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Spatial Autocorrelation of Genetic Variation in Three Stands of *Ophiopogon xylorrhizus* (Liliaceae *s.l.*)

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Using spatial autocorrelation analysis, the spatial distribution of genotypes and gene frequencies at three allozyme loci, as well as the spatial distribution of family outcrossing rates were investigated in three stands of the tropical rainforest endangered perennial *Ophiopogon xylorrhizus* in Southwest China. Similar spatial patterns of the different allozymes were found both in individual stands and among stands, particularly at the whole distance classes. There were slightly different associations between like-homozygous and like-heterozygous plants. Most like-homozygous plant pairs exhibited a significant positive autocorrelation in shorter distance classes, and a random distribution or slightly negative autocorrelation in the following one or two distance classes. In contrast, a significant positive autocorrelation was found in the last one or two distance classes, indicating that like-homozygous plants were clustered in patches with a diameter of 5–10 m. Most like-heterozygous plant pairs exhibited a random distribution or a slightly negative autocorrelation in shorter distance classes, a significantly positive autocorrelation in the next one or two distance classes, and tended to a random distribution in the last one or two distance classes. Fifty-five per cent of unlike-homozygous plant pairs existed in different patches with similar patch sizes and these patches did not overlap. Highly consistent patterns were found in the spatial distribution of gene frequencies. Different genes existed in different patches, with a highly consistent average patch size in all three stands. Family outcrossing rates were randomly distributed in space. These results imply that it is not possible to sample the genetic variation of a population by merely conserving one part of it because the population is unlikely to be homogenous.

Key words: Spatial genetic structure, spatial autocorrelation analysis, ecological genetics, allozyme electrophoresis, endangered plant, *Ophiopogon xylorrhizus*.

INTRODUCTION

The spatial distribution of genetic variability within natural plant populations may significantly influence evolutionary and ecological processes (Lewontin, 1974; Endler, 1977; Wright, 1982; Epperson and Allard, 1989), and has long been of primary interest in ecological and evolutionary studies (Epperson and Clegg, 1986; Legendre, 1993). The importance of spatial structures can be separated into three components (Epperson, 1993). First, spatial structure is sometimes inextricably linked to evolutionary and ecological genetics (Epperson, 1990*a*). Second, because spatial patterns change in a substantial, cumulative and sometimes characteristic manner due to the past effects of natural selection and other factors, spatial pattern analysis can help to detect the action of these factors (Epperson, 1993). Third, knowledge of the spatial structure of populations is important when selecting natural populations for conservation, or sampling them for breeding programs. It should be taken into account in order to maximize diversity, and to avoid misrepresenting species and population diversity (Epperson and Allard, 1989; Shapcott, 1995).

Spatial structuring, or non-randomness, can strongly influence, and be strongly influenced by, many other important aspects of population genetics, including mating system, individual fitness, inbreeding depression, and the action of various other forms of natural selection (Sokal, 1979; Epperson, 1990*a*). Isolation by distance, limited gene flow or seed dispersal should cause neighbouring individuals in plant populations to be genetically related (Wright, 1946; Schaal, 1975). Spatial autocorrelation analysis has been used to detect and interpret spatial patterns of genetic variation in plant populations (Sokal and Oden, 1978). Spatial autocorrelation analysis makes no assumptions about the scale of the structure (Dewey and Heywood, 1988); because individuals can be used for the analysis, any scale of pattern can be analysed. Recently, many studies have described microgeographic differentiation of genetic variation within plant populations and, in populations of many species, these studies have revealed a local structure over short distances (Epperson and Clegg, 1986; Schoen and Latta, 1989; Wagner *et al.*, 1991; Shapcott, 1995; Tani *et al.*, 1998). However, other workers have demonstrated a random or nearly random distribution of genetic variability in some species (Waser, 1987; Dewey and Heywood, 1988; Epperson and Allard, 1989; Leonardi *et al.*, 1995). Differences in spatial patterning among species and populations may result from the differences in life histories, dispersal characteristics, breeding systems, and stand histories (Shoen and Latta, 1989; Tani *et al.*, 1998).

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Ophiopogon xylorrhizus Wang *et* Tai (Liliaceae) is endemic to Mengla County of Yunnan Province, the tropical area of Southwest China. Although *O. xylorrhizus* is a herbaceous perennial with rhizomes, no asexual clones have been found in field investigations. The seed of *O. xylorrhizus* is capable of long-distance dispersal by birds or other animals because of its fleshy exopleura, but most seeds fall beneath the parent plant (He *et al.*, 1999). Analysis of the spatial distribution of individuals revealed that it was closely related to the mean individual age in the stand and that young plants were always assembling, whereas old ones were randomly distributed (He *et al.*, 1999). *F* statistics based on allozyme variation indicated a high level of genetic variation both within and among populations, and showed that most populations were not in Hardy–Weinberg equilibrium (Ge *et al.*, 1997). Pollen dispersal in *O. xylorrhizus* is restricted because this species is pollinated by thrips with high rates of autonomous self-pollination (He *et al.*, 2000). In addition, high levels of inbreeding were demonstrated in populations using allozyme analysis (He *et al.*, 1998*a*). These findings suggest that populations of *O. xylorrhizus* are most likely to exhibit genetic substructuring. Therefore this study aimed to investigate the spatial distribution of genotypes and gene frequencies at three allozyme loci within three stands of *O. xylorrhizus* using spatial autocorrelation analysis. The spatial distribution of family outcrossing rates is also studied using autocorrelation statistics.

MATERIALS AND METHODS

Spatial autocorrelation analysis tests whether observations of a variable at one point or quadrat are independent of observations at neighbouring points or quadrats. If such dependence exists the variable is said to exhibit spatial autocorrelation (Sokal and Oden, 1978).

We calculated the spatial autocorrelation of genotypes of maternal individuals within three stands for three allozyme loci (*Aat*, *Adh* and *Pgm*; He *et al.*, 1998*a* or *b*), and compared the results. In this study, all the seeds from one maternal individual were referred to as a seed family. We obtained allozyme data of the series of seeds by seed embryo electrophoresis, and the genotypes of the maternal individuals at the relevant loci were analysed (He *et al.*, 1998*a*). These data are shown in Table 1. Detailed information about the three stands (P3, P4, and P6) were given by He *et al.* (1998*a*). The genotypes of maternal individuals and the allele frequencies of seed families at all three loci in the three stands are given in the Appendix.

TABLE 1. *General information on the three stands of* O. xylorrhizus *studied*

Stand	No. of maternal plants	Stand area	Individual density (individuals m^{-2})	Mean distance between individuals (m)
P3	16	20 m × 35 m	0·023	5·3
P4	16	20 m × 35 m	0·023	4·2
P6	14	30 m × 35 m	0·014	5·9

All plants of *O. xylorrhizus* that produced offspring (referred to as maternal individuals) in three stands were recorded as a locality and given a unique number. Their locations are recorded as (x, y) co-ordinates relative to the stand boundary lines (Fig. 1). In addition, the locations of seed families were recorded as a quadrat. Effectively, the coordinates (x, y) of maternal individuals and their seed family were identical. Unfortunately, the sample size was small because of the rarity of the species, the small population size and the low flowering rate.

To compute spatial autocorrelation, we need to indicate connections among the localities. We chose to employ a Gabriel-connected graph (Gabriel and Sokal, 1969; Sokal and Oden, 1978). In such a graph, any two localities, A and B, are considered connected if no other locality lies on or within the circle whose diameter is the line AB. The actual co-ordinates of *O. xylorrhizus* plants within the sample stand are used for the analysis. Thus three irregular lattices of sample points for three stands were formed.

The spatial structuring of individual genotypes is quantified with spatial autocorrelation statistics of join-counts (Sokal and Oden, 1978). The distance class is 5 m, this being the mean distance between individuals based on field observations. Six classes were formed with a maximum distance for comparison of 30 m. In a Gabriel-connected graph, test statistics are calculated for the null hypothesis,

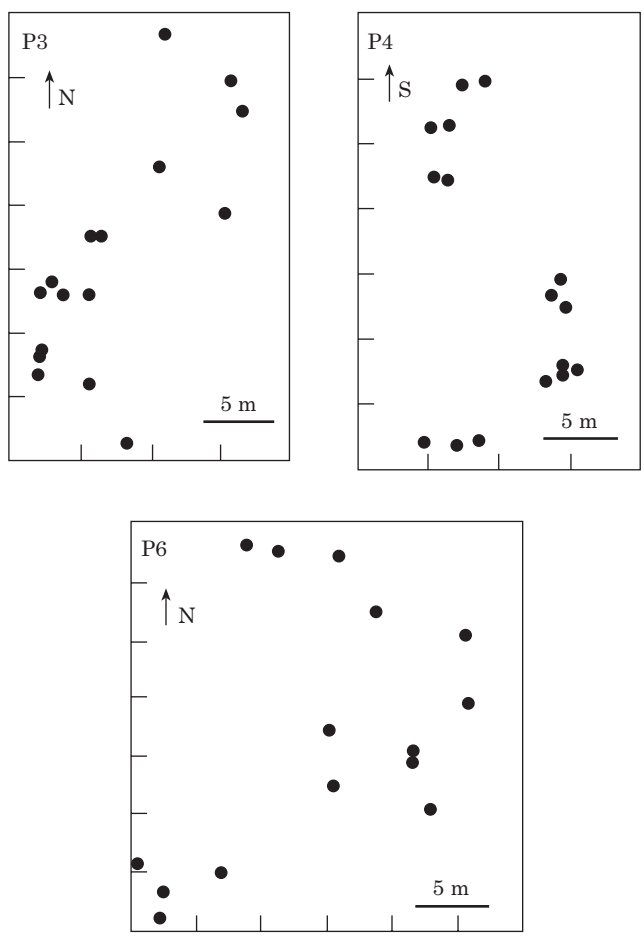

FIG. 1. Sampling map of three stands.

H_0, that the sampling distribution of the numbers of joins is random. Under H_0, the standard errors μ_2 can be found for the expected number of joins μ_1' between genotypes r and s for any distance class k (Sokal and Oden, 1978). In this study, we employed unweighted statistics.

The observed number of joins between the same genotypes is equal to:

$$n_{rr} = \frac{1}{2}\sum_{ij} W_{ij}(rr)_{ij}$$

The observed number of joins between different genotypes is equal to:

$$n_{rs} = \frac{1}{2}\sum_{ij} W_{ij}(rs)_{ij}$$

The expected values of joins between localities of the same genotype and variance are equal to:

$$\mu_1' = \frac{W_{nr}^{(2)}}{2n^{(2)}}$$

$$\mu_2 = \frac{1}{4}\left[\frac{S_1 n_r^{(2)}}{n^{(2)}} + \frac{(S_2 - 2S_1)n_r^{(3)}}{n^{(3)}} + \frac{(W^2 + S_1 - S_2)n_r^{(4)}}{n^{(4)}} - W^2\left(\frac{n_r^{(2)}}{n^{(2)}}\right)^2\right]$$

The expected values of joins between localities of different genotypes and variance are equal to:

$$\mu_1' = \frac{W n_r n_s}{n^{(2)}}$$

$$\mu_2 = \frac{1}{4}\left[\frac{2S_1 n_r n_s}{n^{(2)}} + \frac{(S_2 - 2S_1)n_r n_s (n_r + n_s - 2)}{n^{(3)}} + \frac{4(W^2 + S_1 - S_2)n_r^{(2)} n_s^{(2)}}{n^{(4)}} - 4W^2\left(\frac{n_r n_s}{n^{(2)}}\right)^2\right]$$

where:

- $(rr)_{ij}$ will be equal to 1 when localities i and j are both the genotype r, and will be equal to zero otherwise;
- $(rs)_{ij}$ will be equal to 1 when locality i is genotype r and j is s or when i is s and j is r; it will be equal to zero otherwise;
- n is the number of localities studied;
- n_{rr} is the number of joins between localities of the same genotype, r;
- n_{rs} is the number of joins between localities of different genotypes;
- n_r, n_s are the numbers of the localities of genotype r and s;
- W_{ij} is the weight given to the join between localities i and j; μ_1' and μ_2 are the expected value and variance, respectively;
- W is the sum of the matrix of weights;
- $S_1 = 1/2\Sigma(W_{ij} + W_{ji})^2$, $S_2 = \Sigma(W_{i.}) + W_{.j})$, $W_{i.}$ and $W_{.j}$ are the sums of ith row and jth column of the weight matrix, respectively;
- $n^{(2)} = n(n-1)$, $n^{(3)} = n(n-1)(n-2)$, and so forth.

Under H_0, the test statistics $SND_{rs}(k) = (n_{rs} - \mu_1')/\mu_2$ have an asymptotic standard normal distribution (Sokal and Oden, 1978; Cliff and Ord, 1981). Then the excess (positive value of SND) or deficit (negative value of SND) of each type of join is measured. A set of SND statistics for mutually exclusive distance classes forms an SND correlogram (Sokal and Oden, 1978). The x-intercept of the correlogram measure the size or diameter of the patch (Epperson, 1993).

Moran's I-value is computed for the gene frequency data of seed families. Each seed family was defined as a quadrat. In this study, we employed unweighted Moran's I-statistics (Epperson, 1993). For each distance class k, Moran's I-value is obtained (Sokal and Oden, 1978; Epperson, 1993). Under the random hypothesis, I has an expected value of μ_1' and a variance of μ_2. In addition, a critical I value, Ic, for each correlation (I value) is also obtained.

$$I = \frac{n\sum_{ij} W_{ij} z_i z_j}{\sum_{i=1}^{n} z_i^2}$$

$$\mu_1' = -(n-1)^{-1}$$

$$\mu_2 = \frac{n[(n^2 - 3n + 3)S_1 - nS_2 + 3W^2] - b_2[(n^2 - n)S_1 - 2nS_2 + bw^2]}{(n-1)^{(3)}w^2} - \frac{1}{(n-1)^2}$$

$$Ic = t_{\alpha[\infty]}\mu_2^{1/2}{}_2 - k_\alpha(n-1)^{-1}$$

where:

- $Z_i = X_i - X'$, X_i is the value of the x variable for localities for i, and x' is the mean of x for *all* localities;
- b_α is a coefficient of kurtosis;
- $\alpha = 0{\cdot}05$ or $0{\cdot}01$ (significant level);
- $k_\alpha = (10\alpha)1/2$ for one-tailed testing or $k_\alpha = (5\alpha)1/2$ for two-tailed testing;
- $t_{\alpha[\infty]}$ indicated a normal deviated or Student's distribution for infinite degrees of freedom;
- (other symbols are as described above).

Then $(I - \mu_1')/\mu_2^{1/2}$ has an approximate standard normal distribution under the null hypothesis that the sample values are located randomly (Sokal and Oden, 1978; Cliff and Ord, 1981). Thus an I-correlogram is formed from a set of unweighted I-statistics for mutually exclusive distance classes. In this study, we draw the I-Ic correlogram to show the significance of I-values more directly and clearly. If $I > 0$, the value of I-Ic was plotted; if $I < 0$, then the value of Ic-I was plotted. I-$Ic > 0$ (or Ic-$I > 0$ if $I < 0$) indicates the I-value is significant under H_0.

The spatial distribution pattern of family outcrossing rates was also investigated with Moran's I-statistics as interval data. Family outcrossing rates were estimated by assaying

enzyme loci, and were obtained with Ritland and Jain's (1981) mixed mating model method and Ritland's (1990) MLT computer program. Detailed information about family outcrossing rate estimation is given in He *et al.* (1998*a*).

Using the Bonferroni criterion of simultaneous testing (i.e. obtaining a significance measure for each subset, $i = 1, \ldots, k$ and rejecting overall if, for any subset, $p_i \leqslant 0{\cdot}05/k$), the significance of the correlogram containing spatial autocorrelation coefficients was tested as a whole for significant deviation from total randomness (Sakai and Oden, 1983; Oden, 1984).

RESULTS

Spatial autocorrelation analysis of plant genotypes

For each distance class, there are nine SNDs for three enzyme loci (*Aat*, *Adh* and *Pgm*), and 54 SNDs for distance classes 1 to 6 in each stand of P3, P4 and P6.

There were several different associations between like-homozygous plant pairs and between like-heterozygous plant pairs. An average 44 % of all like-homozygous plant pairs was significantly positively autocorrelated at the shortest distance class, ranging between 33 to 66 % for different stands. On average, like-heterozygous plant pairs showed significantly positive associations in 33 % of the cases with a range of 0 to 66 % for different stands. Unlike-homozygous plant pairs were significantly negatively autocorrelated at one enzyme locus (*Adh*) in stand P6 and 50 % of other enzyme loci showed significantly positive autocorrelation with a range of 33 to 66 % in three stands.

The SND correlogram visualized the patterns of associations with increasing distances between plant pairs. The results from the different enzymes were more or less consistent in each stand as well as between stands. Correlograms for like-homozygous or like-heterozygous plant pairs were plotted together for each stand. Four out of nine correlograms of homozygous plant pairs exhibited a similar pattern: significant positive autocorrelation at shorter distances, random or negative autocorrelation (either significant or not) at the next one or two distance classes, and significant positive autocorrelation in the last one or two distance classes (Fig. 2). This pattern indicates that homozygous plants cluster in patches with a diameter of 5–10 m (Fig. 3). The other five SNDs were not significant at the shortest distance class. For nine correlograms of like-heterozygous plant pairs, there were three spatial patterns. Five correlograms (*Aat* and *Pgm* in P6, *Pgm* in P4, and *Aat* and *Adh* in P3) exhibited random distribution or slightly negative autocorrelation at shorter distances, then significantly positive autocorrelation in the following one or two distance classes, and random distribution or slightly negative autocorrelation again at the most distant distance classes (Fig. 4). Three correlograms exhibited significantly positive autocorrelation at short to moderate distances, but correlations were not significant for larger distances. Both types of correlograms indicate that heterozygous plants are also clustered, and more probably exist in the zone between homozygous patches, because the homozygous patch size is generally 5–10 m, as shown previously, whereas the

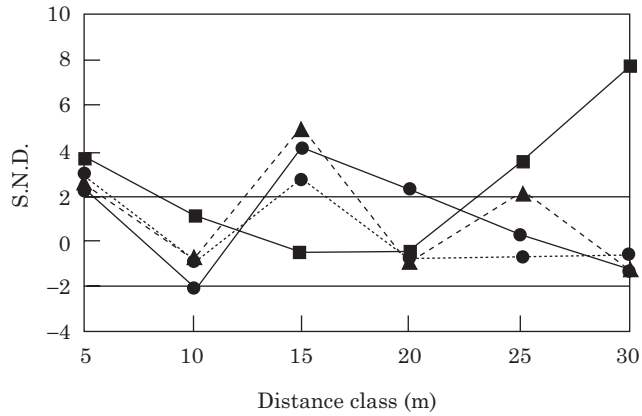

FIG. 2. Correlograms of autocorrelation statistics for like-homozygous plant pairs in three stands. (■) *Aat*; (▲) *Adh*; (●) *Pgm*; ——, P3; – – –, P4; ------, P6.

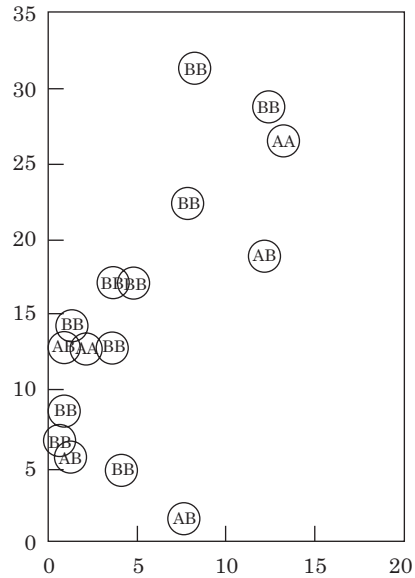

FIG. 3. Genotype distribution map of *Aat* in P3.

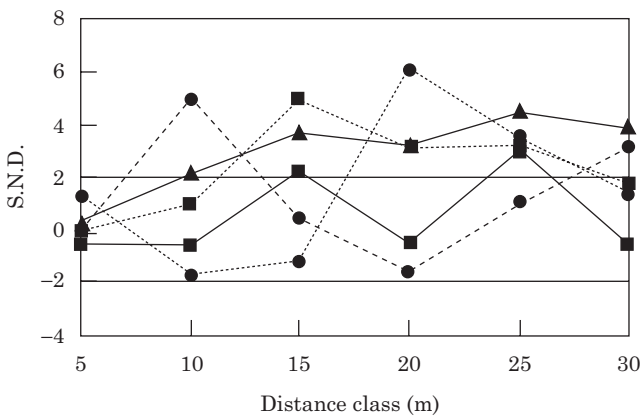

FIG. 4. Correlograms of autocorrelation statistics for like-heterozygous plant pairs in three stands. Symbols as in Fig. 2.

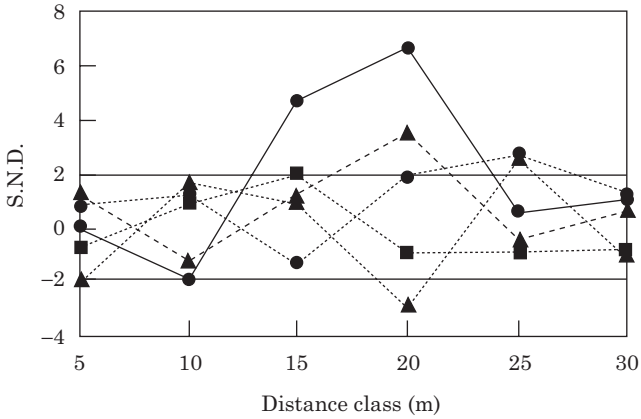

FIG. 5. Correlograms of autocorrelation statistics for unlike-heterozygous plant pairs in three stands. Symbols as in Fig. 2.

heterozygous plants pairs are significantly positively autocorrelated at distances of 10–15 m. The single remaining correlogram of like-heterozygous plant pairs was randomly distributed in all six distance classes.

Of the nine correlograms of unlike-homozygous plant pairs, five exhibited a similar pattern: random distribution or negative autocorrelation (either significant or not) at shorter distances, then significant positive autocorrelation at moderate distances, tending to random distribution in larger distance classes (Fig. 5). This pattern indicates that unlike-homozygous patches did not overlap. Three correlograms of unlike-homozygous plants exhibited a pattern similar to like-homozygous plants, indicating that these unlike-homozygous plant patches overlap in the stands. The remaining correlogram of unlike-homozygous plant pairs was not significant in all six distance classes.

Using the Bonferroni criterion of simultaneous testing, 21 out of 27 correlograms significantly differed from zero for all distance classes. Of these 21 correlograms, seven correlograms were for like-homozygous plant pairs, eight for like-heterozygous plant pairs, and six for unlike-homozygous plant pairs.

Spatial autocorrelation analysis of seed family gene frequency

Using Moran's *I*-value, similar patterns were identified in each stand as well as between stands. Of the 18 *I*-values of three stands at the shortest distance class, 14 were found to be significantly positively autocorrelated. *I-Ic* correlograms visualize the patterns of associations with increasing distance between seed families. The resulting patterns were surprisingly consistent for each allele of the stand (Fig. 6). The *I-Ic* was generally positive and tended to zero at a distance of 15–20 m, indicating that the alleles aggregate and form a patch with a diameter of 15–20 m.

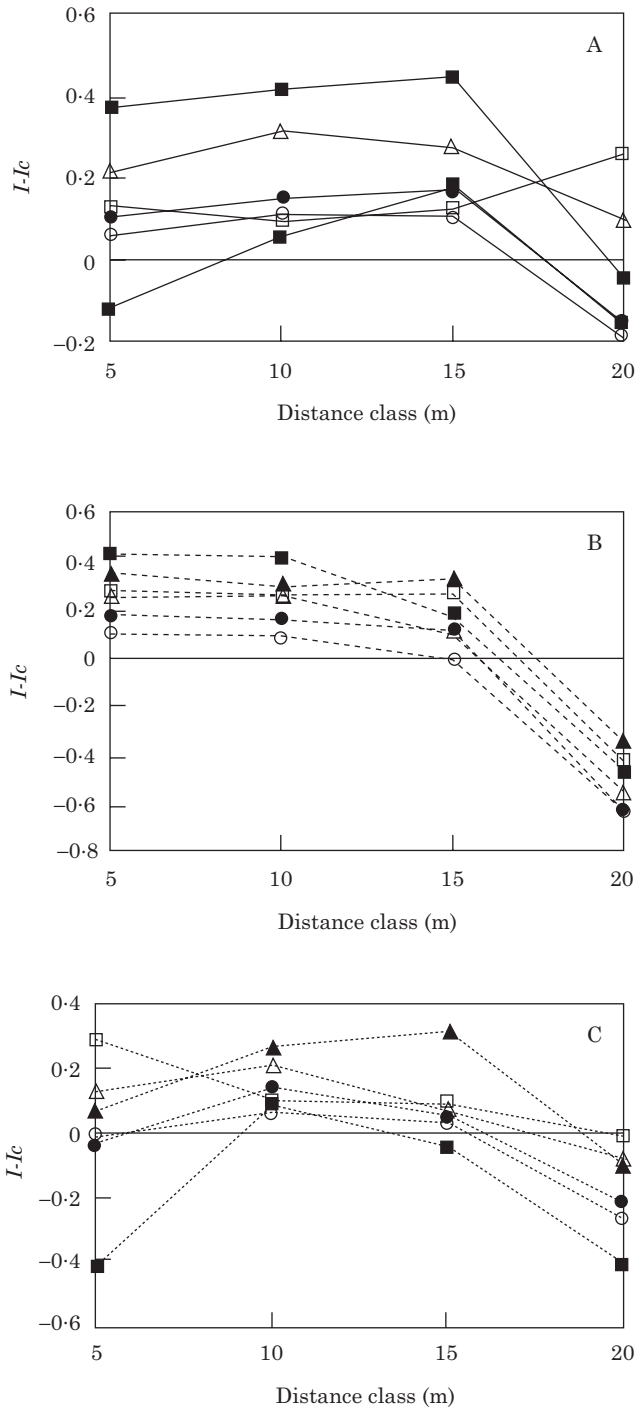

FIG. 6. *I-Ic* Correlograms of *I*-statistics, calculated on seed family gene frequency in three stands (A, stand P3; B, P4; C, P6). *Ic*, Critical *I*-value. If $I > 0$, the value of *I-Ic* was plotted; if $I < 0$, then the value of *Ic-I* was plotted. $I\text{-}Ic > 0$ (or $Ic\text{-}I > 0$ if $I < 0$) indicate the *I*-value was significant under H_0. (■) *Aat*-A; (□) *Aat*-B; (▲) *Adh*-A; (△) *Adh*-B; (●) *Pgm*-A; (○) *Pgm*-B.

Spatial autocorrelation analysis of family outcrossing rates

Similar distribution patterns of family outcrossing rates in space were found using spatial autocorrelation analysis (Fig. 7). Plants with different family outcrossing rates were generally randomly distributed in space, as indicated by the fact that *I-Ic* values are generally negative. In the 12 *I*-statistics of three stands at four distance classes, only one was significantly negatively autocorrelated, indicating that plants with higher family outcrossing rates were closely associated with those having lower family outcrossing rates.

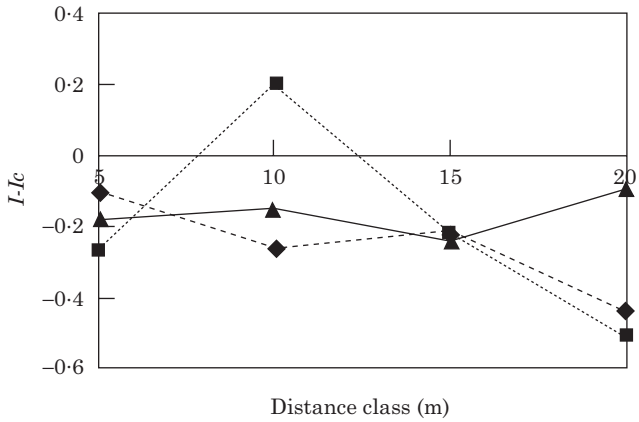

FIG. 7. *I-Ic* Correlograms of *I*-statistics on distribution of family outcrossing rates. *Ic*, Critical *I*-value. If $I > 0$, the value of *I-Ic* was plotted; if $I < 0$, then the value of *Ic-I* was plotted. *I-Ic* > 0 (or *Ic-I* > 0 if $I < 0$) indicate the *I*-value was significant under H_0. ——, P3; – – –, P4; ·····, P6.

DISCUSSION

Spatial patterns of genetic variability found in this study are consistent with the prediction that plant populations are subdivided into local demes or 'neighbourhoods' of related individuals (Ehrlich and Raven, 1969; Bradshaw, 1972; Lavin and Kerster, 1974). In this study, spatial autocorrelation analysis showed that in most cases genotypes and gene frequencies are clustered. Indeed, 21 out of 27 genotype SND-correlograms over the whole distance were significantly different from zero, and 14 out of 18 Moran's *I*-statistics indicating gene frequency were statistically significant at the shortest distance.

The joins between like-homozygotes were generally in excess, but mixed results for joins for like-heterozygotes were found. In a simulation study, Epperson (1995) found that SND statistics for joins between like-heterozygotes depended strongly on the allele frequencies. In particular, when the frequency of either allele carried by a heterozygote is not close to 0·5, SND values are reduced substantially. In *O. xylorrhizus*, very few heterozygotes were detected, and the allele frequencies varied over a wide range, so the variation in SNDs for like-heterozygote joins are expected. On the other hand, large statistical errors as a result of small sample size would be partly attributed to variation in SNDs.

The spatial pattern of gene frequency of seed families was surprisingly consistent. The presence of genetic clustering may be attributed to the reproductive features and pollination characteristics (Sokal *et al.*, 1989). Limited gene flow due to short-distance dispersal of pollen and seed may account for the genetic clustering of most genes in space. *O. xylorrhizus* is thrips-pollinated (He *et al.*, 2000). Thrips are not active pollinators and have weak flight ability. Although outcross-pollination can be mediated by thrips, in most cases, thrips are self-pollinators within flowers (Baker and Cruden, 1991). Based on our results, it is highly likely that thrips contributed partly to the inbreeding. Moreover, autonomous self-pollination in floral buds was also found in *O. xylorrhizus* (He *et al.*, 2000). Limited pollen dispersal resulting from specific pollination characteristics, would be an important cause of consistent genetic clustering. Short-distance seed dispersal could be another factor responsible for genetic clustering. We found that most seeds of *O. xylorrhizus* fell beneath the parent plants (He *et al.*, 1999). Thus family clusters formed and genetic correlation between neighbouring plants occurred.

Partial or predominant selfers are expected to display remarkable local structure (Heywood, 1991). High levels of inbreeding, including selfing and biparental inbreeding, were demonstrated in *O. xylorrhizus* populations. Spatial structure causes substantial biparental inbreeding, thereby causing inbreeding depression. Inbreeding depression itself should change the spatial structure only slightly; the direct effect of selection on the spatial pattern of genotypes is more important (Epperson, 1993). Natural selection (in the form of inbreeding depression) removes inbred individuals, thus changing the spatial structure of genotypes. Significantly positive autocorrelation of alleles was detected among seed families of *O. xylorrhizus*, local gene dispersal increased the genetic identity of neighbouring seed families. A large number of inbred seeds, i.e. homozygous plants, would be removed due to inbreeding depression, and thus the spatial structure of homozygous plant pairs should be reduced. Fourteen out of 18 *I*-statistics were significant, whereas only a third of SNDs of homozygous plant pairs were significant at the shortest distance class. Clearer evidence that selection can modify spatial structure in populations came from a series of simulations (Epperson, 1990*b*) which indicated that selection greatly retards the development of patch structures, and limits them to a much smaller size. These expectations were confirmed in the study of *O. xylorrhizus* using genotype and gene frequency autocorrelation analyses.

The present study confirmed our expectation that the spatial structure of heterozygous plant pairs would not change greatly if we compared genotype and gene frequency. If outcrossing depression does not occur, most heterozygous seeds will develop into adults, and the amount of heterozygous individuals will change little, as will the spatial structure. Apparently, heterozygous plants experience little depression, i.e. weak selection. Monte Carlo simulation revealed that weak selection has a minimal effect on spatial structure and patch size (Epperson, 1990*a*). Although only a third of SNDs for heterozygous plant pairs were significant in the shortest distance class, more SNDs were significant at larger distance classes (15–20 m).

The results of seed dispersal and individual spatial distribution pattern may also partly explain the significant genetic clustering. Most seeds of *O. xylorrhizus* fall near the parent plant, even though *O. xylorrhizus* seed is fleshy and can be dispersed great distances by birds or other animals. Family structure may have generated genotypic structure as a result of clustered cohorts of similar genotype. Perry and Knowles (1991) and Shapcott (1995) suggested a similar scenario, detecting positive autocorrelation of plants of the same genotype existing within a stand, and attributing it in part to limited seed dispersal.

The random distribution of family outcrossing rates was unexpected. Spatial pattern of the sexes of plants is of

considerable importance in determining gene flow within the population, because the pattern influences pollen and seed dispersal and thus the amount of inbreeding (Sakai and Oden, 1983). There is much evidence to suggest spatial segregation of the sexes occurs (Putwain and Harper, 1972; Freeman *et al*., 1976; Grant and Mitton, 1979; Barker *et al*., 1982). In *O. xylorrhizus* about half the individuals are male sterile, i.e. functional female, and the other half hermaphroditic, i.e. bisexual flowers (He *et al*., 1998*b*). We expect that male sterile individuals should be significantly positively autocorrelated with bisexual ones in order to achieve efficient pollination. Family outcrossing rate autocorrelation analysis did not meet our expectation. Families with high outcrossing rates (presumed to be mostly male sterile plants) are not close neighbours of those individuals with low outcrossing rates (presumed to be bisexual plants). Family outcrossing rates are randomly distributed in space. There were no morphological differences between functional female flowers and bisexual flowers—even the flower fragrance was the same. We surmise that random emergence, location and displacment of thrips, the pollinators, shaped the distribution pattern of the sexes.

The results have important implications for conservation management. Strategies of sampling for either *ex situ* gene preservation or *in situ* conservation must consider the spatial structure of genetic variability. Significant patch structure of genetic variation in *O. xylorrhizus* implies that the sampling strategy for conservation should maximize gene diversity and minimize consanguinity. Furthermore, large populations are unlikely to be homogeneous; conserving one part of a large population will not preserve an adequate sample of the genetic variability, since genotype distribution varies between localities and patches in *O. xylorrhizus*.

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APPENDIX

Genotypes of maternal individuals and seed family allele frequency at three loci in three stands

Seed family allele frequency and family outcrossing rates on three loci in three stands

	Amount of seeds	Family outcrossing rates	*Aat*		*Adh*		*Pgm*	
			A	B	A	B	A	B
301	4	0.00	0.04	0.96	0.48	0.52	0.08	0.92
302	5	0.00	0.97	0.03	1.00	0.00	0.00	1.00
303	10	0.69	0.05	0.95	0.55	0.45	0.40	0.60
304	3	0.00	0.25	0.75	0.75	0.25	1.00	0.00
305	4	0.00	0.00	1.00	1.00	0.00	0.51	0.49
306	6	0.00	0.00	1.00	0.48	0.52	0.84	0.16
307	4	0.00	0.00	1.00	1.00	0.00	0.00	1.00
308	14	0.97	0.06	0.94	0.50	0.50	0.86	0.14
309	6	0.26	0.88	0.12	1.00	0.00	0.58	0.42
310	16	1.00	0.08	0.92	0.30	0.70	0.83	0.17
311	11	0.58	0.20	0.80	0.50	0.50	0.95	0.05
312	9	0.73	0.05	0.95	0.17	0.83	0.50	0.00
313	3	0.00	0.00	1.00	0.00	1.00	1.00	0.00
314	11	0.70	0.14	0.86	0.14	0.86	0.00	1.00
315	4	0.00	0.00	1.00	1.00	0.00	0.00	1.00
316	20	1.00	0.45	0.55	0.30	0.70	0.45	0.55
412	7	0.29	0.36	0.64	0.07	0.93	0.14	0.86
413	4	0.00	0.63	0.37	0.05	0.95	0.13	0.87
414	11	0.25	0.40	0.60	0.05	0.95	0.95	0.05
415	12	0.44	0.45	0.55	0.38	0.62	0.17	0.83
416	15	1.00	0.21	0.79	0.93	0.07	0.36	0.64
417	12	0.27	0.08	0.92	0.50	0.50	0.59	0.41
418	6	0.45	0.20	0.80	0.67	0.33	0.33	0.67
419	13	0.85	0.08	0.92	0.60	0.40	0.88	0.12
420	7	0.76	0.29	0.71	0.50	0.50	0.50	0.50
421	9	1.00	0.69	0.31	0.22	0.78	0.29	0.71
422	7	0.48	0.29	0.71	0.33	0.67	0.43	0.57
423	8	0.33	0.05	0.95	0.50	0.50	0.29	0.71
424	12	0.69	0.17	0.83	0.46	0.54	0.43	0.57
425	3	0.00	0.14	0.86	0.50	0.50	0.33	0.67
426	12	1.00	0.05	0.95	0.25	0.75	0.42	0.58
427	7	0.52	0.43	0.57	0.50	0.50	0.95	0.05
601	9	0.44	0.39	0.61	0.95	0.05	0.22	0.78
602	8	0.00	0.50	0.50	0.13	0.87	0.95	0.05
603	4	0.00	0.00	1.00	1.00	0.00	0.00	1.00
605	3	0.00	0.75	0.25	0.95	0.05	0.38	0.62
606	7	0.29	0.21	0.79	0.14	0.86	0.17	0.83
607	6	0.00	0.33	0.67	0.95	0.05	0.95	0.05
608	6	1.00	0.95	0.05	0.95	0.05	0.66	0.34
609	5	1.00	0.40	0.60	0.10	0.90	0.66	0.34
610	9	0.58	0.33	0.67	0.50	0.50	0.38	0.62
611	4	0.00	0.00	1.00	0.00	1.00	1.00	0.00
612	7	1.00	0.25	0.75	0.05	0.95	0.40	0.60
613	8	0.00	0.05	0.95	0.05	0.95	0.05	0.95
614	13	0.39	0.95	0.05	0.48	0.52	0.54	0.46
615	7	0.19	0.36	0.64	0.05	0.95	0.47	0.53

Genotype of maternal individuals in the three stands

Stand P3				Stand P4				Stand P6			
Family	*Aat*	*Adh*	*Pgm*	Family	*Aat*	*Adh*	*Pgm*	Family	*Aat*	*Adh*	*Pgm*
301	BB	AB	BB	412	AB	BB	AB	601	AB	AA	AB
302	BB	AB	BB	413	AB	BB	AB	602	AB	AB	AA
303	AA	AB	AB	414	AB	BB	AA	603	BB	AA	BB
304	BB	AB	AA	415	AB	AB	AA	605	AB	AA	AB
305	BB	AA	AB	416	AB	AA	AB	606	AB	BB	BB
306	BB	AB	AA	417	BB	BB	AB	607	AB	AA	AA
307	BB	AA	BB	418	BB	AB	BB	608	AA	AA	AB
308	BB	AB	AB	419	AB	AB	AA	609	AB	BB	BB
309	AA	AA	AB	420	AB	BB	AB	610	AB	BB	AB
310	BB	AB	AB	421	AB	AB	AB	611	BB	BB	AA
311	AB	BB	AB	422	AB	AB	AB	612	AB	BB	AB
312	BB	AB	AB	423	BB	BB	BB	613	BB	BB	BB
313	BB	BB	BB	424	AB	AB	AB	614	AA	BB	AB
314	AA	AB	BB	425	AB	AB	AB	615	AB	BB	BB
315	BB	AA	BB	426	BB	AB	AB				
316	AB	AB	AB	427	AB	BB	AA				