Journal of Plant Biology, December 1999, 42(4) : 273-278

Spatial Autocorrelation within a Korean Population of *Raphanus sativus* var. *hortensis* f. *raphanstroides*

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Spatial autocorrelation was applied to microgeographic variations in Korean wild radish, *Raphanus sativus* var. *hortensis* Baker f. *raphanstroides*. Separate counts of each type of join (combination of genotypes at a single locus) for each allele, and for each distance class of separation, were tested for significant deviation from random expectations by calculating the Standard Normal Deviation. Moran's *I* was significantly different from the expected value in 16 of 112 cases (14.3%). Eleven of these values (9.8%) were negative, indicating genetic dissimilarity among pairs of individuals in the eight distance classes. Populations of wild radish are small in Korea and are distributed with tractors used for fishing. Occasional cutting of seed- bearing stems as fodder for animals also may bring a high level of gene flow. Thus, disturbance by many anglers, as well as by farmers, may contribute to the fact that the wild radish population of Onsan is unusual in lacking spatial genetic structure.

Keywords: *Raphanus sativus* var. *hortensis* Baker f. *raphanstroides*, spatial autocorrelation, standard normal deviation (SND)

Genetic structure is an integral part of the processes of population genetics (Epperson and Allard, 1989; Huh et al., 1999). Population structure interacts with a number of factors: microenvironmental heterozygosity (Bradshaw, 1984), mortality due to stochastic events (Wright, 1978), and mating systems that feature imited dispersal of seed or pollen (Epperson and Allard, 1989; Huh et al., 1996). The most important factors are gene flow and natural selection, which influence spatial patterns of the genetic population structure (Bradshaw, 1984; Slatkin, 1987; Epperson, 1990). In theory, genetic differentiation over short distances may occur either as a result of spatially variable selection or localized genetic drift, provided that gene flow is sufficiently restricted (Dewey and Heywood, 1988). Many early, direct studies suggested that actual gene-dispersal distances are greater than the observed pollen- and propagule-dispersal distances (Schaal, 1980). The potential for genetic differentiation via genetic drift within populations of outcrossing plant species may be substantially less than was previously thought (Dewey and Heywood, 1988; Chung and Chung, 1994; Chung, 1995).

Indirect evidence for genetic correlations between neighboring plants has been obtained from data on mating systems (Epperson and Allard, 1989). Localized seed and pollen dispersal produced family clusters within these populations (Epperson, 1990; Chung et al., 1995). Several studies revealed decreased seed set and seed survivorship from matings between genetically similar, near- neighbors, which has been interpreted as inbreeding depression (Price and Waser, 1979; Waser and Price, 1983; Levin, 1984).

In this study, spatial autocorrelation (SA) was analyzed in Korean populations of *Raphanus sativus* var. *hortensis* f. *raphannstroides* (wild radish, Brassicaceae), an herbaceous annual. The spatial distribution was described for alleles at polymorphic enzyme loci in a natural population. Compared with studies by Tokugana and Ohnishi (1992) of three wild populations in Japan, relatively long distances were used in the present research. Wild radish occurs only on sand dunes or sandy slopes near beaches in East Asia, and can exhibit gametophytic-sporophytic self-incompatibility (Sampson, 1966; Lewis et al., 1988; Huh et al., 1995). In addition, outcrossing has lower value than should be expected in Korean wild radish (Huh, 1995). Its patchy distribution, self-incompatibility, sparse populations, ease of breeding, and short generation time make it a useful experimental species for genetic structure.

The genetic structure of Korean wild radish is similar to that of the Japanese, but gene flow differs in terms of artificial interference. Although Korean wild radish resembles Japanese wild radish in many respects, seed weight, length of conical beak, and petal color are not alike. Neither Korean nor Japanese wild radishes belong to *R. sativus*, but rather to *R. raphannstroides*. Both flower during April, May, and June.

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MATERIALS AND METHODS

Sampling Procedure and Electrophoresis

The study was conducted from June to July of 1998, at Samam-ri, Onsan-up, Ulsan-si, and involved mapping and sampling of 64 individual plants >30 cm tall. The distance between selected individuals was about 1.0 m, to avoid including those with common lineage. One leaf per plant was sampled. Leaves gathered from natural populations were labeled and then refrigerated in plastic bags for 1 to 2 days, until electrophoresis was carried out.

Homogenization, starch gel electrophoresis, and enzyme assay procedures followed the methods of Soltis et al. (1983). Leaves were homogenized to release enzymes from cellular and organellar membranes by mechanical grinding with a Tris-HCl grinding buffer-PVP solution. Electrophoresis was performed using 10%- starch gels. Seven enzyme systems were assayed: alcohol dehydrogenase (ADH), malate dehydrogenase (MDH), glutamate oxaloacetate transaminase (GOT), peroxidase (PER), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucomutase (PGM), and isocitrate dehydrogenase (IDH).

For enzymes that resolved in more than one zone of activity, the most anodal isozyme was arbitrarily designated as ‘1’, and subsequent isozymes were sequentially assigned higher numbers. Likewise, alleles were designated sequentially, with the most anodally migrating allozyme designated as ‘a’ and progressively slower forms ‘b’, ‘c’, and so on.

Statistical Measures of Genetic Structure

The spatial structuring of allozyme variation was quantified by Moran's *I*, a coefficient of SA (Sokal and Oden, 1978a, 1978b). As applied in this study, Moran's *I* quantifies the genetic similarity of pairs of spatially adjacent individuals relative to the population sample as a whole. The value of *I* ranges between +1 (complete positive autocorrelation, i.e., paired individuals have identical values) and –1 (complete negative autocorrelation). Each plant was assigned a value depending on the presence or absence of a specific allele. If the *i*th plant was a homozygote for the allele of interest, the assigned p*i* value was 1. If the individual was a heterozygote, the value 0.5 was assigned, and if the allele was absent, the value 0 was assigned.

Pairs of sampled individuals [total number of pairs: $(64 \times 63)/2 = 2{,}016$] were classified according to the Euclidian distance d*ij*, so that the class k included d*ij* satisfying $k - 1 < dij < k + 1$, where k takes 1 to 8. The interval for each distance class was 5 m. Moran's *I* statistic for the class k was calculated as follows: $I\ (k) = n\Sigma i\Sigma j(I\neq j)WijZiZj/S\Sigma Zi^2$, where Z*i* is p*i*-p (p is the average of p*i*), W*ij* is 1 if the distance between the *i*th and *j*th plants is classified into class k; otherwise, W*ij* is 0, n is the number of all samples, and S is the sum of W*ij* $\{\Sigma i\Sigma j(i\neq j)Wij\}$ in class k. Under the randomization hypothesis, *I*(k) has the expected value $u1 = -1/(n-1)$ for all k. Its variance, u2, has been given, for example, in Sokal and Oden (1978a). Thus, if an allele is distributed randomly for class k, the normalized *I*(k) for the standard normal deviation (SND) for plant genotype, $g(k) = \{I(k)-u1\}/u2^{1/2}$, asymptotically has the standard normal distribution (Cliff and Ord, 1981). Hence, SND g(k) exceeding 1.96, 2.58, and 3.27 are significant at probability levels 0.05, 0.01, and 0.001, respectively.

For diallelic loci, only those with allele frequencies <0.95 and >0.05 were employed, and then only one allele was considered because the second allele would contribute identical information. For multiallelic loci, all alleles at that locus, regardless of their frequencies, were used for the spatial analysis.

RESULTS

From the individuals sampled, three alleles were found for *Idh-2*, with frequencies of 0.642 (allele *a*), 0.316 (allele *b*), and 0.042 (allele *c*). Three alleles were also found for *Pgm-2*, with frequencies of 0.144 (allele *a*), 0.676 (allele *b*), and 0.180 (allele *c*). *Got-1*, *Mdh-1*, *Pgd-2*, and *Pgm-3* loci also expressed two alleles. ADH and PER were monomorphic at all sites. The SA coefficient, Moran's *I*, for a polymorphic locus is presented in Table 1. Moran's *I* was significantly different from the expected value in only 16 of 112 cases (14.3%). Eleven of these values (9.8%) were negative, indicating genetic dissimilarity among pairs of individuals in the eight distance classes. Only five of the significant values (4.5%) were positive, indicating a partially genetic similarity among individuals in the distance class 7, i.e., pairs of individuals separated by more than 35 m. Overall, the Korean wild radish population lacked significant genetic structure in most spatial classes.

Separate counts of each type of join (combination of genotypes at a single locus) for each allele, and for each distance class of separation, were tested for significant deviation from random expectations by calculating the SND. Figures 1, 2, and 3 show the

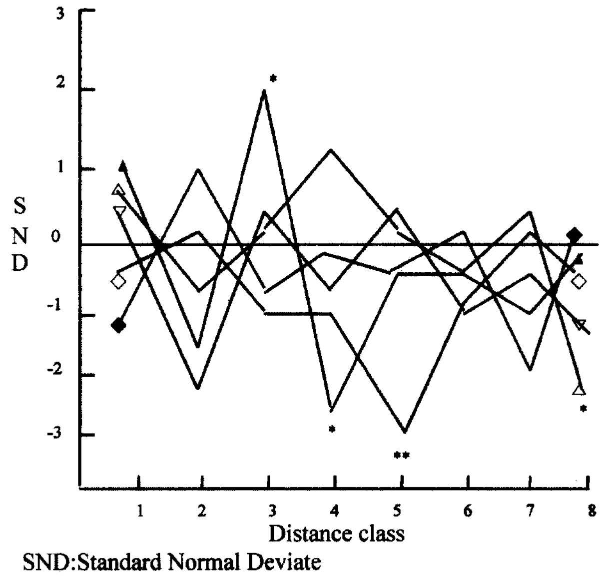

Figure 1. Distance-corrected correlograms for geographic variation patterns of allozyme frequencies (*Got-1* and *Idh-2*) of the wild radish. △ : *Idh-2a*; ▲ : *Idh-2b*; ▽ : *Idh-2c*; ◇ : *Got-1a*; ◆ : *Got-1b*. *$p < 0.05$. The distance classes are the same as in Table 1.

distribution of SA for wild radish across the distance class 8. For all distance classes, only 11 SND statistics were significant. Two alleles, *Idh-2b* and *Pgm-2b*, showed significantly positive SND values for distance class 3 (Figs. 1 and 3). *Mdh-1a* and *Mdh-1b* also had significantly positive SND values for distance classes 6 and 7, respectively (Fig. 2). The aggregation of n identical alleles, called a "patch", resulted in those loci (Tokugana and Ohnishi, 1992). The nine significantly negative SND values indicate an excess of different allele pairs at all eight loci for class 8. This suggests neighbor patches in which different alleles are predominant 35 to 40 m apart, on average.

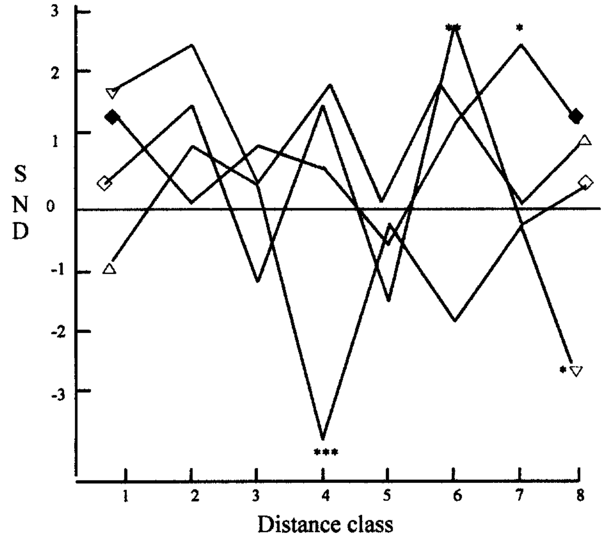

Figure 2. Distance-corrected correlograms for geographic variation patterns of allozyme frequencies (*Mdh-1* and *Pgd-2*) of the wild radish. ◇ : *Mdh-1a*; ◆: *Mdh-1b*; △ : *Pgd-2a*; ▲: *Pgd-2b*. The distance classes are the same as in Table 1. *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$.

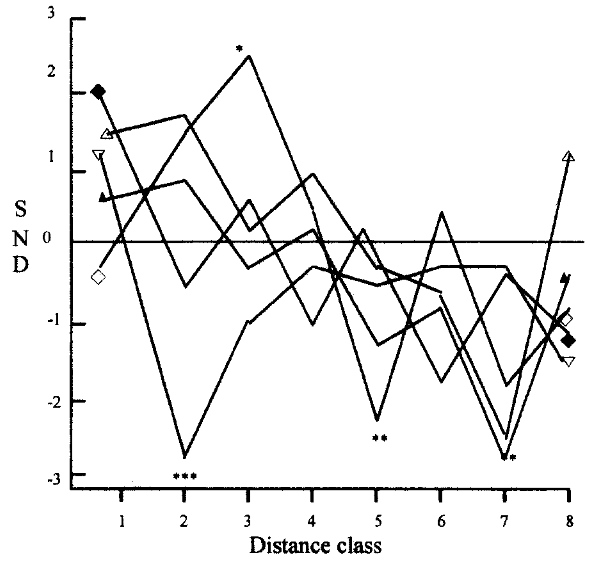

Figure 3. Distance-corrected correlograms for geographic variation patterns of allozyme frequencies (*Pgm-2* and *Pgm-3*) of the wild radish. ◆: *Pgm-2a*; ◇ : *Pgm-2b*; △ : *Pgm-3a*; ▽ : *Pgm-3b*; ▲ : *Pgm-3c*. *$p < 0.05$, **$p < 0.01$. The distance classes are the same as in Table 1.

DISCUSSION

Although significant aggregation of an identical allele was partially observed at four loci for some classes, no spatial structure of allele frequencies was found for either of 14 polymorphic loci within the natural population of wild radish. The results from this study are not completely consistent with the supposition that plant populations are subdivided into local demes, or neighborhoods of related individuals (Ehrich and Raven, 1969; Bradshaw, 1972; Levin and Kerster, 1974). Previous reports on the local distribution of genetic variability suggested that microenvironmental selection and limited gene flow are the main factors causing substructuring of alleles within a population (Epperson and Clegg, 1986; Epperson and Allard, 1989; Schoen and Latta, 1989; Huh et al., 1995). Local genetic differentiation at isozyme or other marker loci, caused by microenvironmental

Table 1. Spatial autocorrelation coefficients (Moran's *I*) of 14 alleles among populations of *Raphanus sativus* var. *hortensis* Baker f. *raphanstroides* for eight distance classes.

Allele	Distance Classes								
	1	2	3	4	5	6	7	8	AF
Got-1a	−0.003	0.010	−0.030	−0.028	−0.079*	−0.022	0.012	−0.017	0.917
Got-1b	−0.019	0.044	−0.009	0.006	−0.012	0.004	−0.048*	0.004	0.083
Idh-2a	0.030	−0.072*	0.058*	0.031	0.008	−0.012	0.010	−0.065*	0.642
Idh-2b	0.040	−0.004	0.019	−0.082**	−0.007	−0.005	−0.015	−0.008	0.316
Idh-2c	0.027	−0.062*	0.024	−0.022	0.017	−0.031	−0.003	−0.030	0.042
Mdh-1a	0.007	0.028	−0.015	0.028	−0.023	0.075*	−0.012	0.006	0.921
Mdh-1b	0.012	0.008	0.021	0.015	−0.013	0.029	0.049*	0.003	0.079
Pgd-2a	0.005	0.017	0.010	0.034	−0.000	0.032	−0.001	0.021	0.028
Pgd-2b	0.034	0.047	0.010	−0.101***	−0.001	−0.037	−0.006	−0.054*	0.079
Pgm-2a	0.049*	−0.007	0.018	−0.027	0.003	−0.048	−0.019	−0.042	0.144
Pgm-2b	−0.007	0.042	0.061*	0.005	−0.095**	0.005	−0.063	−0.031	0.676
Pgm-2c	0.009	0.023	−0.011	−0.002	−0.003	−0.024	−0.082**	−0.010	0.180
Pgm-3a	0.042	0.047	−0.001	0.028	−0.043	0.043	−0.004	−0.025	0.069
Pgm-3b	−0.012	−0.101***	−0.039	−0.010	−0.015	−0.009	−0.008	−0.044	0.704

AF : Allele frequencies.

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

The distance classes are 0-5.0 m (class 1), 5.0-10.0 m (class 2), 10.0-15.0 m (class 3), 15.0-20.0 m (class 4), 20.0-25.0 m (class 5), 25.0-30.0 m (class 6), 30.0-35.0 m (class 7), 35.0-40.0 m (class 8).

heterogeneity, has been observed in a variety of plant species (Bradshaw, 1984). Those loci showed significant aggregation of an identical allele, which persisted for generations, as long as the same microenvironmental conditions continued. This persistence was demonstrated, for example, in the *Got-1* locus in lodgepole pine (Epperson and Allard, 1989) and for the genetic variation in quantitative traits of *Impatiens capensis* (Argyres and Schmit, 1991). In the present study, the *Mdh-1* locus showed significant aggregation in the Korean wild radish population. This aggregation, however, did not persist at all loci, thereby excluding microenvironmental selection as being the main cause for the allele aggregation.

The average Moran's *I* values for each distance class in this study indicate that Korean wild radish populations apparently are less structured than for the Japanese wild radish (Tokugana and Ohnishi, 1992). Possible contributing factors include differences in density, topography, and human interference. The Japanese wild radish populations were located on a beach. A road through the beach ran parallel to the coastline, 100 m away. These populations were distributed with tractors used for fishing. If gene flow is limited at most loci where allele aggregation is observed in adult plant populations, allele aggregation also is expected in a pollen cloud (Conner et al., 1996). This phenomenon was observed at the Japanese inland sites, and supports the pattern of limited gene flow found in wild radish (Tokugana and Ohnishi, 1992). In contrast, the Korean wild radish populations were located on sand dunes at a beach and on rocky upland slopes. Wild radishes in Korea are used widely as fodder for animals such as cows and goats. This occasional cutting of seed- bearing stems may cause high levels of gene flow.

Why is the population of Korean wild radish unusual, in that it lacks significant genetic structure at most spatial classes? A likely explanation is that gene flow has been sufficiently extensive to prevent the random divergence of local gene frequencies. Even a small amount of gene flow is enough to counteract the diversifying effects of genetic drift or weak selection (Wright, 1978; Devlin and Ellstrand, 1990). In recent simulations, Ohsawa et al. (1993) and Epperson (1995) showed that local genetic differentiation is very sensitive to the degree of actual gene dispersal. The indirect estimate of gene flow, based on the mean Gst (the proportion of total genetic diversity partitioned among populations), was high (*Nm* = 4.58) in Korean wild radish populations (Huh, 1995). The levels of gene flow calculated in the present study are of sufficient magnitude to counterbalance genetic drift or weak selection, thus playing a major role in shaping the genetic structure of the wild radish population (Wright, 1978; Devlin and Ellstrand, 1990; Chung et al., 1995).

Relatively large amounts of gene flow occur in wild radish populations (Devlin and Ellstrand, 1990; Devlin et al., 1992). For example, honeybees (50%) tend to move most frequently among neighboring plants, while syrphids (14%), hesperids (4%), and, particularly, butterflies (24%) forage at only a few plants within a population (Devlin and Ellstrand, 1990). The high level of gene flow in the Korean wild radish is mainly caused by pollen dispersal via insects, and may be enhanced, in part, by occasional cutting and seed dispersal by farmers. Action by farmers has contributed to the wider distribution of pollen and seed from a source plant that is not leptokurtic for most insect-pollinated species. Thus, high levels of gene flow caused by humans as well as insects may contribute to the lack of spatial genetic structure in the Onsan population of Korean wild radish.

Received August 9, 1999; accepted November 1, 1999.

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