Spatial Genetic Structure of Allozyme Polymorphisms in a Population of *Eurya japonica* (Theaceae)

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Abstract

Eurya japonica THUNB. (Theaceae), a dioecious, insect-pollinated, broad-leaved evergreen tree, is widely distributed in East Asia. We used allozyme loci to examine the temporal and spatial distribution of genetic variation in the study population which was contained in a 60-m X 100-m area. The population had high levels of allozyme variation, but mean expected heterozygosity differed among three size classes: 0.277 for seedlings and 2-yrs old juveniles; 0.310 for juveniles; and 0.337 for adults. Differences in allelic frequencies among the size classes were significant for four out of eight loci. In addition, the population was spatially structured: values of MORAN's *I*-statistics were statistically significant in 65 (23.2%) of 280 cases for the entire population. There is substantial genetic similarity among individuals separated by less than 30 m distances, and this degree of similarity is consistent with the pollination system and seed dispersal mechanism. The results reveal that genetic diversity within a local population of *E. japonica* is not uniform in time and space.

Key words: allozymes, demographic genetics, *Eurya japonica,* MORAN's *I*, spatial autocorrelation, spatial genetic structure, Theaceae.

Introduction

Spatial structure of genetic variation and limits to pollen dispersal are the primary determinants of inbreeding in dioecious plants. The spatial distribution of genetic variation in populations is in turn determined by seed and pollen dispersal, habitat distribution, and microenvironmental selection (LEVIN and KERSTER, 1974; EPPERSON, 1993). Spatial genetic structure can be quantified using spatial autocorrelation analysis (SOKAL and ODEN, 1978; EPPERSON, 1989; HEYWOOD, 1991). For woody plants or other long-lived perennials, genetic and demographic factors such as gene flow from neighboring adults, past major reproductive events, and selection might cause stochastic fluctuations in genotypic frequencies over time (RITLAND, 1989).

Eurya japonica THUNB. (Theaceae), a dioecious, broad-leaved woody perennial, is distributed in Taiwan, southern China, Japan (Honshu, Shikoku, Kyushu, and Ryu Kyu Islands), and the southern and southwestern coastal parts of the Korean Peninsula. *E. japonica* is pollinated by bees, mature female trees produce hundreds of fruits (ca. 3 mm to 6 mm in diameter), and each fruit (berry) has ten to 23 small seeds. *E. japonica* grows on hillsides with *Pinus thunbergii* PARL., *Quercus* spp., *Camellia japonica* L., *Rhaphiolepis umbellata* (THUNB.) MAKINO, *Neolitsea* spp., *Persea* spp., etc. *E. japonica* is an economically important species in Korea, as the branches are widely used in floral tributes and wreaths. In recent years, such collectings have accelerated disturbance of many natural populations of Korean euryas (M. CHUNG, pers. obs.). In addition, until just the past few years, most Korean forests have been heavily harvested for firewood. Today most Korean forests are revegetated naturally and artificially. As a part of a study of spatio-temporal dynamics in populations of several broad-lived evergreen trees endemic to East Asia, we report the spatial genetic structure in an undisturbed Korean population of *E. japonica.*

Materials and Methods

In a broad sense, there are two habitat types at which *E. japonica* grows. One is relatively disturbed (e.g., hillsides destroyed by fire and logging) areas, where *E. japonica* is a dominant tree. The other is dense, undisturbed broad-leaved evergreen forests, and several of them have been designated as natural monuments in Korea to preserve biodiversity in the forests. In February 1998, all 157 individuals within the population were mapped (34.3 m^2/individual), and one leaf per individuals was collected within a 60-m X 100-m (alt. 15 m to 40 m a.s.l., SEW, 6% grade) area in a broad-leaved evergreen forest, Hakdongri Nature Reserve, Kojae Island, Prov. Gyeongsangnamdo, Korea. Eight other long-lived evergreen woody plants grows in the study population (*Camellia japonica* is a dominant species). For juveniles, diameters at ground level (DGH) rather than diameters at breast height were recorded per each tree because there are several so-called "Oskars" (extremely stunted or branched at the lower part of trees) in the population. For these individuals precise chronological ages could not be determined. Based on the condition of all trees in the population, all sampled trees were classified into three size-classes: seedlings and 2-yrs old juveniles (DGH< 4 mm, 43 individuals), juveniles (4 mm <DGH< 62 mm, 68 individuals), and adults or mature trees (63 mm <DGH< 470 mm, 46 individuals). Criteria included leaf scars, annual rings, and remnants of male flowers and fruit structures. These classes were used for estimating genetic diversity and contrasting alleles among them.

Leaf samples were kept on ice, transported to the laboratory, and stored at 4°C until protein extraction. Leaves were cut finely, and crushed with a mortar and pestle. A potassium phosphate 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. Thirteen putative loci for *E. japonica* from eight enzyme systems were resolved using a Poulik buffer system, a modification (HAUFLER, 1985) of SOLTIS *et al.* (1983) system 6. These were fluorescent esterase (*Fe-1, Fe-2*), leucine peptidase (*Lap-1, Lap-2*), and triosephosphate isomerase (*Tpi-1, Tpi-2*). A discontinuous histidine citrate buffer system 1 (SOLTIS *et al.,* 1983) resolved fructose-1, 6-diphosphatase (*F1, 6*) and diaphorase (*Dia*). A morpholine citrate buffer system by CLAYTON and TRETIAK (1972) was used to resolve formate dehydrogenase (*Fdh*), phosphoglucoisomerase (*Pgi-1, Pgi-2*), and peroxidase (*Per-1, Per-2*). Stain recipes were taken from SOLTIS et al. (1983), except for diaphorase (CHELIAK and PITEL, 1984) and formate dehydrogenase (WENDEL and WEEDEN, 1989). The genetic basis of allozyme banding patterns was inferred from segregation patterns with reference to typical subunit structure (WEEDEN and WENDEL, 1989). Then, putative

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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].

For the analysis of allozyme diversity, a locus was considered polymorphic if two or more alleles were detected. 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) (HAMRICK *et al.,* 1989). The gene diversity was estimated for each size class. A chi-square statistic was used to detect significant differences in allele frequencies among size classes for each locus (WORKMAN and NISWANDER, 1970).

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. Only one allele was considered at a diallelic locus, because the second allele contributes identical information. For a locus having more than two alleles, all alleles at that locus, regardless their frequencies, were used for the spatial analysis. However, alleles that were presented by less than five copies (frequencies < 1.6%) were excluded as non-informative for spatial analysis. Every possible pair of individual trees was considered as a join (a connection between two individuals) and was assigned to one of several distance classes (according to the Euclidean distance separating the pair). Because measures of small-scale autocorrelation more accurately represent the spatial structure, the first of the ten distance classes was designed based on an estimate of the average distance which separates nearest neighbor trees (see *Table 1*). MORAN's I-values (SOKAL and ODEN, 1978) were calculated for each distance class. 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, and hence the presence of spatial structure, for each allele was tested using BONFERRONI'S criterion (SAKAI and ODEN, 1983). All calculations for spatial statistical analyses were performed using the SAAP program (ver. 4.3) written by D. WARTENBERG.

Results

Of the 13 loci examined, eight were polymorphic in the population. *Fdh, F1, 6,* and *Pgi-1* were monomorphic in the

Table 1. – Allele frequencies for polymorphic loci within three size classes. Heterogeneity of allele frequencies among size classes were performed using an x^2-statistic. ^{ns} = not significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

Allele	Size class 1 (N=43)	Size class 2 (N=68)	Size class 3 (N=46)	X^2
Dia				
a	0.163	0.221	0.326	12.07*
b	0.837	0.750	0.674	
c	0.000	0.029	0.000	
Fe-1				8.18ns
a	0.535	0.507	0.500	
b	0.419	0.449	0.478	
c	0.000	0.029	0.022	
d	0.046	0.015	0.000	
Fe-2				9.38ns
a	0.000	0.015	0.000	
b	0.186	0.176	0.163	
c	0.430	0.522	0.380	
d	0.384	0.287	0.457	
Lap-1				10.91**
a	1.000	0.977	0.913	
b	0.000	0.023	0.087	
Per				30.17***
a	0.361	0.706	0.435	
b	0.639	0.294	0.565	
Pgi-2				28.04*
a	0.023	0.029	0.011	
b	0.070	0.029	0.130	
c	0.093	0.088	0.087	
d	0.011	0.052	0.011	
e	0.791	0.721	0.674	
f	0.000	0.037	0.087	
g	0.012	0.029	0.000	
h	0.000	0.015	0.000	
Tpi-1				8.74ns
a	0.884	0.794	0.728	
b	0.105	0.199	0.272	
c	0.011	0.007	0.000	
Tpi-2				5.02ns
a	0.105	0.088	0.087	
b	0.046	0.030	0.044	
c	0.023	0.044	0.000	
d	0.826	0.838	0.869	

population. *Per-2* and *Lap-2* were expressed, but they were not scored because of poor activity and/or resolution. High levels of genetic diversity within the population were evident in the estimated values of *P, A, A_e,* and H_e: 90.9%, 3.18, 1.55, and 0.292, respectively. The gene diversity for seedling and 2-yrs old juveniles (size class 1), juveniles (size class 2), and adults (size class 3) was 0.277, 0.310, and 0.377, respectively. In addition, differences in allelic frequencies among the size classes were significant for four (*Dia, Lap-1*, *Per,* and *Pgi-2*) out of eight loci (*Table 1*).

MORAN's *I*-values were significantly different from the expected value (E [*I*] = -0.006) in 59 (24.5%) of 240 cases, and the overall correlogram was significant for 15 of 24 alleles (62.5%) (*Table 2*). For distance class 1 to 4 (0 < 25 m), 25 (38.5%) significantly positive cases were observed out of 63 significant cases, whereas four significantly negative case were detected among these distance classes, indicating that genetic similarity was shared among individuals within 25 m of each other.

Discussion

The study population of *Eurya japonica* (H_e = 0.292) harbors higher levels of allozyme diversity than do most widespread woody species (mean H_e = 0.228, HAMRICK *et al.,* 1992). CHUNG and KANG (1994) suggested that factors contributing to the high levels of genetic diversity found within populations of *E. japonica* may include large populations, obligating outcrossing (dioecy), high fecundity, and long generation time.

In a previous allozyme study on Korean populations of *E. japonica* indirect estimates of the number of migrants per generation (*Nm*) (3.37, calculated F_{ST}; 3.74, calculated from the mean frequency of eight private alleles) indicated that gene flow among populations is extensive (CHUNG and KANG, 1994). However, fallen fruits have been observed near female plants, indicating that no special seed dispersal mechanism is developed in the species. In addition, pollen gene flow should be limited (primary pollinators are bees and bumblebees). The percentage of statistically significant MORAN's *I*-values in the study population of *E. japonica* is higher than the expected 5% type *I* error, indicating that genetic structuring within the entire population. An excess of significant positive *I* values was detected for distance classes 1 to 4, and there was a substantial excess of significant negative *I* values for distance classes 5 and greater. The observed average value of *I* of 0.07 for the first distance class is roughly comparable to that expected from a stable population with WRIGHT's (1943) neighborhood size (a standardized measure of dispersal) of ca. 80 to 90 (EPPERSON and LI, 1997).

Only 43 seedlings and one-year juveniles were found out of total 157 individuals in the study population (6,000 m²). It is supposed that a large proportion of seedlings have died during the first year of their growth probably due to low light intensi-

Table 2. – Spatial autocorrelation coefficients (MORAN's *I*) for all genotypes in the study population. The expected value of *I* under the null hypothesis of no spatial structure is –0.006. Also shown are the allele frequency, AF.

	Distance class (upper bound, m)											
Allele	1(6)	2(12)	3(18)	4(25)	5(35)	6(45)	7(55)	8(65)	9(75)	10(110)	P[2]	AF[3]
NP[1]	504	570	772	1097	2150	2046	1662	1352	1005	1088		
Dia^a	-0.00	0.10**	0.04	0.04*	-0.02	-0.00	0.03*	-0.01	-0.09**	-0.10**	0.001	0.2357
Dia^b	-0.01	0.06*	0.03	0.07**	-0.00	0.00	0.03	-0.05*	-0.08**	-0.10**	0.001	0.7516
Fe-1^a	0.13**	0.06	0.04	0.01	0.02	-0.05*	-0.06**	-0.03	-0.01	0.00	0.006	0.5127
Fe-1^b	0.08*	0.06*	0.05*	-0.03	0.02	-0.03	-0.07**	-0.02	0.01	-0.02	0.038	0.4490
Fe-1^c	0.10**	-0.04	0.11**	-0.04	-0.02	-0.02	-0.03	0.00	-0.01	-0.00	0.000	0.0191
Fe-1^d	-0.00	0.08**	-0.01	-0.01	-0.02	-0.02	-0.02	-0.03	0.03	0.02	0.040	0.0191
Fe-2^b	0.05	-0.07	0.03	0.02	0.00	-0.05**	0.05**	-0.08**	0.01	0.01	0.022	0.1647
Fe-2^c	0.02	-0.03	0.01	0.02	-0.03	0.05**	-0.01	-0.01	-0.01	-0.09**	0.005	0.4281
Fe-2^d	0.01	-0.04	0.01	0.01	-0.02	0.02	-0.01	0.01	-0.01	-0.06*	0.254	0.4012
Lap-1^a	0.21**	-0.09*	0.07**	-0.04	-0.00	-0.03	-0.05*	-0.02	-0.01	0.03	0.000	0.9650
Per^a	0.10**	0.03	0.01	0.02	-0.04	-0.00	-0.03	-0.04	0.01	0.02	0.064	0.5318
Pgi-2^a	0.23**	0.05	0.02	0.01	-0.04*	-0.04	-0.08**	-0.04	0.04	0.05*	0.000	0.0223
Pgi-2^b	0.02	0.05	0.03	-0.02	0.02	0.01	-0.02	-0.06*	-0.07*	0.00	0.102	0.0701
Pgi-2^c	-0.01	-0.02	0.00	-0.01	-0.03	0.00	0.01	0.02	-0.05	-0.00	0.688	0.0892
Pgi-2^d	0.09**	-0.03	-0.06	0.07**	-0.02	-0.02	-0.05*	0.04*	-0.04	0.01	0.015	0.0287
Pgi-2^e	-0.02	0.02	-0.06	-0.05*	0.03*	-0.02	-0.01	0.02	-0.02	0.01	0.159	0.7261
Pgi-2^f	0.25**	0.09**	-0.02	-0.03	-0.04*	0.02	-0.06**	-0.02	-0.05	0.01	0.000	0.0414
Pgi-2^g	0.01	0.16**	-0.02	0.03	-0.02	-0.03	-0.03	-0.01	-0.03	-0.00	0.000	0.0159
Tpi-1^a	0.14**	-0.03	-0.09**	-0.01	-0.01	0.03*	-0.06**	0.02	0.06**	-0.07**	0.002	0.7994
Tpi-1^b	0.15**	-0.01	-0.10**	-0.02	-0.01	0.01	-0.06*	0.03	0.06*	-0.05*	0.001	0.1943
Tpi-2^a	-0.04	-0.01	0.03	-0.00	-0.01	0.02	-0.06**	0.01	-0.01	0.01	0.079	0.0924
Tpi-2^b	-0.07	-0.03	-0.00	-0.01	0.00	-0.02	0.01	0.02	-0.02	-0.01	0.526	0.0382
Tpi-2^c	0.08*	-0.03	-0.03	-0.02	0.02	-0.02	-0.01	-0.00	-0.01	-0.03	0.104	0.0255
Tpi-2^d	-0.03	-0.01	0.01	-0.01	0.00	0.02	-0.01	-0.03	-0.02	-0.02	0.682	0.8439
Average	0.07	0.01	0.00	-0.00	-0.01	-0.01	-0.02	-0.01	-0.01	-0.02		

[1]) Number of pairs or joins.

[2]) BONFERRONI test for overall correlogram significance was conducted following SAKAI and ODEN (1983).

[3]) Allele frequency.

* = P < 0.05; ** = P < 0.01; *** = P < 0.001.

ty under dense evergreen forest and/or competition with other perennial herbs ("thinning" in seedling stage may reduce the structure). A small proportion of the seedlings would survive and replaces the adults, which affects spatial genetic structure among age classes over generations (EPPERSON and ALVAREZ-BUYLLA, 1997). On the other hand, in a more dense population in which *E. japonica* is a dominant species (density = ca. 2 m^2), adults showed a substantial genetic structure in a small spatial scale (< 7 m) (OH *et al.,* 1996), indicating that density affects shaping in genetic structuring within local populations. It is suggested that differences in ecological parameters (e.g., density, forest gap dynamics, and colonization history, etc.) among the local populations of *E. japonica* may affect on the maintenance of genetic diversity within metapopulations.

Genetic diversity calculated here increases in the order of their size classes (from size classes 1 through 3). Genetic differentiation among size classes is revealed by this study. In addition, as the three alleles (*Dia*^{c}, *Fe-2*^{a}, and *Pgi-2*^{h}) were present only in the juveniles and the four alleles (*Fe-1*^{d}, *Pgi-2*^{g}, *Tpi-1*^{c}, and *Tpi-2*^{c}) in both the seedlings and juveniles (size classes 1 and 2) not in adults (size class 3), propagules containing the alleles originated from adjacent stands, perhaps an infrequent event by frugivorous birds and pollinators. This also suggests that the "reproductive gene pools" might have changed between each reproductive event, thus, may lead to the differences in temporal genetic structure in local populations with overlapping generations.

In summary, the results of this study as well as previous studies suggest that biparental inbreeding due to consanguineous matings or mating between near neighbors coupled with limited seed dispersal are explanatory factors responsible for genetic substructure in populations of *E. japonica.* As revealed in this study, the genetic diversity in the study population is not uniform in time and space. Coupled with the biological and ecological characteristics of *E. japonica* (e.g., dioecy, high fecundity, and abundant, contiguous populations), high levels of genetic diversity accumulated from generation to generation have been maintained in standing populations of *E. japonica.* As revealed in our study, examinations of genetic parameters within limited generations would not sufficiently represent the total picture of genetic diversity within local populations. Thus, this study also stresses the importance of sampling scale in estimating genetic diversity within local populations with overlapping generations (discrimination of age structure).

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References

CHELIAK, W. M. and PITEL, J. P.: Technique for starch gel electrophoresis of enzyme from forest tree species. Petawawa National Forestry Institute, Information Report PI-X-42 (1984). — CHUNG, M. G. and KANG, S. S.: Genetic variation and population structure in Korean populations of *Eurya japonica* (Theaceae). Amer. J. Bot. **81**, 1077–1082 (1994). — CLAYTON, J. W. and TRETIAK, D. W.: Amine citrate buffer for pH control in starch gel electrophoresis. J. Fish. Res. Board Can. **29**, 1169–1172 (1972). — CLIFF, A. D. and ORD, J. K.: Spatial processes-methods and applications. Pion, London (1981). — EPPERSON, B. K.: Spatial patterns of genetic variation within plant population. In: Plant population genetics, breeding, and genetic resources. Edited by A. H. D. BROWN, M. T. CLEGG, A. L. KAHLER, and B. S. WEIR. Sinauer, Sunderland. pp. 229–253 (1989). — EPPERSON, B. K.: Recent advances in correlation analysis of spatial patterns of genetic variation. Evol. Biol. **27**, 95–155 (1993). — EPPERSON, B. K. and ALVAREZ-BUYLLA, E.: Limited seed dispersal and genetic structure in life stages of *Cecropia obtusifolia.* Evolution **51**, 275–282 (1997). — EPPERSON, B. K. and LI, T.: Gene dispersal and spatial genetic structure. Evolution **51**, 672–681 (1997). — HAMRICK, J. L., GODT, M. J. W. and SHERMAN-BROYLES, S. L.: Factors influencing levels of genetic diversity in woody plant species. New Forests **5**, 95–124 (1992). — HAUFLER, C. H.: Enzyme variability and modes of evolution in *Bommeria* (Pteridaceae). Syst. Bot. **10**, 92–104 (1985). — HEYWOOD, J. S.: Spatial analysis of genetic variation in plant populations. Annu. Rev. Ecol. Syst. **22**, 335–355 (1991). — LEVIN, D. A. and KERSTER, H. W.: Gene flow in seed plants. Evol. Biol. **7**, 139–220 (1974). — MITTON, J. B., LINHART, Y. B., STURGEON, K. B. and HAMRICK, J. L.: Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. J. Hered. **70**, 86–89 (1979). — OH, G. S., KANG, S. S. and CHUNG, M. G.: Factors shaping genetic structure within populations of *Eurya japonica* (Theaceae). J. For. Res. **1**, 93–97 (1996). — RITLAND, K.: Gene diversity and the genetic demography of plant populations. In: Plant population genetics, breeding, and genetic resources. Edited by A. H. D. BROWN, M. T. CLEGG, A. L. KAHLER, and B. S. WEIR. Sinauer, Sunderland. pp. 181–199 (1989). — SAKAI, A. K. and ODEN, N. L.: Spatial pattern of sex expression in silver maple (*Acer saccharium* L.): Morista's index and spatial autocorrelation. Am. Nat. **122**, 489–508 (1983). — SOKAL, R. R. and ODEN, N. L.: Spatial autocorrelation in biology. 1. Methodology. Biol. J. Linn. Soc. **10**, 199–249 (1978). — SOLTIS, D. E., HAUFLER, C. H., DARROW, D. C. and GASTONY, G. J.: Starch gel electrophoresis of ferns: A compilation of grinding buffers, gel and electrode buffers, and staining schedules. Amer. Fern J. **73**, 9–27 (1983). — WEEDEN, N. F. and WENDEL, J. F.: Genetics of plant isozymes. In: Isozymes in plant biology. Edited by D. E. SOLTIS and P. S. SOLTIS. Dioscorides Press, Portland. pp. 46–72 (1989). — WENDEL, J. F. and WEEDEN, N. F.: Visualization and interpretation of plant isozymes. In: Isozymes in plant biology. Edited by D. E. SOLTIS and P. S. SOLTIS. Dioscorides Press, Portland. pp. 5–45 (1989). — WORKMAN, P. L. and NISWANDER, J. D.: Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. Amer. J. Hum. Genet. **22**, 24–49 (1970). — WRIGHT, S.: An analysis of local variability of flower color in *Linanthus parryae.* Genetics **28**, 139–156 (1943).