

Temporal aspects of the fine-scale genetic structure in a population of *Cinnamomum insularimontanum* (Lauraceae)

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Cinnamomum insularimontanum Hayata (Lauraceae) is an insect-pollinated, broad-leaved evergreen tree with bird-dispersed seeds. We used allozyme loci, Wright's fixation index, spatial autocorrelation statistics (Moran's I), and coancestry measures to examine changes in genetic structure among four age-classes within a recently founded study population (60 × 100 m area) in southern Korea. There were no significant differences in expected heterozygosity among age classes. However, significant genetic differentiation among age classes was detected ($P < 0.0001$). Fixation indices within age classes showed significant deficits of observed heterozygosity, which may be caused by partial selfing. The homogeneity of genetic structure among four age-classes may reflect similar spatial patterns of seed immigration from surrounding populations occurring year

after year. Finally, the average Moran's I and coancestry estimates indicated essentially random spatial distributions of alleles for each of the four age-classes and between seedlings and 2–4 year juveniles vs adult trees. These findings are very similar to those observed in the same study area for another member of the Lauraceae, *Neolitsea sericea*, which has a very similar life history and ecological characteristics (ie, bird-dispersed fruits, insect pollination, and a similar age structure). Together, these results suggest that the fleshy drupes of lauraceous species represent an adaptation to aid in the independent dispersal of seed by birds, which in turn may increase the genetic diversity of founders colonizing new habitats.

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Introduction

The spatial genetic structure among sexually reproduced individuals within plant populations is determined in large part by gene movement via pollen and seed dispersal, that is, gene movement patterns may be a key factor in the internal spatial genetic structuring of plant populations. Although limits to pollen and seed dispersal distances can both contribute to the buildup of genetic isolation-by-distance, the two do not have the same effects. First, in seed plants, pollen dispersal is solely migration, whereas seed or fruit dispersal could result in a migration or a colonization event (Hamrick and Nason, 1996), a distinction that is particularly important in metapopulations (Wade and McCauley, 1988). Second, gene movement in seed plants is sequential (a two-step process, via pollen then by seed), and the development of genetic structure within populations may be more strongly influenced by seed dispersal than by pollen movement. On the one hand, even if pollen disperses long distances, limited seed movement will result in genetically distinct seed shadows among

maternal plants and the spatial clustering of half-sibs (eg, Berg and Hamrick, 1994). If pollen dispersal is also restricted, this will result in inbreeding and could eventually cause the buildup of more intense genetic structure within populations over generations (Sokal and Wartenberg, 1983; Hamrick and Nason, 1996). In contrast, if seeds are widely and independently dispersed within populations, only weak or 'near-random' spatial genetic structure will result (eg, Dewey and Heywood, 1988; Loiselle *et al.*, 1995; Chung *et al.*, 2000). Even if pollen disperses only short distances, long-distance seed dispersal will effectively randomize the spatial distribution of genetic variation.

Long-lived woody plants allow the study of both long-term temporal and spatial variation in population structure. Age-structured data may allow inferences on historical reproductive episodes, and the extent and patterns of seed dispersal. Recently, studies of genetic structure in terms of demography have revealed sometimes striking changes in genetic structure owing to such demographic processes as changes in density (eg, thinning processes independent of genotypes) and the effects of past major reproductive events by different source trees (eg, Epperson and Alvarez-Buylla, 1997; Schnabel *et al.*, 1998; Parker *et al.*, 2001). Nonetheless, there are still few studies on the spatial distribution of genetic variation in different age or size classes of woody plants

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so that stage-class-specific changes in fine-scale spatial genetic structure and associated causal processes are not well understood.

Cinnamomum insularimontanum Hyata (Lauraceae) exhibits a metapopulation structure with important implications for stage-class-specific genetic structure. A number of field observations have been made in Hakdongri Reserve on Geoje Island in southern Korea to obtain information on where and how populations of the species get established (MY Chung and MG Chung, personal observation). Gap populations in early ecological stages are commonly found near older mature populations of *C. insularimontanum* in Hakdongri Reserve. These early seral stages appeared to be characterized by high light environments and many seedlings and juveniles but few or no adults. Older populations, in contrast, occur in closed canopy broad-leaved evergreen forests, are at a successional stage in which there is little or no regeneration, and thus seem to be in the process of becoming locally extinct. As a result, *C. insularimontanum* would exist only for a few generations at any locality and has to establish new populations continually. Seedlings and juveniles (most with ages <5 years) are commonly found scattered several hundred metres from possible maternal trees in natural habitats on a few islands in southern Korea, indicating that seeds can be dispersed long distances, probably by birds. Many bird species in northeastern Asia consume the fleshy fruits of the members of Lauraceae (*Neolitsea* spp, *Litsea* spp, and *Persea* spp) along coastal areas (Kitamura and Murata, 1987), and have been observed feeding on *C. insularimontanum* in southern Korea (MY Chung and MG Chung, personal observation). Since the fruits of these species are often consumed and dispersed singly or a few at a time, this type of seed dispersal may serve to weaken spatial genetic structuring within and among populations (Hamrick and Loveless, 1986).

One important question arising from the metapopulation structure of *C. insularimontanum* concerns how the founding of populations influences internal spatial genetic structure across age classes. If newly founded populations are populated by the long-distance dispersal of immigrants from adjacent source populations year after year, then the internal spatial genetic structure of young populations is expected to be weak both within- and between-age classes. On the other hand, if population expansion results from local seed production and recruitment in the absence of local adaptational differentiation, then fine-scale spatial genetic structure among seedlings and young individuals is expected to be significant and stronger than among older individuals. To test for evidence of these alternative demographic and genetic processes, in this study, multilocus allozyme genotypes were sampled and mapped from a young local population of *C. insularimontanum* occurring in the undisturbed Hakdongri Reserve on Geoje Island in southern Korea. Wright's fixation index and spatial autocorrelation statistics were calculated and analyzed to examine the dynamics of genetic and spatial genetic structure across age classes in the study population. Finally, the results of the present study were compared with other insect-pollinated woody species with bird-dispersed seeds, in particular *Neolitsea sericea* for which age-class-specific spatial genetic structure data are available for the same study area (Chung *et al*, 2000).

Materials and methods

Study species

C. insularimontanum is distributed in southern China and Japan (western region to Chiba Pref. of Honshu, Shikoku, Kyushu, and the Ryukyu Islands), but on the Korean Peninsula it occurs only in a few isolated populations on the southern coast. These populations have areas ranging from ca 500 m² to several hectares on talus slopes with well-drained, rocky and humus soils (MY Chung and MG Chung, personal observation). *C. insularimontanum* has a mass-flowering pattern from mid-May to mid-June, and diverse insect pollinators such as bees, wasps, flies, and beetles visit its perfect flowers (Yumoto, 1987). Adults typically produce hundreds of single-seeded fruits becoming fully mature about 6 months after flowering. During winter, the black drupes (diameter ca 12 mm) are consumed by birds one or a few at a time and dispersed away from the maternal trees (MY Chung and MG Chung, personal observation).

Population samples

All 423 individuals were mapped and leaf samples were collected within a 60 m × 100 m area (altitude 15–40 m above sea level, 6% grade; Figure 1) in a broad-leaved

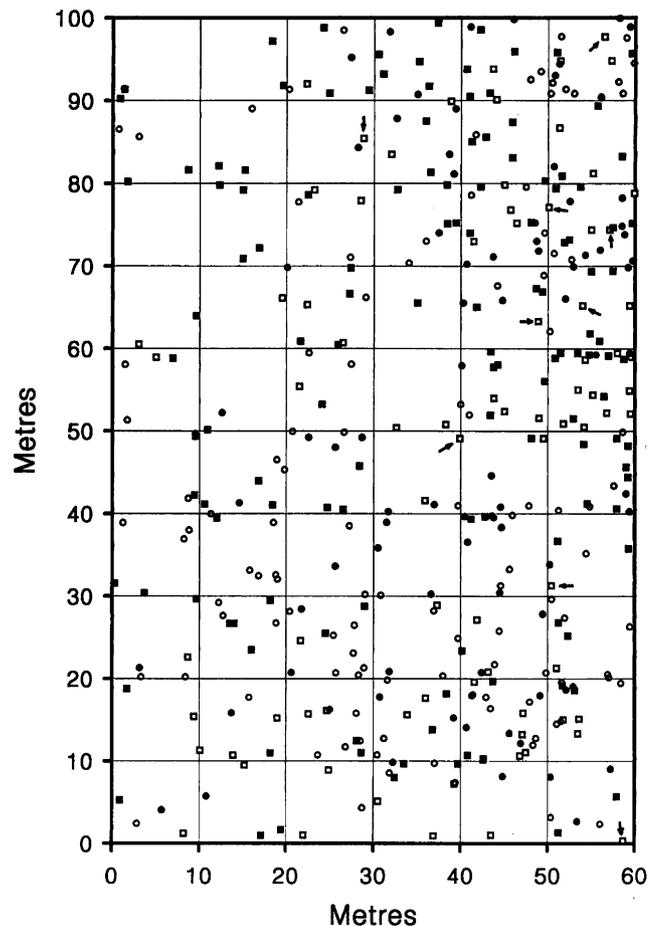


Figure 1 Spatial distribution of trees within the study plot. Trees with different ages are represented by different symbols in the map: ●, age class I; ○, age class II; ■, age class III; and □, age class IV. Symbols are the same as Figure 2. The nine adult trees are indicated by arrows.

evergreen forest with a low density of *Pinus thunbergii* and other deciduous trees (eg, oaks and maples). The study population has no recorded history of fire disturbance, there is no evidence of trees having been planted, and eight other long-lived natural evergreen woody plants coexist at the site. Ages of individuals were determined by counting annual rings (eg, based on cores taken by an increment borer for adults and thin transverse sections of stems and leaf scars for juveniles), and individuals were classified into four age-classes: age class I ($N=85$), seedlings and 2–4 year juveniles; age class II ($N=122$), 5–10 year juveniles; age class III ($N=134$), 11–25 year juveniles; and age class IV ($N=82$), ca 30–40 year juveniles and nine adults. Since *C. insularimontanum* is an early successional species that fruits annually upon reaching maturity, adults were identified as any individuals observed fruiting over a 2-year period (1998–1999). One leaf per individual was stored at 4°C until protein extraction.

Enzyme extraction and electrophoresis

Leaves were 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 wicks, which were stored at –70°C until needed for electrophoretic analysis. Electrophoresis was performed using 11.5% starch gels. Totally, 9 putative loci for *C. insularimontanum* from five enzyme systems were resolved using two electrophoretic buffer systems. A Poulik buffer system of Soltis *et al*'s (1983) 'system 6' was used to resolve diaphorase (*Dia-1*, *Dia-2*), phosphoglucosyltransferase (*Pgm*), and triosephosphate isomerase (*Tpi-1*, *Tpi-2*). A discontinuous histidine–citrate buffer system, a modification (Chung and Kang, 1994) of Soltis *et al*'s (1983) 'system 11', was used to resolve 6-phosphoglucosyl dehydrogenase (*Pgd-1*, *Pgd-2*) and phosphoglucosyl isomerase (*Pgi-1*, *Pgi-2*). Stain recipes were taken from Soltis *et al* (1983), except for diaphorase (Cheliak and Pitel, 1984). The genetic basis of allozyme banding patterns was inferred from segregation patterns with reference to typical subunit structure (Weeden and Wendel, 1989; Wendel and Weeden, 1989) and conceptual methods described in Gillet (1998). 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 with superscript a.

Data analysis

For all data analyses, each of the four age-classes was considered separately as a subgroup (eg, Bacilieri *et al*, 1994; Berg and Hamrick, 1995; Hossaert-McKey *et al*, 1996). A locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95. A total of 4 genetic diversity parameters were estimated using the program POPGENE (Yeh *et al*, 1999): percent polymorphic loci (%*P*); mean number of alleles per locus (*A*); mean observed heterozygosity (H_o); and mean unbiased estimates of expected heterozygosity per locus (H_e). Standard errors for H_o and H_e were calculated over loci. To summarize genetic diversity over age classes, all individuals were pooled into a single

population for which these four genetic diversity statistics were calculated. To make comparisons of H_e across age classes, a complete randomized block ANOVA, with loci as blocks, was conducted because the same loci were sampled from each age class.

For each polymorphic locus in each age class, observed heterozygosity was compared to Hardy–Weinberg (H–W) expected values using fixation index (f_w) of Wright (1922). The proportion (or probability, *P*-value) of randomized data sets that gave larger or smaller f_w values than that observed was estimated for each locus and age class, based on 3200 randomizations of alleles among individuals within age classes. A Bonferroni adjustment was used to achieve an experiment-wide type I error ($\alpha=0.05$) for tests of loci and populations. For each age class, a mean f_w value across polymorphic loci and a 95% bootstrap confidence interval (CI) was calculated using the program GDA (Lewis and Zaykin, 2001). We also calculated the fixation index (and its confidence limits) for all individuals (f_T) by combining age classes into a single, pooled population.

We tested for genetic differentiation among age classes using the exact test of Raymond and Rousset (1995). This test is analogous to Fisher's exact test but uses a Markov chain to explore all potential states of an $r \times k$ contingency table based on r age-classes and k genotypes. This test was conducted using the program ARLEQUIN (Schneider *et al*, 2000) and 10 000 Markov steps.

An analysis of the spatial distribution of individuals in each age class is helpful for understanding whether self-thinning changes the spatial relationships of individuals, and thus genotypes. To assess the spatial distribution of *C. insularimontanum* individuals in each age class, Morisita's (1959) index of dispersion (I_δ) was calculated following the methods of Sakai and Oden (1983). If individuals are randomly distributed per plot, $I_\delta=1$; if individuals are distributed in patches, $I_\delta>1$, and if individuals 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 (999 times) to test, for each age class, whether the slope of I_δ changes systematically with quadrat size (m^2) (Table 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 I_δ decreases with increasing quadrat size, we reject the null if there are fewer than 50 random values at least as large as the actual observed β value. Significant level of type I error was corrected for multiple tests by the method of Šidák's (1967). In addition, in order to test the hypothesis that I_δ decays with increasing age, we regressed β for I_δ on age class.

The spatial distributions of allozyme polymorphisms were analyzed separately for age classes I–IV using both Moran's *I*-statistics as correlations of allele frequencies (Sokal and Oden, 1978) and a pairwise estimate of genetic correlation, f_{ij} ('coancestry' coefficient; see 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). Coancestry coefficient has been used in a number of recent studies (eg, Loiselle *et al*, 1995; Kalisz *et al*, 2001; Parker *et al*, 2001; Chung *et al*, 2002).

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, all alleles at that locus, regardless of their frequencies, were used for the spatial analyses. However, alleles that were represented by less than five copies (frequencies <2.9, 2.1, 1.9, and 3.0% for the respective age classes) were excluded as non-informative for spatial analysis. Every possible pair of individuals was considered as a join or a connection between two individuals and was assigned to one of the 18 distance classes according to the distance separating the two individuals. Since measures of small-scale autocorrelation more accurately represent spatial structure, a 5 m interval was selected. Moran's I -statistic (Sokal and Oden, 1978) was calculated for each of the 18 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.

The coancestry (f_{ij}) was estimated between all pairs of individuals within each age class at a given distance interval and for the total sample (423 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 5 m (as done for Moran's I -statistics) by averaging over all pairs of individuals located within that interval. This measure of coancestry should be near zero when there is no genetic correlation between the frequencies of alleles in individuals at the spatial scale of interest, $f_{ij} > 0$ when individuals in a given distance class are more closely related than expected by chance, and $f_{ij} < 0$ when individuals within a given distance class are less related than expected by chance. Assessment of statistical significance for each f_{ij} estimate per given distance was conducted by the randomization procedures described by Kalisz *et al* (2001).

It is possible that seeds could be produced by adults within the study population and then locally dispersed. In this case, we would expect a significant positive

autocorrelation between seedlings and 2–4 year juveniles and adults. To test this hypothesis, an analysis of coancestry between these juveniles and adult trees was conducted using f_{ij} and 5 m lags. Failure to reject the null hypothesis was interpreted to mean either that seedlings are immigrants or are produced locally but randomly dispersed. All calculations and simulations for f_{ij} were performed using a program developed by J Nason.

Finally, 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 using the program *Permute!* Version 3.4 alpha to construct a distribution of the slope under the null hypothesis $\beta = 0$. Significant level of type I error was corrected by the method of Sidák (1967).

Results

Genetic diversity and structure in age classes

Of the nine loci examined, seven were polymorphic and reliably scored. The locus *Pgi-1* was monomorphic and allele *Pgd-1^a* was detected only in age classes I (allele frequency = 0.006) and II (0.008); thus *Pgd-1* was deleted for data analysis. Levels of genetic diversity averaged across polymorphic loci were high and similar across age classes (Table 1): there was no significant difference among age classes for H_e ($F = 1.563$, $P = 0.228$).

Values of the fixation index (f_w), calculated for each polymorphic locus in each age class, varied among both age classes and loci but showed no clear pattern with respect to age classes (data not shown). Linear regression of f_w on age class for each polymorphic locus was not significant. Only one ($f_w = 0.337$, *Pgi-2* in age class II) of the 28 single locus estimates was statistically significant when type I error was adjusted for multiple tests ($P = 0.0006$, $\alpha' = 0.0018$). Bootstrap confidence limits (95% CI) showed a significant deficit of heterozygosity across polymorphic loci in age classes II–IV but not in age class I (Table 1). When individuals were pooled over age classes into a single population, f_T was significant, indicating deficits of heterozygosity compared to H–W expectations (pooled $f_T = 0.081$; Table 1). Assuming that the estimated fixation index is entirely because of selfing in the mixed mating model at equilibrium, a crude estimate of outcrossing rate (t_m) was 0.85. The exact test of genetic differentiation among age classes was highly significant ($P < 0.0001$).

Table 1 Summary of genetic diversity measures and fixation indices (f_w) for four age-classes of *C. insularimontanum* at Hakdongri population

Age class	N	% P^a	A	H_o (SE)	H_e (SE)	f_w (95% CI)
I	85	75.0	2.30	0.278 (0.057)	0.287 (0.067)	0.030 (–0.020, 0.086)
II	122	75.0	2.30	0.309 (0.058)	0.341 (0.068)	0.094 (0.031, 0.190)
III	134	87.5	2.10	0.315 (0.056)	0.331 (0.065)	0.047 (0.005, 0.096)
IV	82	87.5	2.30	0.321 (0.052)	0.354 (0.062)	0.091 (0.014, 0.174)
Pooled estimates	423	87.5	2.25	0.307 (0.057)	0.334 (0.060)	0.081 (0.054, 0.131)

N , sample size; % P , percent polymorphic loci; A , mean number of allele per locus; H_o , mean observed heterozygosity; H_e , mean expected heterozygosity; SE, standard error; and f_w (95% CI), mean fixation index and 95% confidence intervals. ^aA locus was considered polymorphic if the frequency of the most common alleles does not exceed 0.95. ^bCorresponds to f_T for the pooled sample.

Spatial genetic structuring within age classes

As shown by Morisita's index of dispersion, I_δ (Table 2), individuals in each age class were significantly clumped in all but the largest quadrat size (50 × 60 m). The regression of I_δ on quadrat size was significantly negative for all but age class II ($-0.732 < \beta < -0.920$, $0.001 < P < 0.002$, $0.536 < R^2 < 0.847$; $\alpha' = 0.0128$), and reveals that I_δ decays to a value of 1.00 as quadrat size increases. The correlation coefficient between β with respect to each age class was not significantly positive, indicating that I_δ does not significantly decay with increasing age.

According to the criteria described above, nine (age classes III and IV), 10 (age class I), and 11 (age class II) alleles were used for Moran's I -statistics, analyzed separately for four age-classes. Moran's I -values were significantly different from the expected values in 14 (14.0%, age class I), 13 (11.8%, age class II), 10 (11.1%, age class III), and six (8.8%, age class IV) (data not shown). For distance one (<5 m), only three significantly positive cases were observed, in age classes I (*Dia-1^a* and *Pgi-2^a*) and III (*Pgd-2^a*), and this indicates that spatial genetic structuring in the study population is weak at most. The number of significant overall correlograms was only two in age classes I and III, and one in age class II, and none was significant in age class IV, again indicating essentially random distributions of alleles (data not shown). Moreover, patterns of spatial genetic distributions observed separately for age classes I–IV were very similar, and the overall slope of the correlograms of mean Moran's I values was not significantly negative in each of four age-classes (Figure 2).

Based on 99% confidence limits, pairwise estimates of coancestry were not significantly different from zero within age classes, whereas for the total sample two significant but weak values were detected at distance 5 m (0.0205) and 40 m (−0.0086) (Figure 2). At the 95% level, a significant but weak negative value was also observed at 60 m in age class IV. The significance of values in the total sample is probably the result of increased power to reject the null hypothesis resulting from a larger sample size because the mean f_{ij} value at 5 m for the total sample is similar to that for age classes I and III (Figure 2). As with the Moran's I values, the overall slope of the correlograms of f_{ij} estimates was not significantly negative in four age-classes or the total sample.

Spatial genetic correlation between juveniles and adults

Estimates of coancestry between seedlings and 2–4 year juveniles *vs* adult trees did not reveal significant evidence of spatial autocorrelation. More specifically, none of the estimates for specific distance intervals was significant, nor was the slope of the computed coancestry values on distance significantly less than (or greater than) zero (results not shown).

Discussion

The coastal forests of northeast Asia possess broad-leaved evergreen woody species such as *Camellia japonica*, *Castanopsis* spp, *Eurya* spp, *Ficus* spp, *Ligustrum* spp, *Litsea* spp, *Neolitsea* spp, *Persea* spp, and *Quercus* spp. Some of these species are also important members of coastal forest vegetation in Japan and Korea, and through a series of studies of population dynamics (eg, Yamamoto, 1992), pollination ecology (eg, Yumoto, 1987), allozyme variation in local populations (eg, Wendel and Parks, 1985; Chung and Kang, 1994), and population genetic structure (eg, Chung *et al*, 2000; Ueno *et al*, 2000; Chung *et al*, 2003), the dynamics of these populations are beginning to become elucidated. As mentioned in the Introduction, parts of this study were undertaken to address how the relationships between trees and birds affect the internal spatial genetic structure of newly founded plant populations. Many fruit-eating birds (eg, *Zosterops palpebrosa insularis*) living in warm temperature broad-leaved evergreen forests in southern Korea and Japan feed on insects from spring to autumn. When insect food is scarce in winter, the birds collect fruits (drupes) from members of Lauraceae and also nectar from flowers of understory trees (eg, *Camellia japonica* [Theaceae]) (Yumoto, 1987; MY Chung and MG Chung, personal observation). This situation favors mutualism between the canopy trees (with their drupes having an edible portion) and the associated birds (endozoochory). In the sections that follow, we try to relate internal genetic structure among age classes in the study population to seed dispersal by birds.

Genetic diversity and structure in age classes

Estimates of genetic diversity (H_e) within the study population of *C. insularimontanum* were not significantly different among age classes. If most of the seedlings and juveniles studied are the products of repeated long-

Table 2 Values of Morisita's index of dispersion (I_δ) within four age-classes of *Cinnamomum insularimontanum*

Quadrat		Age class							
Size (m ²)	Number	I		II		III		IV	
		I_δ	F	I_δ	F	I_δ	F	I_δ	F
6.25 × 7.5	128	2.44	1.95***	1.70	1.67***	1.26	1.28*	1.73	1.47***
7.5 × 12.5	64	1.67	1.89***	1.56	2.08***	1.26	1.54**	1.47	1.61**
12.5 × 15.0	32	1.65	2.76***	1.44	2.71***	1.15	1.64*	1.48	2.27***
15.0 × 25.0	16	1.44	3.46***	1.02	1.17ns	1.19	2.33**	1.56	4.03***
25.0 × 30.0	8	1.34	5.10***	1.14	11.67***	1.13	3.43**	1.34	4.96***
30.0 × 50.0	4	1.34	10.49**	1.11	5.36**	1.12	6.30***	1.21	6.72***
50.0 × 60.0	2	0.99	0.29ns	1.09	11.77***	1.02	3.61ns	1.00	1.22ns

The significance was assessed by F tests (Morisita, 1959). ns=not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

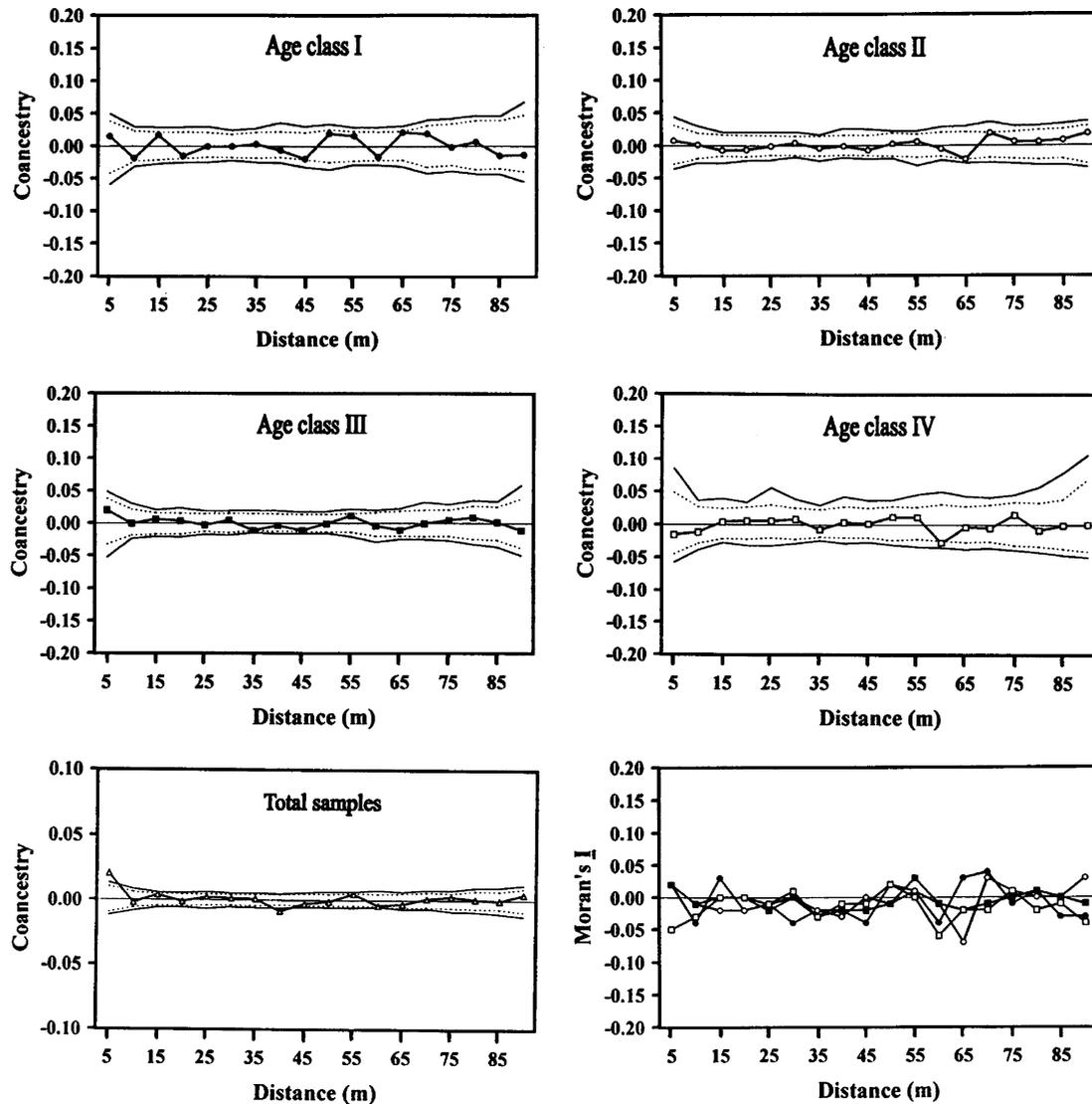


Figure 2 Correlograms of estimated coancestry (f_{ij}) for four age-classes and the total sample and the correlogram of mean values of Moran's I for alleles at seven loci. Symbols for each age class are the same as Figure 1. The solid lines represent the upper and lower 99% confidence envelopes around the null hypothesis of $f_{ij}=0$. The dashed lines give the upper and lower 95% confidence envelopes around the null hypothesis of $f_{ij}=0$. Note that the scale of the Y-axis in the correlogram for the total sample is different from the other correlograms.

distance dispersal of seeds by birds, from other nearby older populations (probably the same source populations), there may be no reason to expect genetic differentiation among the four age-classes. However, significant genetic differentiation among age classes was detected, suggesting temporally varying demographic processes and reproductive events might have operated in the study population.

The estimate of the fixation index ($f_T=0.081$) indicates a significant deficit of heterozygosity for the total sample, which may be indicative of inbreeding (a rough estimate of $t_m=0.85$). *C. insularimontanum* is self-compatible (T Yahara, Kyushu University, personal communication), but the flowers show protandry and bagged inflorescences do not bear fruits (T Yumoto, Kyoto University, personal communication). Although selfing within a flower is not likely, selfing within a tree is possible, because different flowers on the same tree can be found at different stages of development (Yumoto, 1987).

Unfortunately, we do not have any direct information on the level of geitonogamy in this species.

Alternatively, the significant deficit of heterozygosity could be a consequence of spatial or temporal Wahlund effects. A spatial Wahlund effect would be generated by spatial genetic structuring within the study population. Moran's I and coancestry (f_{ij}) spatial autocorrelation analyses, however, indicate little genetic substructuring. In contrast, evidence of a temporal Wahlund effect would be indicated by significant differentiation between age classes (eg, Tonsor *et al*, 1993). As noted above, there is a significant differentiation of genotype frequencies among age classes of *C. insularimontanum*, consistent with a temporal Wahlund effect. In this context, genetic differentiation would be attributable to allele frequency differences among source populations which can be measured hierarchically within- and between-age classes in terms of Wright's (1965) F -statistics (F_{IS} , F_{ST} , and F_{IT}). Using the Weir and Cockerham (1984) estimators of these

parameters, $F_{IS}=0.066$, $F_{ST}=0.019$, and $F_{IT}=0.084$ (calculated using the program FSTAT (ver. 2.9.3.1) by Goudet, 2001). F_{ST} is significant (indicating allele frequency differences among age classes) but small, indicating that the overall deficit of heterozygosity (and similarity of F_{IS} and F_{IT}) must be explained by a process other than a temporal Wahlund effect, such as partial selfing. Further studies on mating system and the degree of population differentiation of *C. insularimontanum* are needed to infer causes of heterozygote deficiencies within local populations.

Spatial genetic structuring within age classes

There are five mature, potential source populations of *C. insularimontanum* within the Hakdongri Reserve (ca 50 ha) on Geoje Island where hundreds of adults exist. Since only nine adults exist in the study population and the range of ages is broad, the study population appeared to have been initially established and then sequentially enlarged by repeated seed movements from maternal trees in nearby older stands (source populations).

As revealed by Moran's I -statistics and correlograms of estimated coancestry (f_{ij}), there was very little or essentially no spatial autocorrelation of genotypes for four age-classes in the population of *C. insularimontanum* (Figure 2). Moreover, although no statistical tests for differences between correlograms have been developed (Sokal and Wartenberg, 1983), the results of this study indicate that the spatial genetic distributions in the four age-classes are similar. One important aspect of seed movement is its role in the initial founding of a population. When populations are founded by long-distance, wind- or bird-dispersed seeds, in many cases the initial spatial distribution of genetic variation may be nearly random, because at long distances the dispersal function relative to distance is 'flat'; hence the relative resting places of seeds become nearly independent of their relative starting places (Epperson, 1990). Our results support these theoretical expectations. The age of a population (ie, how many cycles of regeneration have occurred since population establishment) is also important. Established populations can have much stronger spatial genetic structure because of the opportunity for localized seed dispersal and in older populations increased genetic isolation-by-distance over cycles of regeneration (eg, Epperson, 1993).

Individuals in the study population exhibited a clumped spatial distribution (Table 2), which could have been shaped by limited seed dispersal patterns and habitat heterogeneity. In the case of *C. insularimontanum*, microsite heterogeneity is probably responsible for the clumped distribution. If seeds fall beneath the maternal plants, individuals in younger age classes should exhibit a clumped spatial distribution. As seedlings and younger individuals grow, competition among individuals within cohorts causes thinning, shifting from a clumped to random distribution with age (eg, *Alseis blackiana* and *Platypodium elegans* in Panama: Hamrick *et al*, 1993; *Camellia japonica* in the same study population: Chung *et al*, 2003). This pattern is not observed for *C. insularimontanum*. The homogeneity of spatial genetic structure and similar spatial clumping of individuals

among the four age-classes suggest that patterns of seed dispersal at the population level are similar over time.

Spatial genetic correlation between juveniles and adults
Spatial genetic analyses revealed no significant autocorrelation between seedlings and 2–4 year juveniles *vs* adult trees. This allows us to reject the hypothesis that the youngest age class has been produced by local mating and subsequent dispersal close to the maternal tree. We cannot discriminate from these data alone, however, between local mating and spatially random dispersal *vs* seed production in the surrounding landscape followed by random dispersal into the study population. Evidence for the latter alternative, that is, the role of seed immigration in populating the study site, comes from inspection of the demographic data. In particular, more than half of the population consists of 10–40 year juveniles and sub-adults that are too old to have been produced by the reproductively mature individuals currently at the site. Extrapolating from this pattern of seed immigration over time, the majority of juveniles less than 10 years old are also likely to be immigrants, and local reproduction is likely only beginning to contribute to population growth within the site.

Comparison to other studies

The autocorrelation statistics observed for *C. insularimontanum* are similar to those found for most other insect-pollinated woody species with bird-dispersed seeds. For example, Dewey and Heywood (1988) found little autocorrelation in a population of the shrub *Psychotria nervosa* (Rubiaceae) in south Florida. An exception is the study by Loiselle *et al* (1995) on a population of *Psychotria officinalis* in Costa Rica, where they found significant coancestry among individuals separated by up to 5 m, but not beyond, indicating that some fruits fall underneath maternal plants. They suggested that although some proportion of seed dispersal may be localized, there is substantial mixing of seed shadows as a result of fruit removal by birds. Since *P. officinalis* has the potential for vegetative reproduction (JL Hamrick, personal communication), it is highly probable that the observed significant spatial genetic structure within short distance might be caused in part by clones. More recently, in the same forest as the present study, Chung *et al* (2000) examined changes in spatial genetic structure among five age-classes using four alleles and Moran's I -statistics of *Neolitsea sericea*, a dioecious tree that is insect pollinated, with red, bird-dispersed drupes. Like the present results, the average Moran's I -value for each of the five age-classes revealed little spatial genetic structuring among individuals within the population. Moran's I -values were significantly different from the expected values in five (12.5%, age classes I and V), six (15%, age class IV), and seven (17.5%, age classes II and III) of 40 cases in which only two significantly positive values were found in <14 m. In addition, patterns of spatial genetic distributions for five age-classes were very similar and the overall slope of the correlograms of mean Moran's I -values was not significantly negative in each of the five age-classes. The authors suggested that extensive, perhaps independent, seed dispersal by birds might cause a lack of genetic

structure in an initially regenerative local population. Individual seeds of *C. insularimontanum* and *N. sericea* may move more or less independently because the single-seeded fruits of these species are large (ca 12 mm diameter), and birds can harvest only a few at a time (MY Chung and MG Chung, personal observation). This, combined with the inference that most of the trees in the study site resulted from long-distance seed dispersal from surrounding adult populations, means that we should expect genotypes to be essentially randomly distributed spatially within populations. However, in other species, where birds move seeds in groups (say of half-sibs) owing to seed-caching behavior (eg, *Pinus albicaulis*; Furnier et al, 1987) or the dispersal of intact fruits (eg, *Cecropia obtusifolia*; Epperson and Alvarez-Buylla, 1997), seedlings may exhibit striking spatial structuring within local populations, even if the seeds or fruits move long distances. These studies provide important implications that the amount of spatial genetic structure among seedlings may depend on the type of fruit and the behavior of individual birds or other animals (Hamrick and Loveless, 1986). Particularly important is whether or not birds cause correlated seed dispersal into a given local area or gap.

In summary, the present study of spatial and demographic genetics revealed a lack of genetic structure in a young local population, and we suggest that this is because of extensive gene flow through widespread, and perhaps nearly independent, dispersal of seed by birds. Thus, birds may act to maximize genetic diversity in newly founded populations, which appear to be continually forming and necessarily so because regeneration is limited in older, adult populations of *C. insularimontanum*. Overall, this may also help maintain genetic diversity in the metapopulation of *C. insularimontanum*.

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