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Effects of seed dispersal on spatial genetic structure in populations of *Rutidosis leptorrhychoides* with different levels of correlated paternity

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Summary

Rutidosis leptorrynchoides is a perennial forb endemic to grasslands and grassy woodlands in southeastern Australia. Studies of seed dispersal, spatial genetic structure and clonality were carried out in four populations around the Canberra region that varied in levels of correlated paternity to examine: (1) whether *R. leptorrhynchoides* populations exhibit fine-scale spatial genetic structure and whether this varies between populations as a function of correlated paternity; (2) whether there is a correlation between seed dispersal distance and genetic relatedness within populations; and (3) whether clonal reproduction occurs in this species and to what degree this could account for the observed spatial genetic structure. The results show that there is variation in the magnitude and extent of spatial genetic structure between *R. leptorrhynchoides* populations. The three larger populations, with low to moderate full-sib proportions, showed significant patterns of coancestry between plants over scales of up to one metre, whereas the smallest population, with a high full-sib proportion, had erratically high but non-significant coancestry values. The observed patterns of genetic clumping could be explained by a combination of limited seed dispersal and correlated mating owing to limited mate availability resulting from the species' sporophytic self-incompatibility system. Clonality does not appear to be an important factor contributing to genetic structure in this species.

1. Introduction

Spatial genetic structure – the spatial aggregation of related individuals either within or between populations – is a common feature of many plant species. Recent studies have found significant spatial genetic structure in a wide range of ecological and taxonomic groups including orchids (Chung *et al.*, 1998), herbs (Williams, 1994), shrubs (Franceschinelli & Kesseli, 1999) and temperate and tropical trees (Boyle *et al.*, 1990; Young & Merriam, 1994; Epperson & Alvarez-Buylla, 1997). Such genetic structure is of interest as it represents an endpoint to key evolutionary processes such as reproduction, dispersal, gene flow and selection, processes than can lead to adaptation to new environments, speciation or extinction. Studying spatial genetic structure can provide insight into the spatial dynamics of these processes and, in combination with ecological studies, allow examination of the relative contribution of each to local patterns of genotype distribution.

Several authors have examined the effect of seed dispersal on genetic structure. A study of three sympatric herbs with different seed dispersal strategies by Williams (1994) found that the differences in genetic structure in these species were consistent with their different seed dispersal patterns. Boshier *et al.* (1995) also found a strong correlation between seed dispersal and spatial genetic structure in the neotropical tree *Cordia alliodora* and that this was more influential on genetic structure than the pattern of pollen dispersal was. In a recent simulation study, Doligez *et al.* (1998) showed that changes in pollen dispersal distances had a smaller effect on spatial structure than seed dispersal owing to the differences in ploidy. This relates to the fact that, whereas an increase in seed dispersal distances will lead to an increase in effective pollen dispersal (as half of the genetic material carried by the seed is contributed by the pollen), an increase in pollen dispersal will have no

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such effect. A similar simulation study by Ohsawa *et al*. (1993) found that pollen dispersal did create structure over time but they made no comparison between the effects of pollen and seed dispersal regimes.

The type of breeding system is also likely to influence the spatial genetic structure, as this will affect the pattern of effective gene flow. Strong structure would be expected in obligate self-fertilizers, whereas genetically controlled incompatibility systems will act to decrease genetic structure by promoting disassortative mating. Mixed mating systems are expected to generate intermediate levels of structure. For species that can reproduce both sexually and vegetatively, the effect of vegetative reproduction on spatial genetic structure will depend on the level of intermingling between genets, which can be extensive (Maddox *et al*., 1989) or very limited (Cook, 1983). Recently, there have been several studies that have tried to differentiate between the effect of clonality and limited gene flow on genetic structure (Montalvo *et al*., 1997; Reusch *et al*., 1999; Chung *et al*., 2000; Chung & Epperson, 2000). In a study on *Zostera marina*, Reusch *et al*. (1999) found that the genetic clumping observed between plants up to 7 m apart disappeared when comparisons between ramets within genets were taken out of the analysis, indicating that clonal spread was a major determinant of genetic structure. A similar result was found by Montalvo *et al*. (1997). By contrast, Chung *et al*. (2000) found that the removal of clones from their analysis of spatial genetic structure in *Eurya emarginata* had little effect. In this case, the ramets were randomly distributed throughout the population owing to their 'guerrilla-type' (Lovett Doust, 1981) growth habit.

In the current study, we compare the spatial genetic structure and patterns of seed dispersal and clonality between four populations of the grassland forb *Rutidosis leptorrhynchoides* that differed in the production of full-sib progeny as quantified by levels of outcrossed correlated paternity (Young & Brown, 1999). The general aims of this study were to quantify the level and extent of spatial genetic structure in this self-incompatible plant and to assess the relative roles of mating, clonality and seed dispersal in generating structure. Three specific questions were addressed. (1) Do *R. leptorrhynchoides* populations exhibit fine-scale spatial genetic structure and does this vary between populations as a function of correlated paternity? (2) Within populations, is there a correlation between seed dispersal distance and genetic relatedness? (3) Does clonal reproduction occur in *R. leptorrhynchoides* populations and to what degree could this account for observed spatial genetic patterns?

(i) *Study species*: R. leptorrhynchoides

R. leptorrhynchoides is a multistemmed perennial forb 20–40 cm in height that is endemic to the grasslands and grassy woodlands of southeastern Australia. Plants grown in the glasshouse have lived up to 10 years, although their lifespan in the wild is thought to be much longer (Scarlett & Parsons, 1990). Flowering mainly occurs from November to January, with each plant producing many flower heads. The species is thought to be pollinated by generalist insects and the achenes are wind-dispersed (Morgan, 1995*b*). *R. leptorrhynchoides* has a sporophytic self-incompatibility system that is effective at preventing selfing and, to a lesser degree, mating between relatives (Young *et al*., 2000*a*).

Despite its self-incompatible breeding system, several reproductive characteristics of *R. leptorrhynchoides* suggest that it may exhibit significant spatial genetic structure. First, based on patterns of seedling distributions, seed dispersal is thought largely to be limited to 0·5 m around mature plants (Morgan, 1995*a*). The species has a transient seed bank, relying on seeds from the previous year for recruitment (Morgan, 1995*a*). Second, there is some evidence for the presence of clonality in this species, with many plants grown in pots producing ramets after 2–3 years of growth. Third, reductions in *S* allele diversity in small populations have been shown to reduce mate availability, resulting in both reduced fruit set and increased production of full-sib progeny in populations of < 200 individuals (Young & Brown, 1999).

2. Methods

(i) *Study sites*

Four of the 16 known *R. leptorrhynchoides* populations in the Canberra region were used in this study, ranging in size from 118 to $\sim$ 10000 plants (Table 1). Correlated paternity values had been previously estimated for two of these sites (Queanbeyan and Captains Flat) and inbreeding coefficients were available for three of them (Queanbeyan, Captains Flat and Letchworth) based on studies of this species by Young & Brown (1999) and Young *et al*. (1999).

Table 1. *Population size, fixation coefficients* (F_{IS}) *and correlation of outcrossed paternity* (r_p) *for* R. leptorrhynchoides *study populations*

Location	Number of flowering plants	F_{IS}	r_p
Queanbeyan	10032	0·06	0·37
Letchworth	1171	0·03	0·51
Captains Flat	250	0·11	0·53
Mt Ainslie	118	0·05	0·65

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Values of correlated paternity for Letchworth and of correlated paternity and inbreeding for Mount Ainslie were estimated by Wells (2000). From these studies, it was known that the average proportion of full sibs (r_p) in these four populations varied from 0·37 to 0·65 and the proportion of inbreeding (F_{IS}) ranged between 0·03 and 0·11 (Table 1).

(ii) *Genetic sampling*

In the two largest populations, at Queanbeyan and Letchworth, sampling was based on a 5 m × 5 m square plot gridded at 25 cm intervals. Within each plot, the nearest reproductive plant to each grid intersection was sampled and its coordinates recorded to the nearest 5 cm. Owing to the restricted shape of the Captains Flat population, the dimensions of the sampling plot were 1·75 m × 15 m. This gave approximately the same total area (26·25 m²) as the plots in the two larger populations and extended the full length of the site. In the small Mount Ainslie population, all plants were sampled and their spatial coordinates mapped. This sampling strategy yielded 285 samples at Queanbeyan, 303 at Letchworth, 197 at Captains Flat and 118 at Mount Ainslie.

(iii) *Allozyme analysis*

Two to three leaves were taken from each plant and stored in a cooler with ice packs while in the field and then transferred to a refrigerator (4 °C) overnight until used the following morning for electrophoresis. Leaf tissue (~ 2 g) was then ground in four drops of 0·05 M potassium phosphate buffer (pH 7·0) containing 1 mg ml^{-1} dithiothreitol (Clelands reagent) and 10 mg ml^{-1} polyvinylpyrrolindone (molecular weight 40000; PVP40). Samples were electrophoresed using horizontal starch gel electrophoresis following the methods described for *R. leptorrhynchoides* by Young *et al.* (1999). Five allozyme loci for three enzymes were resolved clearly and consistently using two gel-electrode buffer systems: (1) histidine, pH 8·0, glucose-6-phosphate isomerase (EC 5.3.1.9) (one locus), phosphoglucomutase (EC 5.4.2.2) (two loci); and (2) lithium borate, pH 8·5, aspartate aminotransferase (EC 2.6.1.1) (two loci).

(iv) *Spatial genetic structure*

Spatial genetic structure was quantified using spatial autocorrelation analysis of the coancestry coefficient f_{ij} (Loiselle *et al.*, 1995), which measures the correlation in the frequency of homologous alleles in pairs of samples. The relatedness of each pair of plants was calculated individually at each locus and then the results were averaged over all loci, allowing incompletely genotyped plants still to be used in the analysis. Results from all possible pairs of plants (i,j) within 25 cm distance intervals were averaged to find the mean coancestry for that distance interval. The coefficient f_{ij} is approximately half the value of Wright's fixation coefficient (F_{IS}). Significance of f_{ij} values for each distance interval was assessed by constructing 95% confidence intervals around the mean by bootstrapping ($n = 399$). All analyses were conducted using the computer programs fijAnal and BS-fij (Loiselle *et al.*, 1995).

(v) *Clonality*

Evidence for clonality was investigated by comparing the observed multilocus allozyme genotype frequencies to those expected from the population allele frequencies assuming sexual reproduction following Sydes & Peakall (1998). Any excess of repeated genotypes was attributed to vegetative reproduction. The expected probability of obtaining each of the repeated multilocus genotypes twice by random sexual reproduction (P_{sex}) was calculated as the product of the expected single-locus genotype frequencies corrected for observed levels of inbreeding using the formulae

$$f_{AA} = p^2 + F_{IS}(pq)$$

and

$$f_{Aa} = 2pq - 2F_{IS}(pq),$$

where p is the observed frequency of the 'A' allele and q is the observed frequency of the 'a' allele. The expected probability of obtaining the observed number of repeats (n) of each multilocus genotype (P_{rpt}) was then calculated as $P_{rpt} = P_{sex}^{n-1}$. Significance was assessed with an experimental error rate $P = 0{\cdot}05$, using a Bonferroni correction to calculate critical values for the multiple tests represented by each multilocus genotype examined within a population. Whether P_{sex}, P_{rpt} or both were significant for any particular repeated genotype gave an indication of how the individuals with this genotype had been produced. If neither probability was significant, it was assumed that all repeats of that genotype were due to sexual reproduction whereas, if both probabilities were significant, repeats were assumed to be the result of clonality. If P_{sex} was not significant and P_{rpt} was, a mixture of sexual and asexual reproduction was assumed to be occurring in the population. In the last case, the number of repeats that could be due to sexual reproduction was calculated using the following formula: maximum number of sexual repeats = $\ln(p) \div \ln(P_{rpt})$.

A second issue of interest is the effect of correlated paternity on the likelihood of producing repeated multilocus genotypes sexually. Correlated paternity reduces the expected diversity of sexually produced multilocus genotypes by reducing the paternal diversity contributing to open-pollinated sibships. Un-

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like inbreeding, a simple arithmetical correction cannot be used to adjust the expected multilocus genotype frequencies to take account of correlated paternity. Instead, the potential for correlated paternity to generate putative clones sexually was studied empirically by quantifying the frequency of multilocus genotypes in open-pollinated seed arrays from the four study populations (data from Young & Brown (1999) and Wells (2000)) and subjecting them to the same set of analyses.

(vi) *Seed dispersal*

Distance and pattern of seed dispersal were quantified in three of the four study populations (Queanbeyan, Captains Flat and Mount Ainslie). One to two days prior to dehiscence, flower heads were covered with a slurry of fluorescent dye and water. After dehiscence (7–10 days after painting), the location of these seeds was determined using a handheld ultraviolet light at night. The distance that the seeds travelled from the source plant was measured for four plants at each site and the results from these plants combined. The pattern of seed dispersal in the different sites was compared using *G* tests (Sokal & Rohlf, 1981) between all pairs of populations. Mean dispersal distances were compared between populations using the SAS GLM procedure (SAS Institute, Cary, North Carolina, USA, 1997) to conduct an ANOVA on log-transformed data. To estimate how much of the spatial genetic structure at each site could be accounted for by seed dispersal alone, the proportion of seeds found in each of the distance classes was correlated with f_{ij} for these distances using a linear regression of the proportion of seeds as the independent variable and f_{ij} as the dependent variable.

3. Results

(i) *Spatial genetic structure*

Mean coancestry coefficients (f_{ij}) for each 25 cm distance class up to a maximum of 600 cm, summarized as autocorrelograms, are presented for each of the four populations in Fig. 1, along with 95% confidence limits derived from bootstraps. In general, the results show that there are both significant positive and significant negative structure in the populations, and that the magnitude and scale of spatial genetic structure varied between populations. Pronounced genetic structure was observed in Captains Flat (Fig. 1*b*), Queanbeyan (Fig. 1*d*) and Letchworth (Fig. 1*c*), with positive genetic associations ranging from $f_{ij} =$ 0·02 to $f_{ij} = 0{\cdot}08$ over distances of up to 75 cm in each, and erratically beyond this to 175 cm at Queanbeyan. Queanbeyan also exhibited the only consistent nega-

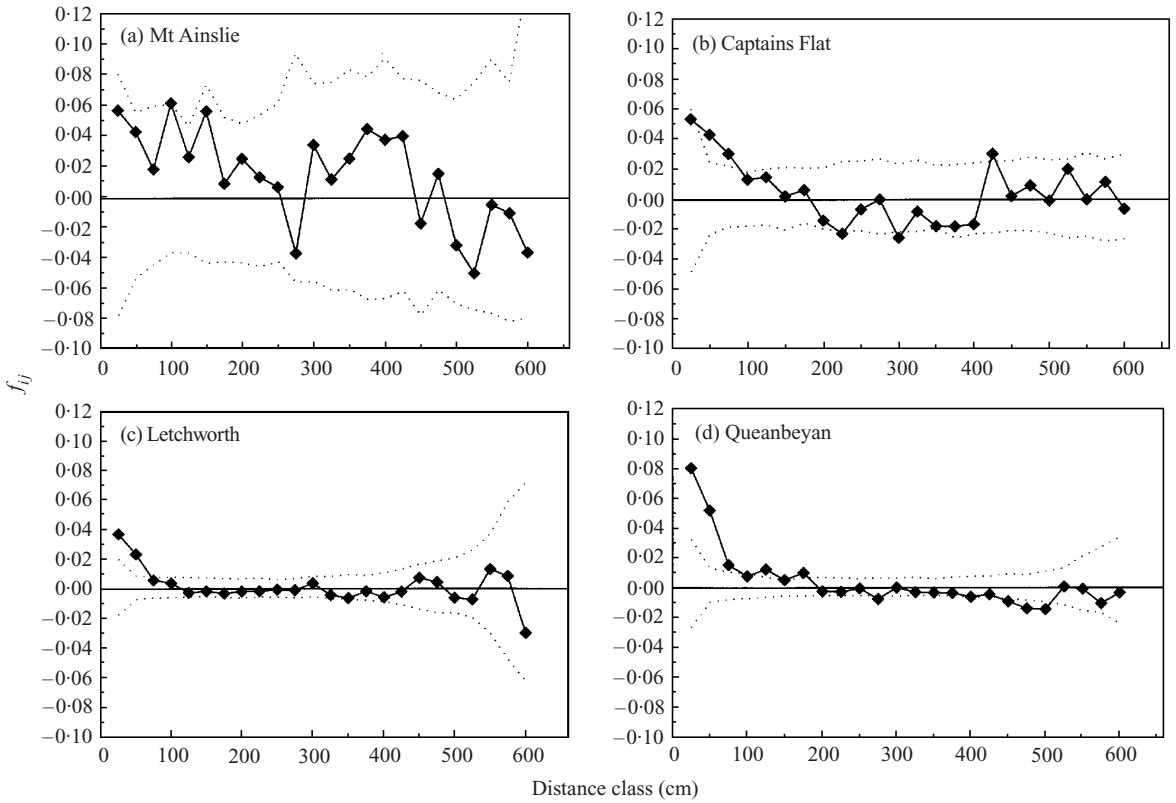

Fig. 1. Spatial autocorrelograms of coancestry coefficients for four populations of *R. leptorrhynchoides*. (*a*) Mount Ainslie, (*b*) Captains Flat, (*c*) Letchworth and (*d*) Queanbeyan. Broken lines represent 95% confidence intervals based on 399 bootstraps.

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Table 2. *Clonality analyses of* R. leptorrhynchoides *populations based on five allozyme loci for vegetative samples and open-pollinated seed samples*

Location	Sample size	Genotypes	Repeated genotypes*†	Mean repeats per genotype‡	Repeated genotypes (vegetative)*§	Repeated genotypes (seed)*§
Queanbeyan	231	49	0·88 (22)	9·2	0·44	0·68
Letchworth	205	79	0·79 (35)	4·6	0·43	0·48
Captains Flat	174	25	0·95 (17)	9·8	0·87	0·76
Mount Ainslie	101	16	0·92 (8)	11·6	0·71	0·99

* Numbers indicate the proportion of the sample that consisted of repeated genotypes.
† Bracketed numbers indicate the actual number of repeated genotypes.
‡ Assumes a single sexual event.
§ Assumes the maximum number of sexual events in each case.

tive association of individuals, with plants separated by 450–500 cm being less genetically similar than expected by chance, although only weakly, with $f_{ij} = -0{\cdot}005$ to $-0{\cdot}01$. Mount Ainslie, the smallest population, with the highest level of correlated paternity, showed the least genetic structure (Fig. 1*a*). Although coancestry coefficients were often as high in this population as for the other populations, there was no spatial pattern to them and only one was statistically significant, because the 95% confidence intervals were broad owing to the lower overall sample size.

(ii) *Clonality*

Table 2 shows the overall number of multilocus genotypes for each population, the proportion of the total samples represented by repeated genotypes and the mean number of repeats per genotype. All populations contained large proportions ($> 0{\cdot}79$) of repeated genotypes. There was some variation between populations in the numbers of repeats per genotype, although this was mostly due to the low value of 4·6 repeats per genotype for Letchworth, with the other three populations being quite similar (9·2–11·6). Large proportions (0·43–0·87) of repeated genotypes in excess of predictions assuming maximum sexual reproduction were observed in the vegetative data for all populations; again, the lowest value was for Letchworth. Such excesses would normally be attributed to asexual reproduction, but similarly large proportions of repeated genotypes in excess of sexual expectations were observed in the open-pollinated seed array data (0·48–0·99).

(iii) *Seed dispersal*

Histograms of seed deposition combined over plants within three populations are presented in Fig. 2. These data show that, in all three populations, most of the seed falls within 1 m of the mother plant. Pairwise G tests between the three populations indicated that there is no significant difference between the shape of the seed dispersal curves at Queanbeyan (Fig. 2*c*) and Mt Ainslie (Fig. 2*a*), but that Captains Flat (Fig. 2*b*) was significantly different from both (comparison with Queanbeyan, $G = 19{\cdot}70$, $P < 0{\cdot}05$; comparison with Mt Ainslie, $G = 21{\cdot}96$, $P < 0{\cdot}01$), owing pri-

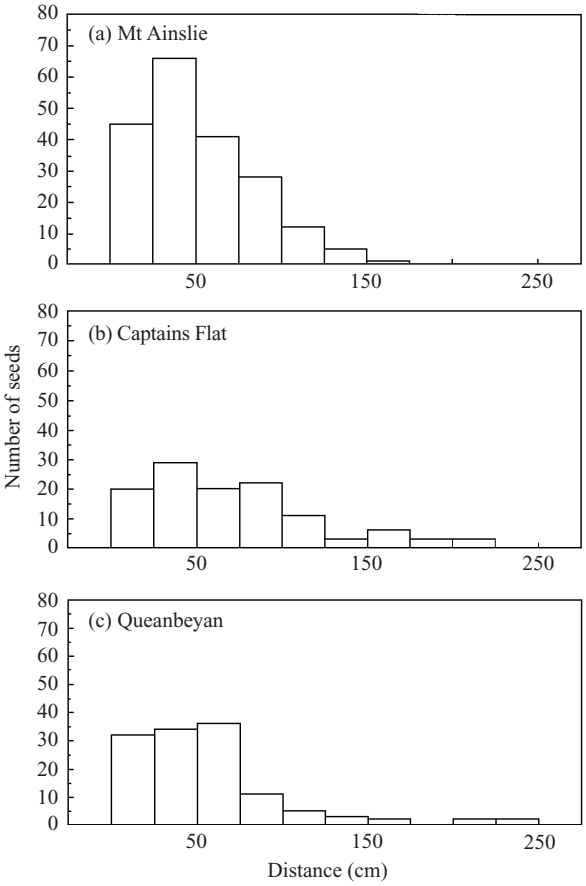

Fig. 2. Histograms of seed dispersal for three populations of *R. leptorrhynchoides*. (*a*) Mount Ainslie, (*b*) Captains Flat and (*c*) Queanbeyan.

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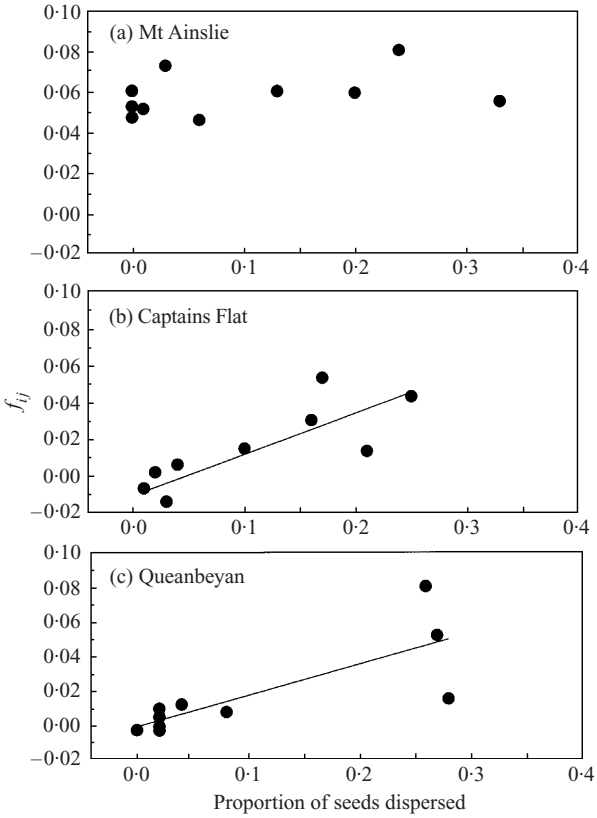

Fig. 3. Relationships between seed dispersal and coancestry for three populations of *R. leptorrhynchoides*. (*a*) Mount Ainslie, not significant; (*b*) Captains Flat, $R^2 = 0{\cdot}63$, $P < 0{\cdot}01$; (*c*) Queanbeyan, $R^2 = 0{\cdot}57$, $P < 0{\cdot}01$.

marily to the flatter structure of the dispersal curve at this site. ANOVA of mean seed dispersal distance showed no significant difference between Queanbeyan (mean = 55·4 cm, standard error = 4·09 cm) and Mt Ainslie (mean = 51·0 cm, standard error = 2·36 cm) but that, on average, seed at Captains Flat dispersed significantly further (mean = 66·4, standard error = 4·87 cm) ($F = 9{\cdot}33$, $P < 0{\cdot}0001$). Correlation analyses between the proportion of seed dispersed to a given distance and the measure of interplant coancestry were significant for Queanbeyan ($R^2 = 0{\cdot}57$, $P < 0{\cdot}01$) and Captains Flat ($R^2 = 0{\cdot}63$, $P < 0{\cdot}01$) but not for Mt Ainslie (Fig. 3).

4. Discussion

The results of the spatial autocorrelation of coancestry coefficients clearly show that populations of *R. leptorrhynchoides* can show positive spatial genetic structure over scales of up to ~ 1 m. The second main finding is that there is interpopulation variation in both the scale and the magnitude of genetic associations, with the small, high-r_p population at Mt Ainslie exhibiting no consistent spatial patterns of genetic similarity among individuals. The magnitude of the positive genetic structure observed for *R. leptorrhynchoides* is at the lower end of the range of values that have been found in studies conducted on other plants over similar spatial scales (Table 3). This is interesting because most of these species are self-compatible and so are expected to exhibit greater genetic structure than an obligate outcrosser like *R. leptorrhynchoides* (Doligez *et al.*, 1998). The build-up of weak spatial genetic structure in *R. leptorrhynchoides* might be due to the sporophytic self-incompatibility system being inefficient at preventing selfing or mating between relatives. This could be due either to frequent dissolution of the system through the generation of null alleles or to a relaxation of the constraints on mating between relatives owing to the presence of dominance in either the pollen or stigma and the presence of dominance hierarchies among *S* alleles (DeNettancourt, 1977). Both of these processes are known to occur in *R. leptorrhynchoides* (Young *et al.*, 2000*a*, *b*).

The second possibility is that inbreeding, either through selfing or mating between relatives, is not the primary force generating spatial genetic structure in this species. Rather, observed coancestry coefficients might be due to either clonality or the limited dispersal of seed arrays, which, in these populations, contain 37–65% full-sibs. The large number of repeated multilocus genotypes observed in all populations, many of which cannot be explained based on sexual reproduction, initially suggests that extensive vegetative reproduction is occurring. However, similarly high frequencies of repeated genotypes in the open-pollinated seed data strongly suggest that sexual reproduction involving significant correlated paternity could account for all of the repeated genotypes in the vegetative data sets. Combined with the strong correlations between seed dispersal and the spatial scale of genetic structure at Queanbeyan and Captains Flat, this indicates that most of the genetic structure in *R. leptorrhynchoides* could result from correlated paternity combined with spatially limited seed dispersal.

To illustrate this point, the observed level of coancestry (f_{ij}) at the 25 cm distance class in Queanbeyan is 0·08, which is roughly equivalent to $F_{IS} = 0{\cdot}16$. This value can be neatly explained by assuming that seed shadows are made up of mixtures of full-sibs and half-sibs in a ratio of 0·37:0·63 (as indicated by $r_p = 0{\cdot}37$), which gives a mean expected progeny array $F_{IS} = 0{\cdot}17$, calculated by multiplying the ratio of full- to half-sibs by their F_{IS} values

$$F_{IS} = 0{\cdot}37 \times 0{\cdot}25 \ (F \text{ for full-sibs}) + 0{\cdot}63 \times 0{\cdot}125 \ (F \text{ for half-sibs}) = 0{\cdot}17.$$

Therefore, it seems that correlated paternity and seed dispersal can generate the appropriate magnitude of genetic relatedness seen between plants, as well as

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Table 3. *A comparison of values of Moran's* I *found in other studies over a 1 m scale relative to* R. leptorrhynchoides

Species	Mating system*	Seed dispersal†	*I* (at 1 m)	Reference
Rutidosis leptorrhynchoides‡	O	G	0·04	Current study
			0·02	
			0·05	
			0·08	
Calluna vulgaris	M/C	G/W	0·05§	Mahy & Neve, 1997
Cryptotaenia canadensis	M	G	0·49	Williams, 1994
			0·14	
Delphinium nuttallianum	M	G	0·09§	Williams & Waser, 1999
			0·02§	
			−0·01§	
			0·02§	
Helicteres brevispira	–	G	0·28§	Franceschinelli & Kesseli, 1999
Lathyrus sylvestris	M/C	G	0·02§	Hossaert-McKey *et al*., 1996
Osmorhiza claytonii	M	A/G	0·34	Williams, 1994
Sanicula gregaria	M	A/G	0·29	Williams, 1994
			0·10	
Silene acaulis	M	G/W	0·30§	Gehring & Delph, 1999
			0·30§	
			0·20§	
			0·13§	
			−0·02§	
Mean (standard deviation)			0·14 (0·14)	

* C, clonal; M, mixed mating; O, outcrossing.
† A, animal; G, gravity; W, wind.
‡ Estimated as $I = 2 \times f_{ij}$.
§ Estimated from graph.

matching the spatial scale of observed genetic structure.

If correlated paternity followed by limited seed dispersal are the main mechanisms generating genetic structure in large *R. leptorrhynchoides* populations, the apparent dissolution of this structure in smaller populations, which have similar seed dispersal patterns and higher correlations of paternity, initially seems counterintuitive. Higher levels of full-sib production should increase fine-scale structure – boosting coancestry values to something of the order of $f_{ij} = 0{\cdot}13$. By contrast, the highest (and only significant) value observed in Mt Ainslie was $f_{ij} = 0{\cdot}07$ for the 1 m distance class.

There are two possible reasons for this result, one analytical and the other biological. First, the lower sample size available for the small Mt Ainslie population reduced the statistical power available to detect spatial genetic structure, as evidenced by the inflated confidence intervals for this population relative to the larger sites. This effect was also evident to a lesser degree at Captains Flat. Clearly, however, this is not the whole explanation for lack of structure at Mt Ainslie because, regardless of their overall magnitude, the mean f_{ij} values themselves show little evidence of a consistent trend with increasing interplant distance: relatively high positive and negative values occur erratically.

A possible biological explanation for this lack of structure despite high r_p lies in the very efficiency of the *R. leptorrhynchoides*' self-incompatibility system. Sporophytic control is the more limiting form of homomorphic incompatibility and its effective functioning relies on the maintenance of high numbers of possible *S* alleles within populations (Richards, 1997). This is normally achieved in large populations through strong frequency-dependent selection (Richman & Kohn, 1996). However, in small populations, erosion of *S* alleles can result in mate limitation as the numbers of possible heterozygous genotypes is reduced. This has been observed in several species including *R. leptorrhynchoides* (Young & Brown, 1999). The result of this is that small populations might be characterized by erratic spatial patterns of mate availability relative to large ones, in which maintenance of high mate availability might allow the development of more classical patterns of isolation by distance.

Overall, this study has shown that populations of *R. leptorrhynchoides* can show significant spatial genetic structure over a scale of up to 1 m but that this structure breaks down in small populations with high levels of correlated paternity. Structure in large populations is unlikely to be due to clonality. The observed levels of repeated multilocus genotypes, which could be seen as evidence for vegetative reproduction, are equally well explained by correlated

paternity combined with limited seed dispersal. Together, these can produce both the magnitude and the observed spatial patterns of genetic relatedness. Loss of genetic structure in very small populations is probably due to a shift from isolation by distance mating to spatially erratic mating events owing to severe mate limitation imposed by erosion of *S* alleles.

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