associated with climatic instability over a relatively long period of time, are all likely to be contributing factors. Comparisons with other long-lived, woody shrub endemics also show that the levels of genetic diversity observed in *L. orbifolia* are not unusually low for a geographically restricted species (Hamrick *et al.*, 1992; Young & Brown, 1998).

Mating system

Analysis of the mating system of *L. orbifolia* showed significant levels of selfing in the four populations studied (Table 3). The difference between multilocus and mean single-locus estimates of outcrossing rate in the high-density Adelaide Springs and Snake Springs populations suggest that at least some biparental inbreeding may be operating at those two sites. This finding is not unexpected given the size and density of those populations. In the other populations inbreeding can be almost entirely attributed to self-fertilization. Bird pollinator behaviour observations indicate that most bird movements are less than 5 m which would involve many intraplant flower visits covering only two or three mature plants (Whitaker & Collins, 1997). This behaviour would ensure significant levels of selfing, and some biparental inbreeding in dense populations with limited seed dispersal.

Mating systems have now been investigated in a number of trees and shrubs of the Australian Proteaceae. Flowering is generally seasonal and often involves mass flowering of many large inflorescences over a relatively restricted period of time. Most of these studies have focused on the genus *Banksia*. With the exception of *Banksia brownii* (Sampson *et al.*, 1994), high outcrossing rates were typical of most populations (see Carthew *et al.*, 1988; Coates & Sokolowski, 1992). Very high outcrossing rates were observed in another member of the family, *Persoonia mollis* (Krauss, 1994). In contrast, consistently low levels of outcrossing were

found in all four populations of *L. orbifolia*. One possible explanation is that the low overall outcrossing rates result from the flowering phenology of this species. *Lambertia orbifolia* flowers throughout the year with flowering peaks in January to February and May to July. Rather than limited mass flowering associated with high pollinator activity, inflorescences are more sparsely distributed over the plants and pollinator activity is likely to be lower, more territorial, but consistent throughout the year.

Interpopulation comparisons of mating system parameters revealed a notably lower level of outcrossing rate in the Spencer Rd population compared with the Scott River Plains populations (Table 3). This was also associated with significantly lower levels of fruit-set (Sage, 1994). Unlike the other three populations, this narrow road-side population has high levels of habitat disturbance. The associated vegetation is degraded in terms of loss of a significant number of plant species, weed invasion and disease caused by the root rot fungus *Phytophthora cinnamomi*. Reduced levels of outcrossing as a result of habitat disturbance have been observed in a number of species, including other members of the Proteaceae such as *Banksia cuneata* and *B. tricuspis* (see Sampson *et al.*, 1996) and *Grevillea barklyana* (Ayre *et al.*, 1994). This was attributed primarily to loss of pollinators or change in pollinator behaviour, concomitant with the loss of many associated plant species important for the maintenance of pollinator populations.

Correlations of outcrossed paternity were high and significantly greater than zero in the four populations studied. Each maternal plant appears to be pollinated by a small number of fathers. This interpretation is consistent with pollinator observations that also indicate the majority of fathers are probably near-neighbours. The small number of potential fathers is clearly reflected in the estimates of the average paternal mating pool (see Sun & Ritland, 1998). Even in the largest population at Adelaide Springs the estimated paternal mating pool is no greater than four.

There was no obvious relationship between population size and correlated paternity. However, a higher correlated paternity was found in the low-density Beenup population. A number of studies have shown that population density will influence pollinator movements and thus mating system parameters (see Karron *et al.*, 1995). Young & Brown (1998) found that in *Daviesia mimosoides* both population size and density were negatively associated with correlated paternity. They proposed that reduced density may increase intraplant pollinator movement and increase the likelihood of a pair of flowers on the same plant receiving the same outcrossed pollen. Estimates of the paternal mating pool indicate that most of the outcrossed pollen

in the low-density Beenup population comes from only one or two fathers.

Conclusions and implications for conservation

Lambertia orbifolia appears to consist of two historically isolated and independently evolving population remnants. It seems likely that these population groups have been isolated and stable for a long period of time. Demographic and genetic data support the view that the Scott Plains and Narrikup populations should be recognized as distinct units for conservation and that they can probably be defined as Evolutionary Significant Units (ESUs) (see Moritz, 1994; Vogler & Desalle, 1994).

The effects of recent habitat fragmentation on the historically disjunct and fragmented population system found in *L. orbifolia* were less than predicted from theoretical and other empirical studies. The smaller Narrikup population at Spencer Rd did have a reduced outcrossing rate which could be attributed both to the small size of the population and increased habitat disturbance. However, there was no evidence to suggest that the smaller populations had less genetic variability. As discussed above, this may be because the decrease in population size was not great enough to affect the level of genetic variation. Also, there may have been insufficient time since recent fragmentation for genetic drift and increased isolation to be effective in reducing genetic variation. Another, more speculative possibility, is that these populations have always been relatively small and isolated, with the capacity to maintain high levels of genetic variability in association with extensive balanced polymorphisms. Such a system would probably also result in a substantial genetic load (see James, 1996).

Lambertia orbifolia has an IUCN ranking of endangered. However, given the large number of critically endangered plants in Western Australia it has a relatively low priority for conservation and recovery actions. Small population size, increased inbreeding and threats posed by disease and habitat degradation indicate that the phylogenetically distinct Narrikup populations are critically endangered. It is proposed that the two population groups be considered separate conservation units and the Narrikup populations be given a high priority for conservation action.

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