Pollination biology of the deceptive orchid *Changnienia amoena*

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The pollination biology of *Changnienia amoena*, an endangered terrestrial orchid, was investigated at two sites in the Shennongjia Mountains, Hubei, central China. The results show that the orchid is exclusively pollinated by bumblebees. In Longmenhe, *Bombus* (*Diversobombus*) *trifasciatus* is the primary effective pollinator, whereas *B.* (*Tricornibombus*) *imitator* is the only pollinator in Guanmenshan. These two bumblebees can be treated as a functional group because they carry pollinaria on the same position on their bodies and have similar pollinating behaviours. The morphological traits of the flower adapt precisely to the pollinators. Visitation by bumblebees is mainly in the first half of the flowering period of *C. amoena*. Pollinarium removal and pollinia deposition take place when bumblebees withdraw from the flower. Crossing experiments show that *C. amoena* is a self-compatible and outcrossing species. Fruit set in this species is low and may result from limited pollinators because 87.5% of individuals set fruits under hand-pollination, but only 6–12% of individuals set fruits under natural conditions. These results imply that it is important to conserve the pollinator community together with the plants when conservation management for this endangered species is undertaken. Artificial pollination would also be a useful measure to facilitate restoration of the small populations. © 2006 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2006, **150**, 165–175.

ADDITIONAL KEYWORDS: adaptation – bumblebee – flowering phenology – fruit set – pollinarium removal – pollinia deposition.

INTRODUCTION

Orchidaceae is one of the most species-rich families of flowering plants, comprising an estimated 800 genera and over 25 000 species (Cribb, 2001a; http://www.kew.org/monocotChecklist/default.jsp). It is one of the few groups of angiosperm whose high diversity can be explained by its possession of a unique association with pollinating insects, which has resulted in extreme radiation (Nilsson, 1992; Gorelick, 2001). In fact, the orchid family is considered as an evolving pollinator-orientated taxon with highly elaborate and diverse interactions with their pollinators (Dressler,

1981, 1993; Nilsson, 1992; Romero, 1996; Soliva & Widmer, 1999). However, not all orchid lineages maintained high species diversity, and some lineages were only diverse for a time or were never diverse. For example, the tribe Calypsoeae includes only 35 species in nine genera, and is distributed in northern temperate and subtropical America (Dressler, 1993). Despite the small number of species, this tribe shows great diversity both in floral morphology and in its pollination system, including *Tipularia* species pollinated by noctuid moths (Stoutamire, 1978; Whigham & McWethy, 1980; Catling & Catling, 1991), *Aplectrum* by halictid bees (Hogan, 1983), *Corallorhiza* by syrphid flies or self-pollinating (Catling, 1990; Catling & Catling, 1991) and *Calypso* by bumblebees (Mosquin, 1970; Ackerman, 1981; Boyden, 1982; Andrews,

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1988; Proctor & Harder, 1995; Alexandersson & Ågren, 1996). Therefore, an exploration of the low species diversity associated with diversified pollination modes in this tribe becomes crucial and a challenge to the understanding of the evolutionary history of this group and of orchid diversity in general.

Changnienia Chien is a monotypic genus in the tribe Calypsoeae, whose sole species *C. amoena* Chien, is endemic to eastern and central China (Chen, 1999). Based upon gynostemium structure, Freudenstein (1994) suggested that *Changnienia* is a close relative to *Calypso*, *Tipularia* and *Yoania*. Morphologically, however, it is more similar to *Calypso*, another monotypic genus with a circumboreal distribution, in sharing an erect inflorescence with a single, relative large flower. Floral morphology varies greatly in *Calypso bulbosa* and four varieties have been recognized, namely *bulbosa*, *speciosa*, *americana* and *occidentalis*. Of these, *bulbosa* and *speciosa* are distributed in the Old World, whereas *americana* and *occidentalis* are confined to the New World (Cribb, 2001b). Although naive bumblebees are the pollinators of these four varieties, different varieties were pollinated by different bumblebee species (van der Cingel, 2001). The interactive morphologies of flower and pollinator might reflect the degree of flower specialization to the pollinator, and of flower adaptation to a new pollination environment (Nilsson, 1983). Based on investigation on the variation of flower size of *Calypso bulbosa* and the body size of its pollinators, Boyden (1982) suggested that the pollinator–flower fit was imprecise. In contrast, *Changnienia amoena* has a relatively uniform floral morphology and colour, and its pollinators are also native bumblebees according to our preliminary study (Sun, Luo & Ge, 2003). Therefore, it is of interest to understand the process and mechanism of pollination in this species. Until now, however, the pollination processes and functional morphological relationship between the bumblebees and *C. amoena* have been poorly understood.

In this study we observed the pollination biology of *C. amoena* at two sites in the Shennongjia Mountains, western Hubei, China, by characterizing its flowering phenology, interactive morphologies of flower and pollinator, visitation level of pollinators and fruit set. We addressed the following problems: (1) the pollinator species and pollination process; (2) the attracting mechanism of bumblebees; (3) whether the pollinator and flower fit imprecisely or precisely; (4) the mating system and fruit set level. By comparing the pollination modes of *C. amoena* and *Calypso bulbosa*, we hope to provide some insights into the diversity of the two species. Such information might also provide suggestions for the restoration and conservation management of *C. amoena*, which is listed as an endangered species in China (Fu, 1992; Xiong *et al.*, 2003).

MATERIAL AND METHODS

Changnienia amoena is mainly restricted to the mountainous region in eastern and central China (Chen, 1999). It grows in relatively nutrient-rich, partially shaded to moist habitats in mixed deciduous/evergreen forests at altitudes up to 1800 m (Chen, 1999; Xiong *et al.*, 2003). Our study was carried out from March to April in the southern part of the Shennongjia Mountains, Hubei Province (31°21′N, 110°03′E), in 2002 at one site and in 2003 at two sites. The first site, Longmenhe, was located at an altitude of 1290 m. At this site, seven populations were selected on the slopes of different hills. They are separated from each other by a distance of 0.8–2 km. The second site, Guanmenshan, was about 15 km northeast of Longmenhe at an altitude of 1275 m. At this site, three populations were chosen along a stream. They are separated by two woodlands strips (about 280 and 400 m wide, respectively).

The weather at Shennongjia area is cold and humid in early spring. The annual average temperature is 10.6 °C. The coldest month is January with a mean temperature of –0.8 °C; the hottest is July with a mean of 21.1 °C. Annual rainfall is about 1402 mm, with more than half of the total in spring and summer (all data from the weather station of the Biodiversity Station of the Chinese Academy of Sciences). The vegetation of the two sites is secondary deciduous woodland mixed with a few evergreen trees and shrubs. In early spring, the understorey of the woodlands was bright. Concurrently blooming entomophilous species within the *C. amoena* community were not rich; only a few species were found in the vicinity, including *Mahonia bealei* (Fort.) Carr, *Corydalis edulis* Maxim, *Viola gryroceras* A. Gray, *Glechoma biondiana* (Diels) C. Y. Wu & C. Chen var. *glabrescens* C. Y. Wu & C. Chen, two species of *Primula* and one species of *Prunus*.

Observations were made at Longmenhe in 2002, and at both Longmenhe and Guanmenshan in 2003 with the help of trained assistants. Every flowering individual in all populations was visited daily and the date of flower opening and wilting was noted. A flower was judged as 'opening' when the dorsal petals became erect, and visiting insects could enter the flower, and a flower as 'wilting' when its colour changed from purplish-pink or pinkish-white to brown, or its sepals and petals changed from erect and hard to collapsed and soft.

Pollinators were defined as those that not only visited *C. amoena* but also carried its pollinaria, and visitors as those that visited the flowers but did not remove the pollinaria on their bodies. Behaviour of pollinators was observed in detail, including their approach, alighting, entering and leaving, as well as

the time they spent in the flower (recorded by using a stopwatch). Some insects were trapped that directly visited *C. amoena* or frequently appeared in the observation areas for identification. Collected insect specimens were identified by entomologists from the Institute of Zoology, CAS, and the vouchers are lodged at the Herbarium (PE).

In *C. amoena*, the pollen is packaged into four large pollinia. Pollinarium removal and pollinia deposition were readily identified and scored in the field, and could be regarded as an index of effective pollinator visitation. We visited each population on every workday during the whole flowering period, and recorded the number of plants that had pollinaria removed from the anther, and the number of plants that pol-

linia were deposited onto the stigma. In late August, the number of plants that produced a mature fruit was counted.

Morphological measurement was carried out for flowers and pollinators in 2003. Proboscises of collected pollinators were measured immediately after they were captured. The width and length of the head, and the width and height of the mesothorax were measured in the laboratory on pinned specimens by use of a vernier calliper. Twenty plants from each site were randomly selected for measurement of floral morphology in the field. The flower of *C. amoena* has five sepals and petals, and a three-lobed lip (Fig. 1A, B). The side-lobes of the lip are triangular, erect and incurved, and their tips are overlapped more or less over the front of
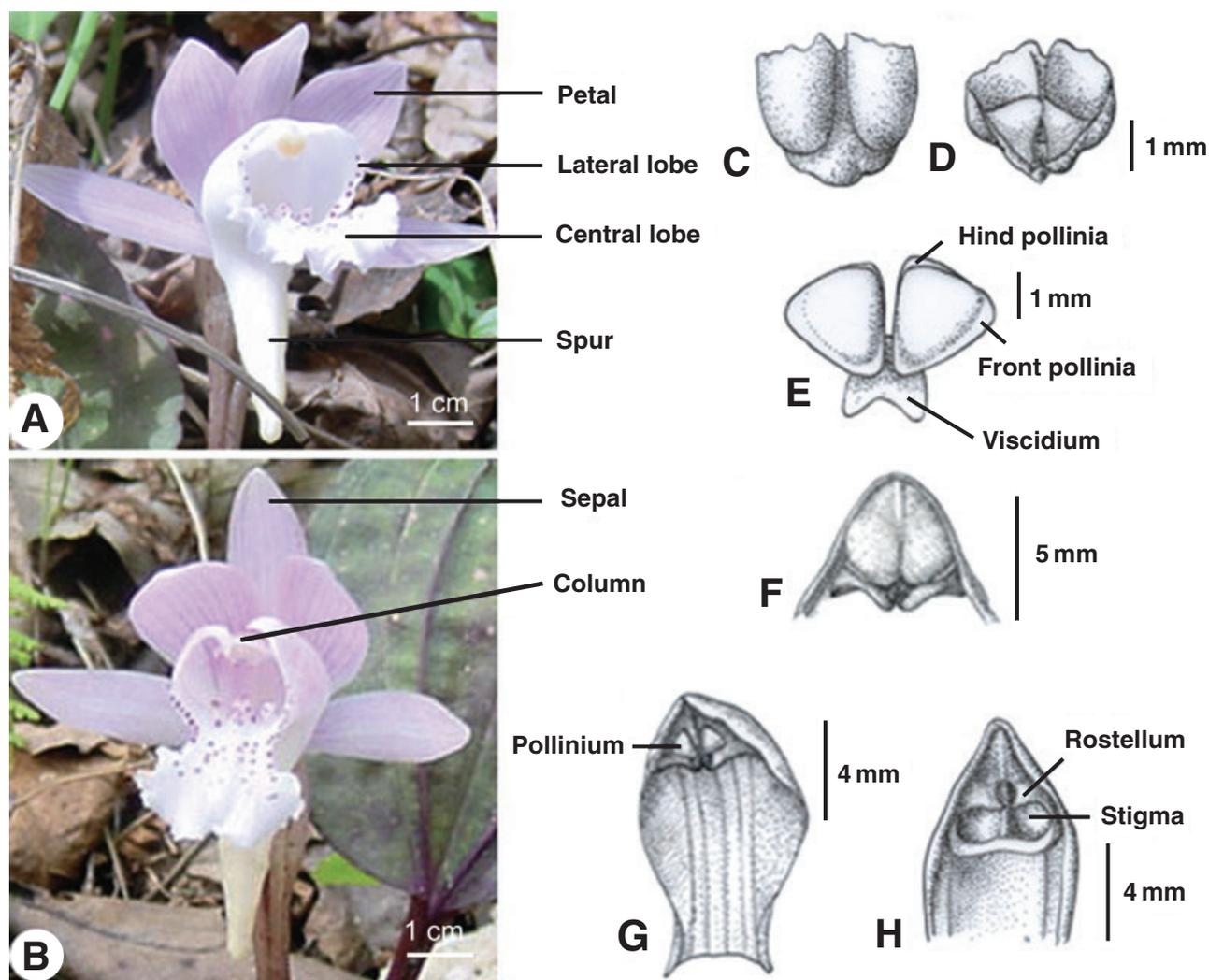

Figure 1. Floral morphology of *Changnienia amoena*. A, individual showing yellow pollinarium covered with the cap. B, individual showing pollinarium removed. C, front view of cap. D, back view of cap. E, front view of pollinarium showing the two pairs of pollinia attached to a common viscidium. F, front view of the column tip with the cap in place. G, front view of the column showing pollinarium with cap removed. H, upward view of column showing the stigma.

the convex column (Fig. 1F,G). Thus, an 'entrance' is formed with the side-lobes at either side, the convex column at the top and the central lobe at the bottom. Sepal and petal width was measured perpendicular to the main vein at the widest point. Sepal and petal length was measured along the main vein from the basal part to the apex. The width of the side-lobes of the lip was measured as the width of 'entrance', while the vertical distance between the tip of column and the upper surface of the central lobe of the lip as the height of 'entrance'. The spur length was measured from its junction with the expanded portion of the lip to the apex, and the width was measured at the transverse of this junction.

We conducted four experiments to investigate the breeding system of *C. amoena* in one population at Longmenhe that was not used for pollinator observation in 2002. Four pairs of individuals were randomly chosen on the first day of anthesis, and each flower was bagged using fine mesh nets followed by the following treatments: (1) cross-pollination of the flowers with the pollinia of the paired plants; (2) self-pollination of the flowers with their own pollinia; (3) no pollination after bagging; and (4) open pollination without treatments as the control. Fruit set was counted in late August.

FLORAL MORPHOLOGY

In early spring, the plant of *C. amoena* produces a solitary flower at the top of the stem up to 10 cm high. The flower is showy with five sepals and petals of a purplish-pink to pinkish-white, and a three-lobed lip (Fig. 1A, B). The mean length and width of lateral sepals, central sepal and petals are 29.3 mm (SD = 2.6, $N = 20$) and 8.2 mm (SD = 1.1, $N = 20$), 29.7 mm (SD = 2.4, $N = 20$) and 9.8 mm (SD = 1.3, $N = 20$), and 28.3 mm (SD = 1.8, $N = 20$) and 14.3 cm (SD = 1.7, $N = 20$), respectively. The base of the lip prolongs into a horn-like spur with no nectar (Fig. 1 A and B). The spur is drooping, arching downwards, and forms an acute angle with the lip (Fig. 1A, B). The lip bears three prominent calli near the spur mouth (Fig. 1A, B). The operculate anther contains two yellow pollinaria covered by a cap (Fig. 1A, C, D, F). The pollinarium consists of two pairs of flat, waxy pollinia (one small and one large pollinium in each pair) that are attached to a common sticky viscidium (Fig. 1E). The stigma is located near the top of the column and below the rostellum. It is elliptic and concave with a sticky secretion (Fig. 1G, H).

FLOWERING PHENOLOGY

The flowering period of *C. amoena* started around the end of March and proceeded for 2–3 weeks until the
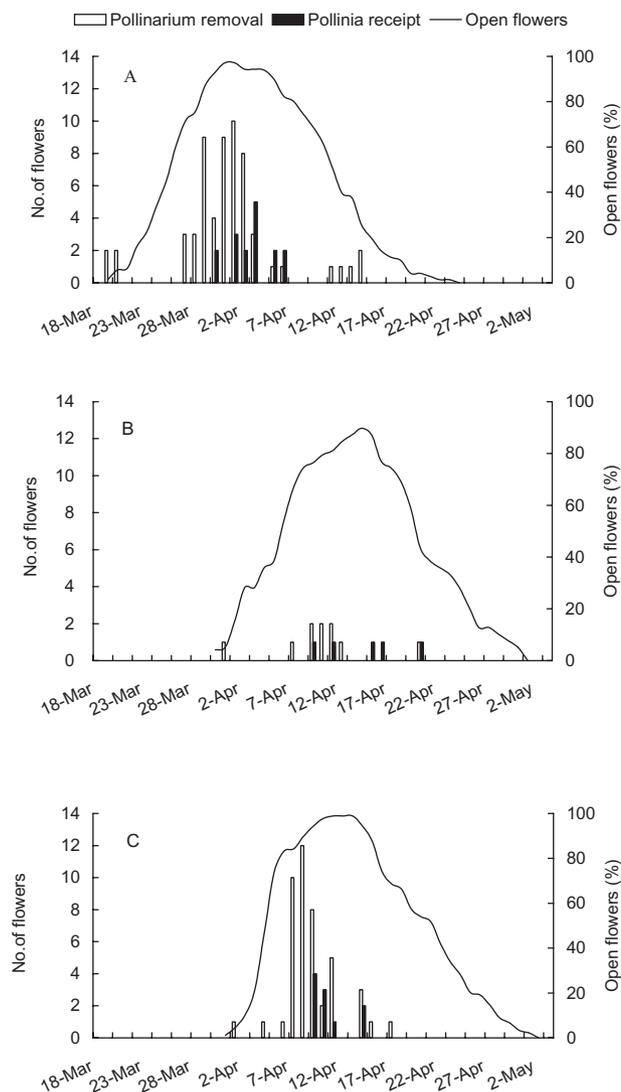

Figure 2. Pollinarium removal, pollinia receipt and number of open flowers during the anthesis of *Changnienia amoena* at Longmenhe in 2002 (A, $N = 76$) and 2003 (B, $N = 43$), and at Guanmenshan in 2003 (C, $N = 92$).

end of April (Fig. 2). All flowers finished blooming when the new leaves of the deciduous trees and shrubs had not fully developed and the understorey of woodlands was still light. The anthesis of an individual flower lasted about 2–3 weeks. Pollinated flowers withered about 1 week earlier than the flowers that were not pollinated. The flowers released a sweet fragrance under direct irradiation or high temperature in the daytime at early anthesis, but the odour was absent at night and on overcast or rainy days. The scent disappeared at later anthesis periods. On average, 5–10% of the flowers were damaged by herbivores and other unknown reasons.

FLOWER POLLINATORS AND THEIR VISITATIONS

A few visitors were found on *C. amoena*, although 15 species of anthophilous insects were found at the two sites (Table 1). *Apis cerana* (Fabricius) and *Lasioglossum* sp. were observed flying out of the entrance of flowers, but neither carried pollinaria. Bumblebees were the exclusive pollinators of *C. amoena* with only one species, *Bombus imitator*, at Guanmenshan, and two species, *B. trifasciatus* and *B. imitator*, at Longmenhe (Table 1; Fig. 4). At the former site, we captured 18 individual bumblebees, and two of them were captured on the flowers of *C. amoena*, 12 on flowers of *Corydalis edulis* and four on other neighbouring plants. The two bumblebees that were captured on *C. amoena* and six bumblebees on *Corydalis edulis* had attached pollinaria of *C. amoena*. At Longmenhe, ten individuals of *B. trifasciatus* were collected, with four of them on the flowers of *C. amoena*, two on flowers of *Corydalis edulis* and four on other neighbouring plants. We found the pollinaria of
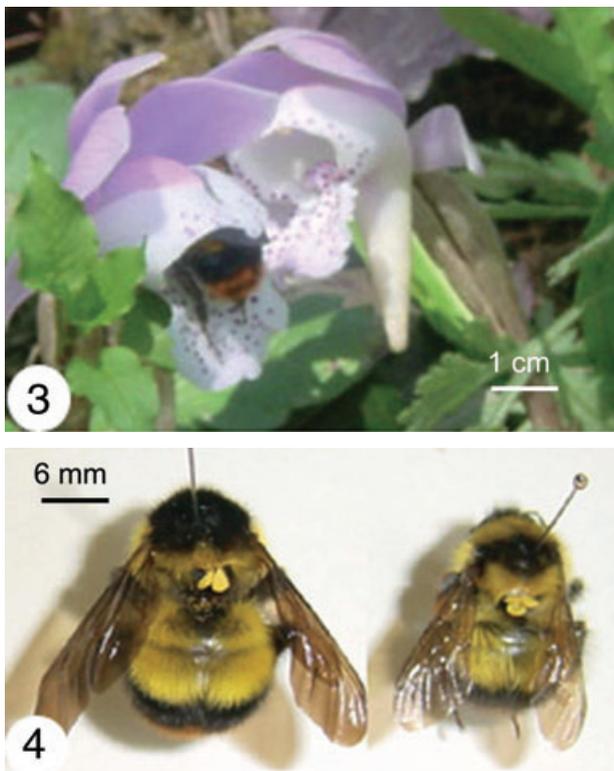
Figures 3 and 4. Pollinators visiting *Changnienia amoena* flowers. Fig. 3. *Bombus* (*Diversobombus*) *trifasciatus* probing into *C. amoena*. Fig. 4. The primary pollinator, *B. trifasciatus*, in Longmenhe (left) and the only pollinator, *B. imitator*, in Guanmenshan (right), showing pollinarium of the orchid attached on the thorax (to the same scale).

C. amoena on two individuals of *B. trifasciatus* that were collected on the flowers of *C. amoena* and one individual that was collected on the flower of *Corydalis edulis*. In addition, two individuals of *B. imitator* were captured in Longmenhe, one on the flower of *C. amoena* and the other on *Corydalis edulis*. However, neither carried the pollinaria of *C. amoena*. Taken together, of the 28 captured individuals of the two bumblebee species, only 11 (39%) carried the pollinaria of *C. amoena* on their bodies, indicating that the bumblebees visited *C. amoena* infrequently. We recorded seven visitations during 150 h of observation at Longmenhe, and two visitations during 39 h of observation at Guanmenshan.

Visitations of bumblebees to *C. amoena* occurred mostly within the first half of its flowering period near the peak flowering time (Fig. 2). In 2002, visits by bumblebees were mainly between 27 March and 1 April, when 85% of the flowers were open (Fig. 2A). In 2003, the maximum number of visitations was from 7 to 12 April at Longmenhe, and 6 to 11 April at Guanmenshan, when 75 and 85% of the flowers were blooming, respectively (Fig. 2B, C). Approaching the end of anthesis, we observed only *B. imitator* patrolling the communities of *C. amoena*, but did not document any visits to *C. amoena*.

BEHAVIOUR OF POLLINATORS

Bumblebees usually appeared on fine or warm days, few on rain or on overcast days. Visits of bumblebees occurred mainly between 11:30 and 16:00 h. Their visits to *C. amoena* were infrequent and very short, with an average of 10.3 s (SD = 1.4, $N = 9$). Based on the observed visitations, we found that *B. trifasciatus* came into the area of *C. amoena* in fast flight, stopped for 1–2 s at about 7–8 m above the ground, and then alighted unhesitatingly and directly on the flower of *C. amoena*. As soon as the bumblebee landed on the central lobe of the lip, it entered the flower and probed into the spur cavity (Fig. 3). The bumblebee stayed on the flower for a short time and then retreated from the flower. During the visit, the pollinarium was attached on the body of bumblebees, coupling with the anther cap falling. Among the seven observed visitations, on three occasions we found that *B. trifasciatus* turned to neighbouring orchids after they left flowers of *C. amoena*.

Compared with *B. trifasciatus*, *B. imitator* has low flying height while foraging, no more than 50 cm above the ground. It flew slowly in an indefinite direction, i.e. following an 'S' pattern or a right and left pattern. When *B. imitator* flew near the flowers of *C. amoena*, it also landed directly. The subsequent behaviour of *B. imitator* on the flower was similar to that of *B. trifasciatus*. After leaving the flower,

Table 1. Insects recorded as flower-visitors to *Changnienia amoena* (L: Longmenhe; G: Guanmenshan)

Flower-visitor	Localities	No. of insects captured on *C. amoena*	No. of insects captured on other flowers	No. of insects captured except on flowers	Total no. captured insects	No. of insects with pollinarium	No. of pollinaria
Diptera							
Eristalis tenax (lin.)	L/G	0/1	1/2	0/1	5		
Bombylius majar Lin.	L/G			0/1	1		
Bombylius sp.	L			1	1		
Hymenoptera							
Anthophora plumipes (Pallas)	L		1		1		
Apis cerana (Fabricius) ♀ ♂	L/G	1/4	2/2	1/2	12		
Bombus (*Diversobombus*) *trifasciatus* Smith ♀	L	2	2	4	8	3	12
B. (*Tricornibombus*) *imitator* Pittioni ♀	L/G	1/2	1/12	0/4	20	0/8	0/24
B. (*s. str.*) *ignites* Smith ♀	G		2		2		
Ceratina sp. ♀ ♂	L/G		1/1	0/1	3		
Lasioglossum sp. ♀	L/G	0/1	0/2		3		
Lasiogossum yunnanense Fan *et* Wu	G		4		4		
Osmia cornifrons Rad. ♀ ♂	G		3		3		
Tetralonia chinensis Sm. ♀	L		1		1		
Xylocopa appendiculata Sm. ♀	G		2		2		
X. rufipes Sm. ♀ ♂	L		1		1		
Total		16	40	11	67	11	

B. imitator also turned to other neighbouring orchids to continue its exploration.

POLLINATION PROCESS

Based on our direct observation of bumblebees visiting the flowers of *C. amoena* and the position of the pollinarium on the body of pollinators, the pollination process is described as follows. As a flower-visiting bumblebee landed on the central lobe of the labellum, its front entered the flowers (Fig. 3). When the bumblebee was probing the spur cavity, the tip of the convex column of the flower exactly fitted the dorsal crevice between the mesothorax and the abdomen of the insect (Fig. 5A). The acute angle between the slanting downward spur and the sloping central lobe of the lip made the bumblebee arch its body, and thus the hind edge of the mesothorax rose up and reached a position towards the anther and stigma when the bumblebee lowered its head to probe into the cavity of the spur (Fig. 5A, E). After a brief and unrewarding exploration, the bumblebee withdrew from the flower. When it retreated, the hind hairless edge of the mesothorax contacted the viscidium and removed the pollinarium from the anther (Fig. 5B, E). The pollinarium attached on the hind edge of the mesothorax originally pointed to the back (Figs 4, 5C). If a bumblebee carrying a pollinarium revisited a flower (Fig. 5D), the pollinia changed their direction passively and pointed towards the stigma as the hind edge of the mesothorax rose up when the insect lowered its head to probe deep into the spur (Fig. 5A, E). As the bumblebee withdrew from the flower, the pollinia were stuck on the stigma, and pollination was completed. Synchronously, the pollinaria on the anther, if still present, would attach on the hind hairless edge of the mesothorax, almost in the same position as where the pollinarium was attached to the body before the visitation (Fig. 5F).

FUNCTIONAL MORPHOLOGY

When bumblebees landed on the central lobe of the lip of *C. amoena* in search of the spur cavity, the front half part of their body entered through the flower entrance that was formed by the three-lobed lip and the convex column (Fig. 3). A morphometric comparison between *C. amoena* and its pollinators is presented in Table 2. The mean width and height of the flower entrance are 9.8 mm (SD = 1.5, *N* = 20) and 17.6 mm (SD = 1.1, *N* = 20), respectively, and the mean width and height

of the mesothorax of bumblebees are slightly less than those of the entrance (Table 2). The mean length of the spur was longer than that of the proboscis plus the head of bumblebees. The mean width of the basal spur (spur mouth) is 7.6 mm (SD = 1.8, N = 20), wider than that of the bumblebee's head, but narrower than that of the mesothorax (Table 2). This means that a bumblebee can insert its proboscis as well as its head into the spur cavity to touch the bottom of the spur, but the slightly broad mesothorax hinders the whole body of the bumblebee from entering the spur. These data indicate that the floral structure of *C. amoena* is adapted precisely to its pollinators.

MATING SYSTEM AND FRUIT SET

After pollination, the flower stalk of *C. amoena* became green and elongated, which lasted about
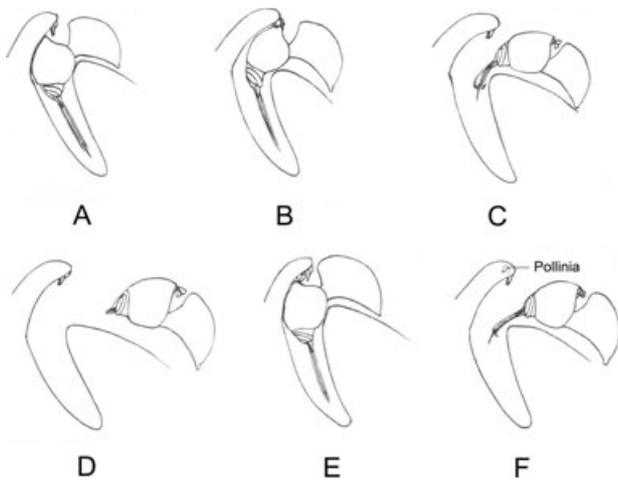

Figure 5. The pollination process of *Changnienia amoena* by a bumblebee (for details see text). A. The bumblebee probes into the spur cavity. B. The thorax of the bumblebee touches the viscidium. C. The bumblebee removes pollinarium off the anther. D. The bumblebee carrying pollinarium visits the orchid. E. The bumblebee probes into the spur cavity with pollinia pointing toward the stigma. F. Pollinia are smeared onto the stigma and pollinarium is removed from the anther and attached to the mesothorax of the bumblebee.

$1^1/_2$ months. When the stalk stopped elongating, the ovary began to swell and developed into a fruit. The stalk elongation could thus be considered an indication of pollination success. Our experiments indicated that autogamy did not occur in the species because no individuals that were bagged and unpollinated (N = 8) exhibited stalk elongation. In artificial cross- and self-pollination, all stalks of the treated flowers showed elongation, indicating that this species is self-compatible. However, several flowers with elongated stalks failed to develop into the fruit due to rain or herbivore damage. Under natural conditions, the percentage of elongated stalks was low, 24 and 15% at Longmenhe in 2002 and 2003, respectively, and 12% at Guanmenshan in 2003 (Fig. 6). The rain or herbivore damage varied between years and sites. At Longmenhe, 22% of the pollinated flowers were destroyed in 2002 and 33% in 2003. At Guanmenshan this proportion was 10% in 2003 (Fig. 6). Therefore, the average fruit set was very low, only 20 and 9% at Longmenhe in 2002 and 2003, respectively, and 11% at Guanmenshan (Fig. 6).

However, the number of pollinaria removed was much higher than that of the pollinia deposited on stigmas and than that of flowers with fruit set (Fig. 6). At Longmenhe, the pollinarium of 82 and 42% flowers has been removed in 2002 and 2003, respectively, and
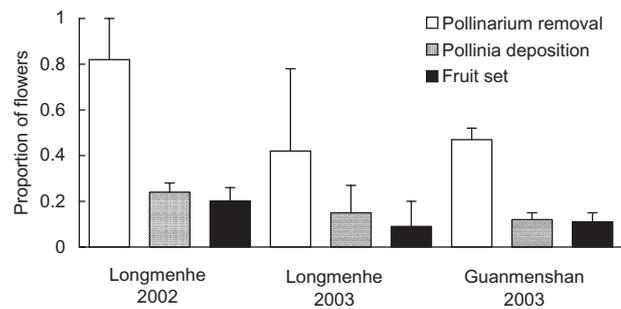

Figure 6. Proportion of plants that have their pollinarium removed, received pollinia and that produced a mature fruit (mean ± SE) in two sites of *Changnienia amoena* (Longmenhe in 2002: N = 76; Longmenhe in 2003: N = 43; Guanmenshan in 2003: N = 92).

Table 2. Morphometry of functional traits in *Changnienia amoena* and its pollinators (mm; mean ± SE)

Taxon	N	Width of the entrance/ mesothorax	Height of the entrance/ mesothorax	Length of the spur/ proboscis + head	Basal width of the spur/ width of head
Changnienia amoena	20	9.8 ±1.5	15.6 ±1.1	20.7 ±1.7	6.34 ±0.11
Bombus trifasciatus	6	6.97 ±0.33	6.97 ±0.33	16.6 ±1.1	5.03 ±0.05
Bombus imitator	12	6.526 ±0.82	6.526 ±0.82	14.3 ±0.6	4.73 ±0.1

at Guanmenshan the proportion was 47%. Consequently, on average, 60–75% of the flowers in which the pollinarium was removed had not been pollinated successfully.

DISCUSSION

Among 15 species of anthophilous insects present in the *Changnienia amoena* community, only bumblebees were found to act as pollinators. The primary effective pollinators are *B. trifasciatus* at Longmenhe and *B. imitator* at Guanmenshan, but the pollinarium was attached on the same position of the insect body and the behaviours of the two bumblebees on flowers of *C. amoena* were essentially the same. Therefore, the two bumblebees could be considered as a functional group (Waser *et al*., 1996; Armbruster *et al*., 1999; Armbruster, Fenster & Dudash, 2000; Fenster *et al*., 2004). In other words, *C. amoena* is an orchid with a specialist pollination system.

Changnienia amoena supplies no reward to its pollinators, and thus is pollinated by deception. Because this species blooms before the trees and shrubs have developed new leaves, its flower is conspicuous despite its short stalk. Bumblebees are normally attracted to flowers by optical cues, i.e. colour and size (Boyden, 1982; Giurfa & Lehrer, 2001), and purplish-pink is one of the most attractive colours to bumblebees (Heinrich, 1975). The flower colour of *C. amoena* falls into the range of the optical maximum sensitivity of bumblebees. The showy floral display of *C. amoena* therefore effectively attracts browsing bumblebees from a distance. Moreover, the flowers of *C. amoena* bloom aggregately and are highly attractive to bumblebees; therefore, as suggested by Keasar (2000), nectarless flowers with synchronized and aggregated flowering patterns could attract bumblebees more effectively.

For orientation close-in, bumblebees rely on scent to adjust their approach and landing (Boyden, 1982; Raguso, 2001). For close attraction of pollinators, the coloured spots on the upper surface of the lip are considered as false nectar guides, and can stimulate visiting and food-searching reactions (Nilsson, 1980). In *C. amoena*, the floral fragrance is only perceptible at a close distance during the whole flowering process. The mauve spots on the upper surface of the lip, however, are conspicuous and could stimulate the food-searching reactions of bumblebees more effectively than does the fragrance, which may be supported by the direct approach and landing of the bumblebees on the lip of *C. amoena*.

Some deceptive orchids attract insects to pollinate by mimicking the co-flowering rewarding flowers in the community (Dafni, 1986; Nilsson, 1992). In our study, however, there is no evidence that a model for visual mimesis by *C. amoena* is present. The concurrent blooming plants, light-yellow inflorescence of *Mahonia bealei* and spurred purple inflorescence of *Corydalis edulis*, which were frequently visited by bumblebees, are different from the flowers of *Changnienia amoena* in size, shape and odour. The magnet-species theory suggests that a nectarless plant could benefit from growing in the vicinity of nectar-containing species (Thomson, 1978; Rathcke, 1983; Laverty & Plowright, 1988; Laverty, 1992; Lammi & Kuitunen, 1995). *Changnienia amoena* often grows within or near the community of *Corydalis edulis*, a nectar-bearing species that is frequently visited by bumblebees during the anthesis of the orchid. Whether it is magnet species for *C. amoena* requires further study.

The average fruit set of *C. amoena* ranged from 9 to 20% and is comparable with other non-rewarding orchids (Montalvo & Ackerman, 1987; Gill, 1989; Zimmerman & Aide, 1989; Calvo, 1990, 1993). Compared with 87.5% fruit set by hand-pollination, low fruit set in natural conditions is considered to be an attribute of pollinator limitation. This is consistent with other deceptive plants (van der Cingel, 1995; Alexandersson & Ågren, 1996). Although 42–82% of flowers had their pollinarium removed, 60–75% of them had not been successfully pollinated. This result suggests that few re-visitations (pollination) take place although there are relatively high first visitations to *C. amoena*. Besides pollinator limitation, additional factors may contribute to the low efficiency of pollination of *C. amoena*. For example, the food-deceptive plants could not sustain foraging pollinator interest (Peakall & Beattie, 1996); floral fragrance, colour and form perhaps cue bumblebees to avoid revisiting the deceptive flower (Boyden, 1982); even odours left by previous visitors on the flowers might make bumblebees avoid repeatedly visiting the same non-rewarding flower (Schmitt & Bertsch, 1990; Valterova & Urbanova, 1997; Raguso, 2001; Stout & Goulson, 2001).

Based on limited morphological features, Freudenstein (1994) considered that *Changnienia* was closely related to the genera *Calypso*, *Tipularia* and *Yoania*. In the tribe Calypsoeae, both *Changnienia* and *Calypso* have a large and showy single flower, while the remaining genera have a raceme with laxly spaced small flowers. However, these two genera are different in the mechanism of pollinator attraction. Gumprecht (1977), cited by van der Cingel (1995, 2001), claimed that *Calypso bulbosa* var. *bulbosa* was doubly deceptive, the pollinator first deceived by the yellow hairs on the lip which were assumed to be pseudostamens, and then by false nectar spurs. This hypothesis was doubted by Boyden (1982) because there was no direct observation of bumblebees on *Calypso* flowers and no study had been conducted to compare the attractive or deceiving efficiency between different varieties (e.g.

var. *americana* with yellow hairs on the lip and var. *occidentalis* without yellow hairs). However, Ackerman (1981) and Cribb (2001b) described that there were hairs on the lip of var. *occidentalis*. Stoutamire (1971) hypothesized that the *Calypso* flowers deceived the bumblebees by conspicuous coloration. Whether *Calypso* flowers attract the bumblebees by yellow hairs on the lip or by conspicuous coloration of flowers, the bright colour could be considered as the main attraction mechanism of this species. By contrast, the attraction mechanism of *C. amoena* is mainly the large and showy flowers with a horn-like spur rather than coloration of flowers. *Calypso bulbosa* is a circumboreal species, inhabiting coniferous woods (Stoutamire, 1971; Ackerman, 1981; Alexandersson & Ågren, 1996; Cribb, 2001b), whereas *C. amoena* is distributed in the subtropical zone, growing in deciduous woodlands mixed with evergreen trees and shrubs at relatively low elevation (Xiong *et al.*, 2003). In early spring, during the flowering time of both species, the understorey of coniferous woods is certainly darker than that of deciduous woodlands. Therefore, we speculate that the bright colour of *Calypso* flowers is an adaptation to the relatively dark habit for attracting pollinators.

The pollinator–flower fit of *Calypso bulbosa* var. *americana* was considered as imprecise because of the variation in both pollinator size and flower size (Boyden, 1982). Stoutamire (1981) reported that queens of four *Bombus* species and females of *Psithyrus crawfordi* were the pollinators of *C. bulbosa* var. *occidentalis* at one location and queens of seven different *Bombus* species at other localities, implying that the variety has not developed an optimal pollination system. Nilsson (1983) suggested that a difference in pollinator faunas of *Orchis ascula* between biotopes indicated that the orchid is anthecologically functional with alternative pollination environments, and the post-glacial colonization by the orchid of new pollination environments is too recent in evolutionary time for a complete shift to specialized pollinators. Similar to *O. mascula* in Central and North Europe, we speculate that the post-glacial evolution of pollination races has not taken place in the circumboreal *Calypso*. The disparate and disruptive selection exerted by different pollinators on the population of *Calypso* could create and preserve a series of genotypes in a state of polymorphic equilibrium. By contrast, *C. amoena* in the centre of China suffered less from glacial movement (Ying, 2001; Zhang, 2004), and its pollination environment is relatively stable. During its long evolutionary period, *C. amoena* developed a stabilized pollination system and a precise pollinator–flower fit relationship.

Many plant species have been at risk of extinction because of direct or indirect human activities (Schemske *et al.*, 1994; Young, Boyle & Brown, 1996; Kwak, Velterop & van Andel, 1998). Plant–pollinator interactions should not be neglected when determining protective measures for endangered plants, independent of whether they have a generalist or specialist pollination system, for ensuring their sexual reproductive success (Ashworth *et al.*, 2004). As an endangered species listed in the Chinese Red Book (Fu, 1992), *C. amoena* is confined to central China. Recent expeditions have shown that the extent and density of extant *C. amoena* populations have decreased gradually because of decades of destruction of habitat from agriculture and silviculture, and because of its overcollection for medicinal products (Xiong *et al.*, 2003). The present study shows that rewardless *C. amoena* is exclusively pollinated by local bumblebees, indicating that these insects are crucial for the reproduction, maintenance and restoration of this orchid species. Therefore, it is necessary to conserve the pollinator community together with food-resource plants around the populations when conservation management is undertaken for this species. Moreover, appropriate artificial cross-pollination would be helpful for increasing the reproductive efficency and increasing the fruit set, and eventually enhancing the recovery of this endangered orchid.

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