

The breeding systems of *Cremastra appendiculata* and *Cymbidium goeringii*: high levels of annual fruit failure in two self-compatible orchids

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Little is known about the breeding systems and pollination biology of terrestrial orchids in northeastern Asia. We investigated the breeding system of *Cremastra appendiculata* and *Cymbidium goeringii* in populations (26 individuals, 120 × 120 m for *Cremastra appendiculata*; 87 individuals, 200 × 500 m for *Cymbidium goeringii*) on Oenaro Island, South Korea. The two orchid species showed little fruit set in natural conditions (1.3%–2.0% for *Cremastra appendiculata* and 0.4%–0.6% for *Cymbidium goeringii*). However, a great increase in fruit set was achieved by hand-pollinations: artificial self-pollination and geitonogamous pollination, with fruit set of 95.7% and 96.6% for *Cremastra appendiculata*, respectively, and 95.5% and 95.6% for *Cymbidium goeringii*, respectively, indicating that the two species are highly self-compatible. Fruit set for pollinator-excluded flowers of *Cremastra appendiculata* and *Cymbidium goeringii* was 0%, indicating that the two species were neither autogamous nor agamosperous. The extremely low fruit set observed in the two species under natural conditions may be attributable to pollinator limitation.

Key words: breeding system, *Cremastra appendiculata*, *Cymbidium goeringii*, Orchidaceae

Introduction

Members of the family Orchidaceae occupy a wide range of temperate and tropical habitats, both terrestrial and epiphytic. The breeding systems of the group are also diverse: (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). According to a recent review by Neiland and Wilcock (1998), fruit-set is generally low both in nectarless orchids (11.5%) and nectariferous orchids (24.9%). They reported two causal relationships between pollinator visitation and fruit set. First,

nectariferous orchids are more successful in setting fruit than are nectarless species, and 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, doubled 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 ten species have been studied, including three *Platanthera* species (Inoue 1985, 1986a, 1986b), *Eulophia sinensis*, *Spiranthes hongkongensis*, and *Zeuxine strateumatica* (Sun 1997), *Goodyera procera* (Wong & Sun 1999), *Pogonia japonica* (Matsui *et al.* 2001, Ushimaru & Nakata 2001), and two *Liparis* species (Oh *et al.* 2001). Understanding this type of causal relationship between pollinator visits, fruit set, and rarity is important for orchid conservation. Because breeding systems in plants often have a profound effect on the levels and partitioning of genetic diversity and the genetic structure of populations (Hamrick & Godt 1995), it is important to obtain information on the breeding system of orchid species in northeastern Asia.

This study is part of a larger project investigating breeding system, genetic diversity, and population genetic structure of orchids in South Korea. In this study we selected two terrestrial orchid species: one is nectariferous, *Cremastra appendiculata*, and the other is nectarless, *Cymbidium goeringii*, which live in the same communities on Oenaro Island in southern Korea. Our initial questions are: (1) What are the natural levels of fruit set in these two species? and (2) Are there any differences between them (Does the nectariferous *Cremastra appendiculata* set more fruit than the nectarless *Cymbidium goeringii*)? To answer these questions, we observed pollination success by counting fruit set in natural habitats and investigated the breeding system of the two species using hand-pollination experiments.

Materials and methods

Cremastra appendiculata (D. Don) Makino is 30–50 cm tall, including the inflorescence. The species grows in the warmer parts of the north-

eastern Asia (Japan, China, Taiwan, and southern Korea to Himalaya; Kitamura *et al.* 1986). *Cremastra appendiculata*, growing in both mature and successional deciduous oak-maple forests, is extremely rare in southern Korean islands (M. Y. Chung & M. G. Chung, pers. obs.). In early summer, individuals normally produce one leaf per year from a newly formed corm which is connected by a rhizome (about 2 cm long) from the corm made in the previous year. Corms are normally shallow rooted and persist for several seasons so that the underground component of any individual includes corms of several ages. Leaves are present throughout summer, autumn, and winter and begin to wither when inflorescence grows and flowers in May to June in southern Korea. Inflorescences are relatively large and produce 20–50 greenish-brown flowers with both nectar and fragrance which are visited by at least three species of bees and two of syrphid flies (Sugiura 1996). However, among these visitors only queens of a long-tongued bumble bee *Bombus diversus diversus* were the probable pollinators. Fruits (about 2.0–2.5 cm long) contain large numbers of small seeds.

Cymbidium goeringii Reichb. f., 10–20 cm tall, including the inflorescence, is distributed throughout southern China, Japan (Honshu, Shikoku, and Kyushu), and southern Korea (Kitamura *et al.* 1986). *Cymbidium goeringii* flowers in March to early April in southern Korea. Old individuals have 3–5 scapes, and each scape has a relatively large, greenish yellow flower which does not secrete nectar. Fruits (about 5 cm long) contain large numbers of small seeds.

The study site was at Oenaro Island located on the southern Korea coast, which is a part of Dadohaesang National Park and has been well preserved. The two species co-occur on the island and there has been no apparent human interference at the site. On 12 March 1999 we set a study area (10 ha, 200 × 500 m) to study breeding systems and spatial genetic structure at the landscape level.

Since fruit-set is the most widely used measure of reproductive success for orchids (Proctor & Harder 1994, Neiland & Wilcock 1998), we obtained information on the mating system of *Cremastra appendiculata* and *Cymbidium goeringii* from field observations of fruit-set and polli-

nator activities and experimental pollination tests.

To calculate the fruit set of *Cymbidium goeringii*, we marked flowering individuals with yellow ribbons and counted the number of flowering individuals and the number of flowers during a three-year period (12–13 and 24 March 1999, 11–13 and 28 March 2000, and 15–17 and 29–30 March 2001).

In the study area, we recorded the total number of fruits on 23 May 1999, 19 May 2000, and 2 June 2001. For *Cremastra appendiculata*, we also censused the number of flowering individuals and the number of flowers during a three-year period (23–24 May 1999, 19–20 May 2000, and 2–4 June 2001) and total number of fruits on 26 August 1999, 15 August 2000, and 29 August 2001.

On 15–17 March and 2–4 June 2001, we conducted breeding system analyses for *Cymbidium goeringii* and *Cremastra appendiculata*, respectively, at the study site. As *Cremastra appendiculata* also can reproduce asexually via a newly formed corm each year, we marked 19 sexually reproduced individuals and seven ramets based on multilocus allozyme genotypes to establish the xenogamous hand pollinations (M. Y. Chung *et al.* unpubl. data). Pre-anthesis inflorescences from 87 and 26 flowering individuals of *Cymbidium goeringii* and *Cremastra appendiculata*, respectively, were bagged with a fine mesh plastic net to exclude pollinators. A total of 307 and 331 flowers for *Cymbidium goeringii* and *Cremastra appendiculata*, respectively, were assigned randomly to one of five pollination treatments as described in Dafni (1992): (1) emasculation without pollination to test for presence of agamospermy and to evaluate the rate of nonsexual reproduction, (2) a test for spontaneous self-pollination to measure autogamy and the need for pollinators, (3) artificial self-pollination to test for self-compatibility by placing pollinia on the stigmas of the same flowers, (4) artificial geitonogamy to evaluate self-compatibility between different flowers 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. After the flowers withered, the bags were removed to minimize any artificial effects.

Statistical tests for differences in fruit set between samples were performed by obtaining 95% confidence limits (95% CL: *see* Eq. 1) because the data are binomially distributed.

$$95\% \text{ CL} = \pm 2\sqrt{\frac{P(100-P)}{n}} \quad (1)$$

where P = percentage of fruit set, and n = total number of flowers examined.

Results and discussion

Cremastra appendiculata and *Cymbidium goeringii* showed very low fruit set in natural conditions (mean 1.6% and 0.5%, respectively; Table 1) although the former had set three times as many fruit as the latter. Our results support the general trend that nectariferous orchids are more successful in setting fruit than are nectarless species (*see* tables 1 and 2 in Neiland & Wilcock 1998). However, the percentage of fruit set found in *Cremastra appendiculata* and *Cymbidium goeringii* are far below the average for the nectariferous and nectarless orchids in various temperate areas (average 43%–74% and 28%–41%, respectively; Neiland & Wilcock 1998). Sugiura (1996) reported a similar low fruit set (1.8%) of *Cremastra appendiculata* in a local population from Japan. Low percentage of fruit set in natural habitats was also reported in two *Liparis* species in South Korea (*L. kumokiri*, self-compatible, 10.2%–12.2% and *L. maki-noana*, self-incompatible, 0.1%–0.2%; Oh *et al.* 2001). Mendel (1995) suggested that fruit set in outcrossing orchids is generally low and limited by pollinator availability in natural habitats.

The 95% CL for data combined across three years indicates that *Cremastra appendiculata* had significantly higher fruit set than *Cymbidium goeringii* (Table 1). For each species, percentage fruit set was similar from year to year. Artificial self-pollination (95.7% and 95.5%), artificial geitonogamy (96.6% and 95.6%), and cross-pollination (artificial xenogamy, 97.2% and 97.8%) produced fruits for *C. appendiculata* and *C. goeringii*, respectively (Table 1). The hand pollination, however, were not significantly different between the two species (Table 1). There were no significant differences in fruit set between

induced autogamy and artificial geitonogamy or between the latter and artificial xenogamy (Table 1), indicating that the two species are highly self-compatible. Although the two species showed very low fruit set in natural conditions, a significant increase in fruit set was achieved through hand-pollination.

No emasculated flowers produced fruits and no automatic pollination was found in *Cremastra appendiculata* and *Cymbidium goeringii*. Since agamospermy and spontaneous autogamy were not detected in this study, pollinia vectors are essential for fruit set in natural habitats.

Sugiura (1996) conducted pollination observations on *Cremastra appendiculata* in May and June 1994 in Japan, and found that queens of bumble bees *Bombus diversus diversus* were probably pollinators of flowers of *C. appendiculata* among the three species of bees and two species of syphid flies that visited the flowers with extremely low frequencies. During a seven-day field study (23–24 May 1999, 19–20 May 2000, and 2–4 June 2001), we also observed only one and two bumble bee queens in 1999 and 2001,

respectively, which visited flowers of *Cremastra appendiculata*. However, we failed to observe any visitors of *Cymbidium goeringii* during the 12-day observations (12–13 and 24 March 1999, 11–13 and 28 March 2000, and 15–17 and 29–30 March 2001). If bumble bees are the only pollinators in *C. appendiculata*, then the presence of bumble bees to this species may explain the significantly higher fruit set than *C. goeringii*. It is highly likely that the very low fruit set may be due to pollinator limitation as a result of the lack of pollinators because a high fruit set was found following artificial pollination in both species.

Although *Cymbidium goeringii* is self-compatible and set very low fruit set in natural habitats, populations of the species have high levels of allozyme variation both in Korea (mean expected heterozygosity, $H_e = 0.240$, Chung & Chung 1999) and in Japan ($H_e = 0.230$, Chung & Chung 2000) compared to most other orchids (reviewed in Sun & Wong 2001). The mean H_e in the study population was 0.225 (Chung & Chung 1999). Since we faced practical difficulties in germinating orchid seeds to estimate outcrossing rate

Table 1. Experimental pollination scheme and results of the breeding system of *Cremastra appendiculata* and *Cymbidium goeringii* with 95% CL (confidence limits) of percentage fruit set.

Test	Treatment	No. of samples (no. of flowering individuals)		No. of flowers fruited (% fruit set)	
		<i>C. appendiculata</i>	<i>C. goeringii</i>	<i>C. appendiculata</i>	<i>C. goeringii</i>
Open pollination					
1999 (95% CL)	None	678 (32)	1407 (567)	9 (1.3%) (1.3% ± 0.85%)	6 (0.4%) (0.4% ± 0.33%)
2000 (95% CL)	None	616 (28)	1062 (325)	12 (2.0%) (2.0% ± 1.11)	4 (0.4%) (0.4% ± 0.38%)
2001 (95% CL)	None	633 (32)	1323 (419)	10 (1.6%) (1.6% ± 0.98%)	8 (0.6%) (0.6% ± 0.42%)
95% CL (totals)		1927 (92)	3792 (1311)	(1.61% ± 0.57%)	(0.48% ± 0.22%)
Hand pollination (2001)					
Agamospermy	Emasculated	48	53	0	0
Spontaneous autogamy	None	72	76	0	0
Induced autogamy (95% CL)	Emasculated	46	44	44 (95.7%) (95.7% ± 5.86%)	42 (95.5%) (95.5% ± 6.13%)
Artificial geitonogamy (95% CL)	Emasculated	59	45	57 (96.6%) (96.6% ± 4.62%)	43 (95.6%) (95.6% ± 5.99%)
Artificial xenogamy (95% CL)	Emasculated	106	89	103 (97.2%) (97.2% ± 3.14%)	87 (97.8%) (97.8% ± 3.05%)
95% CL (totals)		211	178	(96.68% ± 2.42%)	(96.63% ± 2.65%)

based on the progeny genotype arrays of family, we infer the mating system of *Cymbidium goeringii* based on the previously estimated Wright's (1922) fixation index (F). The mean F in the study site was 0.330, indicating a substantial heterozygote deficiency (Chung & Chung 1999), and a similar trend was observed in 15 other populations examined in southern Korea. A significant deficit of heterozygosity may be indicative of inbreeding and/or population substructure (Hartl & Clark 1997). These deficits of heterozygosity are likely to be due to inbreeding via self-fertilization because *Cymbidium goeringii* is capable of self pollination. On the other hand, considerable heterozygote deficiencies could be due to a spatial Wahlund effect. Such an effect is expected if genetically divergent subpopulations are unwittingly included in a population sample and would be indicated by heterozygote deficiency coupled with significant intrapopulation spatial genetic structure. Although *Cremastra appendiculata* and *Cymbidium goeringii* have the potential for self pollination, the situation for *Cremastra appendiculata* may be different from *Cymbidium goeringii*. The fixation index estimated from multilocus allozyme genotypes of *Cremastra appendiculata* in the study site ($F = 0.049$, 95% confidence limits = $-0.063 - 0.157$, $N = 35$; M. Y. Chung *et al.* unpubl. data) was not statistically different from zero, indicating near random mating. To determine whether mating is truly random, further studies on pollination ecology at both daytime and night are needed.

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