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GENETIC STRUCTURE OF AGE CLASSES IN *CAMELLIA JAPONICA* (THEACEAE)

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Abstract.—*Camellia japonica* L. (Theaceae), an insect- and bird-pollinated, broad-leaved evergreen tree, is widely distributed in Japan and the southern Korean peninsula. The species has a relatively even age distribution within populations, which may influence the spatial genetic structure of different age classes relative to species with typical L-shaped age distributions. To determine whether the internal spatial genetic structure found in seedlings and young individuals carries over into adults, we used allozyme loci, *F*-statistics, spatial autocorrelation statistics (Moran's *I*), and coancestry measures to examine changes in genetic structure among seven age classes in a population (60-m × 100-m area) in southern Korea. In seedlings, weak but significant positive values of Moran's *I*-statistics and coancestry measures were found for distances less than 14 m, which is consistent with a mechanism of limited seed dispersal combined with overlapping seed shadows. This spatial structure, however, dissipates in older age classes, and in adults genetic variation has an essentially random spatial distribution. Morisita's index of dispersion of individuals in each age class showed that seedlings and juveniles are more highly clustered than are older individuals. These results suggest that self-thinning changes the spatial relationships of individuals, and thus genotypes. A multilocus estimate of F_{ST} (0.008) shows a small but statistically significant difference in allele frequencies among age classes. In summary, intrapopulation genetic structure within and among age classes of *C. japonica* was significant but weak. Despite presumably limited seed dispersal, weak spatial genetic structure in juveniles suggests overlapping seed shadows followed by self-thinning during recruitment. The present study also demonstrates that studies of spatial genetic structure focusing on limited numbers of generations may not be sufficient to reveal the entire picture of genetic structure in populations with overlapping generations.

Key words.—Allozymes, *Camellia japonica*, spatial and temporal genetic structure, spatial autocorrelation, Theaceae.

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Theoretical models have shown that the genetic structure of local populations is affected by various nonequilibrium processes (Slatkin 1977; Wade and McCauley 1988). Genetic and demographic factors, such as pollen and seed dispersal from neighboring parents, past major reproductive events, selection, and other processes determining the life history of a species, influence population genetic processes (Jain and Bradshaw 1966; Schaal and Levin 1976; Linhart et al. 1981; Hamrick and Loveless 1986; Ritland 1989; Schoen and Latta 1989; Epperson 1993; Smouse and Peakall 1999; Kalisz et al. 2001). More recently, studies of spatial population genetic structure with respect to distinct age or stage cohorts in plant populations have been stressed because this permits the detection of genetic dynamics over the life cycle and thus provides clues regarding the various potential ecological and evolutionary causes of spatial population genetic structure (Kalisz et al. 2001). Furthermore, analyses of fine-scale genetic structure across age classes, coupled with analyses of breeding structure and dispersal, should greatly enhance our understanding of how demographic and evolutionary processes act to produce the next generation of reproductive adults (Hamrick et al. 1993; Kalisz et al. 2001). For example, some studies that have taken a life-stage approach have found significant fine-scale genetic structure in seedlings (attributed to limited seed dispersal and habitat heterogeneity) that is greatly reduced or absent in adults, probably due to the loss of individuals from the family patches as seedling cohorts thin (e.g., *Alsesis blackiana* and *Platypodium elegans*: Hamrick et al. 1993; *Cecropia obtusifolia*: Epperson and Alvarez-Buylla 1997; *Pinus clausa*: Parker et al. 2001). In these cases, inbreeding resulting from mating among relatives should be low because patch structure decays before reproductive maturity. A few other studies have revealed increased genetic structure with increasing life-history stage (e.g., *Plantago lanceolata*: Tonsor et al. 1993; *Trillium grandiflorum*: Kalisz et al. 2001), probably due to historical factors (e.g., founder effects) or local selection. In these cases, in contrast, the level of inbreeding should be increased.

Within populations, the initial template of spatial genetic structure established in seedlings depends on patterns of seed dispersal and adult density. This genetic structure will be stronger under restricted seed dispersal and low adult density, and will weaken as increased seed dispersion and adult density act to increase the overlap of seed shadows around maternal trees (e.g., Hamrick et al. 1993; Young and Merriam 1994; Hamrick and Nason 1996; Parker et al. 2001). Temporal changes in genetic structure across age classes depend on both this initial variation as well as subsequent demographic processes. For example, in many plants, especially woody species, seedlings exist in much higher density than adults, resulting in a typical L-shaped age distribution. Under this age distribution, strong spatial genetic structure present in seedlings may largely disappear in adults due to stand thinning, such as seen in three neotropical trees (Hamrick et al. 1993; Epperson and Alvarez-Buylla 1997). In contrast, few empirical or theoretical studies have been conducted on the change of internal spatial genetic structure within populations of woody angiosperms whose age distribution is a relatively even. In these species the internal spatial genetic structure found in seedlings and young individuals may persist into adults, due to a relatively low mortality rate. In other words, it is predicted that even-age structure may prevent (or slow) the loss of genetic structure that is initially established by limited pollen and seed dispersal in the seedling cohort.

Camellia japonica L. (Theaceae) provides a good model system for testing these predictions. *Camellia japonica* is a subcanopy tree, which germinates and establishes sapling banks beneath a closed canopy. Adult trees exist in relatively high densities and ultimately recruit in gaps from saplings established before gap formation (Yamamoto 1992). In contrast, the density and mortality of seedlings and early stage juveniles are both low compared to the coexisting broad-leaved evergreen trees, such as *Neolitsea sericea* and *Persea thunbergii* (Sato et al. 1994; Chung et al. 2000; M. Y. Chung, and M. G. Chung, unpubl. data), resulting in a relatively flat age distribution (Manabe et al. 2000).

Detailed studies of woody angiosperms involving genetic changes in time and space are rare (e.g., Hamrick et al. 1993; Kitamura et al. 1997a,b; Chung et al. 2000; Chung et al. 2003). Thus, a detailed study of spatio-temporal genetic structure in natural populations may aid our understanding of the mechanisms influencing spatial genetic structuring within populations. In this study, multilocus allozyme genotypes were sampled and mapped from a population of *C. japonica* in the undisturbed, Hakdongri Reserve on Geoje Island in southern Korea. We then used Wright's *F*-statistics, spatial autocorrelation statistics (Moran's *I*), and coancestry measures to test two predictions: first, because of high adult density, fine-scale genetic structure in seedlings will be significant but weak; and second, the fine-scale genetic structure established in seedlings will persist into adult-stage class, as expected for species with even age distribution.

MATERIALS AND METHODS

Study Plant

Camellia japonica is widely distributed in Japan (Honshu, Shikoku, and Kyushu) and the southern Korean peninsula. In Korea, *C. japonica* predominantly exists in old-growth forests on several islands near the coast of the southern regions, where it typically grows with other broad-leaved evergreen trees such as *Eurya japonica*, *Persea thunbergii*, *Neolitsea sericea,* and *Cinnamomum insularimontanum*. *Camellia japonica* flowers in late January through early March. *Camellia japonica* is almost entirely self-incompatible under garden conditions (C. R. Parks, unpubl. data, cited in Wendel and Parks 1985), and the flowers have red, tough petals, several elevated whorls of fused stamens, dilute nectar, and are pollinated by birds (*Zosterops palpebrosa insularis*) and syrphid flies (Yumoto 1987; M. G. Chung, pers. obs.). Adults usually produce small numbers of fruits (on the order of tens), each of which contains one to three large seeds (ca. 1.3 cm long). It appears that most seeds simply fall directly underneath maternal plants, because there are no specialized mechanisms for primary seed dispersal (Ueno et al. 2000; M. Y. Chung and M. G. Chung, pers. obs.).

Population Samples

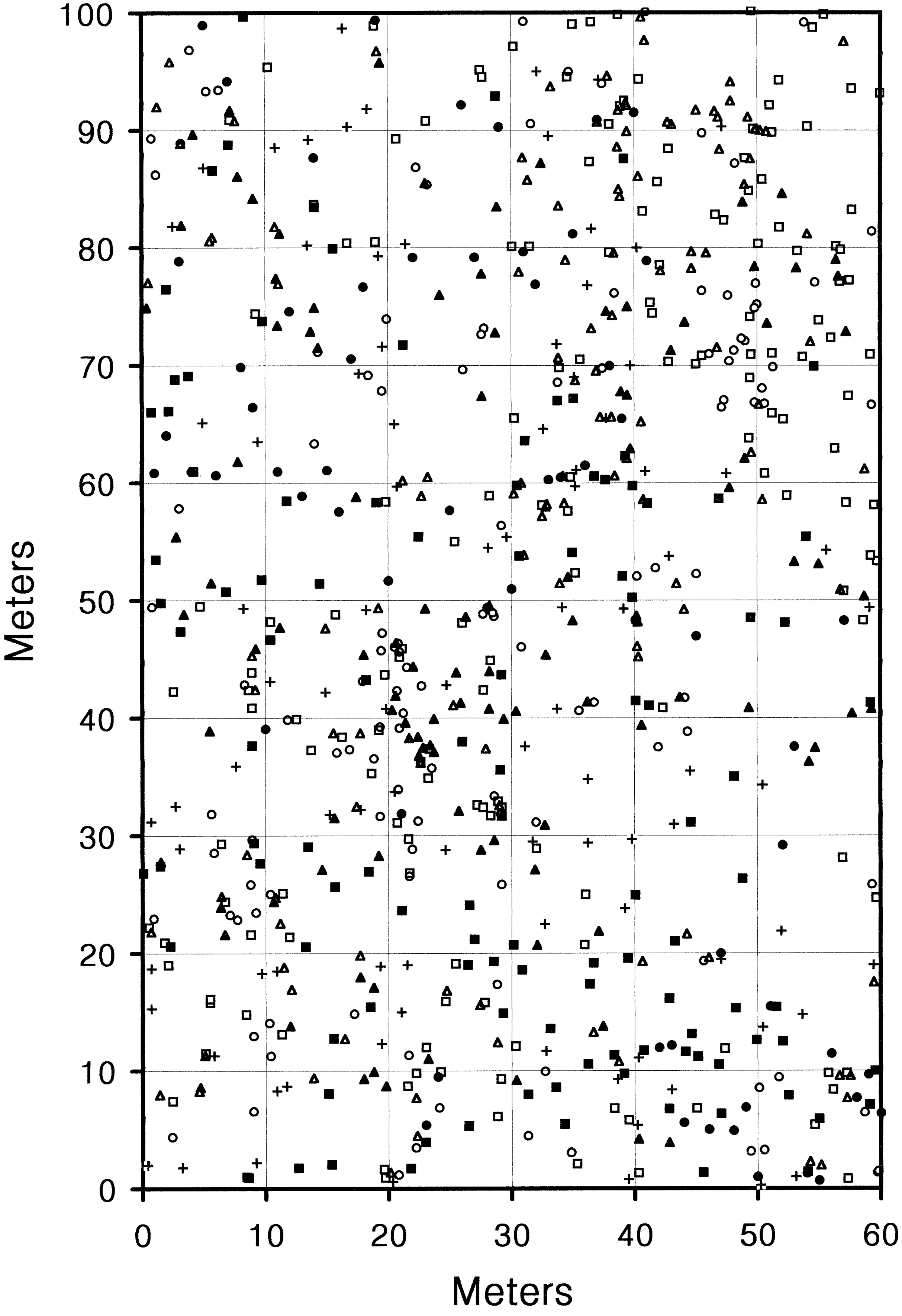
In February 1998, all 833 individuals of *C. japonica* were mapped (Fig. 1) within an area dimensioned 60-m × 100-m (altitude 15–40 m above sea level) of a *Camellia japonica*-dominant, broad-leaved evergreen forest. The site also had low densities of *Pinus thunbergii* and several deciduous trees (e.g., oaks and maples). For the juveniles, diameters at ground level (DGH) were recorded, whereas diameters at breast height (DBH) were measured for the adults. The study site had no recorded history of fire disturbance, nor was there any evidence of trees having been planted, and the eight other long-lived, evergreen, woody plants present are also naturally occurring species in the region. Ages of individuals were determined by counting annual rings and leaf scars, and individuals were classified into seven age-classes (Table 1). Several adults reached ages of a few hundred years. One leaf was collected from each individual of *C. japonica* and stored at 4°C until enzymes were extracted.

Electrophoresis

The leaf material was cut into small pieces 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 (Whatman International, Maidstone, England). The wicks were stored at -70°C until needed for analysis. Electrophoresis was performed using 11.5% starch gels. Ten putative loci for *C. japonica* from eight enzyme systems were resolved using four electrophoretic buffer systems. A Poulik buffer system, a modification (Haufler 1985) of Soltis et al.'s (1983) system 6, resolved alcohol dehydrogenase (*Adh*), fluorescent esterase (*Fe*), and triosephosphate isomerase (*Tpi*). A discontinuous histidine citrate buffer, system 1 (Soltis et al. 1983), resolved fructose-1, 6-diphosphatase (*F1,6*). Buffer system 11 (Soltis et al. 1983) resolved isocitrate dehydrogenase (*Idh*) and phosphoglucomutase (*Pgm-1*, *Pgm-2*). A morpholine citrate buffer system developed by Clayton and Tretiak (1972) was used to resolve 6-phosphogluconate dehydrogenase (*Pgd*) and phosphoglucoisomerase (*Pgi-1*, *Pgi-2*). Stain recipes were taken from 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, with the most anodally migrating alleles denoted with superscript *a*. All *C. japonica* 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). In addition, the genetic inference and description of enzyme systems employed here were reported in Wendel and Parks (1982).

Data Analysis

Spatial genetic structure within age classes.—The spatial distributions of allozyme polymorphisms were analyzed separately for age classes I through VII using both Moran's *I*-statistics (Sokal and Oden 1978) and a pairwise estimate of genetic correlation, f_{ij} (''coancestry'' coefficient; sensu Kalisz et al. 2001), which measures the correlation between the frequency of a random allele from one individual with that of a random allele from another (Cockerham 1969). f_{ij} has been used in a number of recent studies (e.g., Loiselle et al. 1995; Peakall and Beattie 1996; Foster and Sork 1997; Burke


Meters
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10
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60

TABLE 1. Age classes discriminated in this study. Age estimation was made by counting leaf scars and growth rings. DGH, diameter at the ground level; DBH, diameter at breast height.

Age class	Range	No. of individuals
I	Seedlings	113
II	2–3 years (juveniles I)	126
III	4–5 years (juveniles II DGH $<$ 8 mm)	163
IV	6–8 years (8 mm $<$ juveniles III DGH $<$ 25 mm)	121
V	about 10–20 years (25 mm $<$ adults I DBH $<$ 195 mm)	109
VI	about 22–40 years (200 mm $<$ adults II DBH $<$ 230 mm)	109
VII	$<$ about 41 years (230 mm $<$ adults III DBH $<$ 380 mm)	92

et al. 2000; Kalisz et al. 2001; Parker et al. 2001; Chung et al. 2002; Chung et al. 2003). Values of *I*-statistics averaged over alleles allowed indirect estimation of Wright's (1943) neighborhood size (Epperson and Li 1996; Epperson et al. 1999; Chung and Epperson 1999; Epperson and Chung 2001).

For Moran's *I*-statistics, only one allele was considered at diallelic loci, because in this case the second allele contributes identical information. For a locus having more than two alleles, alleles at that locus that were represented by less than five copies (frequencies <2.2%, 2.0%, 1.5%, 2.1%, 2.3%, 2.3%, and 2.7% for the respective age classes) were excluded as noninformative for spatial analysis (Chung and Epperson 1999). Every possible pair of individuals was considered as a join or connection between two individuals and was assigned to one of 14 distance classes according to the distance separating the two individuals. The upper and lower bounds for the distance classes were selected so that the number of pairs (joins) was equal in each class. Moran's *I*-statistic (Sokal and Oden 1978) was calculated for each of 14 distance classes.

Each *I*-value was used to test for significant deviations from the expected values, $E(I) = -1/(N - 1)$ (Cliff and Ord 1981), where N is the sample size. A significant positive value of I indicates that the pairs of individuals within a given distance class have more alleles in common than would be expected by chance, whereas a significant negative value indicates that such individuals have fewer alleles in common than expected. Overall significance of individual correlograms was tested using Bonferroni's criteria (Sakai and Oden 1983). All spatial calculations and statistical analyses were performed using the SAAP program (ver. 4.3; written by D. Wartenberg, Dept. of Environmental and Community Medicine, University of Medicine and Dentistry of New Jersey, Piscataway, NJ).

The coancestry (f_{ij}) was estimated between all pairs of individuals within each age class and in the total sample (833 individuals) following the methods of Loiselle et al. (1995) and Kalisz et al. (2001). Mean values of f_{ij} were obtained for distance intervals (lags) of 7 m (as determined by Moran's *I*-statistics) by averaging over all pairs of individuals located within that interval. The results were combined over loci by weighting each locus by its polymorphic index ($\Sigma\ \mathrm{p}_i\ (1 - \mathrm{p}_i)$). When $f_{\mathrm{ij}} = 0$, there is no significant correlation among individuals at the spatial scale of interest; when $f_{\mathrm{ij}} > 0$, individuals in a given distance class are more closely related than expected by chance; and when $f_{\mathrm{ij}} < 0$, individuals within a given distance class are less related than expected by chance with respect to the local population.

To assess statistical significance, f_{ij} estimates were compared to 95% and 99% confidence envelopes generated under the null hypothesis of no spatial genetic structure. Sample multilocus genotypes were drawn at random with replacement and assigned to occupied map locations within the study population. Resampling was repeated 399 times, and the observed f_{ij} represented the 400th statistic, for each distance class. For a given distance class, f_{ij} is significantly different from zero at $P < 0.05$ (or $P < 0.01$) if the observed value falls above or below the 95% (or 99%) range of these bootstrapped statistics. All calculations and resampling were performed using a program developed by J. Nason (Iowa State University, Ames, IA).

To test whether the slope (β) of the correlograms obtained using Moran's I and f_{ij} for each age class was statistically significant, I and f_{ij} estimates were permuted (999 times) with respect to upper bound (m) of each distance class (Table 2 and Fig. 2) using the program Permute!, version 3.4 alpha (Casgrain 2001) to construct a distribution of the slope under the null hypothesis $\beta = 0$. Under the one-tailed test of the hypothesis that slope of the correlogram decreases with increasing distance class, we rejected the null if there were fewer than 50 random values at least as large as the actual observed β value. Significance levels were corrected for multiple tests by Šidák's (1967) method. Also, to test the hypothesis that I and f_{ij} decay with increasing age we regressed the β for I and f_{ij} on age class.

Spatial patterns of individuals in age classes.—A statistical analysis of the spatial distribution of individuals in each age class is necessary to understand how self-thinning changes the spatial relationships of individuals, and thus genotypes. To assess the spatial distribution of *C. japonica* individuals in age classes, Morisita's (1959) index of dispersion (I_δ) was calculated following the methods of Sakai and Oden (1983). If individuals are randomly distributed within the plot, $I_\delta = 1$; if individuals are patchily distributed, $I_\delta > 1$; and if in-

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FIG. 1. Spatial distribution of *Camellia japonica* trees within the study plot. Trees with different ages are represented by different symbols in the map: ●, age class I; △, age class II; □, age class III; ○, age class IV; ▲, age class V; ■, age class VI; and +, age class VII.

TABLE 2. Summary of Moran's I-statistics and the number of significant positive and negative coefficients per allele at each distance class for *Camellia japonica.* A, number of alleles used. SC, number of significant correlograms.

		Distance class (upper bound, m)											
Age class		1(7)	2(14)	3(21)	4(30)	5(35)	6(40)	7(50)	8(65)	9(80)	10(100)	A	SC
I	Average	0.08	0.02	0.01	−0.01	−0.01	−0.01	−0.02	−0.04	−0.02	−0.00	15	5
	+, −	5, 0	2, 1	1, 1	0, 1	1, 0	0, 1	2, 1	0, 3	1, 1	2, 1		
II	Average	0.02	0.02	0.00	−0.02	0.02	−0.02	−0.01	−0.01	−0.03	−0.02	16	5
	+, −	2, 1	2, 0	2, 1	0, 4	2, 0	0, 2	2, 1	0, 1	1, 4	1, 5		
III	Average	0.05	0.01	0.01	0.00	0.00	−0.01	−0.03	−0.02	−0.02	−0.01	17	4
	+, −	5, 0	3, 2	3, 1	2, 4	3, 1	0, 1	0, 3	2, 3	0, 4	2, 4		
IV	Average	0.01	−0.01	−0.01	−0.01	0.00	−0.02	−0.01	−0.01	−0.02	−0.03	15	2
	+, −	2, 0	1, 0	0, 0	1, 1	2, 1	0, 1	1, 1	1, 1	1, 1	1, 2		
V	Average	−0.01	−0.01	0.02	−0.01	0.00	−0.02	−0.03	−0.02	−0.01	0.03	14	3
	+, −	1, 0	1, 1	3, 0	1, 0	0. 0	0, 1	0, 4	0, 1	1, 0	1, 0		
VI	Average	−0.00	−0.01	−0.00	−0.00	−0.01	−0.03	−0.01	−0.00	−0.00	−0.00	16	3
	+, −	1, 0	1, 0	2, 1	1, 0	−1, 2	0, 0	1, 3	1, 0	1, 0	1, 1		
VII	Average	−0.00	−0.01	−0.02	−0.01	−0.01	−0.01	−0.02	−0.00	0.01	−0.03	18	3
	+, −	2, 0	2, 1	0, 2	1, 2	1, 0	1, 1	1, 2	1, 0	5, 2	0, 0		

dividuals are uniformly distributed (hyperdispersed), $I_\delta < 1$. The significance of departures from randomness was assessed by F tests (Morisita 1959). Further, we used permutation methods to test, for each age class, whether the slope of I_δ changes systematically with quadrat size (m^2) (Fig. 3). Significance levels were corrected by Šidák's (1967) method.

Genetic diversity and structure within and between age classes.—For all data analyses, each age class was considered separately. Five standard genetic diversity parameters were estimated using the program POPGENE (Yeh et al. 1999): percent 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_e); and Nei's unbiased gene diversity per age class and locus (H_e). To make comparisons of H_e across age classes, a complete randomized block analysis of variance (ANOVA), with loci as blocks, was conducted because the same loci were sampled from each age class.

Observed heterozygosity for all polymorphic loci in each age class was compared to Hardy-Weinberg (H-W) expected values using Wright's (1922) fixation indices (F). Statistical significance (P-value) of these values was determined based on 7000 randomizations of alleles among individuals within age classes. A Bonferroni adjustment was used to achieve an experimentwide type-I error (α) of 0.05 for tests of loci and age classes (Rice 1989). For each age class, mean F-value across polymorphic loci and 95% bootstrap confidence intervals were calculated using the program GDA (Lewis and Zaykin 2001).

Wright's (1965) F-statistics (F_{IS}, F_{IT}, and F_{ST}) were calculated following Weir and Cockerham's (1984) multilocus estimators (f, F, and θ, respectively). These fixation indices were used to measure deviations from H-W equilibrium within individuals in age classes (F_{IS}), among age classes (F_{ST}), and over all levels (F_{IT}). The significance of F_{IS}, F_{ST}, and F_{IT} per locus was tested based on 1000 permutations of allele among individuals within samples, genotypes among samples, and alleles among samples, respectively. Means and standard errors were obtained by jackknifing over polymorphic loci. Bootstrap confidence intervals (95% CI) were constructed around jackknifed means of the F-statistics; and observed mean F-statistics were considered significant when confidence intervals did not overlap zero. These calculations were made using the program FSTAT (ver. 2.9.1; Goudet 2000; see also Goudet 1995). Finally, linear regression via permutation test (999 times) of pairwise F_{ST}-values on the number of lags (e.g., age classes I and II differ by one lag, whereas I and IV differ by three lags, etc.) among age classes was conducted to determine whether a consistent pattern of differentiation between age classes exists.

RESULTS

Spatial Genetic Structuring within Age Classes

Moran's I.—Of the ten loci examined, six were polymorphic and reliably scored; the locus *Pgm-2* was expressed but not scored because of poor activity and/or resolution; and the loci *F1,6*, *Idh*, and *Pgi-1* were monomorphic. As a result, 14–18 alleles were used for single locus spatial autocorrelation analysis of the seven age classes (Table 2). The regression of mean Moran's I-values on distance was significantly negative in age classes I, III, and IV ($-0.769 < \beta < -0.698$, $0.001 < P < 0.006$, $0.487 < R^2 < 0.590$; $\dot{\alpha} = 0.0073$), whereas linear regression of the slope of the correlogram for each age class on age class was significantly positive. That is, strength of fine-scale genetic structure decreased with age ($\beta = 0.766$, $P = 0.022$, $R^2 = 0.587$). It is also noteworthy that significant decreases of mean Moran's I-values were observed for distance class 1 (<7 m) with increasing age class ($\beta = -0.810$, $P = 0.014$, $R^2 = 0.657$).

Coancestry (f_{ij}).—These multilocus results were very similar to those for Moran's I-statistics. At the 99% level, a significant positive f_{ij} was detected among seedlings (age class I) located up to 14 m apart, and small but significant negative f_{ij} estimates were detected at longer distances in age class I (at 63 m) and age class III (at 77 m and 84 m; Fig. 2). Analysis of the total sample (833 individuals) revealed that a small but significant positive correlation extended to a distance of approximately 23 m (Fig. 2). The significance of values for the total samples may be a consequence of the larger sample size and increased power to reject the null hypothesis since f_{ij} estimates at less than 14 m for the total

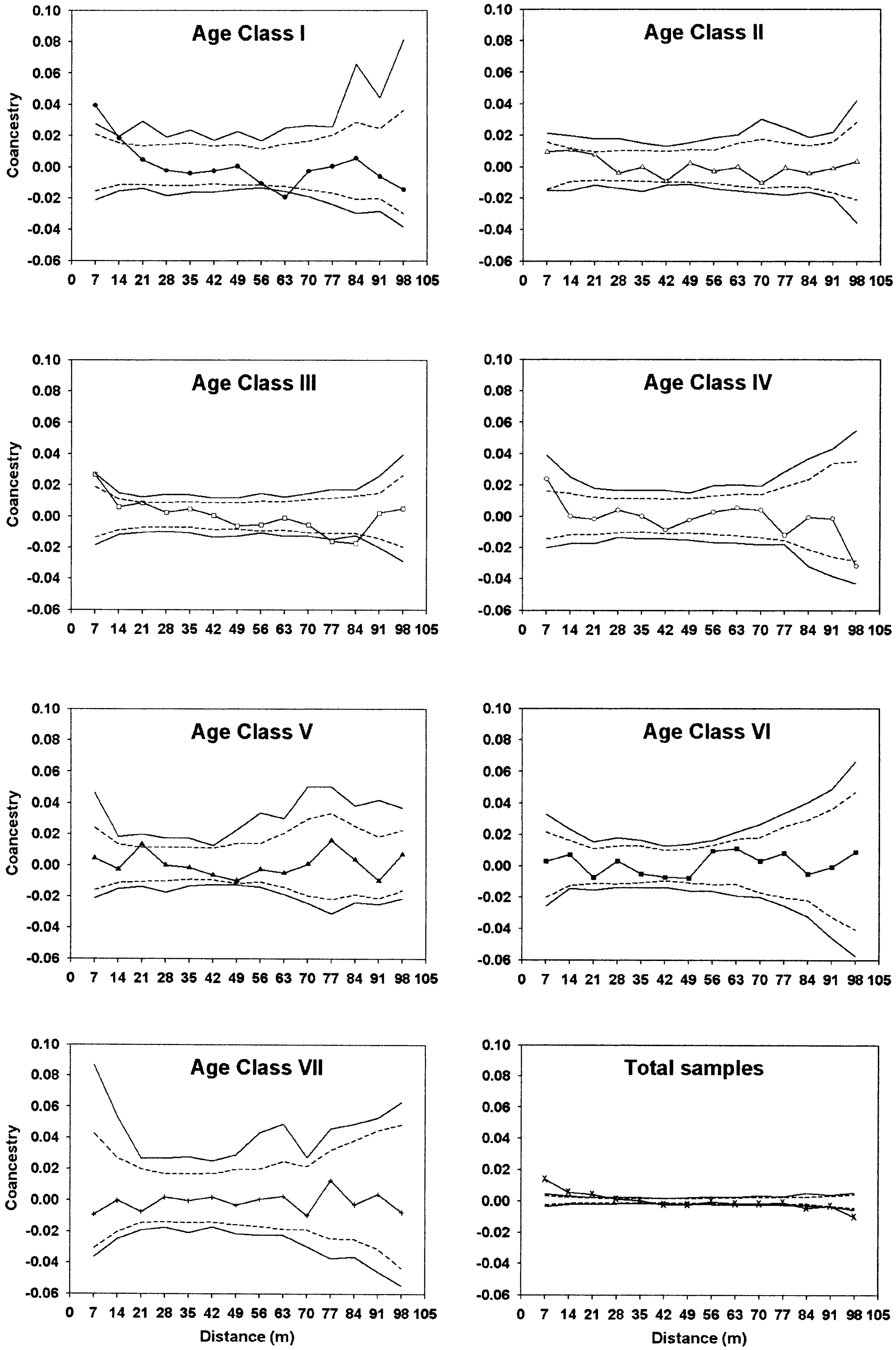

FIG. 2. Correlograms of estimated coancestry (f_{ij}) for seven age classes and total samples. Symbols for each age class are as in Figure 1. The solid lines represent the upper and lower 99% confidence envelopes around the null hypothesis of $f_{ij} = 0$. The dashed lines give the upper and lower 95% confidence envelopes around the null hypothesis of $f_{ij} = 0$.

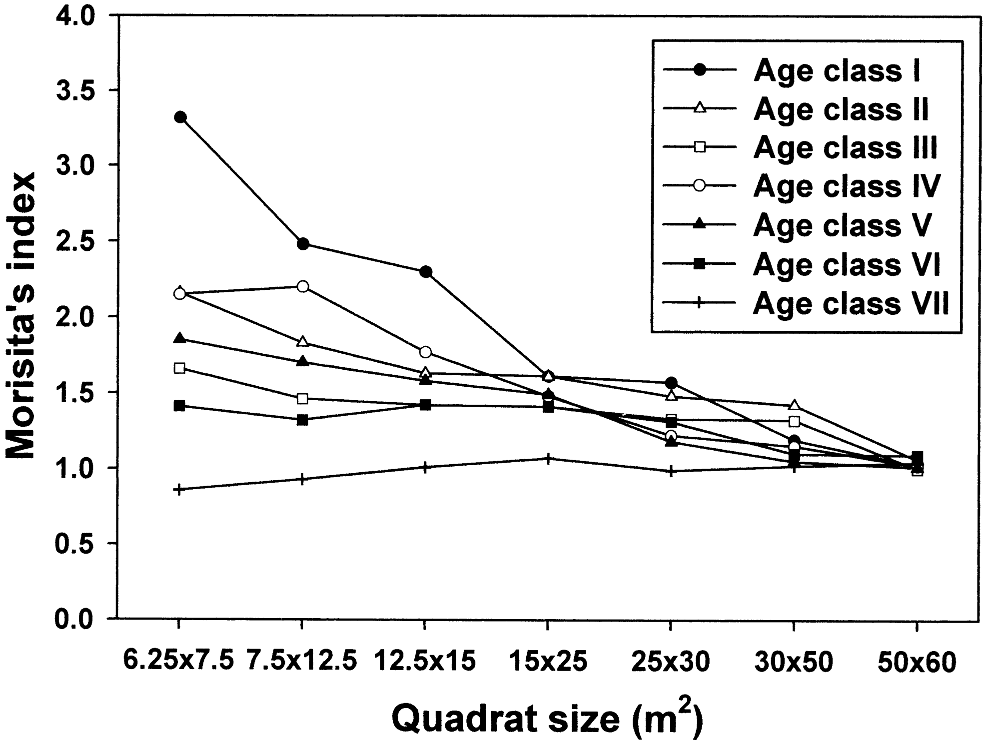

FIG. 3. Morisita's index (I_δ) calculated for seven age classes against seven quadrat sizes. Symbols for each age class are as in Figure 1.

samples are similar to those for age classes II and VI (Fig. 2). At the 95% level, significant positive coancestry values were also observed in age classes III and IV at smaller spatial scales, and one significant negative value was detected at 98 m in age class IV. Other coancestry values were not significantly different from zero across these age classes and none were significant in age classes II, V, VI, and VII, indicating essentially random distributions of genotypes (Fig. 2). The overall slope of the correlograms was significantly negative in age classes I, III, and IV ($-0.633 < \beta < -0.595$, $P = 0.006$, $0.357 < R^2 < 0.401$) and a similar trend was observed in the total samples ($\beta = -0.852$, $P = 0.001$, $R^2 = 0.726$), but not for the other stages. The regression of β (the overall slope of the correlograms) on age class was significantly positive ($\beta = 0.882$, $P = 0.004$, $R^2 = 0.777$). The regression of f_{ij} in the first distance class on age class was significantly negative ($\beta = -0.835$, $P = 0.010$, $R^2 = 0.697$).

Spatial Patterns of Individuals in Age Classes

All values of Morisita's index, I_δ, were significant except those for age class VII and most of the older age classes at the largest quadrat size (data not shown). The regression of I_δ on quadrat size was significantly negative for all but the two oldest age classes, VI and VII ($-0.897 < \beta < -0.767$, $0.001 < P < 0.002$, $0.588 < R^2 < 0.805$; $\dot{\alpha} = 0.0073$) and reveals that I_δ decays to a value of 1.00 as quadrat size gets larger (Fig. 3). In general, there was a decreasing pattern of I_δ values with increasing age class ($\beta = -0.874$, $P = 0.005$, $R^2 = 0.763$ for the first quadrat size; Fig. 3). These results suggest that seedlings and younger age classes are more clumped than older individuals, particularly at smaller quadrat sizes. In other words, individuals in younger age classes form small aggregates whose spatial distribution is random at larger spatial scales, whereas individuals in the oldest age class are randomly distributed over the entire plot.

Genetic Diversity and Structure within and between Age Classes

Levels of genetic diversity averaged across loci were high and very similar among age classes (Table 3): there was no significant difference among age classes for H_e (a complete randomized block ANOVA; $F = 0.784$, $P = 0.590$).

Individual values of fixation indices (F), calculated for all polymorphic loci in each age class, varied among both age classes and loci, but showed no clear pattern across age classes (data not shown): linear regressions on age class of F calculated for individual polymorphic loci and averaged over loci were not significant. At the age class level, bootstrap confidence limits (95% CI) showed a significant deficit of heterozygosity across polymorphic loci in age classes I, III, IV, and VII (Table 3). At the individual locus level, a significant deficit of heterozygosity was found at *Fe* and *Pgi-2* within age classes and in the samples as a whole, whereas remaining loci did not deviate from H-W expectations at either level (Table 4). A multilocus test revealed small but significant levels of deficits of heterozygosity within age classes and for the samples as a whole (mean $F_{IS} = 0.074$; mean $F_{IT} = 0.081$; Table 4).

Overall, about 99% of the total variation in the population is common to all age classes ($F_{ST} = 0.008$); however, small

TABLE 3. Summary of allozyme variation and mean *F*-values observed in seven age classes of *Camellia japonica*. *N*, sample size, *%P*, percentage of polymorphic loci; *AP*, mean number of alleles per polymorphic locus; *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; SE, standard error; and *F*, fixation index.

Age class	*N*	*%P*	*AP*	*A*	A_e	H_o (SE)	H_e (SE)	*F* (95% CI)
I	113	66.7	3.83	2.89	1.60	0.221 (0.013)	0.240 (0.092)	0.092 (0.012, 0.175)
II	126	66.7	3.67	2.78	1.62	0.244 (0.013)	0.242 (0.095)	−0.004 (−0.179, 0.148)
III	163	66.7	3.67	2.78	1.59	0.221 (0.011)	0.236 (0.092)	0.072 (0.025, 0.115)
IV	121	66.7	3.67	2.78	1.61	0.219 (0.013)	0.235 (0.093)	0.075 (0.010, 0.124)
V	109	66.7	3.50	2.67	1.57	0.249 (0.014)	0.248 (0.091)	0.005 (−0.098, 0.119)
VI	109	66.7	3.67	2.78	1.53	0.215 (0.013)	0.238 (0.088)	0.091 (−0.085, 0.230)
VII	92	66.7	3.83	2.89	1.53	0.190 (0.014)	0.228 (0.090)	0.173 (0.010, 0.284)
Mean	119	66.7	3.55	2.79	1.58	0.210 (0.005)	0.238 (0.035)	0.072
Total	833	66.7	4.17	3.11	1.60	0.224 (0.005)	0.241 (0.092)	0.073 (0.017, 0.139)

but significant differences in allelic frequencies among age classes were found for four of six loci (Table 4). Bootstrapped confidence limits (95% CI) showed that jackknifed estimates of mean differentiation among age classes were significantly different from zero (Table 4). The linear regression of pairwise F_{ST} values on the number of lags between age classes was significantly negative ($\beta = -0.349$, $P = 0.038$, $R^2 = 0.122$), indicating that differentiation decreases as the difference in age between classes increases.

DISCUSSION

Spatial Genetic Structuring within Age Classes

The degree of spatial genetic clustering within a population depends on a variety of potential genetic and demographic factors including genetic drift, microenvironmental selection, gene dispersal, plant density, temporal variation in populational reproductive rates, thinning processes and mortality rates, spatial scale of gap formation, and possibly other details of the regeneration process (Harper 1977; Frankel et al. 1995). In particular, the magnitude and spatial scale of genetic structure is strongly influenced by the seed dispersal mechanisms and adult densities that characterize individual species (Hamrick et al. 1993; Kalisz et al. 2001; but see Doligez and Joly 1997).

As was predicted in the introduction, a weak pattern of spatial autocorrelation among seedlings of *C. japonica* was detected. Why is the pattern of spatial genetic structure so weak to begin with? Since adults are abundant in the study population (310 stems/0.6 ha), the low degree of relatedness observed among neighboring seedlings may be caused by overlapping seed shadows, even though *C. japonica* is expected to have poor seed dispersal ability (Ueno et al. 2000). The coancestry between individuals who are not themselves inbred is a measure of the inbreeding coefficient of their hypothetical offspring, with expected values of 0.25 for full-sibs and 0.125 for half-sibs (Cockerham 1969; Kalisz et al. 2001). However, since seedlings, juveniles (age classes III and IV), and adults (age class VII) are not randomly mating ($F = 0.072$ to 0.173; Table 3), the expected f_{ij} values are 0.268–0.293 for full-sibs and 0.134–0.147 for half-sibs (calculated from Crow and Kimura 1970, equation 3.3.1, p.69). Within the 7-m interplant distance class, the mean coancestry for seedlings at 0.04 is much less than expected for half-sibs. This pattern of genetic structure is consistent with a large overlap of seed shadows and/or probable secondary seed dispersal, despite the report that seeds of *C. japonica* are gravity-dispersed and fall near the maternal plant (Ueno et al. 2000).

Another prediction, that initial spatial genetic structure developed in seedlings and young individuals of *C. japonica* would carry over into adults, is not supported by the results. In general, spatial genetic autocorrelation decreased with age class (Table 2, Fig. 2). Further, individuals in younger age classes exhibited clumped spatial distribution that dissipated with age (Fig. 3). Given evidence of substantial overlap in seed shadows at approximately the same scale at which juveniles are clumped, this clumping probably reflects the patchiness of microenvironmental conditions suitable for recruitment (Yamamoto 1992; Manabe and Yamamoto 1997) more than highly localized seed dispersal. Individuals in the older age classes (age classes V through VII) exhibited approximately random spatial distributions, probably due to

TABLE 4. *F*-statistics (Wright 1965) following the method of Weir and Cockerham and *P*-values (test at $H_o = 0$) for six polymorphic loci from all age classes of *Camellia japonica* combined.

Locus	No. of alleles	F_{IS}	*P*	F_{IT}	*P*	F_{ST}	*P*
Adh	2	−0.001	0.999	−0.005	0.988	−0.003	0.983
Fe	3	0.072	0.029	0.075	0.015	0.004	0.002
Pgd	5	0.014	0.310	0.015	0.247	0.001	0.482
Pgi-2	7	0.159	0.001	0.167	0.001	0.010	0.001
Pgm-1	4	0.022	0.251	0.033	0.154	0.012	0.001
Tpi-2	2	0.004	0.464	0.007	0.395	0.004	0.001
Mean		0.074		0.081		0.008	
± 1 SE		0.049		0.050		0.002	
95% CI		(0.011, 0.133)		(0.014, 0.141)		(0.002, 0.010)	

thinning processes that are more or less genotype-independent. As seedlings and younger individuals grow, competition among individuals within cohorts may promote thinning, causing the change from clumped to random distributions as they age, and resulting in a concomitant erosion of spatial genetic structure. An alternative untested hypothesis is that the observed decrease in spatial genetic structure with increasing age may be attributable to different histories of establishment or patterns of overlap in seed shadows between age classes. This hypothesis may explain why age class II (2–3 years) showed weaker spatial genetic structure than age classes I (seedlings), III (4–5 years), and IV (6–8 years). Another possibility is that secondary seed dispersers (as yet unknown) have varied in abundance or diversity during the history of the population. Further studies on breeding dynamics and seed dispersal are needed to test these hypotheses.

Age-specific change in the pattern of internal spatial genetic structure in the population of *C. japonica* is similar to that observed for populations of neotropical canopy trees with more L-shaped age distributions such as *Alseis blackiana*, *Cecropia obtusifolia*, and *Platypodium elegans* (Hamrick et al. 1993; Epperson and Alvarez-Buylla 1997), despite differences in their reproductive biology (e.g., *A. blackiana* and *P. elegans* are bisexual, with wind-dispersed seeds; *C. obtusifolia* is dioecious with bat- and bird-dispersed seeds). In contrast, our results differ from those for a population (more than 100 adults per ha) of the neotropical understory tree *Swartzia simplex* var. *ochnacea*, a bisexual, bird-dispersed species with a relatively even size distribution comparable to *C. japonica* (Hamrick et al. 1993). In *S. simplex* var. *ochnacea* the largest size class (adults $>$4 cm at DBH) retained the substantial fine-scale genetic structure found in younger stages. In addition, in contrast with the results of Hamrick et al. (1993) for *Swartzia*, we found no consistent decrease in the fixation index with age in our population. The *S. simplex* var. *ochnacea* study site was disturbed, so that favorable recruitment conditions may have promoted high adult density and permitted initial fine-scale genetic structure within maternal seed shadows to persist into the adult stage (''disturbance-based hypothesis;'' Hamrick et al. 1993). A disturbed population of *Cordia alliodora* (123 adults in 5.9 ha), a bisexual, wind-dispersed, and moth-pollinated neotropical tree with a more or less even age distribution, has also been found to exhibit significant spatial genetic structure among adults (Boshier et al. 1995). In contrast, the study population of *C. japonica* is undisturbed, and *C. japonica* is the predominant, shade-tolerant, sub-canopy species in broad-leaved evergreen forests in northeastern Asia (Yamamoto 1992).

Based on the adult sample size and density in our study population of *C. japonica*, the observed values of Moran's *I*-statistics (particularly, the average value for distance class one) corresponds to that for a quasi-stationary population with a Wright's (1943) neighborhood size of over 600 (expected Moran's *I*-statistic is approximately zero for adults; Epperson and Li 1996; see also Epperson et al. 1999, Table 5). Because no specialized seed dispersal mechanism is known in *C. japonica*, it appears that pollen dispersal by birds contributes the most to total gene dispersal; indeed the large neighborhood size suggests that pollen flow encompasses essentially the entire study population. This inference conflicts somewhat with the significant *F*-values on age classes I, III, IV, and VII (see the next section, below). Because insect food is scarce in winter and early spring, the birds (*Zosterops palpebrosa insularis*) in warm temperate broad-leaved evergreen forests in northeastern Asia collect nectar from the flowers of *C. japonica* (Wendel and Parks 1985; Yumoto 1987; M. Y. Chung and M. G. Chung, pers. obs.). However, we cannot rule out the possibility that unobserved secondary seed dispersal could be occurring, especially by moving downhill on the sloping hillside of the site or perhaps being moved by animals. A previous allozyme study on 17 Korean populations of *C. japonica* distributed over 300 km in the southern coastal parts of the Korean peninsula revealed rather high indirect estimates of the number of migrants per generation *between* populations (*Nm*; 1.69, calculated from F_{ST}; 2.14, calculated from the frequencies of eight private alleles; Chung and Kang 1996), indicating that historical, presumably pollen-based, gene flow has been relatively extensive among Korean populations of *C. japonica*.

Genetic Diversity and Structure within and between Age Classes

Previous studies have shown that temporal variation in genetic diversity among cohorts within populations is generally weak (e.g., *Pinus ponderosa*: Linhart et al. 1981; *Gleditsia triacanthos*: Schnabel and Hamrick 1990; *Cecropia obtusifolia*: Alvarez-Buylla and Garay 1994; *Lathyrus sylvestris*: Hossaert-McKey et al. 1996; but see *Plantago lanceolata*: Tonsor et al. 1993). Similarly, estimates of the genetic diversity (e.g., expected heterozygosity) were not found to vary significantly among age classes of *C. japonica*, either in this study or in the study of Oh et al. (1996). Nevertheless, we did find weak but significant genetic differentiation among age classes ($F_{ST} = 0.008$), suggesting unique reproductive, colonization, and gene flow events in the history of the study population. Fixation indices also varied among age classes though not as a linear function of age class. These observations suggest that reproductive gene pools may differ among reproductive episodes (unless there is selection affecting the allozyme markers): genetic variation within local populations of *C. japonica* may be chronologically non-uniform, which would increase genetic diversity in populations of *C. japonica*.

The small but significant deficit of heterozygosity within age classes (mean $F_{IS} = 0.074$) may indicate inbreeding and/or population substructure (Hartl and Clark 1997). Ueno et al. (2000) also investigated genetic structure among 518 trees (DBH $>$ 5 cm) of *C. japonica*, using four microsatellite loci in a 4-ha old-growth forest in Tsushima, Japan (about 26 km from our study plot in southern Korea). Similar to our results, the mean F_{IS} across loci within the population was small but significant (0.045). These significant fixation indices could be due to a combination of a Wahlund effect, biparental inbreeding, and self-fertilization. A spatial Wahlund effect is expected if genetically divergent subpopulations are unwittingly included in a population sample, and would be indicated by heterozygote deficiency coupled with significant intrapopulation spatial genetic structure in adults (Crow and Kimura 1970). Moran's *I* and f_{ij} analyses do not indicate significant fine-scale genetic structure of *C. japonica* adult stage (age classes V–VII; Table 2 and Fig. 2), which implies large effective population size and substantial pollen flow within the population. Thus the significant *F*-value in older adults (age class VII) is not indicative of a spatial Wahlund effect (Barbujani 1987). Similarly, the same observations also argue against biparental inbreeding, which requires significant fine-scale genetic structure and localized pollen dispersal. On the other hand, these observations argue that the heterozygote deficiency is due to mating system (some selfing). *Camellia japonica* is, however, almost self-incompatible, and highly outcrossing ($t = 0.98$) under garden conditions (C. R. Parks, unpubl. data, cited in Wendel and Parks 1985). Assuming that the population is at equilibrium and the estimated fixation index in adults ($F = 0.005$–0.173; Table 3) is entirely due to mating system, crude estimates of outcrossing rate suggest some levels of selfing ($t = 0.827$–0.995). In contrast, the absence of both a significant heterozygote deficiency and significant fine-scale genetic structuring in age classes II, V, and VI suggests temporal variation in mating system. If selfing is a major source of fixation index, age class variation in *F* is likely due to temporal variation in selfing versus outcrossing rate. Alternatively, if *C. japonica* is self-incompatible in natural habitats, the deficit of heterozygosity in adults is the result of different, as yet unsolved, processes.

Most studies of intrapopulation spatial genetic structure have examined only adult individuals or at most compared adults with seedlings or juveniles (e.g., Linhart et al. 1981; Roberds and Conkle 1984; Gregorius et al. 1986; Schnabel and Hamrick 1990; Geburek and Tripp-Knowles 1994; Berg and Hamrick 1995; Boshier et al. 1995; Doligez and Joly 1997; Ueno et al. 2000). This study demonstrates, however, that the intensity of the internal spatial genetic structure can vary among age classes (i.e., seedlings, juveniles, and adults). This suggests that studies of spatial genetic structure in species with overlapping generations must focus on a range of age classes to reveal the total picture of genetic structure in local populations (Tonsor et al. 1993; Alvarez-Buylla et al. 1996; Kitamura et al. 1997a,b; Kalisz et al. 2001; Chung et al. 2003).

In summary, by combining results obtained from several different analyses in multiple age classes (analysis of spatial patterns of individuals, spatial autocorrelation analysis, and *F*-statistics), the present study revealed that spatial genetic structure tends to dissipate with increasing age class, and we suggest that this may be the result of thinning processes that are largely genotype-independent. In addition, this study provides a detailed example of a population of *C. japonica* undergoing a dynamic process of spatial and temporal substructuring. Finally, because detailed demographic studies in the genetic analysis of woody angiosperms are rare, theoretical and empirical studies of other forest trees whose age distributions are more or less even would allow us to better understand the dynamics of internal spatial genetic structure within natural populations.

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