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Notes on spatial genetic structure in populations of *Cymbidium goeringii* (Orchidaceae)

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The fine-scale population genetic structure of *Cymbidium goeringii* Rchb. f. (Orchidaceae), a small herbaceous perennial, was analyzed using spatial autocorrelation statistics in a severely disturbed population (60 × 100 m area). All visible individuals (72) were sampled and their locations were mapped. Individual plants were genotyped for 14 allozyme loci, and Moran's spatial autocorrelation statistics were calculated for 19 alleles. The pattern of spatial genetic structuring is very weak in the population, though over 500 out of 598 individuals have been removed during the past two years by collectors. However, the average values show essentially no spatial autocorrelation, but there is some autocorrelation for some alleles. This indicates that mass collectings by human beings have not affected the genetic structure (thinning-like effect). The mean correlogram of the population suggest that total gene flow may be considerable. As pollen movement by bumblebees is leptokuretic, it is highly likely that large numbers of very small seeds travel through the population by wind.

Key words: allozymes, *Cymbidium goeringii*, Moran's *I*, Orchidaceae, spatial autocorrelation

INTRODUCTION

The spatial distribution of genetic variation in populations is primarily determined by the effects of factors such as limited seed and pollen dispersal, isolation in small patches, and microhabitat selection (Levin & Kerster 1974, Epperson 1993, Hamrick *et al*. 1993). In addition, density in local populations, intraspecific competition among dense seedlings, past reproductive events, the history of establishment of populations, and asexual reproduction by rhizomes or root suckers have a direct impact on the spatial genetic structure of populations (Epperson 1990, Hamrick *et al*. 1993, Berg & Hamrick 1994, Shapcott 1995, Epperson & Alvarez-Buylla 1997, Chung & Epperson 1999). Spatial genetic structure can be analyzed using spatial autocorrelation analysis (Sokal & Oden 1978).

Cymbidium goeringii Rchb. f. (Orchidaceae)

is a herbaceous perennial, that is widely distributed in China, southern Korea, and Japan. Members of Orchidaceae are known to have very specialized, complex, pollinator-specific ("oligophilic") flowers (Richards 1986), resulting in "all or nothing" pollination events. The primary pollinators of *C. goeringii* are bumblebees (*Bombus diversus* of the Apidae) (M. Y. Chung & M. G. Chung, pers. obs.). Fruits contain large numbers of small seeds, which are wind-dispersed and may travel long distances. Recently, the number of individuals of *C. goeringii* have rapidly decreased in Korea and Japan due to mass collection by plant sellers and enthusiasts, including some previously known populations that appear to have been completely destroyed. It is highly probable that such activity has decreased the effective number and sizes of populations, and thus will lead to the lost of genetic diversity within the species in Korea and Japan in the near future.

In Korea, previous studies on the spatial genetic structure in two undisturbed areas (each 20 × 40 m) with a similar density (one is newly established, the other is an old population) in Korea has revealed that the older population has a stronger spatial structure than that of the recently established one (Chung *et al.* 1998). To our knowledge, little work on the comparisons of spatial genetic structure between or among disturbed and undisturbed populations has been conducted on endangered plant species. This kind of work would give us hints for changes of genetic structure in plant populations affected by human disturbance. In this study, spatial autocorrelation analysis was conducted in a recently disturbed population of *Cymbidium goeringii* using allozymes as genetic markers.

MATERIALS AND METHODS

In December 1992, a total of 149 and 118 individuals were recorded within two 20 × 40 m undisturbed areas — one in Shinjin Ri, Seungju Eup, Prov. Chollanamdo, Korea, and one in Kwangju, Prov. Chollanamdo, Korea, hereafter referred to as SHI and KWA, respectively — and then the areas were marked for further surveys. In March 1997, the areas were revisited and all individuals (138 in SHI and 110 in KWA) were mapped and leaf samples were collected for analysis of spatial genetic structure (Chung *et al*. 1998). In these areas individuals grow under a pine-oak overstory with *Pinus densiflora*, *Alnus* spp., *Quercus* spp., and other herbs. In December 1996, a total of 598 individuals of *Cymbidium goeringii* in a third area (60 × 100 m, on a broad-leaved evergreen forest hillside of haegumkang (Hanryehaesang National Park), Kojae Island, Prov. Gyeongsangnamdo, hereafter referred to as HAG) were counted. This population appears to be old because the size of each adult clump is large (ca. 10–20 cm diameter) and scattered over the population. Again, this area was revisited in February 1998, but only 72 individuals were found (probably due to mass collections since there was evidence of removed plants) and mapped and half a leaf from each individual was collected for spatial analysis. Leaves were kept on ice, transported to the laboratory, and stored at 4°C until protein extraction.

Leaf samples were cut finely, crushed with a mortar and pestle, and phosphate-polyvinylpyrrolidone extraction buffer (Mitton *et al*. 1979) was added. Enzyme extracts were absorbed onto 4 × 6-mm wicks cut from Whatman 3MM chromatography paper, which were then stored at –70°C until needed. Levels and distribution of allozyme variation were determined via horizontal starch-gel electrophoresis. Fourteen loci were screened from nine enzyme systems (*see* gel and electrode buffer systems in Chung *et al*. 1998, Chung & Chung 1999). Based on the multilocus genotypes, the ramets were excluded in each population. Thus, 98, 91, and 72 individuals in SHI, KWA, and HAG, respectively, were selected for the data analysis in this study.

For spatial autocorrelation analysis in population HAG, the genotype for each allele at each location was converted into the values 0, 0.5, or 1.0 according to the numbers (none, one, or two) of that allele that were carried in the genotype (Sokal & Oden 1978). Only one allele was considered at dialellic loci. For multiallelic loci, all alleles at that locus, regardless of their frequencies, were used for the spatial analysis. All possible pairs of individuals was considered as joins (a connection between two individuals) and was assigned to one of ten distance classes (according to the distance separating the two individuals). The ten distance classes were designed based on an estimate of average distance which separates nearest neighbor individuals, equalizing sample sizes per each distance class. Moran's *I*-statistic (Sokal & Oden 1978) was calculated for each of ten distance classes. Each *I* value was also used to test for significant deviations from the expected values, $E(I) = -1/(N - 1)$ under the random distribution null hypothesis (Cliff & Ord 1981). In addition, the overall significance of each correlogram, and hence the presence of spatial structure, for each allele was tested using Bonferroni's criterion (Sakai & Oden 1983). All calculations for spatial statistical analyses were performed using the SAAP program (vers. 4.3) written by D. Wartenberg.

RESULTS AND DISCUSSION

According to the criteria we described, 19 alleles were used for spatial autocorrelation analysis. The spatial autocorrelation coefficients, Moran's *I*, for population HAG as well as the frequencies of these

alleles are presented in Table 1. Moran's *I* values were significantly different from the expected value ($E(I) = -0.014$) in 21 (11%) of 190 cases, and the overall correlogram was significant for two (11%) of 19 alleles (Table 1). For distance classes 1 and 2 (0 < 21 m), four statistically significant positive values were observed and only two significant negative values were detected. The average Moran's *I* values estimated in population HAG for the first distance class was nearly the same as data previously published for population KWA (Chung *et al*. 1998). Based on several empirical studies Hamrick *et al*. (1993) reported that species with high adult densities may have less genetic structure than species with lower densities, partly due to the overlap of seed shadows. As revealed in this study, the spatial genetic structuring is still very weak, though ca. 500 individuals were removed in the study population. The average values show essentially no spatial autocorrelation, but there is some autocorrelation for some alleles. This indicates that mass collectings by human beings have not affected the genetic structure (thinning-like effect).

The mean correlograms of the three populations suggest that total gene flow may be considerable. As *Cymbidium goeringii* is pollinated by bumblebees and the pattern of pollen movement by bees is leptokuretic (Richards 1986), it is highly probable that the large numbers of very small seeds, typical of the Orchidaceae, travel long distances by wind, and with secondary movement by surface water on hillsides. If this is the case, gene flow primarily via seed dispersal may act against genetic drift to result in little or no genetic differentiation among populations *C. goeringii*.

Table 1. Spatial autocorrelation coefficients (Moran's *I*) of 19 alleles in Haekumkang (HAG) population of *Cymbidium goeringii* for ten distance classes. The expected value = –0.014.

DB[1]	14	21	28	35	42	50	58	68	79	106	Bonferroni	Allele
DC[2]	1	2	3	4	5	6	7	8	9	10	test[3]	frequency
Adh-2[a]	0.00	–0.04	–0.13	–0.03	0.04	–0.01	–0.04	0.02	0.10*	–0.10	0.492	0.7328
Dia[a]	0.03	0.09	–0.02	–0.08	0.04	–0.10	–0.07	0.07	–0.11	–0.03	0.690	0.7069
Fe-1[a]	–0.10	0.07	–0.04	0.00	–0.01	–0.05	0.10*	–0.07	–0.07	–0.00	0.446	0.4224
Fe-2[a]	–0.02	–0.07	–0.05	0.00	0.04	–0.04	–0.01	–0.07	–0.08	0.12*	0.114	0.7500
Fe-2[b]	–0.01	–0.00	0.04	–0.06	–0.02	0.03	–0.08	0.08	–0.00	–0.11	0.624	0.0690
Fe-2[c]	–0.03	–0.14*	0.01	0.05	–0.02	–0.03	0.01	–0.02	–0.07	0.05	0.471	0.1810
Lap-1[a]	–0.04	–0.05*	–0.00	–0.06*	–0.01	0.01	0.01	–0.01	–0.01	–0.01	0.150	0.0086
Lap-1[b]	–0.04	–0.05	0.08	–0.08	–0.03	0.01	–0.12	0.01	–0.01	0.07	0.537	0.0690
Lap-1[c]	0.18**	–0.08	0.01	–0.14*	–0.03	–0.06	–0.00	0.08	–0.13*	–0.00	0.024	0.1379
Lap-1[d]	0.15**	0.00	0.07	–0.14*	–0.03	–0.11	–0.08	0.03	–0.13	0.07	0.082	0.7155
Lap-1[e]	–0.02	–0.06	0.06	–0.07	–0.06	0.09*	–0.02	0.06	–0.04	–0.02	0.488	0.0690
Pgm-2[b]	0.01	0.11*	–0.15*	–0.17*	0.06	0.04	–0.01	–0.07	–0.05	0.05	0.185	0.3879
Pgm-2[c]	0.08	0.05	–0.16*	–0.17*	0.06	0.12*	0.08	–0.07	–0.11	–0.05	0.184	0.3879
Pgm-2[d]	0.00	0.05	–0.06	0.05	–0.08	0.22**	0.03	–0.08	–0.12	–0.19**	0.003	0.1293
Pgm-2[e]	–0.13	0.02	–0.04	–0.03	0.03	–0.05	0.02	–0.01	0.01	0.02	0.508	0.0948
Tpi-1[a]	–0.03	0.12*	–0.04	–0.04	–0.06	0.00	–0.09	0.06	–0.09	–0.02	0.299	0.3362
Tpi-1[c]	0.02	0.10	0.03	–0.08	–0.05	–0.06	–0.10	0.09	–0.11	–0.01	0.504	0.6379
Tpi-1[d]	–0.09	0.01	–0.03	–0.05	–0.01	–0.05	0.05	0.06	–0.05	–0.02	1.000	0.0259
Tpi-2[b]	–0.07	–0.03	0.01	–0.13	0.03	0.02	0.06	–0.08	0.07	–0.05	0.624	0.6810
Average	–0.00	0.00	–0.02	–0.06	–0.01	–0.00	–0.01	0.00	–0.05	–0.01		
Average[4]	0.01	–0.03	–0.03	–0.06	–0.01							
Average[4]	–0.00	–0.02	–0.01	–0.03	–0.03							

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

[1] Upper distance bound (m).

[2] Distance class.

[3] Bonferroni test for overall correlogram significance was conducted by following Sakai and Oden (1983).

[4] Recalculated from tables 1 (SHI) and 2 (KWA) from Chung *et al.* (1998) equalizing the same distance intervals to HAG population.

Population HAG maintains high levels of genetic diversity (mean expected heterozygosity, H_e = 0.270) compared to the other populations (0.226 for SHI and 0.199 for KWA), though these are not statistically different (Duncan's multiple comparison). It indicates that the thinning that has occurred in population HAG has been at random in regards to the genotypes of individuals. Little genetic variation would have been expected to have been lost taking a random sample of 72 individuals from a total of 598. However, rapid decrease in the number of individuals should decrease the chance of pollination between individuals. In addition, members of Orchidaceae are known to have very specialized, complex, pollinator-specific ("oligophilic") flowers (Richards 1986). This results in an "all or nothing" dependence on pollination (Richards 1986). In field surveys, fruits (ca. 5 cm long) of *Cymbidium goeringii* are rarely encountered (< 5%, M. Y. Chung, unpubl. data), indicating that successful pollination takes place infrequently. If adjacent populations have suffered from a similar situation, colonization by seeds into the low-density population would be infrequent. Furthermore, this would increase the genetic differentiation among local populations of *C. goeringii* over time. The distribution of genetic variation among populations is of primary importance to the conservation of genetic diversity of plant species (Hamrick *et al*. 1991). Also, seed germination with fungus takes ca. eight years (J. S. Kim of Gyeongsang National University, pers. comm.). It is highly likely that the effective number and size of populations will decrease from generation to generation, and thus will cause genetic erosion of the species in the near future.

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