

# Clonal and spatial genetic structure in *Eurya emarginata* (Theaceae)

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*Eurya emarginata* (Thunb.) Makino (Theaceae) is a dioecious, insect-pollinated tree, which combines sexual reproduction and clonal spread. It is narrowly distributed in coastal areas from southern China, along southern Korea, and extending to central and southern Japan. We used allozyme loci and spatial autocorrelation statistics to examine the clonal structure and the spatial distribution of allozyme polymorphisms of sexually reproduced individuals in a study population (70 m × 120 m area) in Korea. The population maintains moderate levels of allozyme variability (mean  $H_e = 0.133$ ), and multilocus genotypic diversity is high (mean  $D_G = 0.992$ ). The frequency of clones was 0.377 (90/239), and the mean clone-pair distance was very large (35.06 m ± 1.91 m [SE]), indicating that clones can form at long distances away from their ancestors. In addition, it was found that genetic patch width was at least 24 m. However, join-count statistics for the total number of unlike joins showed that, contrary to expectation for such species, clonal reproduction does not contribute substantially to genetic isolation by distance neither among the sexually reproduced individuals nor the whole population. In contrast, limits to seed and pollen dispersal create substantial levels of genetic structure.

**Keywords:** allozymes, clonal structure, *Eurya emarginata*, Moran's  $I$ , spatial autocorrelation, spatial genetic structure.

## Introduction

*Eurya emarginata* (Thunb.) Makino is a dioecious, broad-leaved woody perennial that has both sexual reproduction and clonal spread. It is distributed in southern China, Japan (western region to Chiba Pref. of Honshu, Shikoku, Kyushu, and Ryukyu Islands) and a few localities in the southern coastal area of the Korean Peninsula. *Eurya emarginata* occurs in rocky and humus soils on talus slopes, rocky cliffs, or hillsides in the coastal areas in this region. It is typically found with *Pinus thunbergii* Parl., *Eurya japonica* Thunb., *Quercus* spp., *Camellia japonica* L. and *Rhaphiolepis umbellata* (Thunb.) Makino. Most populations of *E. emarginata* in Japan and Korea are small and isolated (M. Chung, pers. obs.). This may be in part because of: (i) destruction of natural habitat; (ii) ability to spread clonally on rocky cliffs; (iii) narrow-niched habitats; and (iv) historical events. During four years of field surveys seedlings were rarely found in the study population (M. Chung, pers. obs.). *Eurya emarginata* is pollinated by bees and each female plant produces hundreds of

fruits (drupes ≈4–6 mm in diameter), each with 10–20 small seeds.

For plant species capable of reproducing both sexually and asexually, vegetative reproduction (by root suckers or by rhizomes) is generally expected to have marked effects on the spatial genetic structure of combined asexual and sexual regeneration in plant populations (e.g. Berg & Hamrick, 1994; Shapcott, 1995; Hossaert-McKey *et al.*, 1996; Chung & Epperson, 1999). In addition, spatial genetic structure among sexually reproduced individuals 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 microenvironmental selection (Levin & Kerster, 1974; Epperson, 1993). Conversely, spatial genetic structure of both sexual and clonal individuals influences the dynamics of biparental inbreeding, inbreeding depression, and the operation of natural selection (Epperson, 1993). For predominantly outcrossers, if there are barriers to gene flow among 'patches' or local populations, vegetative spread may reinforce initial genetic drift caused by founder effects, and this may lead to the fixation of alleles, thus decreasing genetic diversity (e.g. Shapcott,

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1995). With nearly random distributions of clones in local populations, it is expected that clonal reproduction should not substantially increase the degree of local consanguineous matings. Thus, information on spatial distribution of clones is of importance for understanding the history of clonal vs. sexual reproduction establishment in local populations. In this regard, it is of interest to separate the spatial genetic structure caused by clonal reproduction from that maintained in sexually reproduced individuals. Spatial genetic structure can be quantified using spatial autocorrelation statistics (Sokal & Oden, 1978).

Genetic and demographic factors such as gene flow from neighbouring parents, past major reproductive events, and selection might be detected as genetic differences among age classes (Ritland, 1989). There are few studies of the spatial distribution of genetic variation in different age or size classes of plants (Hossaert-McKey *et al.*, 1996; Leonardi *et al.*, 1996; Epperson & Alvarez-Buylla, 1997). These studies have indicated that the genetic diversity within local populations is enhanced by means of genetic polymorphisms accumulated both spatially and temporally during different generations. In this paper, we examine in detail the clonal and spatial genetic structure in two size classes within a study population of *E. emarginata*.

## Materials and methods

In March 1998, all 239 individuals were mapped and sampled (one leaf per individual was collected) within a 70 m × 120 m (altitude 5–20 m above sea level, 8% gradient facing SSW) area on a pine forest hillside of Haegumkang, Kojae Island, Province Gyeongsangnam, Korea. Diameters at ground level (d.g.h.) rather than diameters at breast height were recorded because there are frequent so-called 'Oskars' (trees that are stunted or branched in the lower part of the tree). For the same reason, chronological ages could not be precisely determined. Instead, the total sample was divided into two size classes: juveniles (d.g.h. < 40 mm, 118 individuals, hereafter referred to as size class 1), and juveniles and adults (41 < d.g.h. < 290 mm, 121 individuals, hereafter referred to as size class 2). 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 × 6 mm Whatman 3MM chromatography paper wicks which were stored at –70°C until needed for analysis. Electrophoresis used 10% starch gels. Twenty-three putative loci for *E. emarginata* from 15 enzyme systems were resolved using three gel/

electrode buffer systems. A Poulik buffer system, a modification (Hauffer, 1985) of Soltis *et al.* (1983) system 6 was used to resolve colorimetric esterase (*Ce-1*, *Ce-2*), fluorescent esterase (*Fe-1*, *Fe-2*, *Fe-3*), glutamate dehydrogenase (*Gdh*), leucine aminopeptidase (*Lap-1*, *Lap-2*) and triosephosphate isomerase (*Tpi-1*, *Tpi-2*, *Tpi-3*, *Tpi-4*). Two discontinuous histidine–citrate buffer systems were used: a modification (Chung & Kang, 1994) of Soltis *et al.* (1983) system 11 was used to resolve isocitrate dehydrogenase (*Idh*), phosphoglucosmutase (*Pgm*), aldolase (*Ald*) and buffer system 1 (Soltis *et al.*, 1983) resolved fructose-1,6-diphosphatase (*F1,6*) and diaphorase (*Dia*). Soltis *et al.* (1983) system 7 resolved aspartate aminotransferase (*Aat*) and menadi-one reductase (*Mnr*). Finally, a morpholine citrate buffer system by Clayton & Tretiak (1972) was used to resolve formate dehydrogenase (*Fdh*), phosphoglucos-isomerase (*Pgi-1*, *Pgi-2*) and peroxidase (*Per*). Stain recipes were taken from Soltis *et al.* (1983), except for diaphorase (Cheliak & Pitel, 1984) and formate dehydrogenase (Wendel & Weeden, 1989). The genetic basis of allozyme banding patterns was inferred from segregation patterns with reference to typical subunit structure (Weeden & Wendel, 1989). 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*.

Five standard genetic diversity parameters were estimated using a computer program developed by M. D. Loveless & A. Schnabel (pers. comm.): percentage polymorphic loci (*P*), mean number of alleles per polymorphic locus (*AP*), mean number of alleles per locus (*A*), effective number of alleles per locus (*A<sub>e</sub>*), and expected heterozygosity (*H<sub>e</sub>*) or gene diversity.

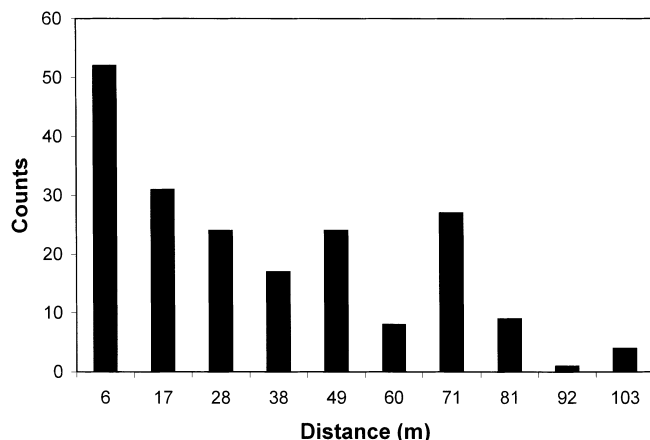
The number of clone-pairs sharing the same multilocus genotype was graphed against distance to infer the spatial distribution of clones within populations. Departures from normality, as measured by skewness (*g<sub>1</sub>*) and kurtosis (*g<sub>2</sub>*), were calculated for distributions of clone-pair distances as described by Sokal & Rohlf (1981). A negative *g<sub>1</sub>* indicates skewness to the left; a positive *g<sub>1</sub>*, skewness to the right. A negative *g<sub>2</sub>* indicates platykurtosis, and a positive *g<sub>2</sub>*, leptokurtosis. In addition, we assessed the amount of clonal diversity within the population (Ellstrand & Roose, 1987). The first measure of clonal diversity was the proportion of distinguishable multilocus genotypes, or *G/N* where *G* is the number of distinct multilocus genotypes in a population and *N* is the number of sampled individuals (Pleasants & Wendel, 1989). A second measure was the probability of obtaining the most common multilocus genotype by chance in each population (*PG*) (Sherman-Broyles *et al.*, 1992). The third measure of multilocus

genotype diversity ( $D_G$ ) was calculated as a modification (Pielou, 1969) of the Simpson index:  $D_G = 1 - \sum \{[n_i(n_i - 1)]/[N(N - 1)]\}$ , where  $n_i$  is the number of individuals of multilocus genotype  $i$  and  $N$  is the total number of individuals in the population. A fourth measure was the probability of randomly drawing two identical multilocus genotypes ( $PR$ ), based on observed single-locus genotypic frequencies and the assumption of linkage equilibrium (Berg & Hamrick, 1994).

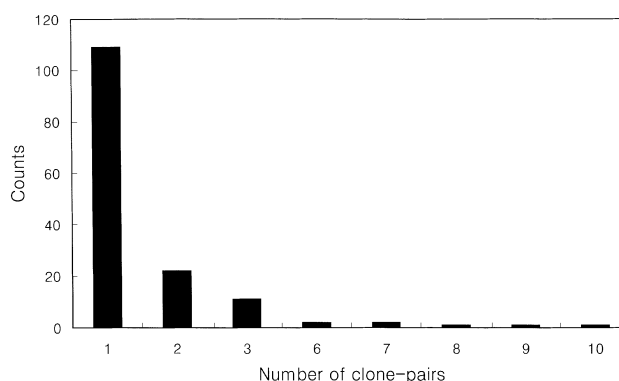
For spatial autocorrelation analysis, the genotypic data were coded so that allele frequency values of 1.0, 0.5 or 0.0 were assigned to each individual homozygous for a given allele, heterozygous for that allele, or genotypes with no copies of that allele, respectively, for each polymorphic locus (Sokal & Oden, 1978). For diallelic loci, only those with allele frequencies less than 0.95 and greater than 0.05 were used, but only one allele was considered, because the second allele contributes identical information. For loci having more than two alleles, all alleles were used for the spatial analysis regardless of their frequencies. Every possible pair of individual trees was considered as a join (a connection between two individuals) and assigned to one of 10 distance classes (according to the Euclidean distance separating the pair). 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 & Oden, 1978) were calculated for each of the 10 distance classes. Each  $I$ -value was also used to test for significant deviations from the expected value,  $E(I) = -1/(N - 1)$  under the null hypothesis of a random distribution of genotypes (Cliff & 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 (a set of  $I$ -statistics for all distance classes) was tested using Bonferroni's approximation (Sakai & Oden, 1983). In addition, the spatial distributions of allozyme polymorphisms were analysed separately for size class 1 (118 individuals) and size class 2 (121), using Moran's  $I$ -statistics as outlined above. In order to examine the spatial distributions of sexually reproduced trees, we discarded multiples of the same multilocus genotypes (because these must be clones, see below) from the total samples, size class 1, and size class 2, after randomly choosing one of each to be retained. This formed a parallel but reduced data set (149, 90 and 88 samples, respectively) in which multiple clonal genotypes were excluded. These data sets were referred to as the data sets of sexually reproduced genotypes, and these were also analysed separately. All calculations of Moran's  $I$ -statistics were conducted using the SAAP program (version 4.3) written by D. Wartenberg.

To test the spatial distribution of the clonal structure (multiple individuals of the same multilocus genotype) for the size classes 1 and 2, additional spatial autocorrelation statistics were calculated for the total number of unlike joins (Sokal & Oden, 1978) among multilocus genotypes using the JCSP program of Epperson. A test statistic (standard normal deviate, SND) was formed which has an asymptotically standard normal distribution under the random spatial distribution null hypothesis. A SND with a value less than  $-1.96$  would indicate a deficit of pairs of unlike multilocus genotypes where pairs are separated by a given range of Euclidean distances (Epperson, 1993). Hence this would indicate an excess of pairs of identical multilocus genotypes (clones) for that distance class and spatial autocorrelation of clonal genotypes.



**Fig. 1** The distribution of distances between pairs (197 pairs) of plants sharing identical multilocus genotypes for the Haegumkang population of *Eurya emarginata*.



**Fig. 2** Number of *Eurya emarginata* individuals with the same multilocus genotype.

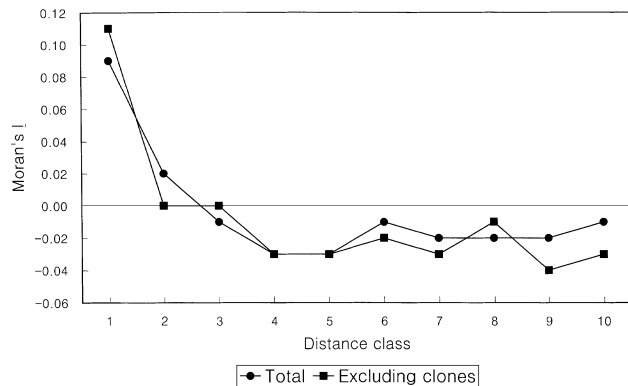
**Table 1** Spatial autocorrelation coefficients (Moran's  $I$ ) of 13 alleles in the Haegumkang population of *Eurya emarginata* for 10 distance classes (239 individuals; the expected value ( $E[I]$ ) is  $-0.004$ )

Allele	Distance class (upper bound, m)										$P$ †	Allele frequency
	1(12)	2(19)	3(25)	4(31)	5(38)	6(45)	7(53)	8(62)	9(74)	10(115)		
<i>Ce-1<sup>a</sup></i>	-0.0	-0.02	0.03**	-0.00	-0.01	0.02**	-0.03*	0.01	-0.00	-0.03*	0.001	0.8975
<i>Did<sup>a</sup></i>	0.17**	-0.01	0.06**	0.01	-0.02*	-0.02	-0.08**	-0.04**	-0.01	-0.10**	0.000	0.5021
<i>Per<sup>a</sup></i>	0.01*	-0.01	-0.01	-0.01	0.02**	-0.02*	0.00	-0.01	-0.02*	-0.01	0.003	0.0105
<i>Per<sup>b</sup></i>	0.12**	0.11**	0.01	-0.02*	-0.06**	-0.06**	-0.03*	-0.04**	-0.07**	-0.01	0.000	0.1360
<i>Per<sup>c</sup></i>	0.22**	0.09**	-0.02	-0.04**	-0.05**	-0.02	-0.06**	-0.11**	-0.06**	0.00	0.000	0.0628
<i>Per<sup>d</sup></i>	0.18**	0.05**	-0.06**	-0.11**	-0.08**	-0.03**	0.02**	0.02*	-0.04**	0.00	0.000	0.7908
<i>Fe-2<sup>a</sup></i>	-0.00	-0.01	0.00	-0.02	0.01	0.01*	-0.03*	0.00	0.01	-0.02	0.129	0.8975
<i>Fe-3<sup>a</sup></i>	0.18**	0.05**	0.06**	-0.06**	-0.09**	-0.06**	-0.10**	0.01	0.00	-0.03*	0.000	0.1590
<i>Tpi-1<sup>a</sup></i>	0.05**	-0.02	-0.05**	0.01	-0.01	-0.00	-0.01	-0.02	-0.03*	0.04**	0.000	0.2782
<i>Tpi-2<sup>a</sup></i>	0.01	0.01	-0.05**	-0.00	-0.01	0.01	-0.01	-0.00	0.01	-0.00	0.000	0.3954
<i>Tpi-2<sup>b</sup></i>	0.12**	-0.00	-0.05**	-0.05**	-0.05**	-0.02*	0.03**	0.03**	-0.04**	-0.01	0.000	0.1548
<i>Tpi-2<sup>c</sup></i>	0.02*	-0.00	-0.00	-0.02	-0.04**	0.02**	0.02*	-0.03*	-0.03*	0.02*	0.001	0.4498
<i>Tpi-3<sup>a</sup></i>	0.06**	-0.04**	-0.01	-0.02*	-0.03*	-0.02*	0.03**	-0.04**	0.04**	-0.01	0.000	0.1715
Average	0.09	0.02	-0.01	-0.03	-0.03	-0.01	-0.02	-0.02	-0.02	-0.01		
Average‡	0.09	0.01	-0.01	-0.04	-0.03	-0.03	-0.02	-0.00	-0.03	-0.02		
	(11)	(18)	(25)	(32)	(40)	(46)	(54)	(62)	(73)	(114)		
Average§	0.08	0.01	-0.01	-0.04	-0.03	-0.01	-0.02	-0.01	-0.02	-0.02		
	(12)	(19)	(25)	(31)	(37)	(44)	(52)	(62)	(76)	(114)		

† Overall correlogram significance (Bonferroni approximation).

‡§ Total sample for size classes 1 and 2, respectively. Number in parentheses indicates upper bound (m).

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



**Fig. 3** Correlograms for the Haegumkang population of *Eurya emarginata* (total sample and samples excluding clones) using mean value of Moran's *I* and 10 distance intervals.

## Results

Of the 23 loci examined, eight were polymorphic; the loci *Aat*, *Ald*, *Ce-2*, *F1.6*, *Fdh*, *Fe-1*, *Gdh*, *Idh*, *Lap-1*, *Mnr*, *Pgi-1*, *Pgi-2*, *Pgm* and *Tpi-4* were monomorphic. In addition, although *Lap-2* was expressed, it was not scored because of poor activity and resolution. Moderate levels of gene diversity within the population were found: mean values of *P*, *AP*, *A*, *A<sub>e</sub>* and *H<sub>e</sub>* were 36.4%, 2.33, 1.57, 1.24 and 0.133, respectively. The estimate of the probability of obtaining two identical multilocus genotypes through sexual reproduction was small (0.0033), indicating that wherever individuals have the same multilocus genotype they are, with very high probability, indeed clones of one another. The population had high estimates of clonal diversity: *G*, 149; *G/N*, 0.62; *D<sub>G</sub>*, 0.992; *PG*, 0.041. The frequency of clones was 0.377 (90 out of 239,  $1 - G/N$ ). The mean interpair distance of plants with identical multilocus genotypes in the population was large, 35.06 m  $\pm$  1.91 m [SE]. The frequency distribution of distances among clones in the population is shown in Fig. 1. It is skewed to the right and platykurtic ( $g_1 = 0.54$ ,  $g_2 = 0.76$ ). Most multilocus genotypes occurred fewer than three times within the population (Fig. 2).

According to the criteria described above, 13 alleles were used for single-locus spatial autocorrelation analysis. For the total samples (239), Moran's *I*-values were significantly different from the expected value ( $E[I] = 0.004$ ) in 75 (57.7%) of 130 cases, and the overall correlogram was significant for all alleles except *Fe-2<sup>a</sup>* (92%) (Table 1). For distance classes 1 and 2 (0–19 m), 14 significantly positive values were observed, whereas only one significantly negative value was detected for the same distance classes, indicating that genetic similarity was shared among individuals within 19 m of each other. The correlogram for the entire sample using

mean values of Moran's *I* and 10 distance intervals is shown in Fig. 3. Moran's *I*-statistics for the population samples excluding multiple clones are shown in Table 2 ( $N = 149$ ).

Patterns of spatial genetic distribution observed separately for size classes 1 and 2 are very similar (see Tables 1 and 2). Most importantly, the average values (over loci) for samples excluding clones (Table 2) are very similar to those calculated for the entire sample (Table 1). This shows that nearly all of the nonrandom, spatial genetic structure is caused by sexually reproduced individuals, not by clones. Join-count statistics for the total number of joins between unlike (nonidentical) multilocus genotypes supports this. The correlograms showed no significantly negative values (Fig. 4), with one minor exception in the first distance class (< 5 m, standard normal deviate test statistic, SND, of  $-3.51$  for size class 1 and  $-7.72$  for size class 2). Beyond 5 m, the distribution of clonal genotypes is nearly random for all distances, spanning the entire area of the population.

## Discussion

The study population of *E. emarginata* ( $H_e = 0.133$ ) harbours levels of allozyme diversity similar to the average for narrow-ranging woody species (0.143, Hamrick *et al.*, 1992). *Eurya emarginata* has higher genotypic diversity ( $D_G = 0.992$ ) than the average (0.61) reported by Ellstrand & Roose (1987) in their review of clonally reproducing species.

The percentage of statistically significant Moran's *I*-values in the population of *E. emarginata* is much greater than the expected five per cent type I error, indicating that a substantial amount of genetic structuring exists within the population. Fourteen significant positive *I*-values were detected in the distance classes 1 and 2, whereas 45 significant negative *I*-values were observed at and beyond distance class 3. For population samples excluding clones (duplicate multilocus genotypes), Moran's *I*-statistics show the same amount of spatial autocorrelation (Table 2). There are no significant differences in means between the total samples and those excluding clones (*t*-test,  $t = 1.85$ ,  $P = 0.09$  in the distance class 1;  $t = 1.23$ ,  $P = 0.24$  in the distance class 2). In addition, spatial autocorrelation for sexually reproduced individuals is very nearly the same as that for the total population, which includes clones. The total number of unlike joins indicated a moderate concentration of clonal individuals within distances less than 5 m but not beyond. This indicates that groups of genotypes caused by clonal reproduction could contribute only slightly, and at small spatial scales, to the degree of consanguineous matings, and hence to development of

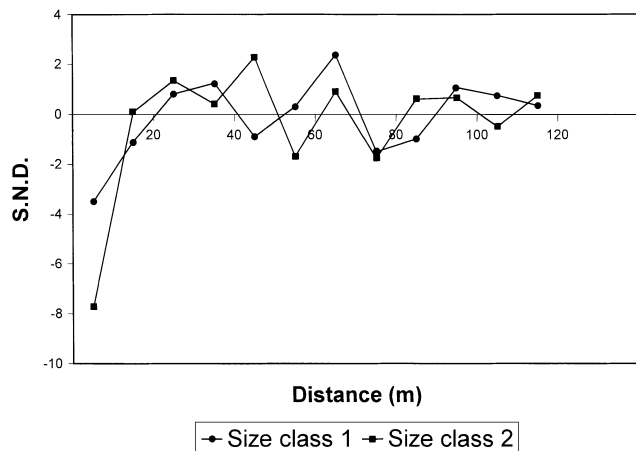
**Table 2** Spatial autocorrelation coefficients (Moran's  $I$ ) of 13 alleles in the Haegumkang population of *Eurya emarginata*, excluding clones, for 10 distance classes (149 individuals, the expected value ( $E[I]$ ) is 0.007)

Allele	Distance class (upper bound, m)										$P$ †	Allele frequency
	1(12)	2(19)	3(25)	4(31)	5(37)	6(44)	7(51)	8(61)	9(73)	10(113)		
<i>Ce-1<sup>a</sup></i>	-0.01	0.01	0.04*	0.01	-0.00	-0.02	-0.01	-0.01	-0.05*	-0.02	0.403	0.8523
<i>Did<sup>a</sup></i>	0.21**	-0.07*	0.13**	0.06**	-0.00	0.01	-0.10**	-0.03	-0.13**	-0.15**	0.000	0.4933
<i>Per<sup>a</sup></i>	0.02	-0.01	-0.01	0.00	0.01	0.01	-0.03	-0.01	-0.03	-0.03	1.000	0.0168
<i>Per<sup>b</sup></i>	0.23**	0.19**	0.08**	-0.06*	-0.07**	-0.09**	-0.10**	-0.11**	-0.06*	-0.08**	0.000	0.1913
<i>Per<sup>c</sup></i>	0.22**	0.03	-0.04	-0.03	-0.01	-0.03	-0.06*	-0.03	-0.10**	-0.02	0.000	0.0671
<i>Per<sup>d</sup></i>	0.30**	0.01	-0.02	-0.16**	-0.09**	-0.10**	-0.09**	0.06**	0.04	-0.01	0.000	0.7248
<i>Fe-2<sup>a</sup></i>	0.04*	0.01	0.01	0.03	-0.00	-0.03	-0.04	-0.07*	-0.07**	0.05*	0.081	0.8523
<i>Fe-3<sup>a</sup></i>	0.10**	-0.01	0.09**	-0.04	-0.09**	0.03	-0.07*	0.01	-0.05	-0.03	0.000	0.2114
<i>Tpi-1<sup>a</sup></i>	0.03	-0.04	-0.01	0.04	-0.09**	-0.02	0.00	0.01	-0.03	0.03	0.013	0.3121
<i>Tpi-2<sup>a</sup></i>	0.08**	-0.04	-0.11**	-0.09**	0.01	0.06*	0.07**	0.03	-0.09**	0.02	0.002	0.4161
<i>Tpi-2<sup>b</sup></i>	0.16**	-0.03	-0.12**	-0.07*	0.01	0.00	0.06**	-0.00	-0.07**	-0.00	0.000	0.1208
<i>Tpi-2<sup>c</sup></i>	0.01	-0.02	0.02	-0.01	-0.04	0.01	0.00	-0.03	-0.00	-0.01	1.000	0.4631
<i>Tpi-3<sup>a</sup></i>	0.10**	-0.02	-0.04	-0.06*	-0.06*	-0.05*	0.01	0.03	0.11**	-0.08**	0.000	0.1711
Average	0.11	0.00	0.00	-0.03	-0.03	-0.02	-0.03	-0.01	-0.04	-0.03		
Average‡	0.10	0.00	0.00	-0.07	-0.03	-0.03	-0.03	0.00	-0.03	-0.03		
	(11)	(18)	(24)	(31)	(38)	(45)	(53)	(61)	(72)	(112)		
Average§	0.10	0.01	0.00	-0.04	-0.03	-0.04	-0.04	-0.01	-0.03	-0.03		
	(13)	(20)	(25)	(31)	(37)	(43)	(51)	(60)	(74)	(113)		

† Overall correlogram significance (Bonferroni approximation).

‡§ Samples excluding clones for size classes 1 and 2, respectively. Number in parentheses indicates upper bound (m).

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



**Fig. 4** Correlograms showing the relationships between pairs of the total number of unlike joins among multilocus genotypes of *Eurya emarginata* in size classes 1 and 2.

genetic isolation by distance, contrary to common predictions about clonal species (e.g. Hossaert-McKey *et al.*, 1996; Chung & Epperson, 1999). In theory, the presence of unisexual (as the species is dioecious) clonal groups, related through the previous generations because of limited seed dispersal (in forming the 'founders' of a clonal patch), could increase attractiveness to pollinators and otherwise increase local concentrations of matings.

The average value of Moran's *I*-statistics for the first distance class (0.11; Table 2) corresponds to the value expected with total dispersal equating to a value of 50 for Wright's neighbourhood size in populations of constant size (Epperson *et al.*, 1999). A neighbourhood size of 50 is consistent with bee-pollinated plants having gravity-dispersed seeds, such as *E. emarginata*. Clustering of sexually reproduced individuals with similar genotypes within such spatial scales develops from the combination of amounts of seed and pollen dispersal. By counting annual rings of the widest stem in the population (290 mm at d.g.h.), the maximum age of the present individuals was determined to be about 120 years and the population probably has existed at the site for many previous generations. Long-existing populations usually have stronger spatial genetic structure, because genetic isolation by distance generally increases over every round of population regeneration (e.g. Epperson, 1993).

The spatial scale of structure can be estimated using the distance at which the mean Moran's *I*-value first intersects the value of  $E(I)$  (Sokal, 1979). The mean correlogram of the population indicates that the minimum genetic patch width is approximately 24 m (Fig. 3). For conservation purposes, sampling methods

for seed stocks could be optimized by utilizing information on spatial genetic structure within populations (e.g. Chung & Park, 1998). As populations of *E. emarginata* are small and isolated in a few localities in Japan and Korea, it is suggested that, overall, the sampling of *E. emarginata* within populations should be conducted at 24 m intervals, in order to extract the genetic diversity efficiently across each population. The scale of spatial genetic structure in a study population of *Eurya japonica* (60 m × 100 m), a widespread congener of *E. emarginata*, was similar (30 m; M. Chung & B. Epperson, unpubl. data). However, the distances over which homogeneous patches of clones occur in *Eurya emarginata* (25–32 m) are much greater than in *Quercus margaretta* (< 5 m; Berg & Hamrick, 1994), but close to *Rhus javanica* (mean 25 m; M. Chung & B. Epperson, unpubl. data). Nonetheless, clonal growth neither significantly increases the spatial genetic structure in standing populations of *E. emarginata* nor apparently contributes to biparental inbreeding, generally expected to be caused by spatial structure in dioecious species with limited pollen and seed dispersal. Why do ramets, as revealed in this study, often establish at long distances from their progenitors? It has been suggested that the extent of spatial spread may be dependent on competition with adjacent clones for light and nutrients, disturbance (e.g. fire), suckering ability of each ramet, and frequency of seedling establishment (Barnes, 1966; Kemperman & Barnes, 1976). As in the study population, if few seedlings are established and there is little competition with other plants, the size of clones may increase. As *E. emarginata* is dioecious, inbreeding except between relatives is not expected. It is suggested that several ecological aspects could be the main reasons for the observed spatial clonal distribution patterns. For this reason, more biological and ecological studies of the species are needed.

In summary, as revealed in this study, clones were found at long distances away from their ancestors. In addition, it was found that genetic patch width was at least 24 m. However, spatial autocorrelation statistics showed that, contrary to expectation for such species, clonal reproduction does not contribute substantially to genetic isolation by distance neither among the sexually reproduced individuals nor the total sample. In contrast, moderate levels of structure are created by limits to seed and pollen dispersal.

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