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Contrasting levels of genetic diversity between the common, self-compatible *Liparis kumokiri* and rare, self-incompatible *Liparis makinoana* (Orchidaceae) in South Korea

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Levels of allozyme variation and intrapopulation spatial genetic structure of the two terrestrial clonal orchids *Liparis kumokiri*, a self-compatible relatively common species, and *L. makinoana*, a self-incompatible rare species, were examined for 17 ($N = 1875$) and four ($N = 425$) populations, respectively, in South Korea. Populations of *L. makinoana* harboured high levels of genetic variation ($H_e = 0.319$) across 15 loci. In contrast, *L. kumokiri* exhibited a complete lack of allozyme variation ($H_e = 0.000$). Considering the lack of genetic variability, it is suggested that current populations of *L. kumokiri* in South Korea originated from a genetically depauperate ancestral population. For *L. makinoana*, a significant deficit of heterozygosity (mean $F_{IS} = 0.198$) was found in population samples excluding clonal ramets, suggesting that pollen dispersal is localized, generating biparental inbreeding. The significant fine-scale genetic structuring (≤ 2 m) found in a previous study, in addition to the moderate levels of population differentiation ($F_{ST} = 0.107$) and the significant relationship between genetic and geographical distances ($r = 0.680$) found here, suggests a leptokurtic distribution of seed dispersal for *L. makinoana*. Although populations of *L. makinoana* harbour high levels of genetic variation, they are affected by a recent genetic bottleneck. This information suggests that genetic drift and limited gene flow could be the main evolutionary forces for speciation of a species-rich genus such as *Liparis*. © 2007 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2007, **153**, 41–48.

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INTRODUCTION

Seeds of members of the Orchidaceae are minute (dust-like) and wind dispersed (Ackerman & Ward, 1999; Arditti & Ghani, 2000) Orchid seeds can enter into the 'air column', thus being dispersed over considerable distances (up to several kilometres) with the aid of a strong wind (Sharma, Clements & Jones, 2000; Trapnell & Hamrick, 2004). However, the existence of significant fine-scale genetic structure in several orchid species indicates that much seed dispersal is highly localized within maternal populations (Peakall & Beattie, 1996; Chung *et al*., 1998; Machon *et al*., 2003; Chung, Nason & Chung, 2004a, b, 2005a, b; Trapnell, Hamrick & Nason, 2004). Although empirical data for population genetic structure, particularly fine-scale genetic structure and direct evidence of seed and pollen dispersal, are still limited, these previous results indicate a leptokurtic distribution of seed dispersal with much recruitment around maternal plants and a very flat tail (reviewed in Cain, Milligan & Strand, 2000).

If this scenario is true for most orchids, one may simply expect moderate or high levels of genetic diversity within widespread orchid species and low or moderate levels of population differentiation. However, although orchids share a trait of tiny, dust-like seeds,

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Orchidaceae is one of the largest families of flowering plants and exhibits a wide array of biological and ecological attributes, such as reproductive strategies, population sizes, habitat specificities, degrees of population isolation, patterns of distribution, and geographical ranges (Arditti, 1992; Dressler, 1993). Probably owing to these factors, a total lack of genetic variation has also been documented in several terrestrial orchids (Scacchi, De Angelis & Corbo, 1991; Bornbusch, Swender & Hoogerwert, 1994; Case, 1994; Sun, 1997; Ramsey & Stewart, 1998). Even within an orchid species, levels of genetic diversity can vary substantially depending on location (Bornbusch *et al*., 1994; Case *et al*., 1998; Wong & Sun, 1999; Gustafsson, 2000). Furthermore, estimates of population differentiation compiled from 76 studies (reviewed in Forrest *et al*., 2004) showed a high variability between species, but also between populations within the same species (Scacchi *et al*., 1991; Hollingsworth & Dickson, 1997; Squirrell *et al*., 2001; Brzosko & Wroblewska, 2003).

Pollinator deception and adaptive radiation for specific pollinators, driven by natural selection for cross-pollination, have been routinely perceived as major explanatory factors for the great variation of floral structure and species diversity in orchids (reviewed in Cozzolino & Widmer, 2005; Tremblay *et al*., 2005). In an attempt to understand the role of orchid diversification from a population genetics perspective, two extreme microevolutionary processes or scenarios have been proposed (Ackerman, 1998; Ackerman & Ward, 1999; Tremblay & Ackerman, 2001; reviewed in Tremblay *et al*., 2005). At one extreme, when populations of orchid species are small, isolated, and discontinuously distributed, coupled with highly restricted gene dispersal between populations, the evolution of orchids will be 'rapid' according to the drift selection model. At the opposite extreme, when populations of predominantly outbreeding species are large, continuous, and widely distributed, and intraspecific gene flow is high, the evolution of orchids will be a 'slow' or 'gradual' process. Some studies appear to fit the first scenario, whereas others do not (reviewed in Forrest *et al*., 2004; Cozzolino & Widmer, 2005; Tremblay *et al*., 2005). In this respect, further studies of population genetic structure (i.e. interplay of evolutionary processes between gene flow, local genetic drift, and selection) of orchid species are needed to achieve a better understanding of the evolutionary processes driving the diversification of orchids (Tupac Otero & Flanagan, 2005).

In this study, we selected two terrestrial orchid congeners, *Liparis kumokiri* F. Maekawa (relatively common and self-compatible) and *L. makinoana* Schlechter (rare and self-incompatible), to compare the levels of genetic diversity within and between populations of the two species in South Korea. Plant populations of common species generally harbour significantly higher levels of genetic diversity than populations of rare congeners, although, in a few instances, opposite findings have been found (reviewed in Gitzendanner & Soltis, 2000). Many terrestrial orchids are relatively rare and occur in small and spatially isolated populations. Such isolation contributes to interrupted gene flow, which increases the effectiveness of genetic drift, resulting in low levels of genetic diversity within populations and a high degree of interpopulation differentiation (reviewed in Forrest *et al*., 2004; Tremblay *et al*., 2005). Considering this information, rare *L. makinoana* is expected to maintain lower levels of genetic diversity than *L. kumokiri*. In the same way, the degree of genetic differentiation between populations of *L. makinoana* is expected to be higher than that between populations of *L. kumokiri*. To test these two predictions and to determine the levels of genetic diversity within and between populations of both species, multilocus allozyme genotypes were sampled across their distribution range in South Korea. The data obtained in this study may be useful to relate the population genetic structure of these species to the diversification processes within the species-rich *Liparis* genus (*c*. 250 species; Mabberley, 1989).

MATERIAL AND METHODS

Plant species and sample collection

Liparis kumokiri and *L. makinoana* are distributed on pine–oak forest hillsides and mountains in Japan and Korea, although the latter is also found in north-eastern China (Kitamura, Murata & Koyama, 1986). *L. kumokiri* is a common species in Korea relative to other *Liparis*, whereas *L. makinoana* is rare in South Korea and Japan (Kitamura *et al*., 1986; Oh *et al*., 2004, 2005; M. Y. Chung & M. G. Chung, pers. observ.).

Liparis species can reproduce both sexually and vegetatively. Each spring, new roots develop from the overwintering corm, and each mature or adult plant usually produces two basal leaves. With the autumn senescence, the parent corm of each plant completely disappears and is replaced by a new corm (Whigham & O'Neill, 1991). Inflorescences of both species bear 3–23 flowers in 10–35 cm tall scapes. The two species can be distinguished easily by the colour and size of the labellum (greenish yellow and width of *c*. 5 mm for *L. kumokiri*, and brownish purple and 8–12 mm for *L. makinoana*) (Chung *et al*., 2005b). The basal part of the dorsal sepal, column, and labellum of the flowers of the two species is shiny, which is thought to function as a nectar mimic (Whigham & O'Neill, 1991). Pollinators of both species are unknown, but breeding systems differ significantly: self-compatible for *L. kumokiri* vs.

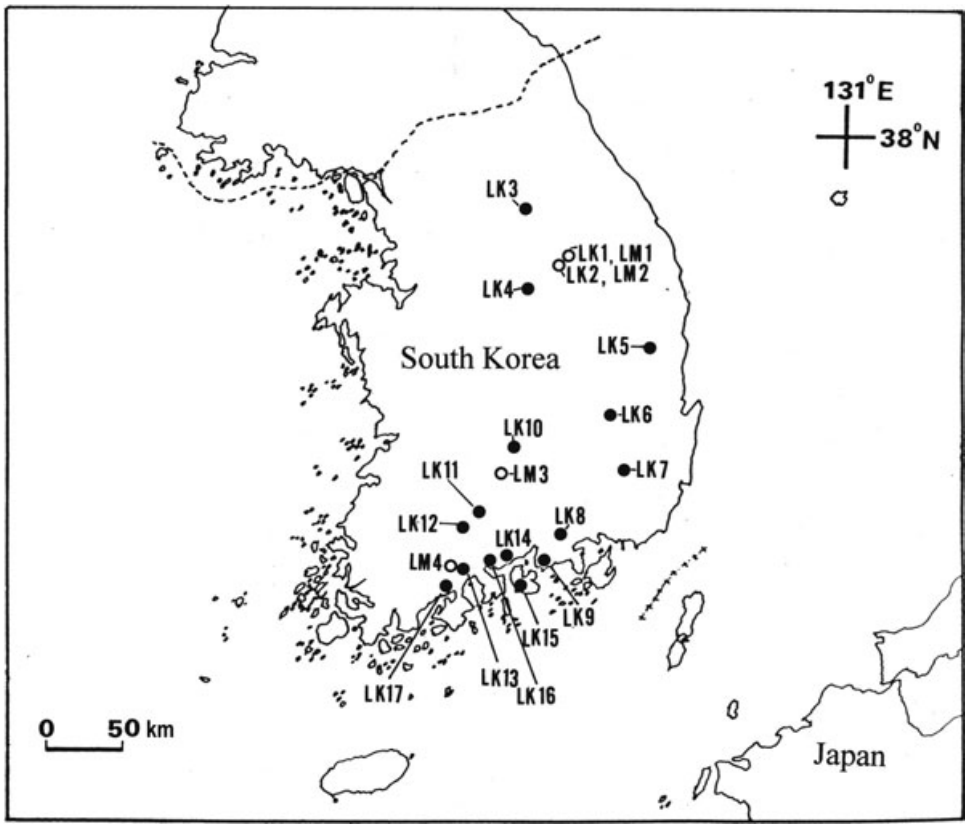

Figure 1. Collection sites of *Liparis kumokiri* (●, 17 populations from LK1 to LK17) and *L. makinoana* (○, four populations from LM1 to LM4) examined in this study. The sample size for each population is given in parentheses. *L. makinoana*: LM1 (374), LM2 (51), LM3 (53), LM4 (61). *L. kumokiri*: LK1 (610), LK2 (184), LK3 (67), LK4 (252), LK5 (59), LK6 (113), LK7 (27), LK8 (60), LK9 (48), LK10 (46), LK11 (79), LK12 (65), LK13 (63), LK14 (68), LK15 (49), LK16 (53), LK17 (58).

self-incompatible for *L. makinoana* (Oh *et al*., 2001). The much lower percentage of fruit set observed in *L. makinoana* (0.1–0.2%) than in *L. kumokiri* (10.2–12.2%) may reflect the combined effects of pollinator limitation and self-incompatibility (Oh *et al*., 2001). Fruits (2.0–2.5 cm long) contain large numbers of minute seeds, as typically found in orchids.

To determine the levels and distribution of allozyme variation within and between populations of the two species, we collected samples from individuals ($N = 1875$) from 17 populations of *L. kumokiri* (LK1 to LK17; Fig. 1) located across the range of the species in South Korea. For *L. makinoana*, we could locate only four populations ($N = 425$) (LM1 to LM4; Fig. 1), including two sympatric populations with *L. kumokiri* (LK1 and LK2) in LM1 and LM2 on hillsides of Mt. Sobaek (Chung *et al*., 2005b). During the past 5 years, we have failed to find this species at other historical localities identified from herbarium records, indicating that this species may be declining and is extremely rare compared with *L. kumokiri* in South Korea (Oh *et al*., 2004, 2005). We collected a 1 cm^2 leaf area from each sample; seedlings and juveniles with a length of the leaf blade of less than 2 cm were not collected to preserve these plants. All sampled leaf material was kept on ice until it could be transported to the laboratory, where it was stored at 4 °C until protein extraction.

Allozyme electrophoresis

Leaf samples were cut finely and then crushed with a mortar and pestle in a phosphate–polyvinylpyrrolidone extraction buffer (Mitton *et al*., 1979). Enzyme extracts were absorbed onto 4 × 6 mm wicks cut from Whatman 3MM chromatography paper, which were then stored at −70 °C until needed. Starch gel electrophoresis details for the two *Liparis* species are described in Chung *et al*. (2005b). Starch gels (12%) were stained for nine enzyme systems (diaphorase, formate dehydrogenase, isocitrate dehydrogenase, leucine aminopeptidase, malate dehydrogenase, 6-phosphogluconate dehydrogenase, phosphoglucoisomerase, phosphoglucomutase, and shikimate dehydrogenase), which resolved 15 putative loci (*Dia-1*, *Dia-2*, *Fdh*, *Idh-1*, *Idh-2, Lap-1*, *Lap-2*, *Mdh-1*, *Mdh-2*, *6Pgd-1*, *6Pgd-2*, *Pgi-1*, *Pgi-2*, *Pgm*, and *Skdh*) using

three different buffer systems [a modification (Haufler, 1985) of Soltis *et al.*'s (1983) system 6, a morpholine citrate buffer system (Clayton & Tretiak, 1972), and a modification (Chung & Kang, 1994) of Soltis *et al.*'s (1983) system 11]. 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 as superscript '*a*'.

Data analysis

To determine whether shoots with identical marker genotypes were clones, we calculated the statistical power ($1 - P_G$, where P_G is the probability that two random, sexually produced genotypes will be identical) for each population of the two species to discriminate between clonal genotypes from identical sexually produced genotypes. A two-locus linkage disequilibrium analysis was conducted, and no significant disequilibria for any combination of alleles were found. Thus, the P_G values were calculated as the product over loci of observed genotypic frequencies of genets (Berg & Hamrick, 1994; Chung *et al.*, 2004a, 2005b). This analysis was performed only for *L. makinoana* because no allozyme variation was detected in *L. kumokiri* (see 'Results').

To estimate the following genetic diversity parameters using the number of genets per population, we used the program POPGENE (Yeh, Yang & Boyle, 1999). The parameters were the percentage of polymorphic loci (%P; a locus was considered to be polymorphic if the frequency of the most common allele did not exceed 0.95), mean number of alleles per polymorphic locus (A_P), observed heterozygosity (H_o), and Nei's unbiased gene diversity (H_e).

To test for significant recent decreases in effective population size (N_e), we used the program BOTTLENECK (Cornuet & Luikart, 1996) with the data for genets at all populations of the two species examined. As alleles are generally lost more rapidly than heterozygosity, recently bottlenecked populations will exhibit an excess of Hardy–Weinberg (H–W) expected heterozygosity relative to that expected from mutation–drift equilibrium of the number of alleles (Luikart & Cornuet, 1998).

To measure the average level of inbreeding within and genetic differentiation between populations of each species, Wright's (1965) F_{IS} and F_{ST}, respectively, were estimated for genets over polymorphic loci according to the method of Weir & Cockerham (1984). These estimates and their 95% bootstrap confidence intervals (CIs) (1000 replicates) were obtained using the program FSTAT (Goudet, 2002). For each population, F_{IS} was also calculated separately with 95% bootstrap CIs (1000 replicates) constructed using the program GDA (Lewis & Zaykin, 2001). Finally, to determine whether genetic differentiation between populations for each species would increase as a function of the geographical distance between populations, we used the method of Rousset (1997), and the Mantel test was conducted using the program PERMUTE! (version 3.4 alpha; Casgrain, 2001).

RESULTS

Allozyme diversity and clonal structure

Of the 33 alleles found at 11 polymorphic loci, 22 were unique to *L. makinoana* and three were unique to *L. kumokiri*; eight alleles were present in both taxa. Moreover, allelic differences at three loci (*Mdh-2*, *Pgd-1*, and *Pgi-2*) were diagnostic, and the frequencies of some shared alleles were also highly skewed (e.g. *Dia-2*^{a}, *Fdh*^{b}, and *Idh-1*^{c}) (data not shown).

All 17 populations of *L. kumokiri* were completely homozygous and allozymically indistinguishable (no polymorphic loci across 15 loci). Patterns of clonal spread could not be quantified for *L. kumokiri* owing to the complete lack of allozyme polymorphism. In contrast, high levels of genetic diversity in populations of *L. makinoana* calculated from genets (excluding clones) were detected, and these levels were homogeneous across populations (Table 1). The percentage of polymorphic loci within populations (%P) ranged from 66.7 to 73.3, and the mean number of alleles per polymorphic locus (A_P), which was similar for the four populations, ranged from 2.27 to 2.73 (Table 1). Genetic diversity (H_e) estimates were also homogeneous across populations (0.304–0.333; Table 1). As our discriminating power was high (close to unity) and similar for the four populations of *L. makinoana* ($1 - P_G = 0.99995$, $1 - P_G = 0.99990$, $1 - P_G = 0.99996$, and $1 - P_G = 0.99994$ at LM1, LM2, LM3, and LM4, respectively), and identical genotypes were spatially clustered as expected for growth via vegetative spread, we identified putative clonal ramets by simple inspection of the genotypic data. We found nearly the same levels of genetic diversity in all samples (data not shown) and samples excluding clones (N_g), probably because of the small number of clonal ramets (the numbers of excluded clonal ramets were 24, three, six, and four at LM1, LM2, LM3, and LM4, respectively).

Finally, we found a significant excess of H–W expected heterozygosity under both the infinite allele and stepwise mutation models for LM1 (Wilcoxon test, $P = 0.001$ and $P = 0.034$, respectively), LM2 (Wilcoxon test, $P = 0.000$ and $P = 0.027$, respectively), LM3 (Wilcoxon test, $P = 0.000$ and $P = 0.002$, respectively), and LM4 (Wilcoxon test, $P = 0.002$ and $P = 0.005$, respectively). These results suggested a recent decrease in N_e of the four populations of *L. makinoana*.

Table 1. Summary of genetic diversity measures and mean fixation (F_{IS}) estimates observed in *Liparis kumokiri* and *L. makinoana*

Species	Population	N_g	%P	A_P	H_o (SE)	H_e (SE)	F_{IS} (95% CI)
L. kumokiri	All 17 populations	1875	0.0	0.00	0.000 (0.000)	0.000 (0.000)	–
L. makinoana							
	LM1	350	73.3	2.73	0.267 (0.059)	0.333 (0.066)	0.199 (0.072, 0.336)
	LM2	48	73.3	2.55	0.278 (0.058)	0.304 (0.058)	0.099 (– 0.003, 0.202)
	LM3	47	66.7	2.27	0.252 (0.056)	0.328 (0.063)	0.235 (0.096, 0.378)
	LM4	57	66.7	2.27	0.232 (0.061)	0.309 (0.048)	0.258 (0.104, 0.389)

A_P, mean number of alleles per polymorphic locus; CI, confidence interval; H_e, Hardy–Weinberg expected heterozygosity or genetic diversity; H_o, observed heterozygosity; N_g, number of genets for *L. makinoana* (numbers for *L. kumokiri* represent the total samples); %P, percentage of polymorphic loci; SE, standard error; –, analysis not conducted because of monomorphism for all loci examined.

Genetic structure

In this paper, we report the results of Wright's *F*-statistics only for genets of *L. makinoana*, because those for all samples and genets were very similar. F_{IS} calculated for the four populations of *L. makinoana* was significantly greater than zero (mean $F_{IS} = 0.198$; 95% CI, 0.070–0.346), with individual population fixation indices from 0.258 (significant) for LM4 to 0.099 (marginally significant) for LM2 (Table 1). Wright's F_{ST} jackknifed over loci across the four populations of *L. makinoana* was moderate and significant ($F_{ST} = 0.107$; 95% CI, 0.068–0.148). F_{ST} for each pair of proximal populations was significant, but low, probably because of their spatial proximity ($F_{ST} = 0.040$; 95% CI, 0.021–0.060 for LM1 vs. LM2; $F_{ST} = 0.028$; 95% CI, 0.018–0.047 for LM3 vs. LM4), whereas that for geographically distant populations was relatively high ($F_{ST} = 0.110$; 95% CI, 0.031–0.260 for LM1 vs. LM3; $F_{ST} = 0.138$; 95% CI, 0.048–0.267 for LM1 vs. LM4; $F_{ST} = 0.184$; 95% CI, 0.053–0.272 for LM2 vs. LM3; and $F_{ST} = 0.220$; 95% CI, 0.118–0.397 for LM2 vs. LM4). Consistent with these findings, the Mantel test revealed significant relationships between genetic and geographical distances ($r = 0.680$, $R^2 = 0.463$, $P = 0.047$). Owing to a number of taxon-specific alleles, F_{ST} between the two taxa was very large and highly significant (e.g. estimated at the two sympatric populations: $F_{ST} = 0.708$; 95% CI, 0.610–0.758 at LK1 and LM1; $F_{ST} = 0.816$; 95% CI, 0.698–0.861 at LK2 and LM2).

DISCUSSION

Contrasting levels of genetic diversity

Our results do not support the first prediction of common species having more genetic variation than restricted species. The common, self-compatible *L. kumokiri* is genetically depauperate in all 17 populations examined. In contrast, populations of the rare, self-incompatible *L. makinoana* possess considerably higher levels of genetic variation within populations (averaged over the four populations: %P = 70, $H_e = 0.319$) than the average within-population genetic diversity of other herbaceous plants (%P = 34, $H_e = 0.090$), as reported by Hamrick & Godt (1989). Moreover, these measures of genetic diversity are amongst the highest values reported for terrestrial orchids. Thus, potential factors underlying this unexpected difference warrant consideration. The contrasting breeding systems of the two species may be an important factor, given that their other life-history and ecological traits appear to be similar. As reported in Hamrick & Godt (1989), the breeding system is strongly associated with the levels of genetic variation found in plant species. In particular, selfing species and animal-pollinated mixed-mating species exhibit lower levels of genetic variation than species with predominantly outcrossing breeding systems. A common feature for widespread species which reveal significantly lower levels (or a complete lack) of genetic diversity compared with their rare congeners is the occurrence of high levels of selfing (e.g. *Lisianthius skinneri*, Sytsma & Schaal, 1985; *Polygonella articulata*, Lewis & Crawford, 1995; reviewed in Gitzendanner & Soltis, 2000). Thus, a scenario is hypothesized to explain the lack of allozyme variation in *L. kumokiri*. Inbreeding via selfing and genetic drift can lead to a loss of genetic variation within populations, but fixation of the same alleles across populations suggests that current populations may have originated from the same genetically depauperate ancestral population following a severe population bottleneck. A total lack of genetic variation (%P = 0) has also been documented in the terrestrial orchids *Cypripedium arietinum* in North America (Bornbusch *et al*., 1994; Case, 1994), *Cypripedium calceolus* in England (Ramsey & Stewart, 1998), *Cephalanthera damasonium* in Italy

(Scacchi *et al*., 1991), and *Zeuxine strateumatica* in Hong Kong (Sun, 1997).

A different scenario is also proposed for *L. makinoana*. As suggested by Sharma *et al*. (2000), *L. makinoana* was probably once much more widely distributed across the Korean Peninsula. If this is true, the number of local populations has declined through recent extinction events, and the high levels of genetic variation observed at the four populations are vestiges of the species' historically large N_e. These four surviving populations might have experienced significant recent decreases in N_e. Consistent with this evidence of population decline, in July 1998, we recorded about 2000 ramets of *L. makinoana* at LM1; however, by July 2003, the numbers had decreased to less than 400 (M. Y. Chung & M. G. Chung, unpublished data), probably as a result of stochastic events, coupled with extremely low fruit production and illegal collection by orchid collectors.

Genetic structure: implications for species diversification in *Liparis*

As seen in most orchids, low fruit production, coupled with a large number of seeds per capsule, may provide a mechanism for diversification in pollination systems and speciation. This indirect and direct evidence confirms that natural selection is responsible for the floral adaptations of orchids (reviewed in Tremblay *et al*., 2005). The two *Liparis* species exhibit low fruit set (10.2–12.2% for *L. kumokiri* and 0.1–0.2% for *L. makinoana*), probably because of pollinator limitation (Oh *et al*., 2001). In addition, a high diversity of breeding systems has been recognized from a limited number of studied species: autogamy in *L. caespitosa*, *L. cleistomama*, *L. longipes*, and *L. loeselii* (Kirchner, 1922; Catling, 1980); self-compatibility in *L. kumokiri* (Oh *et al*., 2001); and self-incompatibility in *L. lilifolia* (Whigham & O'Neill, 1991) and *L. makinoana* (Oh *et al*., 2001). Considering these studies, there is no argument that selection is important for the floral adaptation of the species-rich *Liparis*.

Owing to a complete lack of allozyme variation for 17 populations of *L. kumokiri*, we were unable to calculate the mean F_{ST} value, an important parameter to infer indirectly the amount of gene flow between populations (Bohomak, 1999). Furthermore, the estimation of the number of genets per population of *L. kumokiri* was also not possible. Thus, we failed to test the second prediction about the rate of genetic differentiation between populations of both species. Rather, we focused on the data obtained from *L. makinoana* to infer the extent and patterns of pollen and seed dispersal, and to gain insights about the evolutionary processes of *Liparis*. The significant deficit of heterozygotes relative to H–W expectations in self-incompatible *L. makinoana* suggests that pollen dispersal is localized, generating biparental inbreeding. Our previous study on the spatial distribution of individuals (genets) and fine-scale genetic structure conducted at LM1 and LM2 revealed significant spatial clustering and significant fine-scale genetic structuring (≤ 2 m), suggesting localized patterns of seed dispersal (Chung *et al*., 2005b). Such structure could produce a Wahlund effect, which would increase the apparent rate of inbreeding. The mean value of F_{ST} (0.107) from the four populations of *L. makinoana* was moderate, and pairwise F_{ST} values differed significantly from each other. F_{ST} values for pairs of proximal populations were significantly lower than those for geographically distant populations, leading to a significant relationship between genetic and geographical distances ($r = 0.680$). These fine- and large-scale genetic structures found in *L. makinoana* suggest a leptokurtic distribution of seed dispersal. Genetic drift is an important component of diversification in orchids, because N_e in many orchids is small as a result of pollinator limitation and skewed reproductive success in individuals (Tremblay & Ackerman, 2001; reviewed in Tremblay *et al*., 2005). Populations of many orchids reveal variance in male and female reproductive success (reviewed in Tremblay *et al*., 2005). If this is true for *Liparis*, this process may lead to a further decrease in N_e. More importantly, the four populations of *L. makinoana* have probably been affected by a recent genetic bottleneck.

In conclusion, although the number of populations and species studied may be too limited to draw a full view of the evolutionary scenarios for *Liparis* speciation, we suggest that selection on floral morphology caused by the limitation of pollinators and genetic drift, coupled with limited gene flow ('rapid' process), was the main evolutionary force for speciation of the species-rich genus *Liparis*. As all 17 populations of *L. kumokiri* revealed no polymorphic loci across 15 loci, it is very possible that the *L. kumokiri* populations in Korea resulted from a single long-distance dispersal event. Subsequent spread led to the establishment of additional Korean populations. It is not unreasonable to argue that such colonization events, followed by nearly complete isolation (i.e. the founder effect and its accompanying genetic drift), could be a mechanism for speciation.

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