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Spatial demographic and genetic consequences of harvesting within populations of the terrestrial orchid *Cymbidium goeringii*

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ABSTRACT

Populations of many orchids, especially terrestrial species, have been rapidly decreasing due to mass collection by plant sellers and enthusiasts. Given the presumably negative demographic and genetic consequences, such anthropogenic activity should be taken into consideration for predicting ecological and evolutionary dynamics and for planning conservation strategies. To determine how recent human disturbance alters spatial demographic and population genetic processes, populations of the terrestrial orchid *Cymbidium goeringii* located in South Korea were examined to quantify the spatial distributions of individuals and genotypes with respect to three levels of disturbance: “disturbed” (four populations), “putatively disturbed” (two), and “undisturbed” (two). Undisturbed and putatively disturbed populations were found to possess significantly positive spatial clustering of individuals over a range of spatial scales. In contrast, disturbed populations exhibited little or no spatial aggregation, consistent with the selective removal of plants by collectors from higher density areas within these populations. Although overall genetic differentiation among populations was moderate and significant ($F_{ST} = 0.082$), levels of genetic diversity within populations were similar despite the different disturbance histories (mean $H_e = 0.257–0.324$). Spatial genetic autocorrelation analyses revealed that the undisturbed populations exhibited significant declines in kinship (F_{ij}) with distance, that mean kinship at interplant distances of $\leq 4–6$ m was significantly greater than zero and between plants ≤ 0.5 m apart was in the range expected for first cousins to half-sibs. In contrast, only one putatively disturbed and one disturbed population exhibited significant declines in kinship with distance. These differences between disturbed versus putatively disturbed and undisturbed populations in the spatial distribution of individuals and genetic variation likely reflect the consequences of mass collections. Since these differences (and reduced population density) have important implications for future ecological and evolutionary trajectories, conservation managers of endangered terrestrial orchids may want to analyze the spatial distribution of individuals and their genotypes to infer whether a population with few individuals represents a natural state or the likely outcome of mass collection.

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1. Introduction

The spatial structuring of genetic variation within natural plant populations reflects a complex interplay of ecological and evolutionary processes (Slatkin and Arter, 1991; Sokal and Jacquez, 1991; Hamrick and Nason, 1996; Epperson and Li, 1997; Epperson, 1999, 2003, 2005; Kalisz et al., 2001). Fine-scale genetic structure evolves in conjunction with the population genetic processes of micro-environmental selection, inbreeding, and inbreeding depression: genetic structure influences the strength and scale at which these processes operate within populations while their combined effect on fitness influences the strength and scale of genetic structure in adult plants (Epperson, 2003). The proximal causes of genetic structure, however, are inherently ecological with the restricted dispersal of offspring being of primary importance. Restricted seed dispersal creates seed shadows localized around the maternal plant, which, depending on patterns of recruitment, may generate spatial clustering of full- and half-sibling cohorts (Hamrick and Nason, 1996; Kalisz et al., 2001). If pollen dispersal is also spatially restricted within populations, then this sibling structure will lead to biparental inbreeding and rates of genetic drift will be enhanced. Repeated over generations, restricted dispersal of pollen and seed will lead to the development of fine-scale genetic structure representing the spatial aggregation of complex pedigree structures (Wright, 1943, 1946).

Another ecological factor important to the spatial structuring of genetic variation is a population's demographic structure. Adult density influences the development of genetic structure in two ways. First, at higher mean densities (and within spatial aggregates) there will be greater overlap of seed shadows, creating mixtures of pedigrees that decrease spatial genetic correlations among neighbors (Hamrick et al., 1993; Bleher et al., 2002; Vekemans and Hardy, 2004; Troupin et al., 2006). Second, according to population genetic theory (Wright, 1943; Malécot, 1948; Rousset, 1997, 2000; Vekemans and Hardy, 2004), genetic relatedness is expected to decrease with distance at a rate inversely proportional to the effective population density, in essence because the variance in effective pollen dispersal distance is expected to be lower at higher mean densities (and within spatial aggregates).

Although the theoretical relationship between density, dispersal, and genetic structure are well developed, the theory assumes that the dispersion of adults (density) is uniform within populations. In reality, populations often exhibit significant spatial clustering of reproductive individuals (Sternler et al., 1986; Pancer-Koteja et al., 1998; Dale, 1999) and formal theory linking the interplay of such spatial demographics with fine-scale genetic structure (and *vice versa*) is not well developed. Generalizing from the theory for spatially uniform populations, it is clear that spatial demographic structure can influence spatial genetic structure, and that changes in the former can impact the evolutionary trajectory of the latter.

Of principal interest in this paper are the effects of disturbance-mediated changes in the spatial demography on the strength and scale of fine-scale genetic structure. What are the proximal effects of disturbance on within population genetic structure? Consider, for example, disturbance-mediated

mortality that is random with respect to the spatial demography of a population. In this case it would be predicted that the immediate population genetic impact would be to reduce the effective population size (N_e); the major genetic implications of reduced N_e (increased inbreeding and loss of genetic variability) would be manifested over subsequent generations. In contrast, as with disease or herbivore outbreaks, spatial patterns of anthropogenic disturbance are often likely to be highly non-random. Consider, for example, that when plants of economic value for their medicinal, food, timber, or horticultural properties are removed from natural populations, they are typically selectively harvested from relatively high-density patches (Nantel et al., 1996; Buchert et al., 1997; Robbins, 2000; Cruse-Sanders et al., 2005; Degen et al., 2006). In this case, given the existence of spatial demographic and genetic structure prior to disturbance, several predictions can be made concerning the immediate impacts of such disturbance on the spatial distribution of plants and genetic variation. First, because individuals growing closer together are likely to be more closely related than ones growing further apart, the selective removal of plants from high-density patches should decrease the strength and spatial scale of both demographic and genetic structure. Second, because inter-mating within patches generates inbred individuals, removal of individuals from patches should decrease mean inbreeding for the population. Third, because relatives are more likely to be homozygous for the same allele (autozygous) due to identity by descent, their selective removal from higher density patches should increase population-level observed heterozygosity. Other genetic effects (loss of allelic diversity) are also expected with reduction in N_e but are not specific to spatially structured, density dependent disturbance. Also, these are predictions of the immediate impacts of spatially and density dependent disturbance; if, following disturbance, population growth is slow or absent, then the reduced N_e will result in increased inbreeding and loss of genetic variability.

Compared to other terrestrial orchids in South Korea, *Cymbidium goeringii* is relatively common with relatively large, and continuously distributed populations. Since *C. goeringii* is evergreen and large, however, individuals are easily detected during the period from late Fall to early Spring and populations are increasingly subject to the harvest of entire plants by plant sellers and orchid enthusiasts. As a consequence, there exists a mixture of recently disturbed and undisturbed populations of this species, making it ideal for investigating the effects of selective harvesting on spatial demography and genetics. This paper examines the spatial demographic and genetic structure of South Korean *C. goeringii*, focusing on four "disturbed", two "putatively disturbed", and two "undisturbed" populations. Specifically, it is predicted that in recently highly disturbed populations the spatial aggregation of plants, their fine-scale genetic structure, and inbreeding should be lower, and observed heterozygosity higher, than in putatively disturbed or undisturbed populations. To determine spatial demographic structure, the recently developed O-ring statistic of Wiegand and Moloney (2004) was used. Like genetic autocorrelation methods, the O-ring statistic is annulus-based and non-accumulative (unlike the more commonly used Ripley's L function). To quantify the spatial scale and magnitude of within population genetic structure, spatial

autocorrelation techniques (Epperson, 2003; Vekemans and Hardy, 2004) were used. In closing, the short- and long-term implications of our results for the population biology and conservation of selectively harvested plants, and of terrestrial orchids in particular, are considered.

2. Materials and methods

2.1. Plant species

C. goeringii is distributed in deciduous forests of temperate regions in southern China, Japan (Honshu, Shikoku, and Kyushu), and the southern and southwestern Korean Peninsula (Kitamura et al., 1986). Most individuals do not exhibit clonal growth and in only a few cases do older individuals form clumps (10–20 cm diameter) connected by a short rhizome (M.Y.C., unpublished observations). Yellowish-green, nectarless flowers (3–3.5 cm long) are typically borne one per scape during March to April. The species is self-compatible and exhibits extremely low fruit set in natural conditions (0.4–0.6%), which increases following hand-pollination (Chung and Chung, 2003). During field surveys in South Korea conducted since 1996, the first author has failed to observe any pollinators, suggesting that the very low fruit set may be due to pollinator limitation. Typical of orchids, fruits (5 cm long) contain large numbers of minute, wind-dispersed seeds.

2.2. Study area and history of disturbance

Since this study concerns the degree of disturbance (disturbed vs. undisturbed) and its impacts on demographic and

genetic structure within populations of *C. goeringii*, brief site specific descriptions of habitat disturbance/quality are necessary. Based on a field survey of 20 sites in South Korea since 1996, eight study sites representing: “disturbed”, “putatively disturbed”, and “undisturbed” populations were investigated (Fig. 1). Although collection of terrestrial orchids is illegal, disturbed populations nevertheless were identified, in which recent mass collection has resulted in the removal of 70–90% of plants. One population belonging to this category, HAG, was from an east-facing hillside in Hakdong-ri, Dongbu-myeon on Geojae Island, Province Gyeongsangnam-do ($n = 72$, a 60×100 m area, altitude above sea level, 12 m, on a mixed forest of pines/oaks and broad-leaved evergreen trees). At this site, of a total of 597 individuals that were recorded in December 1996, 525 were removed by collector(s) from January 1997 to January 1998. A second site, HAD, was located on an east-facing slope of a broad-leaved evergreen forest hillside in Yulrim-ri, located near Hangilam, a recreation area on Dolsan Island, Province Jollanam-do ($n = 48$, a 50×50 m area, altitude 78 m a.s.l.). At HAD, of a total of 182 individuals that were censused in January 1997, 134 were removed by collector(s) in January 1999, which were found dead in a pile on site (the first author believes that the collector(s) failed to relocate the harvested *C. goeringii* in the dense broad-leaved evergreen forest). Of note, a few additional *C. goeringii* plants were observed in the vicinity of HAD at low densities that suggest a previous mass collection. A third site, JBJ, was located on a south-facing hillside in Jungpyeong-ri, Bukha-myeon, Jangseong-gun, Province Jollanam-do ($n = 54$, a 80×80 m area on a pine/deciduous oak forest, altitude 340 m a.s.l.). At this site, of a total of 575 individuals that were identified in January 1997, 521 were removed by collector(s) from

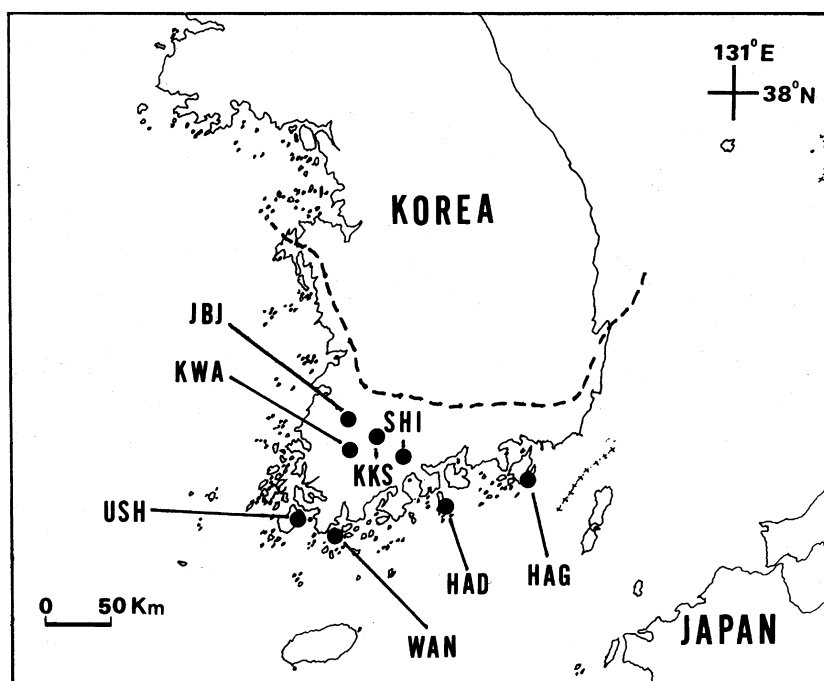


Fig. 1 – Range of *Cymbidium goeringii* in Korea (below dashed line) and locations of the eight populations examined in this study. “Disturbed populations”: HAG, HAD, JBJ, and KKS; “undisturbed populations”: USH and WAN; and “putatively disturbed populations”: KWA and SHI.

December 1998 to January 1999. The fourth disturbed population, KKS, was located on an east-facing slope of a pine/deciduous oak forest hillside in Seogye-ri, Gokseong-eup, Gokseong-gun, Province Jollanam-do ($n = 50$, a 70×90 m area, altitude 220 m a.s.l.). At KKS, of a total of 552 individuals that were counted in January 1997, 502 were removed by collector(s) in January 1999. At JBJ and KKS, the first author found obvious vestiges of mass collection (i.e., traces of digging and presence of dead individuals).

In contrast, two populations were designated as “undisturbed” since no evidence of human-mediated disturbance (e.g., selective harvesting, reforestation, fire, etc.) was detected. These undisturbed populations, belonging to National Parks or Natural Monuments in Korea, occur in old, continuously distributed broad-leaved evergreen forest, and contain relatively high densities of individuals. One study population belonging to this category, USH, was located in the center of a larger continuously distributed population occurring within the Natural Reserve in Uishin-myeon on Jin Island, Province Jollanam-do ($n = 133$, a 20×20 m area, altitude 120 m a.s.l.). The second study population, WAN, was also located in the center of a larger continuously distributed population on a north-facing hillside within Jollanam-do Provincial Arboretum on Wan Island, Province Jollanam-do ($n = 150$, a 20×20 m area, altitude 112 m a.s.l.). USH and WAN are similar to each other in terms of associated plants (*Camellia japonica*, *Cinnamomum insularimontanum*, *Eurya japonica*, *Ligustrum ovatum*, *Neolitsea sericea*, etc.) and density.

Finally, two populations that were suspected of having been subject to human-mediated disturbance were included (Fig. 1). These populations were designated “putatively disturbed”, since they occur adjacent to cities, are easily accessible from these areas, and are suspected of being disturbed by collection (an illegal collector was encountered at one site) or reforestation. One such population, KWA, was located on a south-facing hillside of a pine-oak forest adjacent to a town in Kwangju-shi, Province Jollanam-do ($n = 91$, a 20×40 m area, altitude 220 m a.s.l.). The second population, SHI, was located in recently reforested *Pinus rigida* stand on an east-facing hillside from Shinjin-ri, Seungju-eup, Province Jollanam-do ($n = 98$, a 20×40 m area, altitude 360 m a.s.l.). Most individuals of *C. goeringii* at this site were juveniles. For all eight study populations the first author collected a half-leaf per sample individual, which was kept on ice, transported to the laboratory, and stored at 4 °C until protein extraction for allozyme analysis (see below).

Three of the eight populations were previously studied (HAG, Chung and Chung, 1999; KWA and SHI, Chung et al., 1998). These population data sets were included here to provide replication for comparing demographic and genetic consequences among the three disturbance categories.

2.3. Spatial distribution of individuals

Ripley's *L*-function (Ripley, 1976, 1977, 1981) is commonly used by ecologists as a method for point pattern analysis. Since the Ripley's *L*-function is based on the frequency distribution of points within circles, it is cumulative and confounds effects at larger distances with effects at smaller distances. Like Ripley's *L*-function, Wiegand and Moloney's (2004) O-ring statistic

is a second-order summary statistic of point-to-point distances in a mapped area that is useful for detecting spatial pattern. In contrast, however, the O-ring statistic is non-accumulative as it uses rings to isolate specific distance classes. Consequently, it provides a straightforward biological interpretation of local neighborhood density that is more intuitive than the accumulative measure (Stoyan and Penttinen, 2000). Moreover, use of the O-ring statistic permits a more direct comparison with the results of spatial autocorrelation analyses of genetic structure since the latter also uses rings and is non-accumulative.

To assess and visualize the spatial distribution of plants in the eight *C. goeringii* populations, the univariate O-ring statistic $O(r)$ was calculated from the mean number of individuals in an annulus of radius (r) around each plant and plotted against the spatial scale r . Since the use of ring widths greater than half the shortest plot side introduces bias due to edge effects, the maximal ring width was set less than half the shortest plot side at starting ring width 1 m with a 1 m lag. For testing the significance of $O(r)$ for each r , the common null model, complete spatial randomness, was used. This model is appropriate for this study given the prediction that selective removal of plants from spatial aggregates should erode demographic structure towards spatial randomness. For reference to the point pattern expected under complete spatial randomness the first order intensity, λ was calculated. Ninety-nine percent confidence envelopes about complete spatial randomness (i.e., λ) were constructed from the highest and lowest $O(r)$ of 199 spatial randomizations by Monte Carlo simulation for each study populations. An observed value of $O(r)$ outside of this envelope was judged a significant departure from complete spatial randomness, with an observed value above, within, or below the envelope indicating spatial clustering, spatial randomness, or spatial repulsion (hyperdispersion), respectively, at radius r . All calculations and simulations were conducted using the program PROGRAMITA (Wiegand, 2003).

2.4. Enzyme extraction and electrophoresis

For extraction leaf samples from the eight study populations were finely cut and then crushed with a mortar and pestle in a phosphate-polyvinylpyrrolidone extraction buffer (Mittton et al., 1979). 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 of allozyme variation were determined using horizontal starch-gel (12%) electrophoresis. The 12 putative loci resolved from the eight enzyme systems employed in this study were the same as in Chung et al. (1998) and Chung and Chung (1999). These were acid phosphatase (Acph), alcohol dehydrogenase (Adh-1, Adh-2), diaphorase (Dia), fluorescent esterase (Fe-1, Fe-2), leucine aminopeptidase (Lap), phosphoglucoisomerase (Pgi-1, Pgi-2), phosphoglucomutase (Pgm), and triosephosphate isomerase (Tpi-1, Tpi-2), all of which were resolved using a modification (Haufler, 1985) of Soltis et al.'s (1983) system 6. Putative loci were designated sequentially, with the most anodally migrating isozyme designated as “1”, the next “2”, etc. Likewise, alleles were designated sequentially with the most anodally migrating allele designated “a”.

2.5. Fine-scale genetic structure

To characterize fine-scale genetic structure within populations, spatial autocorrelation procedures and a kinship coefficient (F_{ij} ; Loiselle et al., 1995; Kalisz et al., 2001; Vekemans and Hardy, 2004) were used. Like the O-ring statistic used to measure spatial demographic structure, these procedures are annulus-based and non-cumulative, and so permit isolation of the strength of genetic structure for specific distance classes. To visualize fine-scale genetic structure, mean values of F_{ij} for successive distance intervals (r) were calculated and then plotted against distance. To strike an acceptable balance between spatial resolution and statistical power, in all populations mean F_{ij} estimates were calculated at $r = 2$ m distance intervals (lags) with right-hand binning. Because the two undisturbed populations (USH and WAN) had sufficiently large sample sizes these were also analyzed at a finer scale: for distances of <2 m, $r = 0.5$ m lags were used. For each distance interval, 95% confidence intervals (CIs) were also constructed about the null hypothesis of no genetic structure ($F_{ij} = 0$) with randomization procedures (Loiselle et al., 1995; Kalisz et al., 2001; Hardy and Vekemans, 2002; Vekemans and Hardy, 2004).

To test the overall pattern of genetic structure in each population, the regression slope (b_F) of F_{ij} (the pairwise kinship coefficient between individuals i and j) on the natural logarithm of r_{ij} (the distance between i and j) was calculated. It is expected that b_F will be negative in spatially structured populations and its significance was evaluated using a Mantel test (999 replicates) under the null hypothesis of no genetic structure ($b_F = 0$) (Vekemans and Hardy, 2004). The distance at which average kinship reaches zero is commonly interpreted as indicating the “genetic patch size” or scale of spatial genetic structure (Smouse and Peakall, 1999; Diniz-Filho and Telles, 2002; Epperson, 2003; Escudero et al., 2003). This distance is sensitive to the spatial scale of sampling, however, and is not a characteristic of populations (Fenster et al., 2003; Vekemans and Hardy, 2004). Consequently, because populations in the three disturbance categories were sampled at different scales formal comparisons of this distance were not made between them.

Similarly, because b_F depends to some extent the sampling scheme (Fenster et al., 2003), to make comparisons between disturbance categories a statistic, S_p , which better reflects the rate of decrease of pairwise kinship with distance was used (Vekemans and Hardy, 2004; Hardy et al., 2006). The S_p statistic measures the intensity of fine-scale genetic structure and was calculated for each locus as $-b_F/(1 - F_{(n)})$, where $F_{(n)}$ is the kinship between neighboring plants and is typically estimated by $F_{(l=1)}$, the mean F_{ij} at the smallest distance interval or lag (Vekemans and Hardy, 2004). A mean S_p (and standard error) was also calculated for each population by averaging over loci (Vekemans and Hardy, 2004). Estimation of all spatial genetic autocorrelation statistics were conducted using the program SPAGEDI (Hardy and Vekemans, 2002).

Given specific interest in differences in fine-scale genetic structure among disturbance “treatments”, significant variation in the three treatment-level mean S_p values was tested using a meta-analysis (Hedges and Olkin, 1985) in which each population-level S_p value was weighted by its variance

(square of the standard error). Using the same approach differences in mean S_p for pairs of treatments were tested employing Bonferroni adjustment for multiple tests (three tests total, adjusted $\alpha = 0.0167$). These weighted ANOVA and t-test analogs were conducted using the program MetaWin (Rosenberg et al., 2000).

2.6. Analysis of genetic diversity and structure

In estimating genetic diversity, a locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95. The following 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). Assuming neutral genetic variation and linkage equilibrium among loci, standard errors for H_o and H_e were calculated over loci and populations within disturbance categories. Differences in mean H_o and H_e between disturbance categories were considered to be significant if their 95% confidence intervals did not overlap. To measure deviations from Hardy–Weinberg (H–W) equilibrium at each polymorphic locus, Wright's (1965) F -statistics (F_{IS} , F_{IT} , and F_{ST}) were calculated for the complete set of populations following Weir and Cockerham (1984). These fixation indices were used to measure deviations from H–W equilibrium at the level of individuals relative to their local populations (F_{IS}), local populations relative to the total population (F_{ST} , also a measure of differentiation among local populations), and individuals relative to the total population (F_{IT}). Ninety-five percent bootstrap confidence intervals (1500 replicates) were constructed around means of the F -statistics and the observed F -statistics were considered to be significant when confidence intervals did not overlap zero. Mean F_{IS} and their 95% confidence intervals (1000 replicates) were calculated over populations within disturbance category. These calculations were made using the program FSTAT [version 2.9.3.2 by Goudet (2002), see Goudet (1995)]. Finally, Mean F_{IS} and their 95% confidence intervals (1000 replicates) were also calculated separately for each population using the program GDA (Lewis and Zaykin, 2001).

3. Results

3.1. Spatial distribution of individuals

Analyses of the four disturbed populations using the O-ring function revealed a marginally significant positive spatial aggregation of individuals over a range of spatial scales at HAG and no deviation from random at HAD, JBJ, and KKS (Fig. 2). The putatively disturbed and undisturbed populations, in contrast, exhibited highly significant positive aggregation of individuals at almost all spatial scales and had values of $O(r)$ consistently higher than in the disturbed populations for comparable values of r .

3.2. Fine-scale genetic structure

Among 12 allozyme loci, Acph, Adh-1, Pgi-1, and Pgi-2 were monomorphic across all eight populations and excluded from

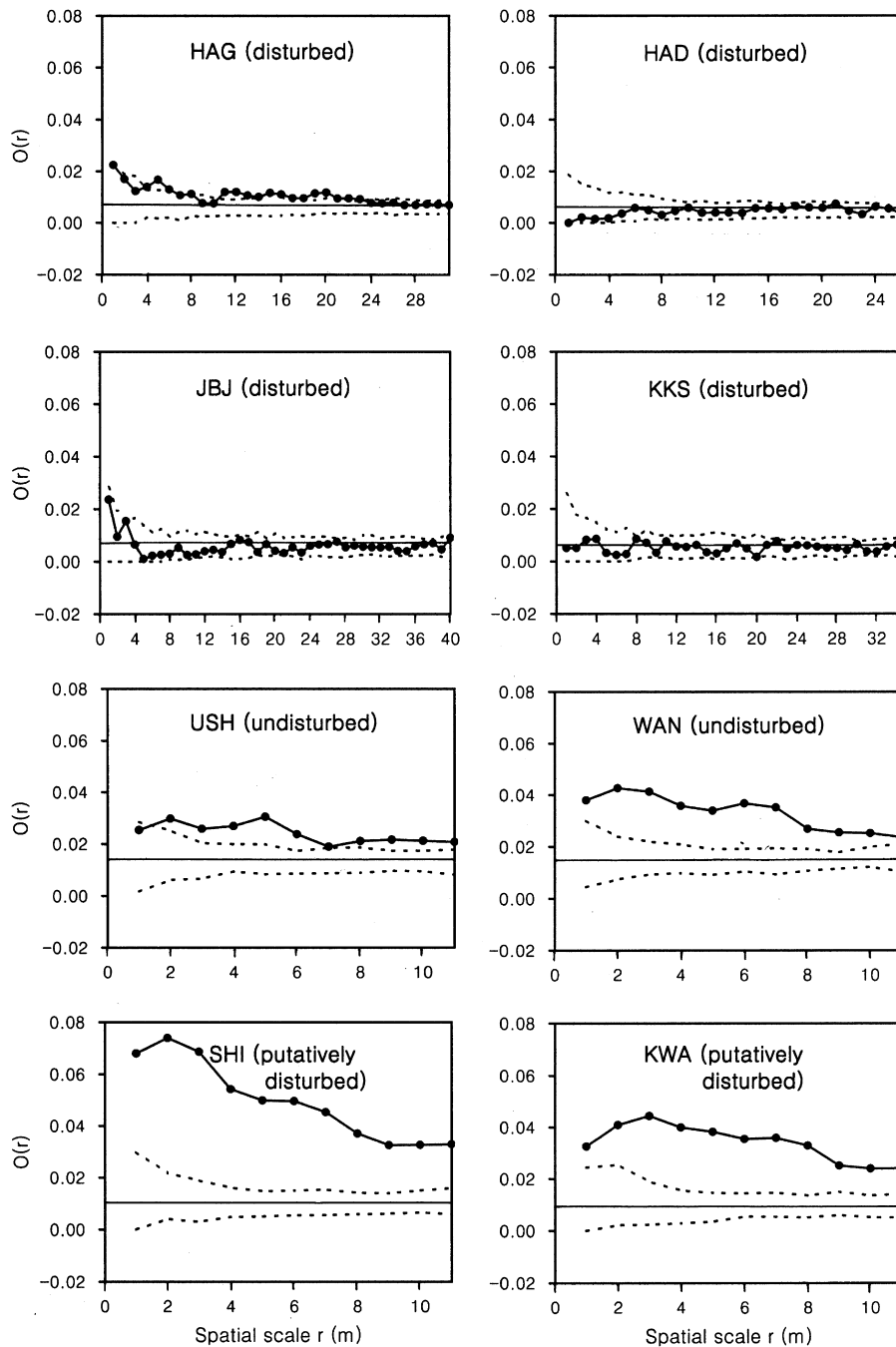


Fig. 2 – Spatial aggregation of individuals within *Cymbidium goeringii* populations as measured using the O-ring statistic $O(r)$. Filled circles indicate the mean $O(r)$ for an annulus of radius r with 1 m lags. Dashed lines indicate confidence envelopes about the null hypothesis of random spatial structure. The solid line indicates the first-order intensity of the point pattern within populations: 0.006 in HAG, 0.004 in HAD, 0.005 in JBJ, 0.004 in KKS, 0.014 in USH, 0.015 in WAN, 0.010 in SHI, and 0.010 in KWA. Note: the x-axis scale varies as a function of the spatial dimensions of the sample population.

analysis of fine-scale genetic structure. Subsequent inspection of multilocus genotypes failed to reveal clones in any populations. Consequently, all genetic analyses describe the population structure of genets.

Significant evidence of fine-scale genetic structure was detected in four of eight populations (Table 1; Fig. 3). For both undisturbed populations (USH and WAN), one putatively dis-

turbed population (SHI), and one disturbed population (HAD), the slope (b_F) of kinship on the natural logarithm of spatial distance (r_{ij}) was significantly less than zero (Table 1). Comparing the strength of fine-scale genetic structure across disturbance categories, two of the three highest Sp values were found in the undisturbed populations while the three lowest values were found among the disturbed populations (Table

Table 1 – Comparison of spatial genetic autocorrelation among eight populations of *Cymbidium goeringii*^a

Population/ parameter	Disturbed				Undisturbed		Putatively disturbed	
	HAG	HAD	JBJ	KKS	USH	WAN	SHI	KWA
n	72	48	54	50	133	150	98	91
b _F	–0.002	–0.031 ^b	–0.003	–0.004	–0.022 ^b	–0.036 ^b	–0.016 ^b	–0.006
Sp	0.0015	0.0363	0.0059	0.0049	0.0279	0.0324	0.0154	0.0063
(SE)	0.0060	0.0143	0.0051	0.0052	0.0050	0.0058	0.0058	0.0039
Mean F _{ij} at ≤2 m	0.051	0.071	0.073	0.051	0.068 ^b	0.084 ^b	0.023	0.008
95% CI	(–0.051, 0.152)	(–0.153, 0.294)	(–0.080, 0.226)	(–0.072, 0.175)	(0.023, 0.112)	(–0.004, 0.173)	(0.000, 0.046)	(–0.040, 0.056)

a Abbreviations: n, sample size; b_F, slope estimated over 2 m distance intervals; Sp, intensity of FSGS; SE, standard error (SE); and F_{ij}, mean kinship coefficients calculated at ≤2 m; and 95% CI, jackknifed 95% confidence intervals.
b Denotes estimates significantly different from zero.

1). Variation in mean Sp values between disturbance “treatments” was highly significant ($P < 0.00001$), as were differences between undisturbed vs. disturbed ($P < 0.00001$) and undisturbed vs. putatively disturbed ($P = 0.00003$) sets of populations (but not disturbed vs. putatively disturbed; $P = 0.453$). In each of these significant tests, the undisturbed populations had the highest mean Sp.

3.3. Genetic diversity and structure

Levels of allozyme diversity, as well as H_o , were similar among populations regardless of disturbance history. Population-level F_{IS} estimates calculated over eight polymorphic loci ranged from 0.081 to 0.300 and were significant for all disturbed and undisturbed, but no putatively disturbed populations (Table 2). Mean F_{IS} values for each disturbance category were significantly greater than zero but were not significantly different from each other (based on broadly overlapping 95% confidence intervals) despite the disturbed category having the three highest population-level F_{IS} values. Viewed over all eight populations, significant deficits of heterozygotes were found within populations (mean $F_{IS} = 0.169$, 95% CI = 0.100, 0.242) and for the population as a whole ($F_{IT} = 0.238$, 95% CI = 0.138, 0.338). Allele frequency differences among populations were also significant, both calculated over polymorphic loci ($F_{ST} = 0.082$, 95% CI = 0.034, 0.134) and individually for each of the eight polymorphic loci (results not shown).

4. Discussion

Using *C. goeringii* as a study species, this study focused on the comparison of disturbed, putatively disturbed, and undisturbed populations, testing *a priori* predictions of how a specific form of anthropogenic disturbance, the selective harvest (removal) of plants, influences spatial demography and, subsequently, diversity and spatial structure of genetic variation. In general, it was predicted that density dependent selective harvest should have the proximal effects of decreasing the spatial aggregation of plants and fine-scale genetic structure while also decreasing within-population inbreeding. Below these predictions are evaluated with respect to data obtained and the implications of results are considered for the conservation of *C. goeringii*, and terrestrial orchids as a whole.

4.1. Effects of disturbance on demographic structure

In the four disturbed populations of *C. goeringii*, the numbers of plants had recently been dramatically reduced by orchid collectors (HAG: 597–72, HAD: 182–48, JBJ: 575–54, and KKS: 552–50). Consistent with predictions, the spatial aggregation of individual plants was much lower in these as compared to putatively disturbed and undisturbed study populations. Specifically, these latter populations exhibited highly significant aggregation of individuals at all values of r whereas spatial structure was marginally significant and positive in one disturbed population (HAG) and entirely absent in the others (Fig. 2). One possible explanation for the apparently lower spatial structure in the disturbed populations is that it is an artifact of their smaller sample sizes (Table 1) and, consequently, reduced power to reject the null hypothesis of complete spatial randomness. This interpretation was rejected based on two observations. First, the 99% confidence limits about complete spatial randomness were similar in all populations (Fig. 2) indicating that the power to reject the null was reduced little (if at all) in the disturbed populations. Second, for every comparable radius r , the measure of spatial aggregation $O(r)$ was consistently lower in the four disturbed populations than in any undisturbed or putatively disturbed population (Fig. 2).

The degree to which spatial structure is reduced by density dependent harvesting (or disturbance related mortality in general) will depend on the intensity of disturbance. In general, the smaller the fraction surviving, the greater the expected loss of spatial demographic and genetic structure (Degen et al., 2006). Of particular importance, however, is the actual number of survivors because it is these individuals that determine the residual spatial structure. For example, prior to disturbance, HAG had many more individuals than HAD (597 vs. 182) though densities were similar (0.100 and 0.073 individuals per m^2). Following disturbance the fraction of survivors and their density in HAG was about half that in HAD (HAG: 12% survivors at density $0.01 m^{-2}$; HAD: 26% survivors at $0.02 m^{-2}$). Nevertheless, HAG had 50% more net survivors (72) than did HAD (48). Thus, the difference between these two populations in the spatial aggregation of individuals (weak in HAG, absent in HAD) is consistent with differences in the number of plants surviving after disturbance. Similarly, like HAD, disturbed populations JBJ and

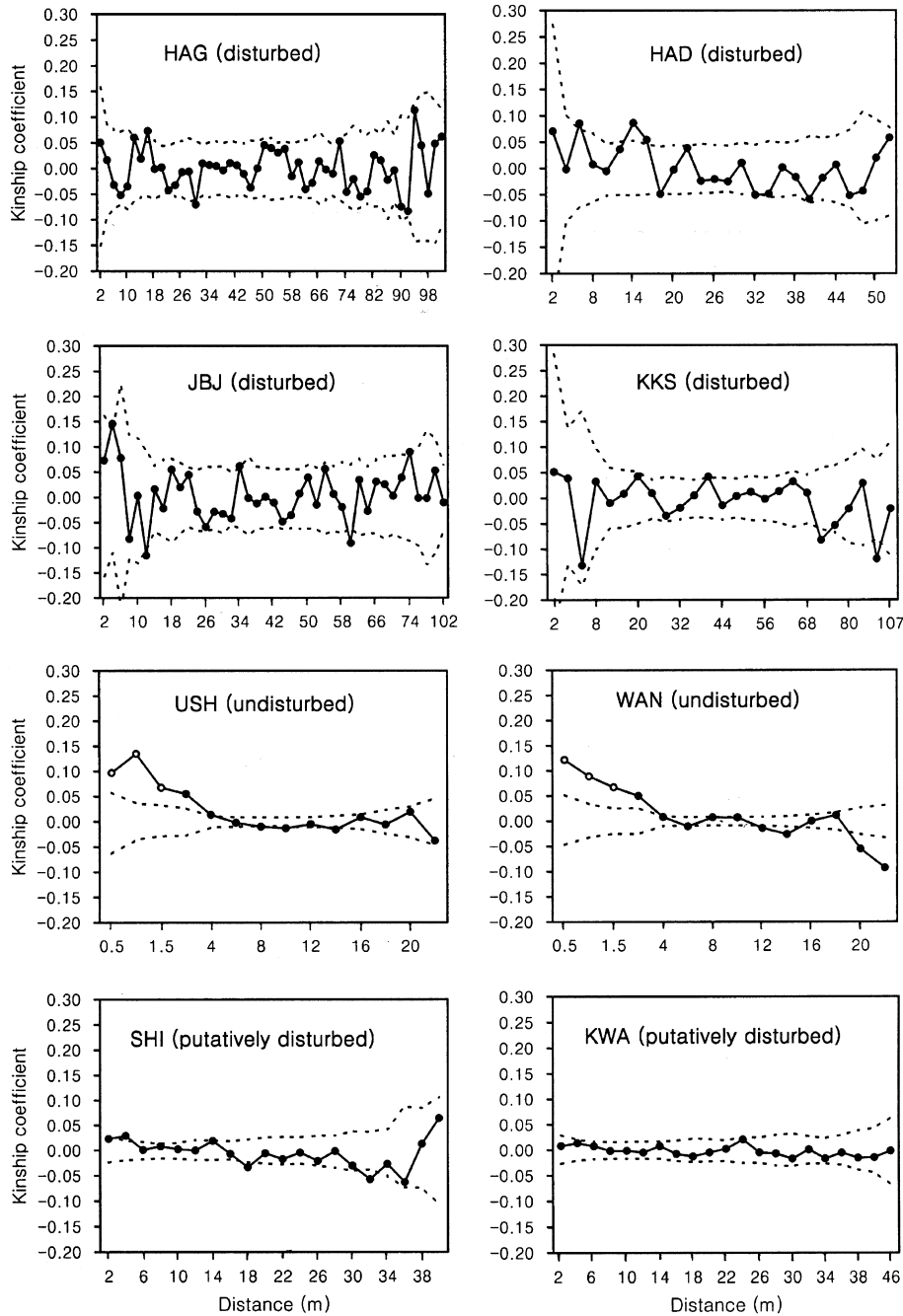


Fig. 3 – Correlograms of estimated kinship coefficients (F_{ij}) over distance within *Cymbidium goeringii* populations. Filled circles (●) indicate mean values of F_{ij} for 2 m distance intervals. Open circles (○) at USH and WAN represent mean F_{ij} for 0.5 m lags at distance intervals of ≤ 2 m. The dashed lines represent the upper and lower bounds of 95% confidence envelopes constructed about the null hypothesis of $F_{ij} = 0$. Note: the x-axis scale varies as a function of the spatial dimensions of the sample population.

KKS had relatively few net survivors (54 and 50, respectively) and exhibited no spatial aggregation of individuals (despite having large numbers of individuals prior to disturbance, like HAG).

4.2. Effects of disturbance on spatial genetic structure

Parallel to the strong differences in spatial demographic structure between disturbed and undisturbed populations,

the impact of disturbance on fine-scale genetic structure is also clear. It was predicted that the removal of plants from higher density patches should decrease both spatial demographic and genetic structure. Indeed, both undisturbed populations, one of two putatively disturbed populations (SHI), and one of four disturbed populations (HAD) exhibited significant evidence of fine-scale genetic structure ($b_F < 0$, Table 1). The apparent lack of overall fine-scale genetic structure in three of four disturbed populations is unlikely to be due to

Table 2 – Summary of genetic diversity measures and mean fixation (F_{IS}) values observed in eight populations of *Cymbidium goeringii*^a

Population	%P	A	H_o (SE)	H_e (SE)	F_{IS} (95% CI)
<i>Disturbed</i>					
HAG	67	2.25	0.234 (0.057)	0.308 (0.066)	0.250 (0.012, 0.455)
HAD	67	2.42	0.252 (0.053)	0.321 (0.067)	0.225 (0.105, 0.321)
JBJ	67	1.92	0.230 (0.050)	0.324 (0.067)	0.300 (0.156, 0.465)
KKS	67	2.00	0.298 (0.064)	0.343 (0.071)	0.141 (0.008, 0.264)
Mean	67	2.15	0.254 (0.016)	0.324 (0.007)	0.233 (0.125, 0.331) ^b
<i>Undisturbed</i>					
USH	67	1.92	0.248 (0.056)	0.296 (0.066)	0.166 (0.060, 0.283)
WAN	67	1.92	0.251 (0.053)	0.311 (0.068)	0.198 (0.051, 0.303)
Mean	67	1.92	0.250 (0.002)	0.304 (0.008)	0.177 (0.094, 0.243) ^b
<i>Putatively disturbed</i>					
SHI	67	2.25	0.227 (0.051)	0.246 (0.054)	0.081 (–0.027, 0.197)
KWA	67	2.42	0.239 (0.057)	0.268 (0.057)	0.115 (–0.010, 0.240)
Mean	67	2.34	0.233 (0.006)	0.257 (0.011)	0.109 (0.023, 0.187) ^b
Mean across					
Eight populations	67	2.14	0.247 (0.008)	0.302 (0.011)	0.169 (0.100, 0.242) ^b

a Abbreviations: %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 95% CI, 95% confidence intervals.

b Means are Weir and Cockerham (1984) estimates of F_{IS} over populations.

small sample sizes and low power to reject the null hypothesis ($b_F = 0$). Consider that sample sizes for the three disturbed populations in which b_F was not significantly less than zero (HAG, JBJ, and KKS) were all larger than the one in which b_F was significantly less than zero (Table 1). Consider too that small sample size is expected to inflate the variance but not bias estimation of b_F or S_p , the latter of which should be used for comparing the strength of overall fine-scale genetic structure between populations. Nonetheless, among all eight populations these three disturbed populations (HAG, JBJ, and KKS) had the lowest estimated S_p values, again indicative of reduced fine-scale genetic structure in these populations and not simply lack of statistical power to detect such structure. Finally, in treatment-level contrasts (meta-analysis of S_p values), overall fine-scale genetic structure was significantly greater for undisturbed than for both disturbed and putatively disturbed sets of populations. Thus, undisturbed *C. goeringii* populations would seem to possess significant fine-scale genetic structure with some of this structure dissipating in putatively disturbed and disturbed populations.

As noted above, reduction in sample size is expected to increase the variance in estimates of fine-scale genetic structure and, indeed, a clear outcome of the relatively small number of surviving individuals in the disturbed populations ($n = 48–72$) is an increase in the variance of F_{ij} estimates across distance intervals (r). Sample size effects are further exacerbated because, relative to undisturbed and putatively disturbed populations, three of four disturbed populations (HAG, JBJ, and KKS) were originally much larger and required sampling over larger spatial scales, which decreased the mean number of pairwise F_{ij} estimates per distance interval. Consequently, there is decreased statistical power to detect subtle, distance-specific evidence of genetic structure in these

disturbed populations should such residual structure exist. Consistent with the effects of smaller sample sizes, disturbed populations exhibited larger 95% confidence intervals about F_{ij} at the smallest distance interval, increased variation in F_{ij} between successive distance intervals, and a broader 95% confidence envelope about the null hypothesis of no genetic structure ($F_{ij} = 0$; Table 1, Fig. 3). Thus a primary effect of selective harvesting is to greatly inflate the variance in distance-specific estimates of fine-scale genetic structure. The larger putatively disturbed populations ($n = 91$ and 98) and especially the undisturbed populations ($n = 133$ and 150), in contrast, exhibited much lower variance in the estimation of F_{ij} (Table 1, Fig. 3). Of particular note, because of this lower variance, we were able to detect significant fine-scale genetic structure down to a distance interval of ≤ 0.5 m in the two undisturbed populations (0.097 in USH and 0.122 in WAN). Taking into account population level inbreeding (F_{IS}), these values are in the range expected for first cousins to half-sibs.

Although studies on spatial demographic and genetic structure comparable to that of *C. goeringii* are unavailable for other terrestrial orchid species subject to selective harvest, Cruse-Sanders and Hamrick (2004) have examined spatial structure in four protected and four unprotected populations of the medicinal herb wild American ginseng (*Panax quinquefolius*). Similar to our findings they found significant demographic and genetic structure within protected ginseng populations. In contrast, however, there was no trend of reductions in this structure in unprotected ginseng populations. Regarding the lack of apparent difference in spatial and demographic structure between protected and unprotected ginseng populations, it should be noted that population sizes and densities were similar between treatments and the actual intensity of disturbance in unprotected populations was not

known. Compared to that for the four disturbed *C. goeringii* populations, it may not have been particularly strong.

Results from the analysis of fine-scale genetic structure and F_{ST} in *C. goeringii* could provide general insight into seed dispersal in terrestrial orchids. On the one hand, the significant fine-scale genetic structure observed for undisturbed populations may be the rule for *C. goeringii* as it has also been detected for four neighboring undisturbed populations on Oenaro Island, South Korea (Chung et al., unpublished data). Significant fine-scale genetic structure has also been observed in the nine other species of terrestrial orchids examined to date (*Caladenia tentaculata*, Peakall and Beattie, 1996; *Spiranthes spiralis*, Machon et al., 2003; *Cephalanthera longibracteata*, Chung et al., 2004a; *Cremastra appendiculata*, Chung et al., 2004b; *Orchis cyclochila*, Chung et al., 2005a; *Liparis makinoana*, Chung et al., 2005b; *Orchis purpurea*, Jacquemyn et al., 2006; *Platanthera aquilonis* and *P. dilatata*, Wallace, 2006). These results suggest that per generational dispersal is sufficiently restricted to generate fine-scale genetic structure within populations of terrestrial orchids (but see Cozzolino and Widmer, 2005).

On the other hand, the modest F_{ST} (0.082) for *C. goeringii* is in accord with the general expectation that significant fraction of the minute wind-dispersed seeds of orchids disperse far (Arditti and Ghani, 2000). Both the existence of fine-scale genetic structure and modest F_{ST} are consistent with a leptokurtic distribution of seed dispersal distances with a very flat tail found in many plant species (reviewed in Cain et al., 2000).

4.3. Effects of disturbance on genetic structure and levels of genetic diversity

Given fine-scale genetic structure in natural populations of terrestrial orchid species, it was predicted that immediate effects of the removal of plants from high density patches should be not only to reduce fine-scale genetic structure but to decrease inbreeding (F_{IS}) and increase observed heterozygosity (H_o). The present results for *C. goeringii* do not strongly support either of these predictions. Population-level F_{IS} estimates ranged from 0.081 to 0.300 and were significant in all undisturbed and disturbed populations but not in either putatively disturbed populations (Table 2). Although pairwise comparisons of F_{IS} calculated for disturbance categories were not significant (Table 2), three of the four highest population-level F_{IS} values were from disturbed populations. Despite the relatively high inbreeding, H_o was also high (range 0.227–0.298, mean 0.247) compared to other long-lived perennial plants (Hamrick and Godt, 1990). Differences among disturbance categories for H_o were not significant, though the two highest estimates were from disturbed populations (HAD and KKS). Other studies too have observed unpredictable variation among conspecific populations in genetic variation and fine-scale genetic structure (Schnabel et al., 1998; Bonnin et al., 2001; Cruse-Sanders and Hamrick, 2004). Consequently, future studies of the proximal genetic impacts of selective harvesting should endeavor to sample relatively large numbers of populations within disturbance categories.

In spite of anthropogenic effects on disturbed populations, *C. goeringii* as a whole harbors high levels of allozyme varia-

tion within populations in Korea and Japan ($H_e = 0.260$; Chung and Chung, 2000). High levels of genetic variation maintained by most populations suggest that disturbance-mediated decreases in the number of individuals are still too recent for long-term effects of genetic drift on variation and inbreeding to be manifested. In contrast, survivors of selective harvesting or regeneration following extensive stand harvesting of forest tree species typically exhibit significant reductions in genetic diversity relative to pristine stands (Ledig, 1992; Buchert et al., 1997; Rajora et al., 2000; Degen et al., 2006) and fine-scale genetic structure (Epperson and Chung, 2001; Walter and Epperson, 2004, but see Cloutier et al., 2007). These contrasting results suggest that the population genetic consequences of disturbance may depend on history of disturbance (time) or probably life-history traits (herbaceous vs. woody perennials, generation time).

4.4. Conservation implications

Cymbidium goeringii is one of the most common terrestrial orchid species in South Korea in terms of extent of occurrence, area of occupancy, and number of individuals within populations (Chung and Chung, 2000). In Japan, in contrast, the species is extremely rare (M.Y.C., unpublished observations in 1998) and listed as “Critically Endangered” (<http://www.biodiv.go.jp>), a consequence of mass collection in past decades (Yahara, Kyushu University, personal communication). Despite the better situation in Korea, however, it has been observed that the number of individuals in many populations (HAG, HAD, JBJ, KKS, and others in the vicinity of cities and near roads) has rapidly decreased, sometimes to near local extinction, primarily due to mass collection by plant sellers and enthusiasts (M.Y.C., unpublished data). Based on the results of this study, it can be concluded that the harvest of *C. goeringii* plants by orchid collectors has impacted demography by reducing the density and spatial aggregation of individuals, as well as their number. While mass collection can be a proximal cause of population extinction, recurrent harvesting from surviving populations too can pose significant impediments to their survival. Long-term monitoring (1991 to present) of two sites in areas where *C. goeringii* was once abundant (initially no individuals at one site and 11 adults at the other) has revealed no new colonists from off-site seed sources in the first location and no regeneration in the second (Chung, Jinju National University, personal communication). These observations suggest that population growth following mass collection is likely to be very low or absent. By further restricting the growth and recovery of small populations, harvesting increases sensitivity to stochastic demographic processes with concomitant negative consequences for population survival.

Populations reduced to small effective population size (N_e) are more susceptible to the effects of random genetic drift and associated losses of genetic diversity and increased inbreeding over generations. Immediate impacts on genetic diversity may be modest, however. Indeed, despite intense collection in recent decades, levels of expected heterozygosity within *C. goeringii* populations in Japan (mean $H_o = 0.181$ – 0.230 ; Chung and Chung, 2000) are comparable those in South

Korea (mean $H_o = 0.182\text{--}0.242$; Chung and Chung, 2000), where harvesting is believed to have been less intense. Similarly, this study's comparison of undisturbed versus recently disturbed South Korean populations indicates that mass collection does not result in strong, short-term reductions in genetic diversity. Such collection of *C. goeringii* has, however, significantly altered fine-scale genetic structure, eroding positive genetic correlations among neighboring plants to levels indistinguishable from random. An unexpected immediate consequence of mass collection is that it may decrease inbreeding arising from the mating of closely related individuals. In the long term, however, if mass collection and recurrent harvesting enforce a low N_e , they will ultimately be responsible for increases in inbreeding and loss of genetic variability due to increased random genetic drift.

Considering the short- and long-term effects on population-genetics aspects and the fate of remnant populations, it is important to know the disturbance history of terrestrial orchid populations. To do this, as clearly presented in this study, the analyses of spatial demographic and fine-scale genetic structure could be a tool for developing conservation strategies. Since mass collection considerably reduces the spatial aggregation of individuals and the intensity of spatial genetic structure (Sp), when small populations of unknown collection history are found to have random or near random spatial demographic and genetic structure they may more likely to be subjected to disturbance than are populations with strong positive spatial demographic and genetic structure. These findings suggest that conservation managers who are interested in endangered terrestrial orchids may be able to analyze the spatial distribution of individuals and their genotypes to infer whether a population with a small number of individuals represents a natural state or is likely to have been affected by mass collection. In addition, the useful outcome of this study is that it also highlights the utility of spatial analytical techniques for other species, particularly in those in highly fragmented landscapes. However, a way of how to apply information generated from the analyses of spatial demographic and genetic structure to help conserve and manage extant populations need to be developed through more studies with creative ideas. One suggestive way is to be used for re-introductions. Since understanding fine-scale genetic structure can provide an indication of the disturbance history of a population, it is possible to locate 'undisturbed populations'. These with high allelic richness could be used as source populations for re-introductions to genetically depauperate or severely disturbed populations because inbred individuals with low allelic richness in 'disturbed populations' over generations will be increased due to small N_e .

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