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Spatial genetic structure in populations of *Chimaphila japonica* and *Pyrola japonica* (Pyrolaceae)

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One hundred and forty and 100 individuals were mapped and sampled in a natural habitat (11 × 45 m) of *Chimaphila japonica* and *Pyrola japonica*, respectively, to analyze the spatial distribution of genotypes using spatial autocorrelation analysis of enzyme polymorphisms. *Chimaphila japonica* is a rhizomatous evergreen subshrub, whereas *P. japonica* is an evergreen herbaceous perennial. Populations of these species have discontinuous distributions and occur in conifer forests in northeastern Asia. For *C. japonica*, Moran's *I* values were significant in 16 out of 40 (40%) cases, and for *P. japonica*, in 26 out of 50 (52%), indicating that a significant small-scale genetic substructuring within a population existed for both species. The mean correlograms of *C. japonica* and *P. japonica* indicate that the patch widths of both species were approximately 5–7 m and 9–10 m, respectively. A nonrandom distribution of genotypes may be a reflection of restricted gene flow, patchy establishment of genetically related individuals, and/or clonal reproduction. The pattern of the average Moran's *I* values of *C. japonica* for each distance class was similar to that of *P. japonica*. The similar pattern of genetic substructuring found in both species reflects their similar life history and ecological traits (e.g., insect pollination, similar habitat and habit, seed dispersal mechanism, and low fecundity).

Key words: Allozyme, *Chimaphila japonica*, gene flow, Moran's *I*, *Pyrola japonica*, spatial autocorrelation

INTRODUCTION

Spatial genetic patterns within populations affect the evolutionary dynamics of populations (Brown 1979). The understanding of spatial patterns of genetic variation within populations could give us more insights into evolutionary and ecological processes in plant species and thus is of continued interest to evolutionary biologists. Recently, spatial genetic structure within plant populations has been analyzed using spatial autocorrelation analysis in an attempt to search for "neighborhood structure" or patches of genetically similar individuals in plants under consideration. Several advantages of the analysis have been noted because the analysis includes all pair comparisons in samples and it makes no assumptions about the spatial scale of the structure within a population (Ep-

person 1989, Heywood 1991). Previous studies, in general, have shown relationships between the spatial genetic patterns of plant populations and their breeding system, seed dispersal mechanism, and spatial distribution of individuals within a population (Linhart *et al.* 1981, Dewey & Heywood 1988, Van Dijk *et al.* 1988, Epperson & Allard 1989, Schnabel & Hamrick 1990, Perry & Knowles 1991, Schnabel *et al.* 1991, Tokunaga & Ohnishi 1992, Maki & Masuda 1993, Geburek & Tripp-Knowles 1994, Berg & Hamrick 1995).

In this study, spatial autocorrelation analysis using allozymes as genetic markers was conducted in populations of *Chimaphila japonica* Miq. (Pyrolaceae), a rhizomatous evergreen subshrub (less than 15 cm tall), and *Pyrola japonica* Klenze, an evergreen herbaceous perennial. Although both species are widely distributed, they have naturally discontinuous distributions, occurring mainly in conifer forests from Taiwan, Japan, and Korea to northern China (Manchuria) and Sakhalin. Usually *C. japonica* has one white flower (ca. 1 cm broad) per peduncle, while *P. japonica* has five to ten flowers. They are visited by *Bombus* spp. (pers. obs.), and like other *Chimaphila* and *Pyrola* species (Kundsen & Olesen 1993), seed-sets via autogamy were made in several individuals of the two species cultivated in a greenhouse (Chung & Kang, unpubl. data). The two species have anthers with poricidal dehiscence (i.e., opening by apical pores) and are known to be buzz-pollinated by bees (Kundsen & Olsen 1993). Fragrant flowers of the species last ca. one week, and offer pollen and/or nectar as a reward to visitors (Kundsen & Olesen 1993). The fruit is a subglobose capsule. Seeds are small (0.5–1.0 mm), and are presumably dispersed by wind and/or surface water movement on hillsides.

MATERIALS AND METHODS

In March 1995, we mapped and collected leaf samples from all individuals (140 of *C. japonica* and 100 of *P. japonica*) within a 11 × 45-m area located on Mt. Yeonwha, Gosung-gun, Prov. Gyeongsangnam-do, Korea to test whether alleles were distributed nonrandomly using a spatial autocorrelation analysis. Samples were placed in plastics bags wrapped with a wet paper towel and stored on ice to prevent protein denaturation prior to returning to a laboratory, where they were stored at 4°C until protein extraction.

Leaf samples were cut finely, and crushed with a mortar and pestle. A phosphate-polyvinylpyrrolidone extraction buffer (Mitton *et al.* 1979) was added to the leaf samples to facilitate crushing and to aid enzyme stabilization. The cellular extract was absorbed onto 4 × 6-mm wicks cut from Whatman 3MM chromatography paper, which were stored at – 70°C until needed for analysis. Electrophoresis was performed using 10.5% starch gels. Twenty-two and 17 putative loci for *C. japonica* and *P. japonica* from 11 and nine enzyme systems were resolved using three systems of electrode and gel buffer. A discontinuous histidine citrate buffer system, a modification (Chung & Kang 1994) of that of Soltis *et al.* (1983) was used to resolve phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), and malate dehydrogenase (MDH) in both species, and peroxidase (PER) in *C. japonica*. A Poulik buffer system, a modification (Haufler 1985) of Soltis *et al.* (1983) system 8, resolved menadione reductase (MNR), glutamate dehydrogenase (GDH), and triosephosphate isomerase (TPI) in both species, and diaphorase (DIA) in *C. japonica*. A tris-citrate buffer system of Soltis *et al.* (1983) system 4 resolved isocitrate dehydrogenase (IDH), shikimic acid dehydrogenase (SKDH), and 6-phosphogluconate dehydrogenase (PGD). Stain recipes were taken from Soltis *et al.* (1983), except for DIA, which were taken from Cheliak and Pitel (1984). Putative loci were designated sequentially, with the most anodally migrating isozyme designated '1', the next '2', etc. Likewise, alleles were designated sequentially with the most anodally migrating allele designated 'a'. Although the genetic bases of the loci were not documented by controlled crosses, the isozymes expressed phenotypes that were consistent in subunit structure and genetic interpretation with most isozyme studies in plants, as documented by Weeden and Wendel (1989). A locus was considered polymorphic in a population only if the most common allele occurred at a frequency of 0.95 or less (a 95% criterion).

For spatial autocorrelation analysis, genotypic data were coded so that allele frequency values of 0.0, 0.5, or 1.0 were assigned to individual plants (Perry & Knowles 1991). Only one allele was considered at diallelic loci as the second allele would contribute identical information. Every possible pair of individuals was considered as a join and was assigned to one of the distance classes, and the ranges of which were selected by equalizing sample sizes. Moran's *I* values were calculated for each of the ten distance classes by:

$$I = N\sum_i\sum_j (W_{ij}Z_iZ_j)(\sum_i\sum_j W_{ij}\sum Z_i^2)^{-1} \qquad (1)$$

(Sokal & Oden, 1978a). Here, N is the number of individuals, W_{ij} is the join on weighting matrix, where W_{ij} is set as one if ith and jth population are in the distance class and zero otherwise, $Z_i = X_i - \overline{X}$, $Z_j = X_j - \overline{X}$, the variables X_i and X_j are the mean allele frequency scores for *i*th and *j*th individual, respectively, and $\overline{X}$ is the mean score for all individuals. Each *I* value was used to test significant deviations from the expected values, $E(I) = -1/(N-1)$ (Cliff & Ord 1981). A significant positive value of Moran's *I* indicates that the neighboring individuals in the distance class con-

sidered tend to have similar gene frequencies, whereas a significant negative value suggests that they tend to have different gene frequencies. Overall significance of individual correlograms was tested using Bonferroni's criteria (Sakai & Oden 1983). All calculations and statistical analyses were performed using the SAAP program (Version 4.3) written by D. Wartenberg.

RESULTS

For *Chimaphila japonica* and *Pyrola japonica*, only four and three of the 22 and 17 loci resolved were polymorphic in the samples examined (*Pgm-2*, *Mnr-1*, *Skdh-1*, and *Skdh-2* for *C. japonica* and *Mnr-1*, *Tpi-2*, and *Skdh-1* for *P. japonica*). Four and five alleles for *C. japonica* and *P. japonica*, respectively, were used for spatial autocorrelation analysis on the basis of a 95% criterion for considering a polymorphic locus. The spatial autocorrelation coefficients, Moran's *I*, for both species are presented in Table 1. For *C. japonica*, Moran's *I* values were significantly different from the expected value ($E(I) = -0.007$) in 16 out of 40 (40%) cases, and the overall correlogram for *Pgm-2* and *Skdh-1* were significant (Table 1). In the short distance classes (Classes 1 to 3, $0 < 5.5$ m), *I* was significantly positive in five out of 12 cases, indicating that genetic similarity was shared among individuals within a 5.5-m distance. Beyond the distance class 6 ($8 < 43$ m), six significant negative values were observed. For *P. japonica*, Moran's *I* was significantly different from the expected value ($E(I) = -0.01$) in 26 out of 50 (52%) cases, and five of six alleles were nonrandomly distributed (Table 1). Again, within Distance class four ($0 < 9.5$ m), nine out of 20 *I* values were significantly positive, indicating that plants within the distance class were more likely to have similar genotypes (i.e., genetic substructure) for *Tpi-2* and *Skdh-1* (Table 1).

DISCUSSION

The rates of significant *I* values in *Chimaphila japonica* and *Pyrola japonica* was higher than the intended 5% type I error, suggesting that genetic structuring within a population existed for both species. Although no statistical test for difference between correlograms is known (Sokal & Wartenberg 1983), the results of this study indicate that the pattern of spatial genetic distributions in both species seems to be similar to each other. Among 40 cases calculated for ten distance classes in a

Table 1. Spatial autocorrelation coefficients (Moran's *I*) over 10 distance classes for allozymes in a location of *Chimaphila japonica* Miq. and *Pyrola japonica* Klenze. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Locus	Distance class[1)]										Significance of correlogram
	1	2	3	4	5	6	7	8	9	10	
C. japonica											
Pgm-2	0.11**	0.05*	0.03	– 0.01	0.04	0.09**	0.03	– 0.12**	– 0.16**	– 0.12**	***
Mnr-1	0.01	– 0.00	– 0.04	– 0.02	– 0.04	0.02	0.00	0.01	– 0.06*	0.03	*ns*
Skdh-1	0.19**	0.14**	0.11**	– 0.04	– 0.06*	– 0.09**	– 0.04	0.11**	– 0.29**	– 0.22**	***
Skdh-2	0.02	– 0.07*	0.03	– 0.04	0.00	0.00	0.00	– 0.03	0.00	0.01	*ns*
Average	0.08	0.03	0.03	– 0.03	0.02	0.00	– 0.00	– 0.01	– 0.13	– 0.07	
$E(I) = -0.007$											
P. japonica											
Mnr-1	0.04	– 0.04	– 0.01	0.01	0.02	– 0.11**	– 0.04	– 0.04	0.03	0.03	*ns*
Tpi-2	0.03	– 0.04	0.10**	– 0.01	– 0.03	– 0.05	– 0.04	– 0.05	– 0.01	– 0.01	**
Skdh-1[a]	0.13**	– 0.02	– 0.14**	0.08*	– 0.07	– 0.15**	0.07*	0.01	0.12**	– 0.13**	**
Skdh-1[b]	0.33**	0.29**	0.10**	– 0.07	– 0.36**	– 0.18**	– 0.15**	– 0.31**	0.07*	0.19**	***
Skdh-1[c]	0.22**	0.28**	0.18**	0.05	– 0.21**	– 0.10*	– 0.33**	– 0.40**	0.04	0.17**	***
Average	0.15	0.09	0.05	0.01	– 0.13	– 0.12	– 0.10	– 0.16	0.05	0.05	
$E(I) = -0.010$											

[1)] Distance classes in meters: *C. japonica* (1, 0.0–2.5; 2, 2.5–4.0; 3, 4.0–5.5; 4, 5.5–7.0; 5, 7.0–8.0; 6, 8.0–10.0; 7, 10.0–22.0; 8, 22.0–28.0; 9, 28.0–31.5; 10, 31.5–43.0) and *P. japonica* (1, 0.0–2.0; 2, 2.0–4.0; 3, 4.0–6.5; 4, 6.5–9.5; 5, 9.5–11.0; 6, 11.0–13.0; 7, 13.0–16.0; 8, 16.0–20.0; 9, 20.0–24.5; 10, 24.5–33.0).

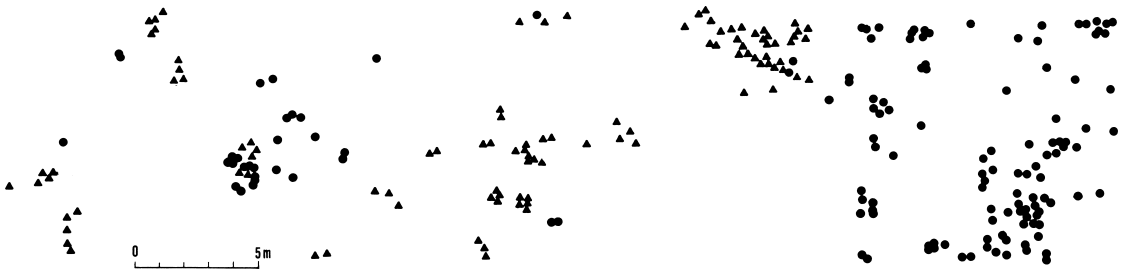

Fig. 1. The relative location of 140 and 100 individuals of *Chimaphila japonica* Miq. (closed circles) and *Pyrola japonica* Klenze (closed triangles), respectively.

C. japonica population, Moran's *I* was significant for 16 (40%) cases, and, for *P. japonica*, 26 out of 50 (52) cases of Moran's *I* were significant. In addition, the pattern of the average Moran's *I* value of *C. japonica* for each distance class was similar to that of *P. japonica*. The average values for *C. japonica* were 0.08, 0.03, 0.03, – 0.03, 0.02, 0.00, – 0.00, – 0.01, – 0.13, and – 0.07, and, for *P. japonica*, these values were 0.15, 0.09, 0.05, 0.01, – 0.13, – 0.12, – 0.10, – 0.16, 0.05, and 0.05. These results indicate that a similar pattern of genetic substructuring within populations examined exists in both species. This is not surprising when we consider the similarity between *C. japonica* and *P. japonica* in terms of a very similar life history and ecological traits (e.g., insect pollination, a very similar habitat and habit, seed dispersal mechanism by wind, and low fecundity).

Clustering of individuals with genotypes similar in distances within an area under consideration may develop from a combination of several evolutionary forces such as seed and/or pollen dispersal, genetic drift, and/or microhabitat selection, and history of plant species. Results from this study seem to be in good agreement with the habits and seed dispersal mechanism of both species. As seeds of *C. japonica* and *P. japonica* are small (ca. 0.5–1.0 mm), they are presumably dispersed by wind and/or surface water movement on hillsides. However, distance of seed dispersal by wind in pine–oak forests would be restricted because the height of *C. japonica* and *P. japonica* ranges only 10 to 20 cm tall. Although we do not know what the standardized dispersal is (e.g., Wright's neighborhood size), this information is necessary because the physical distances of dispersal should be related to the density of individuals in order to relate to the physical scale of spatial structure (Epperson 1995a). As significant spatial autocorrelation within a population exists, gene flow via pollen and seed would be restricted in a small spatial scale within populations of both species. This is consistent with the prediction by Levin and Kerster (1974) that plant populations in general are subdivided into neighborhoods of related individuals. It may be of interest to infer the patch sizes of both species. The distance at which a mean Moran's *I* value first intercepts the *E*(*I*) value may represent the shortest side of an irregularly shaped patch. The mean correlograms of *C. japonica* and *P. japonica* indicate that the approximate patch widths were inferred to be 5–7 m and 9–10 m in *C. japonica* and *P. japonica*, respectively. This seems to be consistent with the distributional patterns of both species within a location (Fig. 1). Thus, the presence of genetic structure in populations of the two species suggests that local genetic drift and spatially variable selection, if present, may play important roles in shaping genetic structuring within populations. If so, genetic drift may be a primary factor in producing monomorphism in several enzyme systems found in *C. japonica* and *P. japonica* (i.e., fixation of one allele or loss of allele). According to more recent theoretical works concerning relationships between dispersal and spatial structure as measured by spatial autocorrelation statistics (Epperson 1995ab), autocorrelation values estimated in this study are expected to be much lower than predicted for neutral loci because amounts of dispersal in *C. japonica* and *P. japonica* are expected to be low or moderate.

The results of spatial autocorrelation is consistent with an overall deficiency of heterozygotes relative to Hardy-Weinberg expectations. Mean Wright's (1965) F_{IS} values for *C. japonica* and

P. japonica were 0.124 and 0.240 (Chung & Kang 1996) respectively. As the two species are considered to be self-compatible (Chung & Kang, unpubl. data), and gene flow restricted, an effective size of subpopulations would be small (Maruyama & Tachida 1992). If this is true, genetic drift may in part result in considerable levels of heterozygote deficiencies (genetic substructure) observed in populations of the two species (inbreeding-like effects, Hartl & Clark 1989).

As negative *I* values in the higher distance classes of *C. japonica* exist, individuals of the species occur on a gradient (Sokal & Oden 1978b). On the other hand, for *P. japonica*, positive *I* values in the higher distance classes suggest that the gradient, if present, is circular (Sokal & Oden 1978b) or patch distribution is somewhat regular (Epperson & Clegg 1986). These results indicate that the possible differences between both species in terms of the extent of gene flow and clonal reproduction, patch sizes, and other unknown factors of their biology.

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