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Notes on spatial genetic structure in a hybrid population between *Aconitum japonicum* subsp. *napiformme* and *A. jaluense* (Ranunculaceae)

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The fine-scale genetic structure in a hybrid population (35 × 55-m area) between the herbaceous perennials *Aconitum japonicum* Thunb. ex Murray subsp. *napiformme* (Lév. & Vaniot) and *A. jaluense* Komarov (Ranunculaceae) was analyzed using spatial autocorrelation statistics. Although individuals of the population have protandrous flowers, the population is highly structured. Individual genotypes exhibited significant spatial autocorrelation on a scale of 4 m due to the limited seed and pollen dispersal, which is consistent with a neighborhood size of about 25. Such neighborhood sizes are about one-fourth of the entire population.

Key words: *Aconitum*, allozyme, gene flow, Moran's *I*, spatial autocorrelation

INTRODUCTION

The spatial distribution of genetic variation in plant populations of non-clonal species is primarily determined by seed and pollen dispersal, habitat distribution, microenvironmental selection, and genetic drift (Levin & Kerster 1974, Epperson 1993). The degree to which selection and drift effects patterns of genetic variation is known to depend on the dispersal ability (gene flow via pollen and seed dispersal) of plant species (Hamrick & Nason 1996). For example, if gene flow is limited to a small spatial scale, populations will have more inbreeding, and are, as a result, more likely to differentiate in response to local selective forces or to genetic drift. Thus, it is important to analyze spatial genetic structure in plant populations, which can be quantified using spatial autocorrelation analysis (Sokal & Oden 1978).

This study is a part of a larger project investigating the spatial genetic structure of several herbaceous perennials growing in the understorey of temperature forests in northeastern Asia (Chung & Park 1998, Chung & Epperson 1999, Chung *et*

al. 1999, Kang & Chung 2000). The previous results showed that individual genotypes of allozymes in populations are substantially structured in an isolation-by-distance manner on a small spatial scale, consistent with the biology of the species (e.g., species with insect-pollination and with seed dispersal by gravity resulting in limited seed and pollen dispersal). Little is known, however, about the fine-scale genetic structure in hybrid populations of herbaceous plants (e.g., Burke *et al*. 2000). In this study, we analyze the fine-scale genetic structure using allozymes as genetic markers in a hybrid population between *Aconitum japonicum* Thunb. ex Murray subsp. *napiforme* (Lév. & Vaniot) Kadota and *A. jaluense* Komarov (Ranunculaceae) (Park *et al.* 1997). Members of aconites are self-compatible, though flowers are protandrous and pollinated by bumblebees (Park *et al.* 1997; Y. Kadota of Tsumura Laboratory, Japan, pers. comm.). Each ellipsoidal follicle (ca. 20 mm long) contains a few small seeds (ca. 3 mm long).

MATERIALS AND METHODS

For spatial analysis, in October 1998, a total of 95 individuals were mapped and leaf samples were collected within a 35 × 55-m area at Nogodan (altitude ca. 1 500 m above sea level, south–southeastern facing 10% slope), Prov. Chollanamdo, Korea. Samples of contiguous individuals can have near-optimal sampling properties for measuring genetic isolation by distance within the population (Epperson & Li 1997). Individuals in the study population grow under the subshrub *Rhododendron schlippenbachii*. Leaves were kept on ice, transported to the laboratory, and stored at 4 °C until protein extraction. Leaf samples were cut finely, and crushed with a mortar and pestle, and phosphate-polyvinylpyrrolidone extraction buffer (Mitton *et al*. 1979) was added. Enzyme extracts were absorbed onto 4 × 6-mm wicks cut from Whatman 3MM chromatography paper, which were then stored at –70 °C until needed. Levels and distribution of allozyme variation were determined via horizontal starch-gel electrophoresis. Electrophoresis was performed using 10.5% starch gels. Four putative loci from four enzyme systems were resolved using two electrode and gel buffer systems. A Poulik buffer system, modified (Haufler 1985) from Soltis *et al.* (1983) system 6, resolved menadione reductase (*Mnr*) and phosphoglucomutase (*Pgm*). A histidine citrate buffer system, a modification (Chung & Kang 1994) of Soltis *et al.* (1983) system 11 was used to resolve isocitrate dehydrogenase (*Idh*) and 6-phosphogluconate dehydrogenase (*Pgd*). Stain recipes followed Soltis *et al.* (1983). Putative loci were designated sequentially, with the most anodally-migrating isozyme designated 1, the next 2, and so on. Similarly, alleles were designated sequentially in alphabetical order, with the most anodally-migrating alleles marked with a superscript ‘a’. Although the genetic basis of each locus was not documented by controlled crosses, the isozymes expressed phenotypes that were consistent in subunit structure and genetic interpretation with other plant isozyme studies, as documented by Weeden and Wendel (1989). For spatial autocorrelation analysis, the genotype for each allele at the different locations was converted into the values 0.0, 0.5 or 1.0 according to the numbers (none, one, or two) of that allele which were carried in the genotype (Sokal & Oden 1978). Only one allele was considered at dialellic loci. All possible pairs of individuals were considered as joins (a connection between two individuals) and assigned to one of ten distance classes (according to the distance separating the two individuals). The ten distance classes were designed to equalize sample sizes per each distance class. Moran’s *I*-statistic (Sokal & Oden 1978) was calculated for each of ten distance classes. Each *I* value was also used to test for significant deviations from the expected values, $E(I) = -1/(N - 1)$ under the random distribution null hypothesis (Cliff & Ord 1981). In addition, the overall significance of each correlogram, and hence the presence of spatial structure, for each allele was tested using Bonferroni’s criterion (Sakai & Oden 1983). All calculations for spatial statistical analyses were performed with the SAAP program (ver. 4.3) written by D. Wartenberg.

RESULTS AND DISCUSSION

Four alleles were used for spatial autocorrelation analysis. Moran’s *I* values were significantly

Table 1. Spatial autocorrelation coefficients (Moran's *I*) of four alleles in a hybrid population (Mt. Chiri) between *Aconitum japonicum* subsp. *napiforme* and *A. jaluense* for ten distance classes. The expected value = –0.011

DB[1]	4	8	11	13	17	21	25	31	38	61	Bonferroni	Allele
DC[2]	1	2	3	4	5	6	7	8	9	10	test[3]	frequency
Idh[a]	0.28**	–0.03	0.14**	0.03	–0.10*	–0.06	–0.12**	–0.09*	–0.11**	0.05	0.000	0.595
Mnr[a]	0.16**	–0.05	0.01	0.04	–0.02	–0.04	0.02	–0.09*	–0.01	0.01	0.027	0.695
Pgm[a]	0.05	–0.08*	0.00	0.01	–0.00	–0.05	0.02	–0.04	–0.02	0.02	0.417	0.745
Pgd[a]	0.08*	0.02	0.05	–0.08	0.05	–0.13**	–0.03	–0.04	0.03	–0.06	0.032	0.855
Average	0.15	–0.04	0.05	0.00	–0.02	–0.07	–0.03	–0.07	–0.03	0.01		

* = $P < 0.05$; ** = $P < 0.01$.

[1] Upper distance bound (m), [2] Distance class, [3] Bonferroni test for overall correlogram significance was conducted following Sakai and Oden (1983).

different from the expected value ($E(I) = –0.011$) in 11 (27.5%) of 40 cases, and the overall correlogram was significant for three out of four alleles (Table 1). For distance class 1 (0 < 4 m), three statistically significant positive values were observed, indicating that most of the genetic similarity is shared among individuals separated by less than 4 m. The distance at which the average Moran's *I*-values first intercepts the E(*I*) value may represent the shortest length of an irregularly shaped patch size (Sokal 1979). In this study, the mean correlogram indicated that the minimum patch widths were approximately 5 m. The observed fine-scale genetic structure in the hybrid population seems to be consistent with its biology. The distance of seed dispersal would be short because no special seed dispersal mechanisms are developed in aconites and thus seeds fall beneath the maternal plants. In addition, pollen travel via bumblebees occurs preferentially among neighbors (M. G. Chung pers. obs.).

Similar results were observed in other herbaceous perennials. Chung & Epperson (1999) studied two populations of *Adenophora grandiflora* having protandrous and insect-pollinated flowers. Individual genotypes in populations of this species are distributed in a substantially structured, isolation-by-distance manner on a scale of less than 10 m. Structured patterns were observed in a population of *Lycoris sanguinea* var. *koreana*, an insect-pollinated herbaceous perennial with individual genotypes exhibiting significant spatial autocorrelation on a scale of 2–6 m due to the limited seed and pollen dispersal (Chung *et al.* 1999). Again, analyses of fine-scale genetic structure in two hybrid populations among *Iris fulva*, *I. hexagona* and *I. brevicaulis* (bumblebee pollination and gravity seed dispersal) revealed a significant positive correlation among neighbors on a scale of approximately 2–4 m (Burke *et al.* 2000). The authors concluded, however, that the observed spatial genetic structure largely results from clonal reproduction rather than the role of limited pollen and seed dispersal. It may be of importance to note that the authors did not separate the spatial genetic structure caused by clonal reproduction from that maintained in sexually reproduced individuals to draw their conclusion (e.g., Chung & Epperson 1999). For *Adenophora grandiflora* and *Lycoris sanguinea* var. *koreana*,

both the entire populations and the sets of sexually reproduced individuals exhibited significant autocorrelation at less than approximately 10 m.

It may be of interest to infer the neighborhood size of the study population. There were no "typical" forms of either *Aconitum japonicum* subsp. *napiforme* or *A. jaluense* in the hybrid population, suggesting that repeated introgression has probably occurred in the population (Park *et al.* 1997) and that the study population may be old. Based on the sample sizes, the observed statistical values indicate a Wright's neighborhood size (Wright 1943), N_e, of ca. 25 for stable populations (Epperson & Li 1997, Epperson *et al.* 1999). Such neighborhood sizes are about one-fourth of the entire population. A neighborhood size of 25 to 50 is consistent with bee-pollinated herbaceous perennials having gravity-dispersed seeds such as *Lycoris sanguinea* var. *koreana* (Chung *et al*. 1999), *Adenophora grandiflora* (Chung & Epperson 1999), *Hosta* spp. (Chung & Park 1998), *Hemerocallis hakuunensis* (Kang & Chung 2000), and *Chinographis japonica* var. *japonica* (Maki & Masuda 1994).

In seed plants, pollen dispersal is solely by migration, whereas seed or fruit dispersal could result in both colonization and gene flow (Hamrick & Nason 1996). Since gene movement in seed plants is a sequential process (a two-step process via pollen and then by seed), observed genetic structure may be most strongly influenced by limited seed dispersal compared to pollen movement (Hamrick & Nason 1996). In other words, limited seed dispersal can proximally cause genetically distinct seed shadows around maternal plants, and if pollen dispersal is also limited, this will eventually lead to the development of a more intense genetic structure within populations over cycles of regeneration (Epperson 1993). This prediction is consistent with the present study as well as with previous results for other herbaceous perennials with insect-pollination and without special mechanisms for seed dispersal.

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