

Contrasting breeding systems: *Liparis kumokiri* and *L. makinoana* (Orchidaceae)

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Received 28 September 2000, accepted 23 March 2001

Oh, G. S., Chung, M. Y., Chung, S. G. & Chung M. G. 2001: Contrasting breeding systems: *Liparis kumokiri* and *L. makinoana* (Orchidaceae). — *Ann. Bot. Fennici* 38: 281–284.

We investigated the breeding systems of *Liparis kumokiri* and *L. makinoana* (Orchidaceae) in Korea. *Liparis kumokiri* is self-compatible, but *L. makinoana* is self-incompatible. The two *Liparis* species showed little fruit set in natural conditions (10.2%–12.2% for *L. kumokiri* and 0.1%–0.2% for *L. makinoana*). However, a great increase in fruit set was achieved by hand-pollinations. The much lower percentage of fruit set observed in *L. makinoana* may reflect combined effects of pollinator limitation and self-incompatibility.

Key words: breeding systems, *Liparis*, nectarless orchids, Orchidaceae

Introduction

The Orchidaceae is one of the largest families of flowering plants. Members of the family occupy a wide range of temperate and tropical habitats, terrestrial and epiphytic. In addition, the group exhibits diverse breeding systems: (1) pollinator-dependent breeding systems (e.g., autogamy, xenogamy by self-incompatibility, and mixed mating systems) with nectar rewarding, nectar mimic or nectar deceit pollination systems and (2) pollinator-independent breeding systems (e.g., nectarless or nectariferous autogamy and agamospermy, nectarless and rain-assisted autogamy, and combinations of these systems) (*see*

references in Neiland & Wilcock 1998). Such diverse breeding systems have attracted the interest of plant population biologists. Knowledge of reproductive and population biology in conjunction with population genetics is also essential for planning comprehensive conservation strategies (Falk & Holsinger 1991).

According to a recent review by Neiland and Wilcock (1998), fruit-set is generally low in nectarless orchids (11.5%) as well as in nectariferous orchids (24.9%). They reported two causal relationships between pollinator and fruit set. First, nectariferous orchids are more successful in setting fruit than are nectarless species, but there is a positive association between orchid

rarity and lack of nectar reward to pollinators. Second, the provision of nectar as a reward to pollinators, on average, doubles the probability of fruit set. However, most studies of the reproductive biology of orchids have been conducted in Europe (37 species), North America (20 species), and temperate southern hemisphere (11 species). In north temperate Asia, only seven species have been studied. These include three *Platanthera* species (Inoue 1985, 1986a, 1986b), *Eulophia sinensis*, *Spiranthes hongkongensis*, and *Zeuxine strateumatica* (Sun 1997), and *Goodyera prosera* (Wong & Sun 1999). Furthermore, little is known about the breeding systems and pollination biology of nectarless orchids in north temperate Asia (Neiland & Wilcock 1998).

Our study is part of a larger project investigating the history of colonization and spatial and temporal genetic structure of metapopulations of *Liparis kumokiri* F. Maekawa and *L. makinoana* Schlechter in Korea. Because breeding systems in plants have a profound effect on the levels and partitioning of genetic diversity and the spatial genetic structure in populations, it may be important in advance to obtain information on the breeding systems. We investigated the breeding systems of *Liparis kumokiri* and *L. makinoana*, which are two nectarless, deciduous, and terrestrial orchids.

Material and methods

Liparis kumokiri and *L. makinoana* grow in pine-oak forests in Japan and Korea. In addition, *L. makinoana* is also found in northeastern China. The plants inhabit primarily areas with pine needle litter and humus soils. The two species usually flower in June–July with 10–35 cm tall scapes. Inflorescences bear 3–23 flowers. The two species can be distinguished easily by color and size of labellum (greenish-yellow and ca. 5 mm wide in *L. kumokiri*, brownish-purple and 8–12 mm wide in *L. makinoana*). The basal part of the dorsal sepal, column, and labellum of the flower is glossy, and thought to function as a “nectar mimic”. Vegetative reproduction through pseudobulblets is common, although the new

plants or ramets remain close to the plant. Thus, it is easy to identify distinct genets in a population. The two species sometimes co-occur, but hybrids are rare in nature (M. G. Chung unpubl. data).

Fruit-set is the most widely used measure of reproductive success for orchids (Proctor & Harder 1994, Neiland & Wilcock 1998). Thus, we obtained information on the mating system of *Liparis kumokiri* and *L. makinoana* from experimental pollinations and emasculation tests, and from field observations of fruit-set and pollinator activities. Our study was initiated in a 50 × 60-m area in a *Pinus densiflora* forest. The study area, which contained ca. 7000 *Liparis* individuals (5200 of *L. kumokiri* and 1800 of *L. makinoana*), is located at Mount Sobaek (altitude ca. 650 m a.s.l., north-facing 3% slope), Prov. Chungchungbukdo, Korea. On 22 June and 3 July 1999, the number of flowers and the number of flowering individuals were censused for both species. The numbers of fruits were recorded on 17 August 1999. In the study plot, the total number of flowers and fruits were also recorded on 26–28 June and 21 August 2000, respectively.

On 19 April 2000, we transplanted 78 and 75 randomly collected individuals of *Liparis kumokiri* and *L. makinoana* into a greenhouse (to exclude pollinators) at Gyeongsang National University, Korea. Plants were acclimatized for a period of two months before manipulation. On 19 June 2000, we began a breeding system analysis of the plants. A total of 524 flowers (211 flowers from 63 individuals of *L. kumokiri* and 313 flowers from 61 individuals of *L. makinoana*) were assigned randomly to one of five pollination treatments as described by Dafni (1992), Sun (1997), and Wong and Sun (1998): (1) emasculation without pollination to test agamospermy and to evaluate the rate of nonsexual reproduction, (2) a test for spontaneous self-pollination or autogamy to measure the need for pollinators, (3) induced or artificial self-pollination or autogamy to test self-compatibility by placing pollinia on the stigmas of the same flowers, (4) artificial geitonogamy to evaluate self-compatibility by placing pollinia on stigmas of adjacent flowers of the same inflorescence or

different inflorescence belonging to the same genet, and (5) artificial xenogamy or cross-pollination to assess cross-compatibility and whether fruit-set is limited by pollinators. All manipulative tests were conducted from 1 to 3 p.m. on 19 June (beginning of flowering period) to 17 July 2000 (end of flowering period) in a screenhouse.

Results and discussion

The two *Liparis* species showed very low fruit set in natural conditions (10.2%–12.2% for *L. kumokiri* and 0.1%–0.2% for *L. makinoana*; Table 1). The lower percentage of fruit set observed in *L. makinoana* may have resulted from the combined effect of pollinator limitation and self-incompatibility (*see below*). Whigham and O'Neil (1991) reported a similar result in a North American congener, *L. lilifolia*, with 0.7%–2.5% fruit set in open pollination. The percentages of fruit set found in the three *Liparis* species are far below the average for the nectarless orchids in temperate areas (28.5% fruit set, $N = 46$; Neiland & Wilcock 1998). Fruit set in nectarless terrestrial orchids is suggested to be pollinator-limited partly because of dependence on deception for pollination (Whigham & O'Neil 1991).

In spite of the low fruit set in natural habi-

tats, it is of interest to note that *L. kumokiri* and *L. makinoana* have different mating systems. Like *L. lilifolia* in North America, *L. makinoana* is self-incompatible. None of the emasculated or intact flowers or induced self-pollination produced fruits in the screenhouse (Table 1). About 91% of the artificially cross-pollinated flowers fruited in *L. makinoana* (Table 1). *Liparis kumokiri*, however, is self-compatible: no emasculated flowers produced fruits, but automatic pollination (autogamy, 17%), induced autogamy (70%), artificial geitonogamy (65%), and cross-pollination (artificial xenogamy, 92.5%) produced fruits (Table 1). A significant increase in fruit set was achieved through hand-pollinations. Fruit set rates differed among automatic-, induced selfed-, and artificial xenogamy ($\chi^2 = 18.21$, $df = 3$, $P < 0.001$): $\chi^2 = 12.86$, $P < 0.01$ for spontaneous autogamy vs. induced-selfing; $\chi^2 = 17.39$, $P < 0.001$ for spontaneous autogamy vs. artificial xenogamy. However, there was no significant differences in fruit set between induced autogamy and artificial geitonogamy ($\chi^2 = 0.05$, $P > 0.90$) and artificial xenogamy ($\chi^2 = 0.53$, $0.25 < P < 0.50$) and between artificial geitonogamy and artificial xenogamy ($\chi^2 = 1.09$, $0.25 < P < 0.50$).

Automatic self-pollination has been reported in other terrestrial orchids (e.g., *Aplectrum hyemale*, Hogan 1983; *Cyperipedium acaule*, Gill 1989 and Primack & Hall 1990; *Isotria mede-*

Table 1. Experimental pollination scheme and results of the breeding system of *Liparis kumokiri* and *L. makinoana*.

Test	Treatment	No. of samples (No. of flowering individuals)		No. of flowers fruited (% fruit set)	
		<i>L. kumokiri</i>	<i>L. makinoana</i>	<i>L. kumokiri</i>	<i>L. makinoana</i>
Open pollination					
1999	None	2580 (465)	2679 (267)	262 (10.2%)	3 (0.1%)
2000	None	2348 (447)	2321 (258)	286 (12.2%)	5 (0.2%)
Hand pollination (2000)					
Agamospermy	Emasculated	38	55	0 (0.0%)	0 (0.0%)
Spontaneous autogamy	None	53	56	9 (17.0%)	0 (0.0%)
Induced autogamy	Emasculated	40	57	28 (70.0%)	0 (0.0%)
Artificial geitonogamy	Emasculated	40	52	26 (65.0%)	0 (0.0%)
Artificial xenogamy	Emasculated	40	93	37 (92.5%)	85 (91.4%)

olordes, Mehrhoff 1994; *Goodyera procera*, Wong & Sun 1999; and references in Catling 1990). Neiland and Wilcock (1994) suggested that spontaneous autogamy can be successful in circumventing dependence on pollinators. If a pollinator is rare in natural populations of *L. kumokiri*, it is highly likely that the fruit set produced in the study plot would be made through spontaneous autogamy.

During the day on a two-week field survey conducted in July 1999 and 2000, we failed to observe the pollinators in the populations. Although small ants and flies sit on the glossy labellums of the two species, they probably neither touched the anther cap nor removed the pollinia. It is highly likely that the very low fruit set is due to pollinator limitation or lack of pollinators, because a high fruit set was found in artificial xenogamy in both species. Although interspecific crosses in the greenhouse produced high fruit set (86%), no natural hybrids were identified based on morphological characters in the study population (M. G. Chung, unpubl. data). This may also be indicative of rarity of pollinators, resulting in a very low chance of pollinia of one species being transferred to stigma of another species (an example of prezygotic isolating mechanism).

The different mating systems should influence levels of genetic diversity, population genetic structure, and spatio-temporal distribution of genetic variation within and among patches of the two species in the study area. To elucidate this prediction, we are beginning to study the population genetics of the two orchid species.

Acknowledgements

The authors thank Eric Myers for reading the manuscript and making helpful suggestions, and D. Whigham for providing the breeding system information of North American *Liparis* species. This research was supported by a Korea Research Foundation Grant (KRF-2001-015-DS0050) to MGC.

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