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Spatial Genetic Structure of Allozyme Polymorphisms Within Populations of *Rhus trichocarpa* (Anacardiaceae)

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Abstract

Rhus trichocarpa MIQ. (Anacardiaceae) is a dioecious, insect-pollinated tree. It has a wide distribution in East Asia, from Japan, China and Korea, north to Sakhalin. In these regions, *R. trichocarpa* is a pioneer tree that, once established, creates conditions favorable for subsequent successional tree species, by providing attractive perching for various bird species, which then drop seed of the later successional species. Later species form pine-oak forests which overgrow *R. trichocarpa.* We used allozyme loci and spatial autocorrelation statistics to examine the spatial distribution of allozyme polymorphisms of individuals in two Korean populations. Populations of the species maintain moderate levels of allozyme variation (mean H_e = 0.173, G_{ST} = 0.064). It was found that genetic patch width was at least 25 m, and this was created by limits to seed or pollen dispersal.

Key words: Allozymes, *Rhus trichocarpa,* spatial autocorrelation, spatial genetic structure.

Introduction

Rhus trichocarpa MIQ. (Anacardiaceae) is a dioecious, insect-pollinated woody species. Flowers are visited by bees (*Bombus diversus diversus* and *Apis mellifera,* M. G. CHUNG pers. obs.). The species occurs widely in East Asia, from Japan, China and

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Korea, north to Sakhalin. *Rhus trichocarpa* is a pioneer tree that initiates new successional communities by encouraging seed dispersal by birds during the early successional stages of pine-oak forests in these regions (KAMITANI et al., 1998). Fruits (drupes, ca. 8 mm to 9 mm long) remain attached throughout the year and attract frugivorous birds (MATSUOKA and KOJIMA, 1979). As the fruits of *R. trichocarpa* attract birds, ingested seeds from other trees are also dispersed into the area. After about 30 years, patches of *R. trichocarpa* are replaced by taller tree species in pine forests in Japan (KAMITANI et al., 1998). Thus, the species has an important role in shifting communities from shade-intolerant pioneer tree species to shade-tolerant species. In Korea, the species commonly grows in pine-oak forests. Despite the ecological importance of *R. trichocarpa,* fires occur frequently during the dry, winter season in the pine-oak forests in which individuals of *R. trichocarpa* usually grow. The Korean government has made efforts to reestablish artificial stands of pines, oaks, sumacs, and other associated plant species in the areas disturbed by fires.

Spatial genetic structure within plant populations is primarily determined by the effects of factors such as limited seed and pollen dispersal, isolation in small patches, differential mortality, and microhabitat selection (LEVIN and KERSTER, 1974; EPPERSON, 1993). Conversely, spatial genetic structure influences the dynamics of biparental inbreeding, inbreeding depression, and the operation of natural selection (EPPERSON, 1993). Spatial genetic structure can be analyzed using spatial autocorrelation statistics (SOKAL and ODEN, 1978). In addition to its inherent interest, analyses of spatial genetic structure within populations of *R. trichocarpa* could be used to provide baseline information for sampling strategies of the species. In this paper, we study the spatial genetic structure within populations of *R. trichocarpa*.

Materials and Methods

In November 1997, all 200 and 135 individuals were mapped, diameters at breast height (DBH) were recorded, and a 10 cm-long branch (one year old) per individuals was collected, within 60-m X 100-m (alt. 200 m a.s.l., SSW, 3% grade) and 30-m X 70-m (alt. 420 m a.s.l., SSW, 4% grade) areas in Mt. Wola (hereafter referred to as WOL) and on a hillside near Gyeongsang National University (GNU), Chinju City, Prov. Gyeongsangnamdo, respectively. Both populations grow in natural pine-oak forests in the southern Korean peninsula. Branches were kept on ice, transported to the laboratory, and stored at 4 °C until protein extraction.

Young bark was cut finely, and crushed with a mortar and pestle. A phosphate-polyvinylpyrrolidone extraction buffer (MITTON *et al.,* 1979) was added and the crushed extract was absorbed onto 4-mm X 6-mm wicks cut from Whatman 3MM chromatography paper, which were stored at -70 °C until needed for analysis. Electrophoresis was performed using 10% starch gels. Twelve putative loci for *R. trichocarpa* from six enzyme systems were resolved using a Poulik buffer system, a modification (HAUFLER, 1985) of SOLTIS *et al.* (1983) system 6. These were diaphorase (Dia), fluorescent esterase (*Fe-1, Fe-2*), peroxidase (*Per-1, Per-2*), phosphoglucoisomerase (*Pgi-1, Pgi-2*), phosphoglucomutase (*Pgm-1, Pgm-2*), and triosephosphate isomerase (*Tpi-1, Tpi-2, Tpi-3*). Stain recipes were taken from SOLTIS *et al.* (1983), except diaphorase (CHELIAK and PITEL, 1984). Putative loci were designated sequentially, with the most anodally migrating isozyme designated 1, the next 2, and so on. Similarly, alleles were designated sequentially with the most anodally migrating alleles 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 other isozyme studies in plants, as documented by WEEDEN and WENDEL (1989).

For the analysis of allozyme diversity, a locus was considered polymorphic if two or more alleles were detected, regardless of their frequencies. Four standard genetic diversity parameters were estimated using a computer program developed by M. D. LOVELESS and A. SCHNABEL (pers. comm.): percent polymorphic loci (P), mean number of alleles per locus (A), effective number of alleles per locus (A_e), and gene diversity (H_e).

For spatial autocorrelation analysis, the genotypic data were coded so that allele frequency values of 1.0, 0.5, or 0.0 were assigned to each individual being homozygous for a given allele, heterozygous for that allele, or genotypes with no copies of that allele, respectively, for each polymorphic locus (SOKAL and ODEN, 1978). If a diallelic locus had allele frequencies less than 0.95 and greater than 0.05, it was used. In any case, only one allele was considered at a diallelic locus, because the second allele contributes identical information. For a locus having more than three alleles, all alleles at that locus, regardless their frequencies, were used for the spatial analysis. Every possible pair of individual trees was considered as a join (a connection between two individuals) and was assigned to one of the ten distance classes (according to the Euclidean distance separating the pair), and the ranges of the distance classes were selected in a way that equalized the total number of joins in each. MORAN's I-values (SOKAL and ODEN, 1978) were 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 and ORD, 1981). A significant positive value of MORAN's I indicates that the pairs or joins of individuals in that distance class have similar gene frequencies, whereas a significant negative value indicates that they have dissimilar gene frequencies. Overall significance of each correlogram was tested using BONFERRONI's criterion (SAKAI and ODEN, 1983). All calculations and statistical analyses were performed using the SAAP program (ver. 4.3) written by D. WARTENBERG.

NEI's (1973, 1977) gene diversity formulae (H_T, H_S, D_{ST}, and G_{ST}) were used to evaluate the distribution of genetic diversity within and among populations. In addition, a X^2-statistic was used to detect significant differences in allele frequencies among populations for each locus (WORKMAN and NISWANDER, 1970). An indirect estimate of gene flow (Nm, the number of migrants per generation) was calculated based on F_{ST}, equivalent to G_{ST} as calculated in this study (WRIGHT, 1951).

Results

The mean DBH for population WOL (3.49 cm ± 0.16 cm [SE]) was significantly larger than that for population GNU (1.26 cm ± 0.09 cm) (unpaired t-test, $t = -12.4$, $P < 0.001$).

Of the 12 loci examined, five were polymorphic in at least one population. Loci *Dia, Fe-1, Fe-2, Per-2, Pgi-1, Tpi-1,* and *Tpi-2* were monomorphic in both populations. Estimates of gene diversity within populations (means of P, A, A_e, and H_e were 41.7%, 1.54, 1.32, and 0.173) are presented in *table 1.*

According to the criteria described above, seven (WOL) and nine (GNU) alleles were used for single locus spatial autocorrelation analysis. The spatial autocorrelation coefficients, MORAN's I, for the two populations as well as frequencies of these alleles are presented in *tables 2* and *3*. For population WOL, MORAN's I-values were significantly different from the expected value ($E[I] = -0.005$) in 44 (63%) of 70 cases, and the overall correlogram was significant for all seven alleles (*Table*

Table 1. – Summary of allozyme variation for 12 loci within two populations of *Rhus trichocarpa*[1]).

Pop[b]	N^c	P	A	A_e	H_o (SE)	H_e (SE)
WOL	200	41.67	1.50	1.34	0.214 (0.008)	0.185 (0.067)
GNU	135	41.67	1.58	1.29	0.162 (0.009)	0.161 (0.064)
Mean	167.5	41.67	1.54	1.32	0.188 (0.006)	0.173 (0.046)

[1]) Abbreviations: P, percentage of polymorphic loci; A, mean number of alleles per locus; A_e, effective number of alleles per locus; H_o, observed heterozygosity; H_e, HARDY-WEINBERG expected heterozygosity or genetic diversity.
[b]) WOL, population in Mt. Wola; GNU, Chinju population.
[c]) Sample size.

2). For distance class 1 to 3 (0 < 29 m), 12 significantly positive cases were observed, whereas only four significantly negative cases were detected among all the longer distance classes, indicating that genetic similarity was shared among individuals within 29 m of each other. A different result was observed in population GNU. MORAN's I-values were significantly different from the expected value (E [I] = -0.007) in 10 (11%) of 90 cases, and the correlograms were significant for only three (33%) of nine alleles (*Table 3*). More importantly, the average values for GNU are much smaller than those for WOL (compare *Tables 2* and *3*). Indeed, average values for GNU indicate an essentially random distribution of alleles.

Table 2. – Spatial autocorrelation coefficients (MORAN's I) of seven alleles in Mt. Wola (WOL) population of *Rhus trichocarpa* for ten distance classes.

	Distance class (upper bound, m)											
Allele	1(14)	2(22)	3(29)	4(35)	5(42)	6(49)	7(57)	8(68)	9(81)	10(111)	$P^{1)}$	AF[2)]
Per-1[b]	0.05**	-0.04*	-0.02	0.02	-0.01	-0.05**	-0.04	0.05**	0.00	-0.02	0.019	0.5200
Pgi-2[a]	0.26**	0.02	-0.04*	-0.09**	-0.08**	-0.07**	-0.07**	-0.05*	0.02	0.05**	0.000	0.0275
Pgi-2[b]	0.17**	0.05**	0.01	-0.03	-0.05**	-0.03	-0.02	-0.04*	-0.00	-0.11**	0.000	0.7150
Pgi-2[c]	0.19**	0.04*	-0.05*	-0.06**	-0.08**	-0.06**	0.01	0.03*	0.04*	-0.10**	0.000	0.2575
Pgm-1[b]	0.17**	0.05**	-0.10**	-0.11**	-0.05*	-0.07**	-0.04*	0.04*	0.09**	-0.02	0.000	0.3500
Pgm-2[a]	0.31**	0.17**	0.06**	-0.07**	-0.14**	-0.16**	-0.24**	-0.14**	0.02	0.13**	0.000	0.4975
Tpi-3[a]	0.06**	0.01	-0.07**	-0.00	-0.05**	0.02	-0.01	-0.02	0.01	0.00	0.005	0.7775
Average	0.17	0.04	-0.03	-0.05	-0.07	-0.06	-0.06	-0.02	0.03	-0.01		

[1]) Overall correlogram significance (BONFERRONI approximation): * = P < 0.05; ** = P < 0.01.
[2]) Allele frequency.

Table 3. – Spatial autocorrelation coefficients (MORAN's I) of nine alleles in Chinju (GNU) population of *Rhus trichocarpa* for ten distance classes.

	Distance class (upper bound, m)											
Allele	1(9)	2(13)	3(17)	4(22)	5(27)	6(34)	7(41)	8(48)	9(55)	10(75)	$P^{1)}$	AF[2)]
Per-1[a]	-0.01	0.02	0.02	-0.04	-0.09**	0.00	0.01	0.01	-0.03	0.02	0.021	0.1037
Per-1[b]	0.03	0.03	-0.01	-0.00	-0.09**	-0.05	0.01	0.02	0.01	-0.02	0.036	0.6667
Per-1[c]	0.01	0.02	-0.07*	-0.06	0.01	0.00	0.01	0.04	0.01	-0.05	0.244	0.2296
Pgi-2[b]	0.04	-0.02	0.00	-0.04	-0.04	0.02	-0.06*	-0.02	0.03	0.02	0.361	0.3148
Pgm-1[a]	0.04*	-0.03	-0.01	-0.01	-0.00	0.00	-0.00	-0.01	-0.02	-0.04	0.156	0.0037
Pgm-1[b]	-0.01	-0.01	0.01	-0.03	-0.02	-0.00	-0.00	-0.00	0.01	-0.01	1.000	0.3963
Pgm-1[c]	-0.02	-0.02	0.01	-0.03	-0.03	0.00	-0.00	-0.00	0.01	-0.00	1.000	0.6000
Pgm-2[a]	0.03	-0.00	-0.00	-0.00	-0.02	-0.03	-0.03	-0.02	-0.06*	0.05*	0.117	0.3000
Tpi-3[a]	0.02	0.02	0.07**	0.06*	-0.00	0.01	-0.01	-0.06*	-0.05	-0.13**	0.000	0.9407
Average	0.02	0.00	0.00	-0.02	-0.03	-0.00	-0.01	-0.00	-0.01	-0.02		

[1]) Overall correlogram significance (BONFERRONI approximation): * = P < 0.05; ** = P < 0.01.
2) Allele frequency.

Significant differences in allele frequencies between the two populations were found for four ($P < 0.001$ in each case) polymorphic loci except *Pgm-1*. The G_{ST} values ranged from 0.002 for *Pgm-1* to 0.163 for *Pgi-2* with a mean of 0.064, indicating that most of the total variation resides within populations (*Table 4*). The indirect estimate of gene flow based on the mean G_{ST} was high ($Nm = 3.67$).

Discussion

The two study populations of *Rhus trichocarpa* ($H_e = 0.173$) harbor levels of allozyme diversity similar to that in two wide-ranging North American species, *R. glabra* L. ($H_e = 0.150$) and *R. copallina* L. (0.150) (SHERMAN-BROYLES *et al.*, 1992) and the Asian species, *R. javanica* L. ($H_e = 0.175$; M. G. CHUNG et al., unpubl. data).

The percentage of statistically significant MORAN's *I*-values in the two populations of *R. trichocarpa* is higher than the expected 5% type I error, suggesting that some genetic structuring within populations exists. Although no statistical test for difference between correlograms have been developed (SOKAL and WARTENBERG, 1983), the results of this study indicate that the pattern of spatial genetic distribution in population WOL differs from that of population GNU. For population WOL, 11 significant positive *I* values were detected in the distance classes one to three, whereas 24 significant negative *I* values were observed beyond the distance class 3. This indicates that there must be significant limits to seed and/or pollen dispersal. The average value of Moran's *I*-statistics for the first distance class (0.17) corresponds to an expected value of 25 for WRIGHT's neighborhood size for stable populations (EPPERSON, HUANG and LI, 1999). In contrast, the mean values for each distance class for population GNU were similar to the expected value, indicating that the pattern is at most very weak and indeed alleles are essentially randomly distributed. Clustering of sexually reproduced individuals with similar genotypes 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, microhabitat selection, and the nature of and time since colonization. As the habitats of the two populations appear to be similar, the differences in observed patterns in the two populations are most simply attributed to differences in history of colonization. Based on the annual rings of the widest stem in populations WOL (DBH = 12.1 cm) and GNU (6.6 cm), the maximum age of populations WOL and GNU is about 26 and 12 years, respectively, suggesting that population WOL was colonized ten years (about one sexual-generation) earlier than was GNU. It is expected that older populations have stronger spatial genetic structure, because genetic isolation by distance generally increases over generations (e.g., EPPERSON, 1993). Moreover, it is clear that in population GNU, the genotypes of the founding seeds (deposited by birds) were essentially randomly distributed spatially.

The spatial scale of structure, even weak structure, can be estimated using the distance at which mean MORAN's *I*-values first intercept the E (*I*) value (SOKAL, 1979). The mean correlogram of population WOL indicates that the minimum patch width is approximately 25 m. The scale of spatial genetic structure in *R. javanica* is similar, 23 m to 25 m (M. G. CHUNG *et al.,* unpubl. data), and the same is true for populations of other trees *Quercus margaretta* ASHE (about 20 m; BERG and HAMRICK, 1994) and *Acer saccharum* MARSH. (about 20 m to 30 m; PERRY and KNOWLES, 1991).

The mean G_{ST} value (0.060) between the two populations (separated by 12 km) of *R. trichocarpa* is similar to averages reported for woody species with similar life history characteristics (HAMRICK *et al.,* 1992), i.e., wide-ranging species ($N = 9$, 0.033), outcrossing, animal pollinated species ($N = 37$, 0.099), species with ingested seed dispersal ($N = 14$, 0.051), and species with both sexual and asexual modes of reproduction ($N = 10$, 0.051). A higher G_{ST} value would be expected if more isolated populations were included in the analyses. The values of the indirect estimate, *Nm*, of gene flow between the two populations was high, 3.67. For neutral genes, a value of *Nm* below 1 indicates that genetic drift causes substantial population differentiation, whereas *Nm* values above 4 indicate that gene flow precludes differentiation via genetic drift (WRIGHT, 1951).

For preservation purposes, sampling methods for seed stocks could be optimized by utilizing information on spatial genetic structure within populations (e.g., MAKI and YAHARA, 1997; CHUNG and PARK, 1998). It is suggested that, overall, the sampling of *R. trichocarpa* should be conducted at 25 m intervals, in order to efficiently extract the genetic diversity across an entire population.

Table 4. – Genetic diversity statistics (NEI, 1973, 1977) for each of five polymorphic loci in *R. trichocarpa*[1]).

Locus	H_T	H_S	G_{ST}
Per-1	0.519	0.496	0.044***
Pgi-2	0.508	0.426	0.163***
Pgm-1	0.467	0.466	0.002ns
Pgm-2	0.487	0.468	0.039***
Tpi-3	0.271	0.252	0.071***
Mean	0.450	0.422	0.064

[1]) Abbreviations: H_T, total genetic diversity; H_S, genetic diversity within populations; G_{ST}, proportion of the total genetic diversity partitioned among populations. A chi-square test for allele frequency heterogeneity between populations: ns = not significant; *** = $P < 0.001$.

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Marker-QTL Linkage Detection in Self-Families of Outbred Populations

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Abstract

In forest trees which are normally outcrossing, inbreeding by self-fertilisation (selfing) generally has deleterious effects including reduced seed set, poor seed germination, and slow seedling growth. Inbreeding depression (ID) is mainly caused by deleterious alleles that will be almost never expressed under panmixis. Until the advent of molecular markers, there has been no way to track most of the individual genes causing ID. In this study, the theory for a single-marker ANOVA method was developed to find the linkage between a marker locus and a gene causing ID in growth traits in self-families of outbred populations. The power of linkage detection, which was at the lower limit because of single-marker method, was calculated for a wide range of progeny sizes and genetic parameters at the quantitative trait locus (QTL). The magnitude of the gene effect was found to have an enormous effect on the power. The situations where the QTL detected in a self-family can be considered as those expressed in normal course of outbreeding are also discussed.

Key words: Selfing, inbreeding, molecular marker, QTL, outbred.

Introduction

Inbreeding, which is reduction in heterozygosity across the genome resulting from mating among relatives including selfing, usually affects the phenotypic performance of inbred offspring. The deleterious effect of inbreeding on the phenotype is termed as inbreeding depression. In outbreeding forest trees, inbreeding by self-fertilisation (selfing) generally has highly deleterious effects which include reduced seed set, poor seed germination, slow seedling growth and abnormal morphology (Williams and Savolainen, 1996). Inbreeding depression (ID) is a complex quantitative phenomena, presumably controlled by many deleterious genes of different magnitudes of effects. Inbreeding depression is common and severe in many tree species, particularly conifers which are believed to have large numbers of recessive embryo lethals and post-germination lethals.

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