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Population genetic diversity and structure in *Goodyera rosulacea* (Orchidaceae), endemic in Korea, and implications for conservation

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We assessed genetic diversity and structure in the outcrossing orchid *Goodyera rosulacea*, endemic to limestone regions in Korea. Using allozymes as genetic markers, standard genetic diversity measures within and among populations as well as genetic structure were estimated. In addition, a regression analysis between pairwise genetic and geographical distances and correlation analyses between population sizes and estimates of genetic variation within populations were conducted to gain insights into the past evolutionary processes. Moderate levels of genetic variation were found in the species ($\%P_s$ = 31.6%, A_s = 1.37, H_{es} = 0.126); at the population level, slightly lower levels were estimated ($\%P_p$ = 27.8%, A_p = 1.31, H_{ep} = 0.100). Overall fixation index was not significantly different from zero (F_{IS} = 0.160). As compared with that in most terrestrial orchids, the measure of genetic differentiation among populations was also moderate (F_{ST} = 0.150). At the regional scale, a weak linear regression between pairwise genetic and geographical distances was found, suggesting that populations are not at equilibrium between gene flow and genetic drift (i.e., little evidence of isolation-by-distance effect). A significant correlation between population size and mean number of alleles per locus was detected (p = 0.006). These results suggest that genetic drift, coupled with limited seed dispersal, has played an increasing role in the genetic dynamics of small populations, though outcrossing breeding system might have in part counterbalanced against these negative effects. Threats to the species are considered to be anthropogenic. *In situ* and *ex situ* conservation strategies are suggested to preserve the genetic variation in the rare *G. rosulacea*.

Key words: allozymes, conservation, endemic, fine-scale genetic structure, genetic diversity, genetic drift, limited seed dispersal, outcrossing

Introduction

Since genetic diversity is the raw material for adaptive evolution of plant species, understanding of causes of the loss of genetic variability in rare, endangered plant species and making conservation decisions to maintain sufficient levels of genetic variation for these species have become important (Falk & Holsinger 1991, Young *et al*. 1996, Frankham *et al*. 2002). According to recent plant allozyme studies, rare plant species share several ecological and genetic factors (reviewed in Gitzendanner & Soltis 2000, Godt & Hamrick 2001, Cole 2003). Since many rare and/or endemic species have narrow habitat preferences, "directional or stabilizing selection" in a restricted set of environmental conditions could lower the genetic variability within such species (Godt & Hamrick 2001). Furthermore, many rare species are geographically restricted or sparsely distributed, with small population sizes. Most of the 44 species in which allozyme variation is lacking are self-compatible, with limited distributions (Cole 2003). Currently, many rare species are endangered, and their population sizes and numbers have declined primarily due to anthropogenic threats (e.g., habitat loss and alteration). In such species with small populations and a self-compatible breeding system, genetic drift and inbreeding play increasing roles in shaping the genetic structure. Loss of genetic diversity through drift and inbreeding should be accelerated via limited gene dispersal across fragmented landscapes.

The Orchidaceae, one of the largest families of flowering plants (up to one tenth of all angiosperms in the world; Dressler 1993), encompass a variety of reproductive strategies, habitats, and distributional patterns. Among these, breeding systems are generally regarded as the important factor in shaping the organization of genetic variability in orchids (Scacchi *et al*. 1991, Wallace & Case 2000, Trapnell *et al*. 2004, Chung *et al*. 2007). This view is based on the fact that population genetic structure is greatly influenced by pollinator behavior (i.e., the fruit set is predominantly dependent on pollinators; Neiland & Wilcock 1998, Tremblay *et al*. 2005). On the one hand, plant allozyme studies have revealed that absence of allozyme diversity prevails in highly inbred terrestrial orchids, despite their broad distribution: *Cephalanthera damasonium* in Italy (Scacchi *et al*. 1991); *Epipactis microphylla* in Italy (Scacchi *et al*. 1987); *Epipactis phyllanthes* in Denmark (Ehlers & Pedersen 2000); *Liparis kumokiri* in South Korea (Chung *et al*. 2007), and *Zeuxine strateumatica* in Hong Kong (Sun 1997). On the other hand, many outcrossing terrestrial orchid species with a broad distribution maintain high levels of genetic variation both at the population and the species levels with moderate or low degrees of genetic divergence between populations. Such orchids are *Calopogon* species in the eastern U.S. (Trapnell *et al*. 2004), *Cymbidium goeringii* and *Cremastra appendiculata* in South Korea (Chung & Chung 2000, Chung *et al*. 2004), *Cypripedium calceolus* in northeastern Poland and southeastern France (Brzosko *et al*. 2009), *Cypripedium parviflorum* in eastern U.S. (Wallace & Case 2000), and *Epipactis helleborine* in Europe and North America (Ehlers & Pedersen 2000, Squirrell *et al*. 2001). Still, there are few population genetic studies on pollinator-dependent, outcrossing, narrowly distributed rare, endemic terrestrial orchids with small population sizes (but *see* Sharma *et al*. 2003, Li & Ge 2006).

Goodyera rosulacea is a predominantly outcrossing, rare, and narrowly distributed terrestrial orchid, endemic to limestone regions in Korea. Presumably owing to its rarity, *G. rosulacea* was only recently described (Lee 2004), and specimens collected prior to the species description were misidentified as *G. repens* or *G. schlechtendaliana*. *Goodyera rosulacea* grows usually on dry humus in mixed forests of pines and deciduous shrubs/trees in central Korea. Most of the known populations are small, isolated within a range of 200 km (Fig. 1). *Goodyera rosulacea* is a pollinator-dependent outcrossing orchid (M. Y. C. unpubl. data).

In this study, we examined the genetic diversity within and among populations, and the genetic structure in *G. rosulacea*. To infer evolutionary processes within and among populations of *G. rosulacea*, we also conducted a linear regression between genetic and geographic distance between populations and further analyzed associations between genetic diversity and population sizes. To gain insights into the causes of rarity and to infer evolutionary history of *G.*

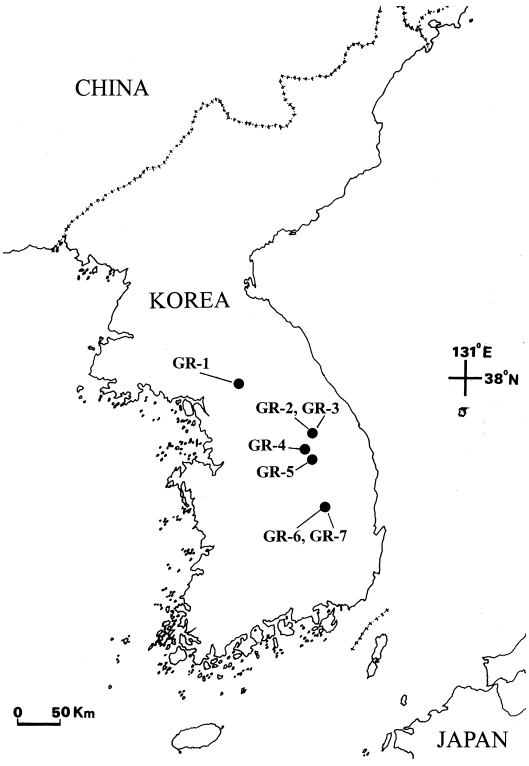

Fig. 1. Locations of the seven populations of *Goodyera rosulacea* sampled.

rosulacea, we compared its genetic diversity statistics with that for its relatively common and widely distributed, and the single investigated (Wong & Sun 1999), congener *G. procera* from Hong Kong. Finally, given the resulting genetic data and extant demographic information, we make recommendations for the development of conservation for this rare orchid.

Material and methods

Plant material

Goodyera rosulacea is a small, rosette-forming terrestrial orchid (ca. 25 cm tall with inflorescence). Ten to 25 small, white flowers (petal, 2.2–2.5 mm long; labellum, 3.0 mm long) on an inflorescence open from June to July. During fruit maturation in August, the basal rosette leaves become senescent, and a new shoot arises from the short rhizome (< 1 cm) to replace the old shoot as seen also in *G. procera* (Wong & Sun 1999). Thus, most individuals within populations are likely distinct genets rather than clonal ramets, with new shoots or ramets remaining close to the original plant (M. Y. C. pers. obs.). Since the individuals are shallowly rooted, they are easily destroyed or removed by trampling.

Goodyera rosulacea is self-compatible, and high rates of fruit sets (55%–68%) were recorded at GR-5 and GR-7 (Fig. 1), but bagged individuals set no fruit (M. Y. C. unpubl. data). This indicates that the species is pollinator-dependent in reproduction, and also the congeners are outcrossers (*G. maximowicziana*, Sugiura & Yamaguchi 1997; *G. procera*, Wong & Sun 1999). Potential pollinators of *G. rosulacea* have not been identified, but small wild bees are presumably pollinators since other goodyeras are pollinated by bumblebees (*Bombus* spp.; Ackerman 1975, Kallunki 1981, Sugiura & Yamaguchi 1997) and the sweat bee *Lasioglossum morio* (Vöth 1992). Mature capsules are small (4 mm long).

Population sampling

A total of 322 leaf samples were collected from seven populations (GR-1 to GR-7), which cover the entire distribution of *G. rosulacea* (Fig. 1 and Table 1). Populations GR-2 and GR-3 are separated 1.2 km in a linear distance. Population GR-6 is located 1.1 km south of GR-7. To minimize the damage to this rare orchid, we used material cut from just one leaf per shoot. All sampled leaf materials were kept on ice until transported to the laboratory, where they were stored at 4 °C until protein extraction.

Allozyme electrophoresis

For enzyme extraction, we ground leaf samples with a pestle in a mortar while adding a crushing buffer (Mitton *et al*. 1979), and absorbed enzyme extracts into 4 mm × 6 mm wicks cut from Whatman 3MM chromatography paper. We then stored them in microtiter plates at –70 °C until needed. We used horizontal starch-gel (13%) electrophoresis to determine levels and distribution of allozyme diversity within and among populations of *G. rosulacea*. We used a modification (Haufler

1985) of the "system 6" of Soltis *et al*. (1983) to resolve alcohol dehydrogenase (*Adh*), diaphorase (*Dia-1*, *Dia-2*), fluorescent esterase (*Fe*), leucine aminopeptidase (*Lap-1, Lap-2*), malic enzyme (*Me*), cathodal peroxidase (*Cpx*), phosphoglucomutase (*Pgm*), and triosephosphate isomerase (*Tpi-1*, *Tpi-2*). We also used the morpholine-citrate buffer system (pH 6.1) of Clayton and Tretiak (1972) to resolve fructose-1,6-diphosphatase (*F1,6-1*, *F1,6-2*), isocitrate dehydrogenase (*Idh*), malate dehydrogenase (*Mdh-1*, *Mdh-2*), 6-phosphogluconate dehydrogenase (*6Pgd*), and shikimate dehydrogenase (*Skdh-1*, *Skdh-2*). We followed stain recipes from Soltis *et al*. (1983), except for diaphorase (Cheliak & Pitel 1984). By inferring genetic basis of the observed enzyme banding patterns from typical subunit structure and subcellular compartmentalization (Weeden & Wendel 1989), we designated putative loci sequentially, with the most anodally migrating isozyme identified by *1*, the next by *2*, and so on. Different alleles within each locus were numbered sequentially, giving the most anodally migrating allele the lowest number.

Levels of genetic variation within populations

To estimate the genetic diversity and structure, we considered a locus to be polymorphic if the frequency of the most common allele did not exceed 0.99 (Godt *et al*. 1995, Young *et al*. 1996). The following genetic diversity parameters were estimated using the program POPGENE (Yeh *et al*. 1997) and the program FSTAT (Goudet 2002): percentage of polymorphic loci (*%P*), mean number of alleles per locus (*A*), mean number of alleles per polymorphic locus (AP), allelic richness (AR) corrected by minimum sample size (n = 13 at GR-1), mean observed heterozygosity (H_o), and mean Hardy-Weinberg (H-W) expected heterozygosity or Nei's (1978) gene diversity (H_e). Except AR and H_o, these parameters were estimated for the species as a whole (subscript 's') as well as within population data (subscript 'p'). To gain insights into the role of the genetic drift in shaping the genetic structure within populations, we conducted a correlation analysis between log-transformed sample size (n, the number of estimated individuals) and several genetic variation statistics. As our sample sizes (n) varied highly among populations (20 to ca. 3000), log-transformation was used to distribute the points more uniformly in a scatter plot (e.g.,Young *et al*. 1999, McGaughlin *et al*. 2002, Hensen & Wesche 2006). Since *A* is generally lost more rapidly than H_e (Nei *et al*. 1975), recently bottlenecked populations will exhibit an excess of H-W H_e relative to that expected (H_{eq}) from the number of alleles under mutation-drift equilibrium conditions (Cornuet & Luikart 1996). To determine any evidence of recent decreases in effective population size of *G. rosulacea* under the null hypothesis of no difference between

Table 1. Population size (n = the number of estimated individuals), population area, and habitat characteristics of *Goodyera rosulacea*.

Population	*n*	Area	Habitat characteristics	Location
GR-1	20	10 × 10 m	Deciduous forest margin	Korea National Arboretum, Prov. Gyeonggi-do
GR-2	828	50 × 70 m	Steep limestome hillside under *Pinus densiflora* forest	Changwon-ri, Yeongwal-gun, Prov. Gangwon-do
GR-3	250	20 × 20 m	Steep limestome hillside under *P. densiflora* forest	Changwon-ri, Yeongwal-gun, Prov. Gangwon-do
GR-4	ca. 3000	100 × 150 m	Under *P. densiflora* forest	Maepo-ri, Dangyang-gun, Prov. Chungcheongbuk-do
GR-5	70	10 × 51 m	Under *P. densiflora* forest	Bobal-ri, Dangyang-gun, Prov. Chungcheongbuk-do
GR-6	42	10 × 10 m	Vertically positioned limestone rocks under *P. densiflora* forest	Geumgye-ri, Andong-shi, Prov. Gyeongsangbuk-do
GR-7	453	20 × 20 m	Steep limestome slope under deciduous oak-pine forest	Geumgye-ri, Andong-shi, Prov. Gyeongsangbuk-do

H-W H_e and H_{eq}, we used the program BOTTLENECK (Cornuet & Luikart 1996).

Population genetic structure and inbreeding

To measure deviations from the H-W equilibrium at each polymorphic locus, we calculated Wright's (1965) F_{IS} and F_{ST} following Weir and Cockerham (1984) for the seven populations, and estimated the jackknifed average. We used these fixation indices (F_{IS}) to measure deviations from the H-W equilibrium at the level of individuals relative to their local populations, and local populations relative to the total population (F_{ST}, also a measure of differentiation among local populations). Using the FSTAT, we constructed 95% bootstrap confidence intervals, CI (999 replicates) around means of the *F*-statistics, and considered the observed *F*-statistics to be significant when 95% CI did not overlap zero. We further calculated average F_{IS} and their 95% CIs (999 replicates) separately for each population using the program GDA developed by P. O. Lewis & D. Zaykin. Using the resulting F_{IS} statistics, we calculated an equilibrium estimate of the outcrossing rates (*t*) as follows:

$$t = (1 - F_{IS})/(1 + F_{IS}) \quad (1)$$

We assume that the estimated F_{IS} is entirely due to nonrandom mating or inbreeding. As a complimentary estimate for F_{ST}, we also estimated Nei's (1973, 1977) average G_{ST} value, a measure of genetic differentiation among populations, over polymorphic loci and across populations using the FSTAT.

To test the overall differentiation pattern of genetic structure at the regional scale (i.e., isolation-by-distance [IBD] effect; Rousset 1997), we conducted the Mantel test (Mantel 1967) (999 replicates) between all pairwise $F_{ST}/(1 - F_{ST})$ (F_{ST} was calculated following Wier & Cockerham 1984) and corresponding pairwise log-transformed distances (as our study area was two dimensional; Rousset 1997) under the null hypothesis of no spatial genetic structure (regression slope, $b = 0$). Finally, to determine genetic associations among populations, we calculated Nei's (1978) genetic distances between pairs of populations, which were used to cluster the populations into a phenogram following unweighted pair groups method using arithmetic averages (UPGMA).

Results

Levels of genetic variation within populations

Of the 19 putative loci resolved from 13 enzyme systems, six loci (*F1,6-2*, *Idh*, *Mdh-2*, *Me*, *6Pgd*, and *Skdh-2*) were polymorphic across the seven populations ($\%P_s$ = 31.6 %; Table 2). The mean numbers of allele per locus (A_s) and per polymorphic locus (AP_s) were 1.37 and 2.17, respectively, and the mean expected heterozygosity (H_{es}) was 0.126 (Table 2). At the population level, slightly lower mean estimates of genetic diversity were observed: $\%P_p$ = 27.8%, A_p = 1.31, AP_p = 2.10, H_{op} = 0.088, and H_{ep} = 0.100 (Table 2). Populations GR-1 and GR-4 harbored the lowest and the highest allelic richness (AR = 1.16 and 1.37, respectively) (Table 2). Populations GR-7 (0.138) and GR-4 (0.128) maintained a higher H_{ep} than the other populations. There was a significant correlation between the population size (*n*) and mean A_p (Spearman's rank correlation coefficient, $r_s = 0.898$, $p = 0.006$). However, we found insignificant and weak correlations for $\%P_p$ ($r_s = 0.657$, $p = 0.109$), AR_p ($r_s = 0.685$, $p = 0.089$), H_{op} ($r_s = 0.321$, $p = 0.482$), and H_{ep} ($r_s = 0.679$, $p = 0.094$). We found a significant excess of H-W H_e relative to H_{eq} under both the infinite allele (IAM) and stepwise mutation models at GR-4 (Wilcoxon test: $p = 0.008$ and $p = 0.008$, respectively) and GR-5 (Wilcoxon test: $p = 0.016$ and $p = 0.031$, respectively), and GR-7 exhibited a significant excess of H-W H_e under IAM (Wilcoxon test: $p = 0.016$).

Population genetic structure and inbreeding

We found a significant deficiency of heterozygotes relative to H-W expectations in populations GR-1 (F_{IS} = 0.376) and GR-3 (0.343;

Table 2); however, overall, pooled F_{IS} was not significantly greater than zero (0.160; Table 2). Estimated outcrossing rates were varied among populations, ranged from 0.708 (GR-4) to 1.304 (GR-6) with a mean of 0.761. It should be noted that the observed fixation indices require the assumption that the observed F_{IS} values are entirely due to inbreeding. However, we found a significant spatial genetic structure within GR-5 (*see* Degree of population differentiation in the Discussion). This within-population structuring would produce a positive F_{IS} value. Thus, we may underestimate the outcrossing rates obtained using Eq. 1. Deviations from H-W expectations due to allele frequency differences between populations were significant (F_{ST} = 0.150, 95% CI for F_{ST} = 0 was from 0.077 to 0.239). The proportion of total genetic variation attributable to differentiation among populations of *G. rosulacea* (G_{ST}), averaged across six polymorphic loci, was 0.164. We found no significant correlation between all pairwise genetic differentiation estimates and between-population log-distance (b = 0.023, r^2 = 0.064, p = 0.119), indicating that about 94% of the variation in genetic differentiation was due to unknown factors other than geographic distance. In a good agreement with this, the UPGMA phenogram showed a very weak association between populations in relation to their geographic locations (Fig. 2). For instance, two adjacent populations GR-4 and GR-5 displayed the most closely related population pairs, but some anomalous relationships were found being mixed among proximally and distantly located populations (e.g., GR-2, GR-3, GR-6, and GR-7).

Discussion

Moderate levels of genetic variation within populations

The levels of genetic diversity found in *G. rosulacea* ($\%P_s$ = 31.6%, A_s = 1.37, H_{es} = 0.126) are comparable to those for *G. procera* from Hong Kong ($\%P_s$ = 33.0%, A_s = 1.33, H_{es} = 0.150 from pooled samples; Wong & Sun 1999). The H_{es} value for *G. rosulacea* is also similar to that for most terrestrial orchids examined (H_{es} = 0.119; Case 2002); however, the $\%P_s$ and A_s estimates are substantially lower than multi-species means of most terrestrial orchids ($\%P_s$ = 46.2%, A_s = 1.83; Case 2002). At the population level,

Table 2. Summary of genetic diversity measures and mean fixation indexes (F_{IS}). Abbreviations: n = sample size; $\%P$ = percentage of polymorphic loci; A = mean number of alleles per locus; AP = mean number of alleles per polymorphic locus; AR = mean allelic richness based on minimum sample size of 13 plants; H_o = observed heterozygosity; H_e = Hardy-Weinberg expected heterozygosity or genetic diversity; SE = standard error; F_{IS} = fixation index within populations; 95% CI = 95% confidence intervals.

	n	$\%P$	A	AP	AR	H_o (SE)	H_e (SE)	F_{IS} (95% CI)
Goodyera rosulacea								
GR-1	13	15.8	1.16	2.00	1.16	0.036 (0.020)	0.055 (0.032)	0.376 (0.023, 0.486)
GR-2	62	31.6	1.37	2.17	1.29	0.087 (0.040)	0.102 (0.047)	0.155 (–0.184, 0.346)
GR-3	33	26.3	1.32	2.20	1.29	0.054 (0.023)	0.081 (0.037)	0.343 (0.012, 0.463)
GR-4	86	31.6	1.37	2.17	1.37	0.107 (0.040)	0.128 (0.047)	0.171 (–0.225, 0.372)
GR-5	68	26.3	1.26	2.00	1.26	0.095 (0.041)	0.103 (0.042)	0.097 (–0.158, 0.246)
GR-6	20	31.6	1.32	2.00	1.31	0.111 (0.050)	0.096 (0.037)	0.132 (–0.246, 0.139)
GR-7	40	31.6	1.37	2.17	1.33	0.124 (0.057)	0.138 (0.054)	0.114 (–0.189, 0.286)
Population average	46	27.8	1.31	2.10	1.29	0.088 (0.032)	0.100 (0.028)	0.160* (–0.208, 0.397)
Species level	322	31.6	1.37	2.17		0.095 (0.037)	0.126 (0.048)	
Goodyera procera (from Wong & Sun 1999)								
Population average	34	21.8	1.22	2.00		0.049 (0.007)	0.073 (0.009)	0.283
Sample total	507	33.0	1.33	2.00			0.150	
Orchidaceae (from Case 2002)								
Population level		33.7	1.46				0.119	
Species level		46.2	1.83				0.119	

* The Weir and Cockerham (1984) estimate of F_{IS} over populations.

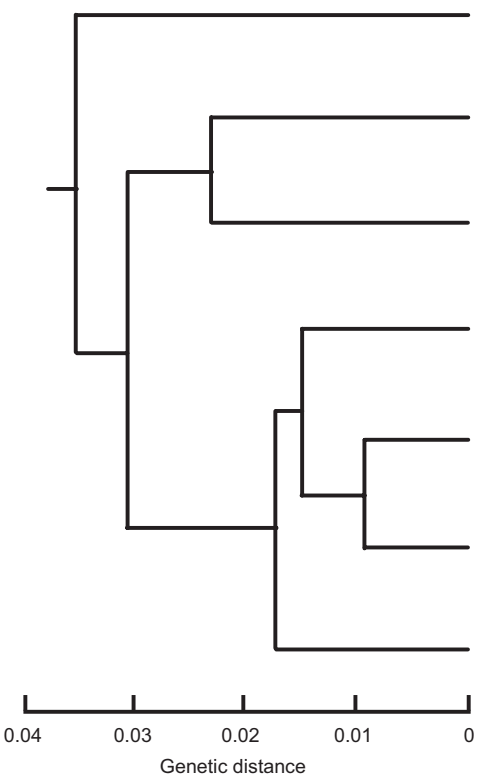

Fig. 2. Dendrogram based on Nei's genetic distances between populations of *Goodyera rosulacea*.

slightly lower estimates were found: $\%P_p$ = 27.8%, A_p = 1.31, H_{ep} = 0.100 for *G. rosulacea*; $\%P_p$ = 33.7%, A_p = 1.46, H_{ep} = 0.119 for most terrestrial orchids; $\%P_p$ = 21.8%, A_p = 1.22, H_{ep} = 0.073 for *G. procera* (Table 2). Populations of *G. rosulacea* maintained slightly higher levels of genetic variation than those of *G. procera* did. Furthermore, *G. rosulacea* harbored more variation than mean within-population statistics for 100 endemic taxa (H_{ep} = 0.063; Hamrick & Godt 1989) and mean species-level genetic diversity for 81 endemic taxa (H_{es} = 0.096; Hamrick & Godt 1989). Therefore, it may be reasonable to conclude that *G. rosulacea* maintains the moderate levels of allozyme variation, despite its rarity, specific habitat preference, and endemism.

Several biological and ecological characteristics as well as historical processes of *G. rosulacea* could be attributable to the present-day levels of genetic diversity in the species. Generally, outcrossing orchid species harbor higher levels of genetic variation than selfing or apomictic orchids (Scacchi *et al*. 1991, Sun 1996, Chung & Chung 2000, Wallace & Case 2000, Sun & Wong 2001, Wallace 2004, Ávila-Díaz & Oyama 2007, Brzosko *et al*. 2009). The moderate or high outcrossing rates found within populations of *G. rosulacea* would be one of main factors in maintaining the current levels of genetic variation within the species.

Population genetics theory predicts that genetic variation within species is related to population size (n), as a consequence of random genetic drift (Soulé 1976, Frankham 1996, Sun 1996). Theory also predicts that the loss of allelic diversity could be much greater than the loss of heterozygosity with decreasing effective population size (Nei *et al*. 1975). Many empirical studies are consistent with this theory; among genetic variation parameters within populations, A_p and P_p are significantly correlated with n, but there is no significant correlation between n and H_{op} or H_{ep} (Sun 1996 and references therein). Although we failed to find a significant correlation between P_p and n, our results support this trend. If the degrees of freedom (i.e., number of populations) were somewhat larger, the correlation values for $\%P_p$, AR_p, and H_{ep} close to being significant would probably be significant.

Allozyme data may also provide insights into population's establishment history (Gonzales & Hamrick 2005, Chung *et al*. 2009). Since we failed to find evidence of recent bottlenecks in the disjunct, small population GR-1 harbored the lowest level of genetic variation in all genetic parameters, the current low levels might be a consequence of recent founder effects. The isolated population GR-5 experienced recent bottlenecks but harbored the second lowest allelic richness. However, the population size of GR-5 has continuously decreased from 421 to 70 as revealed by our field surveys carried out since 1998. It is hypothesized that GR-6 was established from GR-7 via a few seeds because: (1) GR-6 exhibited lower levels of genetic variation than GR-7 (H_{ep} = 0.096 *vs*. 0.138), (2) GR-7 consists of much larger number of individuals than GR-6 (n = 453 *vs*. 42), (3) the two populations are proximally located, and (4) GR-7 contains more alleles than GR-6 (AP_p = 2.17 *vs*. 2.00). Since allozymes have a low power in terms of number of alleles per locus, this hypothesis

could be tested by applying highly variable DNA markers. In contrast to these small populations, large populations GR-4 and GR-7 maintained high levels of allelic richness (Table 2), although they have suffered recent bottlenecks. All these results suggest that the populations of *G. rosulacea*, particularly the small populations, have suffered from random genetic drift.

Degree of population differentiation

At the regional geographic scale, a measure of differentiation among local populations was moderate (F_{ST} = 0.150; G_{ST} = 0.164) and comparable to the reported average for terrestrial orchids (G_{ST} = 0.163; Case 2002). This level, however, was considerably lower than that reported among 15 populations of *G. procera* (maximum distance between populations was 43 km) in Hong Kong (G_{ST} = 0.523; Wong & Sun 1999). This discrepancy might be attributed to different topography, degree of population isolation, presumably different intensity of anthropogenic disturbances, different sample size (the samples in the two populations were seven and eight in Wong & Sun 1999), probable inclusion of another morphologically similar *Goodyera* species in Hong Kong, and other unknown factors. The high G_{ST} estimate in the *G. procera* study is attributable to two times higher H_e in pooled samples (H_{es} = 0.150) than that averaged across 15 populations (H_{ep} = 0.073). To determine the degree of among-population differentiation in other *Goodyera* species, we have initiated to examine their population genetic diversity and structure in Korea.

Population genetics theory predicts that if gene flow is extensive over relatively large areas resulting in a low population differentiation, no apparent pattern of isolation by distance (IBD) would exist (i.e., gene dispersal > genetic drift). Conversely, if gene movement via pollen and seed dispersal is more restricted or moderate and historically population sizes are large, then an IBD effect would be present among populations (i.e., gene dispersal ≈ genetic drift). Alternatively, if gene flow is also restricted between small, isolated populations, then one may expect the absence of IBD equilibrium (i.e., gene dispersal < genetic drift). *Goodyera rosulacea* exhibited insignificant and weak geographic structure, suggesting that its populations may not be at genetic equilibrium balancing between dispersal and drift. Our ongoing study on the dynamics of fine-scale genetic structure (FSGS) in populations of *G. rosulacea* revealed a significant FSGS among individuals at GR-5. Regression slope (b_F) of f_{ij} (the pairwise kinship coefficient between individual *i* and *j*; Loiselle *et al.* 1995) on the natural logarithm between the distance *i* and *j* (Vekemans & Hardy 2004) was significantly negative (b_F = –0.016, p = 0.000, S_p [intensity of FSGS] = 0.0177). This result suggests that most seeds fall close to the maternal plants. As discussed before, small populations of *G. rosulacea* have experienced random genetic drift. Given that, the third scenario appears to fit for *G. rosulacea* presumably caused by a combination of factors such as limited seed dispersal, random genetic drift, and some unknown processes.

To conclude, the distribution of genetic diversity within and among populations of *G. rosulacea* might have been shaped by a combination of several genetic and ecological factors such as pollinator-dependent outcrossing breeding system, random genetic drift in the small populations, restricted gene dispersal, and specific habitat preference in the limestone regions on the Korean peninsula.

Conservation implications

Since *G. rosulacea* is restricted to central Korea, it is of importance to evaluate its current threatened status according to the IUCN criteria (IUCN 2001). The extent of occurrence estimated for the species is about 7500 km^2; however, the area of occupancy is quite small (< 3 km^2). Thus, *G. rosulacea* can be categorized as "endangered" (EN) following the IUCN criteria: [B2ab (I, ii, iii, iv, v); IUCN 2001].

Most terrestrial orchids are threatened by habitat loss/alteration and collection for botanical and horticultural interest (Pillon & Chase 2007). Currently, threats to natural populations of *G. rosulacea* are mainly anthropogenic, such as construction of new roads and resorts, and

a complete clearance of the limestone hillsides for cement production (Lee & Choi 2006). The habitats of *G. rosulacea* characterized by thin, dry, and fragile soils of the limestone rocks make it particularly vulnerable to destruction by trampling. Furthermore, the collection of wild goodyeras continues by hobbyists and plant sellers (Lee & Choi 2006). Since small populations GR-1, GR-5, and GR-6 showed decreased levels of genetic variation and increased inbreeding due to random genetic drift, illegal collections may further pose significant threats to the long-term viability of the species.

Given the current status of *G. rosulacea*, we suggest several management strategies improviung short- and long-term survival chances of the existing populations in Korea. First, we recommend that all known populations should be protected by law to prevent further decrease in their size. Second, we suggest that the habitats occupied by pollinators of *G. rosulacea*, and plants similar to *G. rosulacea* with respect to floral rewards for pollinators, if any, and soils should be protected. Third, since *G. rosulacea* reproduces via seeds, hand-mediated artificial pollination would be an approach to increase the size of small populations. Fourth, since *G. rosulacea* exhibited a moderate degree of inter-population divergence, several populations or all known populations are concerned for *in situ* protection and/or *ex situ* conservation of genetic diversity (Godt *et al*. 1995, Godt & Hamrick 2001). Populations maintaining high levels of genetic variation would have a priority for *in situ* conservation and *ex situ* preservation for sampling strategies (Godt *et al*. 1995, 1997). As populations GR-4 and GR-7 have high allelic richness, these populations might be best suited for sampling for *in situ* preservation and *ex situ* conservation. On the other hand, the genetically depauperate population GR-1 is suggested as a prime candidate for management. Finally, detailed information on the life-history traits and population ecology of *G. rosulacea* are needed for comprehensive conservation actions. These are (1) specific ecological requirements for germination, establishment, and growth; (2) identification of pollinators and their habitat requirements; (3) identification of fungal associates, their habitat requirements, and their roles in the life history of *G. rosulacea*; and (4) determination of viability and stability of small populations over time, including monitoring of reductions in the number of populations and individuals within populations.

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