Heredity **86** (2001) 489–496 *Received 30 August 2000, accepted 3 January 2001*

Low neighbourhood size and high interpopulation differentiation in the endangered shrub *Grevillea iaspicula* McGill (Proteaceae)

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Mating system parameters and genetic diversity were examined for five populations of the endangered shrub *Grevillea iaspicula* (Proteaceae). Controlled pollinations show that *G. iaspicula* has an effective self-incompatibility system and little potential for agamospermy. This is reflected in uniformly high multilocus outcrossing rates ($t_m = 0.96$–1.00). However, average paternal diversity within open-pollinated sibships is low ($r_p = 0.31$–0.54), suggesting that mating within populations is quite restricted. Despite the small size of most populations (four of the five populations studied have fewer than 20 reproductive individuals) the species still possesses moderate to high allelic richness ($A = 1.6$–2.5). Interpopulation genetic differentiation is high ($D = 0.04$–0.32), suggesting that gene flow is limited, even among populations separated by only a few kilometres.

Keywords: allozyme, controlled pollination, endangered, genetic diversity, *Grevillea*, mating system.

Introduction

Plant mating systems incorporate both genetic and ecological factors that affect reproduction and are primary determinants of the level of genetic variation within a population and how this variation is distributed among individuals. They are the result of evolutionary pressures both for and against inbreeding, such that there is a balance between genetic load, fitness and fecundity. Specifically, mating systems are influenced by factors such as plant size and floral display, flowering phenology, pollination syndrome, and pre- and post-zygotic incompatibility (Stephenson, 1981; Goldingay & Carthew, 1998). They can also be affected by population parameters such as population size (Buza *et al*., 2000), density (Watkins & Levin, 1990) and isolation (Young & Brown, 1999).

Understanding the limitations that mating systems place on demographic, genetic and evolutionary dynamics is of particular interest for species endangered by habitat loss, degradation and fragmentation. In such cases, rapid changes in population size, density, isolation and pollinator guilds may mean that species mating systems are maladapted for their current circumstances. For example, for self-compatible but normally outcrossing species, increased self-fertilization will lead to rapid expression of genetic load and reduced fitness. Alternatively, for self-incompatible species, if reduced population size is accompanied by the loss of *S*-allele richness then mate availability and fecundity may be limited (DeMauro, 1993; Vekemans *et al*., 1998). Recently, it has been shown that a combination of such factors is often implicated in reducing the viability of small populations of endangered plants (Oostermeijer, 2000; Young *et al*., 2000).

This study used controlled pollinations and allozyme markers to investigate the mating system and genetic structure of *Grevillea iaspicula* McGill. (Proteaceae). *Grevillea iaspicula* is a species for which population persistence is of critical concern, as the few remaining populations have become fragmented due to land clearing for agriculture and population sizes have declined owing to grazing, flooding and possibly competition with invasive species (Briggs & Leigh, 1990; Butler *et al*., 1991). Four main questions are addressed: (1) Is *G. iaspicula* self-compatible? (2) Do mating patterns change with population size and isolation? (3) How much genetic variation is present in *G. iaspicula* populations? (4) How much genetic differentiation is there among populations?

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Materials and methods

Study species

The Australian shrub *Grevillea iaspicula* (Proteaceae) is listed as nationally endangered and is known from eight populations occurring in remnant eucalypt vegetation on rocky limestone outcrops (ANZECC, 1999). The populations surround the town of Wee Jasper, in south-eastern New South Wales (Fig. 1) and range in size from 20 to several hundred individuals. The vegetation at all but one of these sites has been highly disturbed by the conversion of land for stock grazing. It is predicted that the species will disappear from natural environments within 10–20 years if present land use and other threats, such as grazing and competition with invasive exotics, persist (Briggs & Leigh, 1996).

The plants are straggling to erect bushy shrubs, 1.2–2.5 m in height (occasionally up to 4 m) with light green, leathery leaves. They generally have terminal, or occasionally subterminal inflorescences, that are often pendant and always branched. There are about 12 cream-yellow flowers per branch with red protruding styles. Fruits are thin-walled ovoid follicles about 10–15 mm long that split opposite the style to release, generally, two grey-brown arillate seeds about 8 mm in length. McGillivray (1993) provides a full taxonomic treatment.

Given the flower coloration, *G. iaspicula* is probably bird pollinated (Olde & Marriott, 1995). Field observations have identified five honeyeater species visiting the flowers: *Lichenostomus penicillatus* (White-plumed Honeyeater), *L. leucotis* (White-eared Honeyeater), *L. chrysops* (Yellow-faced Honeyeater), *Acanthorhychus tenuirostris* (Eastern Spinebill), and *Phylidonyris novaehollandiae* (New Holland Honeyeater) (personal observations). The European honeybee (*Apis mellifera*) also visits the species.

Controlled pollinations

In *Grevillea* species, pollen adheres to modified style-ends (pollen presenters) following anther dehiscence. As a result, many species are protandrous (Carolin, 1961; Johnson & Briggs, 1975). Therefore, artificial manipulation of flowers is relatively easy as pollen can be removed from the pollen presenter and subsequently applied to the stigmatic papillae once receptive. Inflorescences of *G. iaspicula* may contain up to 10 branches (McGillivray, 1993), with flowers on a branch generally opening after flowering on the preceding branch has finished. Ovaries of fertilized flowers swell within five to seven days after pollination, whereas unfertilized flowers wither and fall from the rachis at this stage (Harriss & Whelan, 1993). Fruit development takes between six and 14 weeks, depending on environmental conditions.

Pollinations were conducted from August–October 1998 and seed were harvested in January 1999. The experimental design consisted of two populations (Slyney 1 and 2), five plants per population and five treatments applied at the inflorescence level (see Table 1 for a description of these treatments). Each treatment was replicated five times within a plant. Within each inflorescence, all flowers from one, two or three branches were pollinated; other branches were removed, resulting in an average of 20 flowers manipulated per inflorescence. The total number of inflorescences in the experiment was 250. Following seed set, both seed and fruit were collected in mesh bags.

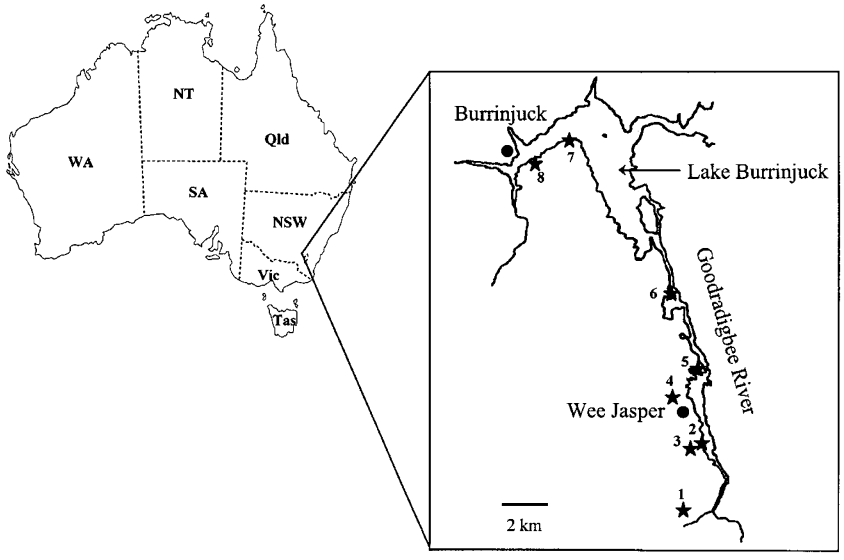

Fig. 1 Distribution of *Grevillea iaspicula* populations around the townships of Wee Jasper and Burrinjuck, New South Wales. Populations are labelled 1–8: 1, Punchbowl; 2, Slyney 1; 3, Slyney 2; 4, Ashley; 5, Carey (inaccessible); 6, Cathles (inaccessible); 7, Dutton's Bluff ; and 8, Burrinjuck.

Table 1 Pollination treatments applied to inflorescences of *Grevillea iaspicula*. Inflorescences in closed treatments were 'bagged' prior to opening and subsequently to exclude all floral visitors. All pollen manipulation was conducted using a paintbrush. Outcross treatments were crossed using pollen from two other plants within the population

Treatment type and name	Description
Open-pollination treatment	
Open	Open to all natural pollinators
Closed-pollination treatments	
Agamospermy	Pollen removed from pollen presenters within 2–3 days of opening
Spontaneous autogamy	No manipulation
Geitonogamy	Pollen removed and flowers brushed with pollen from a different flower on the same plant within 48 h of opening
Outcross	Pollen removed and flowers brushed with pollen from another plant in the same population within 48 h of opening

The effects of pollination treatment, population and maternal plant were assessed in relation to fruit and seed set, using an analysis of variance (ANOVA), with pollination treatment as a fixed effect and population and maternal plant as random effects, using the SAS General Linear Models Procedure for uneven sample sizes (PROC GLM) at $P < 0.05$ (SAS Institute, 1990).

Fruit and seed set

During the 1998 fruiting season, between two and 19 inflorescences with swollen ovules were bagged on 7–15 plants from each of five populations. One, population (Burrinjuck) was excluded because the combined effects of drought and grazing severely limited its reproductive output and two other populations (Carey and Cathles) were excluded due to inaccessibility. In January 1999, after seed were shed, bags were collected and the number of mature fruit, the number of filled seed and the number of flower scars per inflorescence were recorded. Differences in fruit per flower and filled seed per flower were analysed as a nested ANOVA, with population and maternal plant as random effects, using the SAS PROC GLM at $P < 0.05$ (SAS Institute, 1990). All populations had damage to the mesh bags used for seed collecting as a result of bird predation. Effects of this predation were analysed as a nested ANOVA with predation (bag torn or intact) as the fixed effect and population and maternal plant as random effects, using SAS PROC GLM as above.

Allozyme markers

Seed were collected from up to eight inflorescences (depending on availability) from six to eight adult plants from the same five populations of *G. iaspicula* as were assessed for fruit and seed set. Allozyme assays using starch gel electrophoresis were conducted on 8–15 seed sampled as evenly as possible across inflorescences. Seed were allowed to imbibe for 12 h, seed coats were removed and individual seed were ground in five drops of pH 9.0 borate buffer containing 1 mg mL^{-1} dithiothreitol (DTT) and 20 mg mL^{-1} polyvinylpyrrolidone (PVP, mol. wt. = 40 000). The resulting solution was absorbed onto 6 × 4 mm filter paper wicks and inserted into horizontal 9.6% starch gels, with morpholine citrate buffer. The gels were run at 48 mA for 5 h and were then sliced horizontally into three 2 mm slices. Each slice was stained for a different enzyme. The enzyme systems examined were shikimate dehydrogenase (SDH, E.C. 1.1.1.25), malate dehydrogenase (MDH, E.C. 1.1.1.37), phosphoglucomutase (PGM, E.C. 5.4.2.2), triose-phosphate isomerase (TPI, E.C. 5.3.1.1), isocitrate dehydrogenase (IDH, E.C. 1.1.1.41) and phosphogluconate dehydrogenase (PGD, E.C. 1.1.1.43). In total, eight putative loci were examined.

Estimation of mating system and genetic diversity parameters

Estimates for multilocus and single locus outcrossing rates (t_m and t_s, respectively), correlation of outcrossed paternity (r_p; Ritland, 1989) and maternal fixation indices (F_{IS}) were calculated from allozyme data using MLTR version 0.9 (Ritland, 1994). Standard errors for these estimates were based on 100 bootstraps with resampling among maternal plants. The divergence between estimates of allele frequencies in the pollen pool and their corresponding estimates in the maternal pool was estimated using the Pollak estimator (F_k; Waples, 1989) following Young & Brown (1999).

Polymorphism (P), allelic richness (A), expected and observed heterozygosity (H_e and H_o, respectively) were

estimated for all populations. Genetic divergence among populations was measured using Wright's (1951) F_{ST}. Nei's (1972) genetic distance was also calculated among all possible pairs of populations. A Mantel test (Mantel, 1967) was conducted to assess correlation between the genetic and geographical distances among populations. All diversity and differentiation statistics were calculated using POPGENE (Yeh *et al.*, 1998). Gene flow among populations, as measured by *Nm* (the number of migrants per generation), was estimated using Slatkin & Barton's (1989) modification to Slatkin's (1985) private alleles method for a sample size of 50 individuals.

Results

Controlled pollinations

Both fruit and seed set differed significantly among the pollination treatments ($F_{4,36} = 50.19$, $P < 0.0001$ for fruit and $F_{4,36} = 8.57$, $P < 0.0001$ for seed). Open and outcross treatments were comparable and exhibited significantly higher fruit and seed set than the other three treatments for which seed set was uniformly close to zero (Fig. 2).

Populations did not differ significantly for either fruit set or seed set; nor was there any population-by-treatment interaction. There were significant effects of maternal plant for fruit set ($F_{9,36} = 2.21$, $P < 0.05$) but not for seed set.

Fruit and seed set

There was no significant difference in open-pollinated fruit and seed set among the five populations studied. In general, fruit and seed production per flower were low (Table 2). All populations experienced high levels of damage to seed collecting bags (range of 0.56–0.98) and damaged bags contained significantly fewer seed than undamaged ones ($F_{1,79} = 49.92$, $P < 0.0001$). However, the damage did not significantly affect the number of mature fruit collected. This is reflected in the higher fruit numbers, as opposed to seed. There were significant among-plant differences in both fruit and seed set for some populations (Table 2).

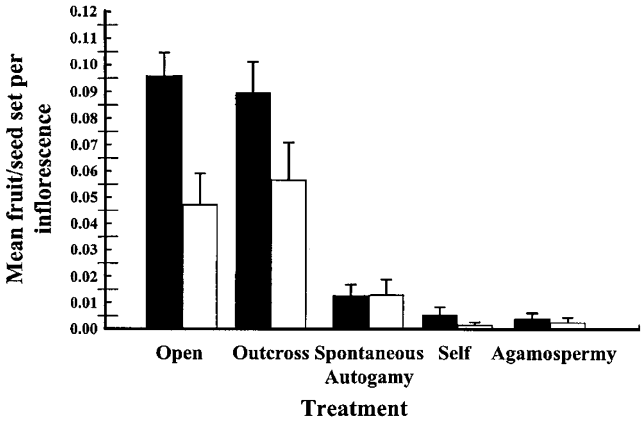

Fig. 2 Mean fruit (solid bars) and seed (open bars) set per *Grevillea iaspicula* inflorescence under different pollination treatments. Errors bar represents one standard error.

Genetic variation and differentiation

There was 100% polymorphism across the eight allozyme loci assayed although the degree of polymorphism within populations differed ($P = 50$–100%; Table 3). Allelic richness was moderate to high (Table 3) for all populations regardless of size, ranging from $A = 1.63$–2.50 alleles per locus, with the lowest value observed in the Dutton's Bluff population. Additionally, both observed and expected heterozygosities were high (mean $H_o = 0.30$ and mean $H_e = 0.27$; Table 3), though again Dutton's Bluff was the least variable in this respect.

Wright's F_{ST} was high at 0.204 (SE = 0.040). Genetic distances among populations were also generally high, with a minimum of $D = 0.04$ (between Punchbowl and Slyney 2) and a maximum of $D = 0.32$ (between Slyney 1 and Ashley). Average genetic distances of the popula-

Table 2 Levels of seed and fruit set for *G. iaspicula* populations

	Proportion of seed per flower		Proportion of fruit per flower	
Population	Mean (SE)	Effect of maternal plant	Mean (SE)	Effect of maternal plant
Punchbowl	0.14 (0.03)	$F_{6,34} = 1.36$	0.12 (0.02)	$F_{6,34} = 1.32$
Slyney 1	0.06 (0.02)	$F_{7,40} = 12.01$***	0.21 (0.02)	$F_{7,40} = 2.35$
Slyney 2	0.08 (0.02)	$F_{10,65} = 2.13$	0.19 (0.01)	$F_{10,65} = 3.79$**
Ashley	0.07 (0.01)	$F_{13,99} = 7.60$***	0.17 (0.01)	$F_{13,99} = 3.93$***
Dutton's Bluff	0.10 (0.02)	$F_{11,79} = 2.78$*	0.19 (0.02)	$F_{11,79} = 0.92$

Asterisks indicate significance corrected for multiple tests using the Bonferroni procedure.
Experiment-wise error rates are *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 3 Genetic variation and differentiation statistics for *Grevillea iaspicula* populations based on eight allozyme loci

Population	Size†	*P* (SE)	*A* (SE)	H_o (SE)	H_e (SE)	*D* (SE)
Punchbowl	11	100	2.13 (0.13)	0.30 (0.02)	0.24 (0.02)	0.10 (0.03)
Slyney 1	10	88	2.13 (0.29)	0.35 (0.02)	0.31 (0.02)	0.19 (0.06)
Slyney 2	17	88	2.50 (0.42)	0.32 (0.02)	0.29 (0.02)	0.09 (0.03)
Ashley	95	75	2.13 (0.35)	0.35 (0.02)	0.30 (0.02)	0.19 (0.05)
Dutton's Bluff	~15‡	50	1.63 (0.26)	0.20 (0.02)	0.22 (0.02)	0.16 (0.03)
Mean	—	80.2 (9.53)	2.10 (0.16)	0.30 (0.03)	0.27 (0.02)	0.15 (0.03)

P, percent polymorphic loci; *A*, average number of alleles per locus; H_o, observed heterozygosity; H_e, expected heterozygosity; *D*, average genetic distance to all other populations.
†Number of reproductive individuals.
‡Not all plants were accessible for sampling.

tions from all other populations are presented in Table 3. The estimate of interpopulation gene flow, based on the frequencies of nine private alleles, was very low ($Nm = 0.136$). There was no significant relationship between geographical and genetic distances between populations.

Mating system parameters

Multilocus outcrossing rates were consistently high for the five populations assayed ($t_m = 0.96$–1.00) regardless of population size (Table 4). Values of $t_m - t_s$ were all low (Table 4); this is indicative of low levels of biparental inbreeding. Maternal and progeny fixation indices were uniformly low (Table 4).

The correlations of outcrossed paternity estimates were all significantly greater than zero ($r_p = 0.31$–0.54; Table 4) suggesting that a large proportion of open-pollinated seed are full-sibs. Neighbourhood sizes based on $1/r_p$ (Sun & Ritland, 1998) averaged 2–3 plants. Overall, F_k estimates were relatively low, suggesting little deviation between allele frequencies in the pollen pool and their corresponding frequencies in the maternal pool, though there was some variation among the populations.

Discussion

Grevillea species have highly variable mating systems. For example, *G. linearifolia* is predominantly self-incompatible but *G. longifolia* is self-compatible (Hermanutz *et al*., 1998). In this study, the lack of both fruit and seed set from self-pollinations and the uniformly high multilocus outcrossing rates observed indicate that *G. iaspicula* is self-incompatible. Gametophytic incompatibility is the only form of self-incompatibility that has so far been reported in the Proteaceae (Kalinganire *et al*., 2000) and families generally exhibit a single form of incompatibility (Matton *et al*., 1994). Therefore, it is likely that gametophytic incompatibility is also the system operating in *G. iaspicula*.

The observation of low maternal fixation indices for all *G. iaspicula* populations suggests that plants within the populations are not inbred. The negative fixation values for progeny may be indicative of disassortative mating that might be driven by the incompatibility system. However, the large standard errors associated with the progeny estimates suggest they should be interpreted cautiously.

The estimates of correlated paternity were substantially higher than those observed for many other species.

Table 4 Mating system parameters of *Grevillea iaspicula* populations: multilocus outcrossing rate (t_m), an indicator of current levels of biparental inbreeding ($t_m - t_s$), levels of correlated paternity (r_p), neighbourhood size ($1/r_p$; Sun & Ritland, 1998), inbreeding coefficients for both the maternal and progeny cohorts (F_{IS}), and the divergence of allele frequencies between the pollen and maternal pools (F_k)

					F_{IS} (SE)		
Population	t_m (SE)	$t_m - t_s$ (SE)	r_p (SE)	$1/r_p$ (SE)	Maternal	Progeny	F_k (SE)
Punchbowl	1.00 (0.00)	0.05 (0.00)	0.40 (0.01)	2.51	0.00 (0.00)	−0.15 (0.08)	0.08
Slyney 1	0.96 (0.00)	0.03 (0.00)	0.44 (0.01)	2.27	0.01 (0.00)	−0.13 (0.05)	0.10
Slyney 2	0.96 (0.00)	0.04 (0.00)	0.46 (0.01)	2.19	0.06 (0.00)	−0.09 (0.08)	0.08
Ashley	1.00 (0.00)	0.04 (0.00)	0.31 (0.01)	3.24	0.00 (0.00)	−0.15 (0.11)	0.12
Dutton's Bluff	0.97 (0.01)	0.06 (0.01)	0.54 (0.02)	1.84	0.07 (0.01)	0.07 (0.19)	0.03
Mean	0.98 (0.01)	0.04 (0.01)	0.43 (0.04)	2.41 (0.26)	0.03 (0.02)	−0.09 (0.05)	0.08 (0.02)

For example, the extent of correlated paternity between flowers on the same plant of *Mimulus guttatus* was $r_p = 0.20$ (Ritland, 1989), $r_p = 0.26$ within fruits of *Eucalyptus rameliana* (Sampson, 1998), $r_p = 0.20$ for *Daviesia mimosoides* and $r_p = 0.25$ for *D. suaveolens* (Young & Brown, 1998) and $r_p = 0.19$ for *Centaurea solstitialis*, although levels up to $r_p = 0.64$ were reported in some populations (Sun & Ritland, 1998). However, they were similar to estimates from another bird pollinated Proteaceae, *Lambertia orbifolia*, for which $r_p = 0.39$ (Coates & Hamley, 1999).

High levels of correlated paternity and low derived estimates of neighbourhood size may arise in two ways. First, they could be due to mating between pairs or small groups of plants. This has also been suggested for *L. orbifolia* where bird movements are generally less than five metres and restricted to only two or three mature plants (Coates & Hamley, 1999). Alternatively, there may be a few males within populations that are particularly successful at siring seed. These mating scenarios have very different implications for effective population size. However, since low F_k estimates are also observed in *G. iaspicula* populations, the former explanation is more likely in this instance (Young & Brown, 1999). In other words, since *G. iaspicula* populations show little allozyme divergence between pollen and maternal pools, it is unlikely that a single plant is, or few plants are, dominating paternity.

For *G. iaspicula*, seed set is pollinator dependent, since the exclusion of pollinators from inflorescences (by bagging) resulted in negligible seed set. Moreover, since levels of seed set were comparable between the open and outcross pollination treatments, it is unlikely that either pollen availability or pollinator service is limiting in these two populations (Slyney 1 and 2). Given that there were no interpopulation differences in fruit or seed set it seems likely that this is generally the case and that low mate availability, suggested by high r_p, has not yet translated into reproductive failure. The significant interplant variation in fruit and seed set that was observed for several of the populations might reflect differences arising from several factors that are known to affect plant mating systems, such as plant size and isolation, floral display and incompatibility genotype.

The very low levels of fruit and seed set per flower in *G. iaspicula* is a phenomenon that is apparently common within the Proteaceae (Ayre & Whelan, 1989; Harriss & Whelan, 1993). For this study, fruit set is likely to be a more reliable measure of reproductive output as it was less affected by damage to mesh bags associated with seed predation by parrots. However, as flowering can continue for up to six months in most *Grevillea* species, with seed setting continuously during this period (Olde & Marriott, 1995), it is plausible that during such an extended reproductive season plants can produce large quantities of viable seed regardless of low flower: fruit ratios.

The genetic diversity estimates of percentage polymorphic loci, average number of alleles per locus and observed and expected heterozygosities for *G. iaspicula* are high in comparison to such estimates for other proteaceous species and other taxa with similar life-history characteristics (Hamrick & Godt, 1990). For example, in other woody proteaceous species, $H_e = 0.11$ for *G. robusta* (Harwood *et al*., 1997), $H_e = 0.16$ for *Hakea carinata* (Starr & Carthew, 1998), expectations for *Lomatia polymorpha* and *L. tinctoria* are both $H_e = 0.17$, while *L. tasmanica* has no detectable variation at all (Lynch *et al*., 1998). Ignoring *L. tasmanica*, as it is believed to be clonal (Lynch *et al*., 1998), the other species are apparently genetically more depauperate than the endangered *G. iaspicula*. In part, this may be due to both the breeding system and history of the species. For example, *H. carinata* populations are substantially selfing, and small and isolated populations appear to be a long-term evolutionary condition in this species (Starr & Carthew, 1998). On the other hand, *G. robusta* is a predominant outcrosser but some plants do produce a small proportion of self-fertilized seed (Harwood *et al*., 1992) and these apparently low levels of selfing may still be sufficient to affect heterozygosity levels within *G. robusta* populations. Indeed, F_{IS} estimates for *G. robusta* ($F_{IS} = 0.121$–0.152) indicate significant levels of inbreeding (Harwood *et al*., 1992, 1997).

It appears that current population size is unrelated to the level of genetic variation within *G. iaspicula* populations. This may be a sampling effect caused by sampling only a few populations that do not differ greatly in size; however, the populations chosen do represent the majority of all *G. iaspicula* populations and include the current size range. The lack of relationship may also be because *Grevillea* species are relatively long-lived (generally 10–30 years; Olde & Marriott, 1995) and the populations may have only been recently reduced in size, so that insufficient generations have passed for any effect on allozyme diversity to be detected. Therefore, the extant stand may still represent a greater part of the natural genetic variation. Additionally, the likely presence of a soil-stored seed bank may help to buffer the effects of population decline, in terms of genetic variation, at least for the short term (Levin, 1990).

Interpopulation genetic distances are large, considering that the populations are often separated by only a few kilometres. Similarly large genetic distances have been found between populations of another proteaceous shrub, *Hakea carinata* (Starr & Carthew, 1998), though this was associated with a relationship between

geographical distance and genetic differentiation across a range of about 500 km. Large genetic distances between populations are indicative of genetic isolation and are possibly due to founder effects, differential selection or limited gene flow among populations. Slatkin's (1985) rare alleles procedure indicated that there was indeed limited gene flow between *G. iaspicula* populations.

In summary, populations of *Grevillea iaspicula* show significant interpopulation genetic differentiation that is associated with low levels of gene flow among them. Mating within populations appears to be similarly restricted, with neighbourhood sizes of 2–3 plants, though populations are not yet experiencing reproductive limitation. Despite the apparent restrictions on mating and immigration, genetic diversity both at the individual and population level is high. Maintenance of heterozygosity in this species is perhaps not surprising, as it may well be a function of disassortative mating arising from the self-incompatibility system. The high allelic richness is more difficult to explain. One possibility is that there is a substantial seed bank that is acting as a temporal reservoir of genetic variation, providing small populations with diversity that was present in previous generations. Alternatively, though not exclusively, current populations may have only recently become small and therefore too few generations have passed for significant loss of alleles to have occurred due to genetic drift.

Acknowledgements

We thank Tony Brown and David Coates for comments on an earlier version of this manuscript, Elizabeth Gregory for assistance with allozyme electrophoresis, Bernard Pfeil and Jane Pulford for collections requiring abseiling and NSW National Parks and Wildlife Service for providing collection permits. Sites were accessed with the permission of property owners. SEH was supported by a CSIRO Plant Industry Student Scholarship and the research was funded in part by an Environment Australia Natural Heritage Trust grant to AGY.

References

AUSTRALIAN AND NEW ZEALAND ENVIRONMENTAL AND CONSERVATION COUNCIL (ANZECC). 1999. Threatened Australian Flora. Prepared by the Endangered Flora Network.

AYRE, D. J. AND WHELAN, R. J. 1989. Factors controlling fruit set in hermaphroditic plants: studies with the Australian Proteaceae. *Trends Ecol. Evol.*, **4**, 267–272.

BRIGGS, J. D. AND LEIGH, J. H. 1990. *Delineation of Important Habitats of Threatened Plant Species in South-Eastern New South Wales*. Research Report to the Australian Heritage Commission.

BRIGGS, J. D. AND LEIGH, J. H. 1996. *Rare or Threatened Australian Plants*. Revised Edition. CSIRO Publishing, Collingwood.

BUTLER, G., RICHARDSON, M. AND GANTER, W. 1991. *Recovery Plan for* Grevillea iaspicula *McGillivray* (*Wee Jasper Grevillea*). Prepared by the Australian National Botanic Gardens, Canberra.

BUZA, L., YOUNG, A. AND THRALL, P. 2000. Genetic erosion, inbreeding and reduced fitness in fragmented populations of the endangered tetraploid pea *Swainsona recta*. *Biol. Cons.*, **93**, 177–186.

CAROLIN, R. 1961. Pollination of the Proteaceae. *Aust. Museum Mag.*, **13**, 371–374.

COATES, D. J. AND HAMLEY, V. L. 1999. Genetic divergence and the mating system in the endangered and geographically restricted species, *Lambertia orbifolia* Gardner (Proteaceae). *Heredity*, **83**, 418–427.

DEMAURO, M. M. 1993. Relationship of breeding system to rarity in the Lakeside daisy (*Hymenoxys acaulis* var. *glabra*). *Conserv. Biol.*, **7**, 542–550.

GOLDINGAY, R. L. AND CARTHEW, S. M. 1998. Breeding and mating systems of Australian Proteaceae. *Aust. J. Bot.*, **46**, 421–437.

HAMRICK, J. L. AND GODT, M. J. W. 1990. Allozyme diversity in plant species. In: Brown, A. D. H., Clegg, M. T., Kahler, A. L. and Weir, B. S. (eds) *Plant Population Genetics, Breeding and Genetic Resources*, pp. 43–63. Sinauer, Sunderland.

HARRISS, F. AND WHELAN, R. J. 1993. Selective fruit abortion in *Grevillea barklyana* (Proteaceae). *Aust. J. Bot.*, **41**, 499–509.

HARWOOD, C. E., BELL, J. C. AND MORAN, G. F. 1992. Isozyme studies on genetic variation and the breeding system in *Grevillea robusta*. In: Harwood, C. E. (ed.) Grevillea robusta *in Agroforestry and Forestry*, pp. 165–176. ICRAF, Nairobi.

HARWOOD, C. E., MORAN, G. F. AND BELL, J. C. 1997. Genetic differentiation in natural populations of *Grevillea robusta*. *Aust. J. Bot.*, **46**, 669–678.

HERMANUTZ, L., INNES, D., DENHAM, A. AND WHELAN, R. 1998. Very low fruit: flower ratios in *Grevillea* (Proteaceae) are independent of breeding system. *Aust. J. Bot.*, **46**, 465–478.

JOHNSON, L. A. S. AND BRIGGS, B. G. 1975. On the Proteaceae – the evolution and classification of a southern family. *Bot. J. Linn. Soc.*, **70**, 83–182.

KALINGANIRE, A., HARWOOD, C. E., SLEE, M. U. AND SIMONS, A. J. 2000. Floral structure, stigma receptivity and pollen viability in relation to protandry and self-incompatibility in Silky Oak (*Grevillea robusta* A. Cunn.). *Ann. Bot.*, **86**, 133–148.

LEVIN, D. A. 1990. The seed bank as a source of genetic novelty in plants. *Am. Nat.*, **135**, 563–572.

LYNCH, A. J. J., BARNES, R. W., CAMBECÈDES, J. AND VAILLANCOURT, R. E. 1998. Genetic evidence that *Lomatia tasmanica* (Proteaceae) is an ancient clone. *Aust. J. Bot.*, **46**, 25–33.

MANTEL, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Res.*, **27**, 209–220.

MATTON, D. P., NASS, N., CLARKE, A. E. AND NEWBIGIN, E. 1994. Self-incompatibility: how plants avoid illegitimate offspring. *Proc. Nat. Acad. Sci. U.S.A.*, **91**, 1992–1997.

MCGILLIVRAY, D. J. 1993. Grevillea, *Proteaceae: A Taxonomic Revision*. Melbourne University Press.

NEI, M. 1972. Genetic distance between populations. *Am. Nat.*, **106**, 283–292.

OLDE, P. AND MARRIOTT, N. 1995. *The Grevillea Book*, vol. 1–3. Kangaroo Press, Kenthurst.

OOSTERMEIJER, J. G. B. 2000. Population viability analysis of the rare *Gentiana pneumonanthe*: the importance of genetics, demography and reproductive biology. In: Young, A. G. and Clarke, G. M. (eds) *Genetics, Demography and Viability of Fragmented Populations*, pp. 313–334. Cambridge University Press, Cambridge.

RITLAND, K. 1989. Correlated matings in the partial selfer *Mimulus guttatus*. *Evolution*, **43**, 848–859.

RITLAND, K. 1994. MLTR *Multilocus mating system program*, version 0.9. Available from author.

SAMPSON, J. F. 1998. Multiple paternity in *Eucalyptus rameliana* (Myrtaceae). *Heredity*, **81**, 349–355.

SAS INSTITUTE. 1990. *SAS*, *version 6.12*. SAS Institute, Cary.

SLATKIN, M. 1985. Rare alleles as indicators of gene flow. *Evolution*, **39**, 53–65.

SLATKIN, M. AND BARTON, N. H. 1989. A comparison of three indirect methods for estimating average levels of gene flow. *Evolution*, **43**, 1349–1368.

STARR, G. J. AND CARTHEW, S. M. 1998. Genetic differentiation in isolated populations of *Hakea carinata* (Proteaceae). *Aust. J. Bot.*, **46**, 671–682.

STEPHENSON, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. *Ann. Rev. Ecol. Syst.*, **12**, 253–279.

SUN, M. AND RITLAND, K. 1998. Mating system of yellow starthistle (*Centaurea solstitialis*), a successful colonizer in North America. *Heredity*, **80**, 225–232.

VEKEMANS, X., SCHIERUP, M. H. AND CHRISTIANSEN, F. B. 1998. Mate availability and fecundity selection in multi-allelic self-incompatibility systems in plants. *Evolution*, **52**, 19–29.

WAPLES, R. S. 1989. A generalized approach for estimating effective population size from termporal changes in allele frequency. *Genetics*, **121**, 379–391.

WATKINS, L. AND LEVIN, D. A. 1990. Outcrossing rates as related to plant density in *Phlox drummondii*. *Heredity*, **65**, 81–89.

WRIGHT, S. 1951. The genetical structure of populations. *Ann. Eugen.*, **15**, 313–354.

YEH, F. C., YANG, R.-C. AND BOYLE, T. J. B. 1998. POPGENE, version 1.31. University of Alberta and CIFOR.

YOUNG, A. G. AND BROWN, A. H. D. 1998. Comparative analysis of the mating system of the rare woodland shrub *Daviesia suaveolens* and its common congener *D. mimosoides*. *Heredity*, **80**, 374–381.

YOUNG, A. G. AND BROWN, A. H. D. 1999. Paternal bottlenecks in fragmented populations of the grassland *daisy Rutidosis leptorrhynchoides*. *Genet. Res.*, **73**, 111–117.

YOUNG, A. G., BROWN, A. H. D., MURRAY, B. G., THRALL, P. H. AND MILLER, C. H. 2000. Genetic erosion, restricted mating and reduced viability in fragmented populations of the endangered grassland herb *Rutiolosis leptorrhynchoides*. In: Young, A. G. and Clarke, G. M. (eds) *Genetics, Demography and Viability of Fragmented populations*, pp. 335–359. Cambridge University Press, Cambridge.