

SPATIAL GENETIC STRUCTURE IN POPULATIONS OF THE TERRESTRIAL ORCHID *CEPHALANTHERA* *LONGIBRACTEATA* (ORCHIDACEAE)¹

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Orchid seeds are unusual for being the smallest among flowering plants. These dust-like seeds are wind-borne and, thus, would seem to have the potential for long-distance dispersal (a common perception); this perception has led to a prediction of near-random spatial genetic structure within orchid populations. Mathematical models (e.g., simple ballistic model) for wind-dispersed seeds and wind-tunnel experiments, in contrast, indicate that most seeds of orchids should fall close to the maternal plant (<6 m), supporting a prediction of significant fine-scale genetic structure within populations. In reality we do not know much about seed dispersal in orchids. To determine which of these two predictions is more appropriate, Wright's F statistics and spatial autocorrelation analysis were used to examine the genetic structure within two adult populations of the terrestrial orchid *Cephalanthera longibracteata* (Orchidaceae) in southern Korea. In results comparable to those of other self-compatible, mixed-mating plant species, *C. longibracteata* populations exhibited low levels of genetic diversity (mean $H_e = 0.036$) and a significant excess of homozygosity (mean $F_{is} = 0.330$), consistent with substantial inbreeding via selfing and/or mating among close relatives in a spatially structured population. Spatial autocorrelation analysis revealed significant positive genetic correlations among plants located <10 m, with relatedness at <3 m comparable to that expected for half sibs and first cousins. This genetic structure supports the prediction that the majority of seed dispersal occurs over distances of less than 10 m and is responsible for generating substantial overlap in seed shadows within *C. longibracteata* populations.

Key words: allozymes; *Cephalanthera longibracteata*; Orchidaceae; seed dispersal; spatial genetic structure; spatial autocorrelation analysis.

Fine-scale genetic structure is evident within plant populations when the spatial distribution of genetic variation among individuals is nonrandom. A number of evolutionary and ecological processes affect the development and maintenance of genetic structure within plant populations, including limited seed and pollen dispersal (Wright, 1943; Schoen and Latta, 1989; Sokal and Jacquez, 1991; McCauley, 1997), adult density (Hamrick et al., 1993; Hamrick and Nason, 1996), thinning among cohorts (Berg and Hamrick, 1995; Parker et al., 2001; Chung et al., 2003a), spatial and temporal patterns of seedling establishment (Ellstrand, 1992; Schnabel and Hamrick, 1995; Parker et al., 2001), colonization and disturbance history (Schnabel et al., 1998; Epperson and Chung, 2001; Parker et al., 2001), and, potentially, microenvironmental selection (Linhart et al., 1981; Slatkin and Arter, 1991; Kalisz et al., 2001).

Among these factors, probably the most widely studied influence on fine-scale genetic structure is seed dispersal (Hamrick and Nason, 1996). Under possible combinations of seed and pollen dispersal within plant populations, four general scenarios are possible (Kalisz et al., 2001). If, at the scale of investigation, seed dispersal is localized while pollen disperses long distances or randomly, spatial clustering of full and half-sibs will result in the development of significant fine-scale genetic structure in the absence of inbreeding (e.g., Peakall and Beattie, 1996; Kalisz et al., 2001). If pollen dispersal is

also restricted, this will result in inbreeding, reinforcing the buildup of more intense genetic structure and subdividing the population over time under a process of isolation by distance (Wright, 1943; Sokal and Wartenberg, 1983; Barbujani, 1987; e.g., Maki and Yahara, 1997). In contrast, if seeds are widely and independently dispersed then, regardless of whether pollen disperses long or short distances, seed dispersal will effectively randomize the spatial distribution of genetic variation within populations (e.g., Dewey and Heywood, 1988; Loiselle et al., 1995; Chung et al., 2000, 2003b).

Empirical studies of seed dispersal and population genetic structure in orchids are few. On the one hand, as orchid seeds are minute, dust-like, and wind-dispersed, it is generally assumed that they would be dispersed over long distances (Ackerman and Ward, 1999). This assumption leads to the prediction of near random spatial genetic structure within populations of orchid species. On the other hand, using mathematical models (e.g., a simple ballistic model) for wind-dispersed seeds and wind-tunnel experiments, Murren and Ellison (1998) showed that mean expected seed dispersal distances for the neotropical epiphytic orchid *Brassavola nodosa* L. were less than 6 m under conditions approximating those found in its natural habitats (mangrove island in Belize, Central America). The long-distance tail of the seed dispersal distribution is also important because even small amounts of gene flow have significant consequences for the homogenization of genetic variation among populations. At the within-population level, however, localized seed dispersal can generate significant fine-scale genetic structure, even in the face of evolutionarily significant rates of interpopulational gene flow. If coupled with limited pollen dispersal or selfing, then, one would expect significant fine-scale genetic structure and inbreeding within orchid populations.

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Fine-scale genetic structure has been widely studied using spatial autocorrelation analysis to investigate population genetic processes (Sokal and Oden, 1978; Epperson, 1989; Heywood, 1991). Studies of fine-scale genetic structure within populations of orchids may allow us to understand the extent and patterns of gene movement within plant populations (e.g., Peakall and Beattie, 1996; Chung et al., 1998). To date, however, only two studies of fine-scale genetic structure within populations of terrestrial orchids have been conducted, both indicating significant genetic clustering on scale of less than 10 m within 20 × 40 m plots (*Caladenia tentaculata* Schidl., Peakall and Beattie, 1996; *Cymbidium goeringii* Reichb.fil., Chung et al., 1998). Peakall and Beattie (1996) attributed evidence of the significant fine-scale genetic structure to limited seed dispersal because pollen movement within populations of *C. tentaculata* was extensive (a mean dispersal distance of 17 m [maximum = 58 m] by male wasps). Although pollen dispersal patterns were not quantified, *C. goeringii* showed a similar pattern of genetic structure. Based on these two studies, one may expect significant fine-scale spatial genetic structure at short interplant distances in other terrestrial orchids, suggesting that most seeds fall in the immediate vicinity of the maternal plant. Nevertheless, it is difficult to generalize these results, given the limited number of studies of these species.

To further understanding of whether seed dispersal generates near random or significant fine-scale spatial genetic structure, we investigated the terrestrial orchid *Cephalanthera longibracteata* Blume, a sexually reproducing species. Natural populations of *C. longibracteata* are relatively large, making them suitable for the analysis of spatial genetic structure. To quantify this structure, multilocus allozyme genotypes were sampled and mapped from three undisturbed populations in southern Korea. Wright's *F* statistics and spatial autocorrelation statistics were then calculated to examine the distribution of spatial genetic structure both within and among populations.

MATERIALS AND METHODS

Study plant and sites—*Cephalanthera longibracteata* is a terrestrial orchid, 30–50 cm tall, with 3–10 flowers per inflorescence. The species grows in the warmer parts of Korea and Japan (Kitamura et al., 1986). In Korea *C. longibracteata* grows in humus soils under deciduous pine–oak overstory (M. Y. Chung and M. G. Chung, personal observation). The relatively small (ca. 1.0 cm long) white flowers bloom in May and June. Although we observed a small bee (*Lasioglossum* sp.: Halictidae) visiting flowers, *Cephalanthera longibracteata* is highly self-compatible (M. Y. Chung and M. G. Chung, personal observations), and autogamy is likely because fruit-set was observed in a pollinator-free screened greenhouse. The mature fruits (ca. 2.0 cm long) contain large numbers of small seeds.

In September 2001, a total of 391 adults were mapped and sampled from three populations in South Korea. The first location (SOB, a 50 × 70 m area, *N* = 90, altitude 850 m asl) is on a north-facing slope of a hill under pine (*Pinus densiflora* Sieb. et Zucc.) forests, located at Mt. Sobaek, Province Gyeongsangbuk-do. The second site (JIR, a 45 × 140 m area, *N* = 88; Fig. 1, altitude 1050 m asl) is on an east-facing hillside under oak forests, located at Mt. Jiri, Province Gyeongsangnam-do. For the analysis of spatial genetic structure, the population JIR was divided into two clusters JIR-1 (*N* = 38, 35 × 40 m plot) and JIR-2 (*N* = 50, 45 × 70 m, plot), which were divided by a dense stand of small bamboos (*Sasa chirisanensis* (Nakai) Y. Lee) (Fig. 1). Thus, three sets of data (JIR-1, JIR-2, and JIR) were used for spatial genetic structure analysis. The third site (JON, a 45 × 100 m area, *N* = 213; Fig. 1, altitude 380 m asl) is on a north-facing hillside under pine forests, located at Mt. Jongnam, Milyang-shi, Province Gyeongsangnam-do. Leaf samples were

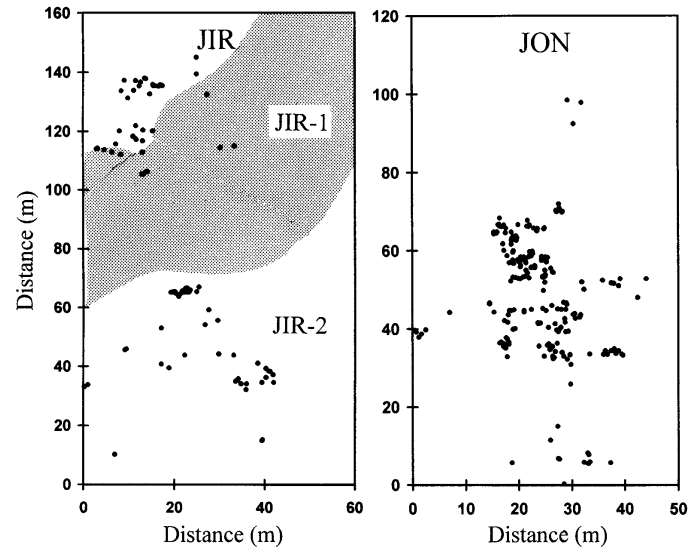


Fig. 1. Distribution of 88 (JIR; 38 of JIR-1 and 50 of JIR-2) and 213 (JON) *Cephalanthera longibracteata* individuals. Cover of *Sasa chirisanensis* (small bamboos) in JIR is indicated by shaded area.

kept on ice, transported to the laboratory, and stored at 4°C until protein extraction.

Allozyme electrophoresis—Leaf samples were cut finely and crushed with a mortar and pestle in a phosphate-polyvinylpyrrolidone extraction buffer (Mitton et al., 1979). Enzyme extracts were absorbed onto 4 × 6 mm wicks cut from Whatman 3 MM chromatography paper (Whatman International, Maidstone, UK), which were then stored at –70°C until needed. Allozyme variation was determined via horizontal starch-gel electrophoresis. Gels (11%) were stained for 13 enzyme systems, which produced 20 putative loci. Stain recipes were taken from Soltis et al. (1983), except diaphorase (Cheliak and Pitel, 1984). The genetic basis of enzyme banding patterns was inferred from observed segregation patterns in light of typical subunit structure and sub-cellular compartmentalization (Weeden and Wendel, 1989). Putative loci were designated sequentially, with the most anodally migrating isozyme designated “1,” the next “2,” etc. Likewise, alleles were designated sequentially with the most anodally migrating allele designated “a.” A modification (Hauffer, 1985) of Soltis et al.’s (1983) system 6 was used to resolve aconitase (*Aco*), alcohol dehydrogenase (*Adh*), diaphorase (*Dia-1*, *Dia-2*, *Dia-3*), fluorescent esterase (*Fe-1*, *Fe-2*), leucine aminopeptidase (*Lap*), malic enzyme (*Me*), peroxidase (*Per*), phosphoglucosomerase (*Pgi-1*, *Pgi-2*), phosphoglucosomutase (*Pgm-1*, *Pgm-2*), and triosephosphate dehydrogenase (*Tpi*). A morpholine citrate buffer system, a modification (Chung and Kang, 1994) of that of Clayton and Tretiak (1972), was used to resolve isocitrate dehydrogenase (*Idh*), malate dehydrogenase (*Mdh-1*, *Mdh-2*), and 6-phosphogluconate dehydrogenase (*6Pgd-1*, *6Pgd-2*).

Analysis of genetic diversity and inbreeding—To estimate genetic diversity and genetic structure, a locus was considered polymorphic if the frequency of the most common allele does not exceed 0.95. Genetic diversity parameters were estimated using the program POPGENE (Yeh et al., 1999): percent polymorphic loci (%*P*), mean number of alleles per locus (*A*), observed heterozygosity (*H_o*), and Nei’s unbiased gene diversity (*H_e*).

For each polymorphic locus in each population, observed heterozygosity was compared to Hardy-Weinberg (H-W) expected values using Wright’s (1922) fixation indices (*f_w*). Proportion (or probability, *P* value) of randomizations, based on 1800 randomizations, that gave larger and smaller *f_w* values than the observed were estimated for each locus and in each local population using the program FSTAT (version 2.9.1 by Goudet [2000], see Goudet [1995]).

To measure deviations from H-W equilibrium at each polymorphic locus,

TABLE 1. Summary of genetic diversity measures and mean fixation (F_{IS}) values observed in three populations of *Cephalanthera longibracteata*.

| Population | <i>N</i> | % <i>P</i> | <i>A</i> | H_o (SE) | H_e (SE) | F_{IS} (95% CI) |
|------------|----------|------------|----------|---------------|---------------|----------------------|
| SOB | 90 | 0 | 1.00 | 0.000 (0.000) | 0.000 (0.000) | — |
| JIR | 88 | 30 | 1.45 | 0.044 (0.017) | 0.065 (0.028) | 0.329 (0.119, 0.469) |
| JON | 213 | 25 | 1.35 | 0.029 (0.020) | 0.042 (0.027) | 0.320 (0.264, 0.544) |
| Mean | 130.3 | 18 | 1.27 | 0.024 (0.006) | 0.036 (0.005) | 0.325 |

Note: *N*, sample size; %*P*, percentage of polymorphic loci; *A*, mean number of alleles per locus; H_o , observed heterozygosity; H_e , Hardy-Weinberg expected heterozygosity or genetic diversity; SE, standard error; and CI, confidence intervals. Dash indicates monomorphic for all loci examined.

Wright's (1965) *F* statistics (F_{IS} , F_{IT} , and F_{ST}) were calculated following Weir and Cockerham (1984). These fixation indices were used to measure deviations from H-W equilibrium attributable to individuals in local populations (F_{IS}), variation among local populations (F_{ST} , an indicator of the degree of differentiation among local populations), and individuals relative to the total population (F_{IT}). The significance of F_{IS} , F_{ST} , and F_{IT} per locus was tested based on 1800 permutations of alleles among individuals within samples, genotypes among samples, and alleles among samples, respectively. Means and standard errors were obtained by jackknifing over six loci. Bootstrap confidence intervals (95% CI) were constructed around jackknifed means of the *F* statistics with 1500 replicates and the observed mean *F* statistics were considered significant when confidence intervals did not overlap zero. These calculations were also made using the program FSTAT (Goudet, 2000). We also calculated F_{IS} separately for each population with 95% bootstrap confidence intervals (1000 replicates) constructed using the program GDA (Lewis and Zaykin, 2001).

Spatial autocorrelation analysis of genetic variation—To obtain a multiallelic, multilocus mean measure of spatial genetic structure per a given distance, the coancestry (f_{ij}) between all pairs of individuals within each age class and total sample from their multilocus genotypes was estimated following the methods of Loiselle et al. (1995) and Kalisz et al. (2001). Mean values of f_{ij} were obtained for distance a interval of 3 m over all pairs of individuals located within that interval. When $f_{ij} = 0$, there is no significant correlation among individuals at the spatial scale of interest; when $f_{ij} > 0$, individuals in a given distance class are more closely related than expected by chance; and when $f_{ij} < 0$, individuals within a given distance class are less related than expected by chance. To assess statistical significance at each distance interval, mean f_{ij} estimates were compared to 95% and 99% confidence intervals estimated via bootstrapping under the null hypothesis of no spatial genetic structure ($f_{ij} = 0$). To generate these intervals, intact multilocus genotypes were drawn at random with replacement, assigned to occupied map locations within the study population, and used to calculate f_{ij} . This procedure was repeated 399 times with the bootstrap f_{ij} estimates for each distance class ranked from lowest to highest. For a given distance class, the observed f_{ij} represents the 400th statistic and is significantly different from zero at $P < 0.05$ (or $P < 0.01$) if it falls in the 2.5% (or 0.5%) tails of this ranked distribution. All calculations and simulations were performed using a program developed by J. Nason. Finally, to test whether the slope (β) of a correlogram is statistically significant, f_{ij} estimates were permuted with respect to distance (999 times) using the program Permute! (version 3.4 alpha; Casgrain, 2001) to construct

the distribution of the slope under the null hypothesis $\beta = 0$. When testing for negative spatial autocorrelation (one-tailed test), we reject the null if there are fewer than 50 random values at least as large as the actual observed β value.

RESULTS

Genetic diversity and inbreeding—Of the 20 loci examined, six (*6Pgd-2*, *Pgi-2*, *Pgm-1*, *Dia-2*, *Mdh-1*, and *Mdh-2*) and five (*Pgi-2*, *Pgm-1*, *Dia-2*, *Mdh-1*, and *Mdh-2*) were polymorphic in JIR and JON, respectively, while SOB was completely devoid of allozyme variation.

Allozyme variation was low: the mean percentage of polymorphic loci within populations was 18% and mean genetic diversity (H_e) was 0.036 (Table 1). Except for *6Pgd-2* in JIR, all single locus fixation indices (f_w) were positive, ranging 0.058 to 0.721. In populations JIR and JON, five of the 11 single locus estimates were statistically significant when type I error was adjusted for multiple tests ($\alpha = 0.0023$), indicating a heterozygosity deficit in comparison with H-W expectations (data not shown). The mean fixation indices across all loci (F_{IS}) were both very similar for JIR and JON, ranging 0.320–0.329 and significantly greater than zero (Table 1). In calculating F_{IS} over populations, with the exception of *6Pgd-2*, a significant deficit of heterozygosity was found at all loci but *6Pgd-2* and in the sample as a whole (Table 2). Finally, allele frequencies were significantly different among populations for all polymorphic loci; 95% CI for the mean F_{ST} (0.247) showed that differentiation among populations was significantly different from zero (Table 2). When SOB was included, the mean F_{ST} was increased to 0.335 (95% CI = 0.033 to 0.415).

Spatial genetic structure—Spatial genetic structure analyses for JIR-2, JIR (total samples), and JON indicated significant positive autocorrelation at shorter distance intervals (3–6 m) with f_{ij} at 3 m of 0.110 in JIR-2, 0.094 in JIR (total), and 0.060 in JON (Fig. 2). In JIR-1 f_{ij} was not significant at short distances, however, this may be a consequence of relatively small sample sizes and low power to reject the null hypothesis

TABLE 2. *F* statistics (Wright, 1965) following the method of Weir and Cockerham (1984) for six polymorphic loci from two populations of *Cephalanthera longibracteata*.

| Locus | F_{IS} | <i>P</i> | F_{IT} | <i>P</i> | F_{ST} | <i>P</i> |
|---------------|----------------|----------|----------------|----------|----------------|----------|
| <i>Dia-2</i> | 0.333 | 0.0005 | 0.549 | 0.0005 | 0.324 | 0.0005 |
| <i>Mdh-1</i> | 0.443 | 0.0005 | 0.503 | 0.0005 | 0.107 | 0.0005 |
| <i>Mdh-2</i> | 0.233 | 0.0005 | 0.292 | 0.0005 | 0.077 | 0.0005 |
| <i>6Pgd-2</i> | 0.055 | 0.4060 | 0.170 | 0.0125 | 0.121 | 0.0005 |
| <i>Pgi-2</i> | 0.304 | 0.0005 | 0.319 | 0.0005 | 0.021 | 0.0005 |
| <i>Pgm-1</i> | 0.368 | 0.0005 | 0.383 | 0.0005 | 0.025 | 0.0005 |
| Mean | 0.330 | | 0.495 | | 0.247 | |
| ±1 SE | 0.025 | | 0.124 | | 0.166 | |
| 95% CI | (0.227, 0.373) | | (0.271, 0.534) | | (0.024, 0.302) | |

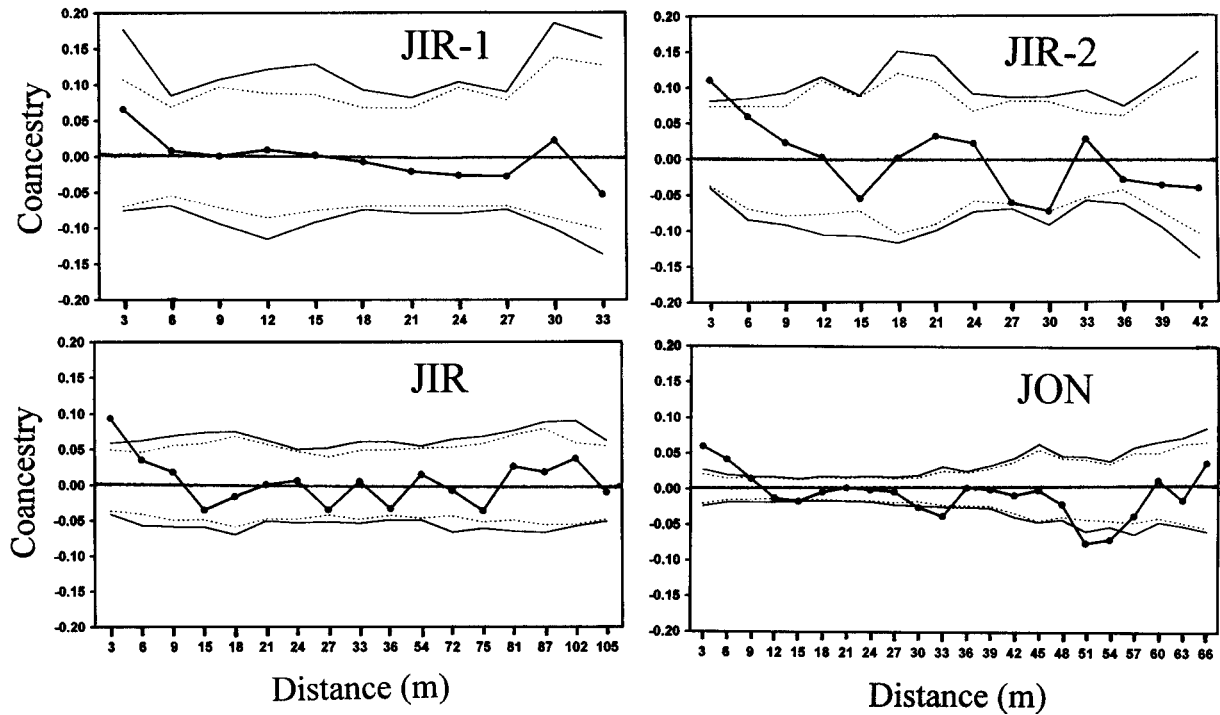


Fig. 2. Correlograms of estimated coancestry (f_{ij}) at 3-m lags for populations JIR (including JIR-1 and JIR-2) and JON. Closed circles indicate mean coancestry values for successive distance classes. The solid and dashed lines represent upper and lower 99% and 95% confidence envelopes, respectively, around the null hypothesis of $f_{ij} = 0$.

since f_{ij} estimates at less than 6 m for JIR-1 samples are similar to those for JON that were significant. Moreover, the overall slope of the correlogram for each study site was significantly negative, indicating spatial genetic structuring in all populations (Table 3).

DISCUSSION

Genetic diversity and inbreeding—Relative to H-W expectations, a deficiency of heterozygotes observed within plant populations indicates inbreeding and/or population substructure (Hartl and Clark, 1997). If the small bee *Lasios glossus* sp. is the primary pollinator of *Cephalanthera longibracteata*, long-distance pollen movement may be uncommon. It is likely that the significant deficit of heterozygosity found in populations of *C. longibracteata* (mean $F_{IS} = 0.330$) is indicative of inbreeding, which is consistent with a mixed-mating system coupled with proximity-dependent mating among relatives. If heterozygote deficiencies are associated with significant intra-population spatial genetic structure, they may also be attributable to a spatial Wahlund effect, which will arise when genetically divergent subpopulations are unwittingly included in a population sample. Coancestry analyses do indicate signifi-

cant fine-scale genetic structure at distances less than 9 m within *C. longibracteata* populations (Fig. 2). However, the correlograms do not continue to decline after crossing the x-axis. In addition, allele frequency differentiation between neighboring subpopulations JIR-1 and JIR-2 was small and not significantly different from zero ($F_{ST} = 0.006$, 95% CI = $-0.015-0.014$). Thus the significant F_{IS} values for JIR and JON appear to reflect inbreeding per se and not a spatial Wahlund effect (Barbujani, 1987).

In plants, the breeding system has a profound effect on the genetic composition of natural populations (Brown, 1979). Under the assumption of no selection between fertilization and the adult stage and a mixture of selfing and random outcrossing, the selfing rate can be estimated from the population inbreeding coefficient, f_w , as $s = 2 f_w / (1 + f_w)$ (Jain, 1979). Based on this equilibrium estimator, the selfing rates for JIR and JON are about 0.5, suggesting a mixed-mating system in *Cephalanthera longibracteata*.

Relative to outcrossers, selfing plants have generally lower allozyme diversity within populations and higher genetic divergence among populations (Hamrick and Godt, 1989). The levels of allozyme diversity observed within and among pop-

TABLE 3. Summary of significant tests for the slope (β) of spatial correlograms.

| Population | 3-m intervals (lags) | | | | 6-m intervals | | | |
|------------|----------------------|---------|-------|-------|---------------|---------|-------|-------|
| | RT | β | R^2 | P | RT | β | R^2 | P |
| JIR-1 | 0-33 | -0.696 | 0.485 | 0.006 | 0-30 | -0.895 | 0.802 | 0.024 |
| JIR-2 | 0-42 | -0.648 | 0.420 | 0.004 | 0-42 | -0.679 | 0.462 | 0.038 |
| JIR | 0-36 | -0.686 | 0.471 | 0.010 | 0-36 | -0.750 | 0.562 | 0.046 |
| JON | 0-66 | -0.401 | 0.161 | 0.025 | 0-60 | -0.647 | 0.419 | 0.010 |

Note: RT, range of test (in meters); R^2 , square of slope; and P , probability.

ulations of *C. longibracteata* ($H_e = 0.036$ and $F_{ST} = 0.335$) were found to be comparable to the means for other selfing ($H_e = 0.074$, $F_{ST} = 0.510$) and mixed-mating ($H_e = 0.090$, $F_{ST} = 0.216$) plant species (Hamrick and Godt, 1989). In contrast, populations of *Cephalanthera longifolia*, a predominantly outcrossing terrestrial species, exhibit genetic diversity within and among populations ($H_e = 0.168$, $F_{ST} = 0.104$; Scacchi et al., 1991) comparable to that observed in other animal-pollinated outcrossing species ($H_e = 0.124$, $F_{ST} = 0.197$; Hamrick and Godt, 1989). Lack of allozyme diversity in SOB suggests that this population is genetically isolated and has drifted to fixation or has undergone a recent genetic bottleneck, perhaps in association with a founding event (e.g., Sun and Wong, 2001; Cheon et al., 2002). The complete lack of allozyme variation within (and among) populations has been observed in other highly inbreeding orchids, including the terrestrial *Cephalanthera damasonium* (Scacchi et al., 1991) and the epiphyte *Spiranthes hongkongensis* (Sun, 1997).

Spatial genetic structure—Nonrandom gene dispersal is the key factor in establishing the internal spatial genetic structuring of plant populations. Although gene movement in seed plants involves both pollen and seed, a variety of arguments and empirical data indicate that the development of spatial genetic structure within populations is more strongly influenced by seed than pollen dispersal (Hamrick and Nason, 1996; Kalisz et al., 2001). The positive coancestry values at the short interplant distances likely reflect spatially localized family structures generated by the limited seed dispersal. The genetic patch width thus reflects the clustering of genetically related individuals as well as the distance of seed dispersal (Sokal and Wartenberg, 1983). The point at which the coancestry correlogram first intersects the x-axis provides an approximate estimate of this genetic patch width and was found to be 9–12 m within populations of *Cephalanthera longibracteata*. Similarly, spatial autocorrelation analysis of genetic structure within populations of two other terrestrial orchid species, *Caladenia tentaculata* (Peakall and Beattie, 1996) and *Cymbidium goeringii* (Chung et al., 1998), have revealed genetic patch structure with widths of approximately 7 and 14 m, respectively. These distances inferred from genetic data are all consistent with the results of Murren and Ellison (1998) whose models indicate the mean wind-mediated seed dispersal distance of the epiphytic orchid *Brassavola nodosa* to be less than 6 m. Together these studies suggest that seed dispersal is likely to be restricted and to generate significant fine-scale spatial genetic structuring in populations of both terrestrial and epiphytic orchid species.

Under random mating, the coancestry between individuals is a measure of the inbreeding coefficient of their hypothetical offspring, with expected values of 0.250 for full-sibs, 0.125 for half-sibs, and 0.0625 for first cousins (Cockerham, 1969; Kalisz et al., 2001). However, as populations JIR and JON are not randomly mating ($F_{IS} = 0.329$ and 0.310, respectively; Table 1), the expected f_{ij} values are 0.188–0.190 for full-sibs, 0.094–0.095 for half-sibs, and 0.047–0.048 for first cousins (calculated from Crow and Kimura, 1970: equation 3.3.1, p. 69). For the 0–3 m interplant distance class, the mean coancestry values for JIR and JON were 0.094 and 0.060, respectively, which are comparable to that expected for half-sibs and first cousins, respectively.

Structure that is less than that expected for full or half sibs can arise from overlap of seed shadows about maternal plants.

Accordingly, plant species with high adult densities have been found to exhibit weaker fine-scale spatial genetic structure than species with lower densities (Hamrick et al., 1993; Hamrick and Nason, 1996). Indeed JIR-1 and JON have higher population density than JIR-2 as well as weaker genetic structure (Figs. 1 and 2). Differences in spatial genetic structure between adjacent subpopulations may also arise from variation in local environmental conditions (e.g., Stanton et al., 1997; Gram and Sork, 1999) and may be eroded by the process of standing thinning during recruitment (e.g., Hamrick et al., 1993; Epperson and Alvarez-Buylla, 1997; Parker et al., 2001; Chung et al., 2003b). Given the large number of orchid seeds produced per plant and the requirement of mycorrhizal infection for successful germination and establishment thinning is likely to be extensive in orchid populations (Warcup, 1981).

In summary, levels of genetic diversity in *C. longibracteata* populations were comparable to mean values for other selfing and mixed-mating plant species. Multilocus estimates of Wright's F_{IS} were significantly deviated from H-W expectations, indicating substantial inbreeding. Spatial autocorrelation analyses revealed moderate but significant local spatial structure in populations of *C. longibracteata*, suggesting that restricted seed dispersal coupled with overlap of seed shadows and probable thinning among individuals around maternal plants have contributed to shaping spatial genetic structuring in *C. longibracteata*.

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