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# A Preliminary Study on Pollination Biology of an Endangered Orchid, *Changnienia amoena*, in Shennongjia

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**Abstract**: *Changnienia amoena* Chien is a monotypic species and endemic to China, and was listed on the Chinese Red Book in 1992. The species was once abundant but has become rare and endangered in recent years because of the habitat fragmentation and unduly commercial collections. Previous observation showed that this species has very low and even no fruit set, and the pollinators are not observed before this report. The present observation was conducted at the Shennongjia, Hubei Province during the spring of 2002. The results showed that *Bombus* (*Diversobombus*) *trifasciatus* Smith ,¡*B. imitator* Pittion and one species of *Apis* visited the flowers of the orchid, but only *B. trifasciatus* could carry pollinaria on its body and was the legitimate pollinators of *C. amoena*. During 113 h of observation, only nine visitations were recorded. The bumblebees mainly appeared during 12:00-15:00 during the day. Bumblebees stayed in a flower only a few seconds and never more than ten seconds. The flowers would persist in fresh for about three weeks when they were not pollinated, but 3 or 4 d after pollinated, the pollinated flowers underwent a series of color and morphological changes including stalk elongation and ovary swelling. Therefore, stalk elongation can be considered an index of fruits set. Artificial pollination indicated that *C. amoena* is a highly self-compatible and outcrossing species, but dependent on pollinators for fruit set. Based on the field observations, we concluded that pollination system of *C. amoena* is deceptive. The fruit set in nature is not very low (26.98% on average) compared to other deceptive orchids, which may be related to small population sizes. The number of pollinia removal is much higher than that of fruit set, indicating that there are some degrees of pollinia wasting in *C. amoena*.

**Key words**: *Changnienia amoena* ; *Bombus* (*Diversobombus*) *trifasciatus* ; deceptive pollination; stalk elongation

Orchidaceae is one of the largest families of flowering plants, with an estimated 800 genera and conservatively 20 000 species (Cribb, 2001). Orchids are considered as a rapidly evolving pollinator-oriented family, and rely on mechanical or ecological factors for barriers to hybridization, such as different pollinators, different microsites on the same pollinator, and different phenologies (Romero, 1996). *Changnienia* is a monotypic genus of the tribe Calypsoeae, Orchidaceae (Dressler, 1993). The species *Changnienia amoena* is endemic to China, and was listed on the Chinese Red Book in 1992 (Fu, 1992). Although investigation on the biology, ecology, phenological and reproductive characters of this species has been undertaken (Wang *et al.*, 1994; Xiong *et al.*, 2003), the information on the pollination biology of this species is still limited. Previous Sobservation showed that this species has very low and even no fruit set (Wang *et al.*, 1994; Xiong *et al.*, 2003). Wang *et al.* (1994) speculated that this phenomenon is attributed to the unique flower structure and the lack of suitable visiting insects.

Generally, orchids are pollinated mainly by insects and birds with some of them being auto-pollination (Catling, 1990). Most orchid species provide only nectar or oil as a reward, and many advertise false rewards, reaching pollination through deceit. Within the tribe Calypsoeae there exists great diversity in terms of pollination system. For example, *Tipularia* is pollinated by noctuid moths, *Aplectrum* by halictid bees, *Corallorhia* by syrphid flies, but *Calypso* offers no rewards and deceives bumblebees by the clump of anther-like hairs on the lip (Dressler, 1993). In orchids, particularly in deceptive ones, a large proportion of individuals has no fruit set at all and a minority contributes the population’s fruit set (Nilsson, 1992). In other words, the average fruit set of orchids is generally low. For instance, the fruit set of a food deceptive orchid, *Hemipilia flabellata*, is 12% (Luo and Chen, 1999). Therefore, it is possible that the low fruit set in *C. amoena* is related to its pollination system. In the present paper, we report our preliminary survey on the pollination biology of this species.

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## 1 Materials and Methods

*Changnienia amoena* Chien is mainly restricted to the mountainous region in the eastern and central parts of China. The plants grow primarily at moist or shady place in the forest with the elevation no more than 1 800 m. The species was once abundant but its number has been greatly reduced in recent years because of the habitat fragmentation and unduly commercial collections. The present observation was conducted at the Shennongjia, Hubei Province during the spring of 2002 (Table 1).

Of the total seven populations, three populations were chosen for observation, two of which (populations 1, 2) were located at the garden of the Biodiversity Station of The Chinese Academy of Sciences where the orchids were transplanted from the field two years ago. In another three populations (populations 3-6), the numbers of plants and flowers, the flowers with pollinia removed, and the flowers pollinated were recorded in the field (Table 1). In the last population (population 7), the breeding system was investigated following Dafni's method (1992). For this population, three experiments were designed: (1) five plants were bagged before flowering with fine mesh nets to test whether this orchid needs pollinators or not; (2) six flowers were emasculated, then pollinated with the pollinia from different flower; and (3) seven flowers were pollinated with the pollinia from the same flower to evaluate self-compatibility.

## 2 Results and Discussion

### 2.1 Floral traits and flowering phenology

The floral morphological traits in all populations showed variation. The color of sepals and petals varied from purplish-pink to pinkish-white. The leaf color was green in upper-side and purple lower side, or purple on both sides. The stalk height varied from 4.8 to 13.5 cm. The labellum of *C. amoena* was 3-lobed with purplish-red spots and the side-lobes erect and incurved at the front tip over the convex column. The side-lobes, mid-lobe and the convex column formed an entrance, which guides pollinators into flowers. The size of the entrance varied from 7 to 16 mm in height and from 1.2 to 2.0 cm in width. No nectar was found in the spur at all populations. However, we found that the flowers released perfume-like odor from about 9:30 am to 5:30 pm during the day when air temperature was high, but the odor disappeared beyond this period and on overcast or rainy day.

The longevity of flowers of *C. amoena* under natural condition varied among different flowers, but as a whole, there were two cases. One was that when pollinia were untouched or removed by bumblebee, the flower would persist in fresh for about three weeks until they wilted. The other was that *C. amoena* flowers underwent a series of color and morphological changes in three or four days after pollinated. The flower with originally expanding and fresh sepals and petals and an ovary that projected out from the stalk gradually developed a drooping ovary that approached the stalk, and sepals and petals that became brownish-yellow and collapsed over the column and lip. Sequentially, the light-brown stalk became green and elongated, which was different from those in *Calypso* (Proctor and Harder, 1995). As the stalk elongated, the flower rose again until it stood almost upright to the stalk, but its petals and sepals remained collapse over the lip and withered gradually. Eventually, the stalk stopped elongating, and the ovary began to swell.

### 2.2 Flower visitors, pollinators and their behavior

Two species of bumblebee, queen of *Bombus* (*Diversobombus*) *trifasciatus* Smith and *B.* (*Tricornibombus*) *imitator* Pittion, and one species of *Apis*, were captured on the flowers of *C. amoena*. Based on our observation, honeybee infrequently entered into the flowers, and *B. initator* visited the flowers but no pollinium was found on its body. *B. trifasciatus* could carry pollinaria on its body when visiting the flower, thus can be considered as the legitimate pollinators of *C. amoena*. Besides this orchid, *Mahonia bealei* and one species of *Prunus* were also visited by *B. trifasciatus*.

Usually, *B. trifasciatus* visit orchid at the beginning of its blooming period, and rarely at the late stage of flower. The visiting frequency of bumblebees to orchids was very

**Table 1** Population size, habitat of *Changnienia amoena*

| Populations | Habitat | Altitude (m) | No. of plants |
|---|---|---|---|
| Population 1 | Growing with shrubs at the garden of the Station | 1 290 | 18 |
| Population 2 | Covered with black nets at the garden of the Station | 1 290 | 16 |
| Population 3 | On a sunny-slop, *Carpinus* sp. spare woodlands | 1 300 | 22 |
| Population 4 | On a shady-slop, *Carpinus* sp. spare woodlands | 1 380 | 14 |
| Population 5 | On a half-shady-slop, *Carpinus* sp. spare woodlands | 1 420 | 33 |
| Population 6 | On a slop of valley, *Carpinus* sp. spare woodlands | 1 000 | 17 |
| Population 7 | On a shady slope, mixed woodlands | 1 370 | 22 |

**Fig.1.** *Changnienia amoena*, attachment of pollinaria on pollen vector. **A**. *Bombus* (*Diversobombus*) *trifasciatus* on the flower of *C. amoena*. **B**. *B. trifasciatus* queen with pollinaria attached on the scutellum.

low. During 113 h of observation, only nine visitations were recorded. No individuals of the bumblebees appeared in rain or overcast weather. The bumblebees mainly appeared during 12:00 - 15:00 o'clock in a day when the flowers have a strong and perfume-like fragrance, rarely before or after this period. Usually, bumblebees stayed only a few seconds and never more than ten seconds in a flower (Fig.1A). After finding no reward, bumblebees would quickly get away. When the bumblebee was back out of the flower, the pollinaria attached to the hairless region of the scutellum (Fig.1B), or it brushed the stigma where it had deposited pollinaria already on its scutellum. Occasionally, we observed that the bumblebees had a short stay on labellum of the neighbor flower after previous visitation, but they did not strivingly enter into the flower. Bumblebees appeared only one or two times, even none within one day except once we observed four times within one day at population 3.

### 2.3 Breeding system and fruit set

After artificial self- and cross-pollination, all of *C. amoena*'s flowers exhibited the elongated stalk. On the contrary, the flowers bagged before flowering have not shown stalk elongation although they wilted too at last. Therefore we took stalk elongation as an index of pollination success (fruits set) of *C. amoena* (in fact, those with the elongated stalk developed fruit finally according to Xiong's observation in consecutive years and our observation in July). The functions of stalk elongation presented by Xiong *et al.* (2002) include (1) to promote capsule development because the green and long stalk will increase the area of photosynthesis; and (2) to be beneficial to wind-mediated seed dispersal (Xiong *et al.*, 2002). Manual pollination indicated that *C. amoena* is a highly self-compatible and outcrossing species, but dependent on pollinators for fruit set.

In orchids, especially deceptive ones, fruit set is generally lower under natural condition. In *Cypripedium acaule*, 20 (2%) fruits were set from 895 flowers over a ten-year period (Nilsson, 1992). The explanation for low fruit set in deceptive *Calypso* species, is that native bumblebee queens probably learn to avoid flowers after one to a few attempts (Mosquin, 1970; Stoutamire, 1971; Ackerman, 1981; Cribb, 2001). Fruit set of *C. amoena* exhibit congregative distribution pattern. The fruit set (26.98% in average in populations 3 - 6, Table 2) is not very low considering the fact that fruit set in outcrossing orchids is generally low under natural conditions (Mendel, 1995). Based on our observation that low visiting frequency, distribution of fruit set in

**Table 2** Fruit set of six populations of *Changnienia amoena* in Shennongjia

| Populations | No. of flowers | No. of flowers with pollinia removal | No. of flowers with stalk elongation |
|---|---|---|---|
| Population 1 | 13 | 9 | 3 |
| Population 2 | 9 | 7 | 0 |
| Population 3 | 15 | 15 | 4 |
| Population 4 | 11 | 9 | 2 |
| Population 5 | 13 | 11 | 3 |
| Population 6 | 10 | 9 | 4 |
| Total | 71 | 60 | 16 |

population, particularly, no nectar was found in the flowers of all populations, we conclude that pollination system of *C. amoena* is deceptive. In addition, the way that pollinia of *C. amoena* attached to the bumblebee (Fig.1B) was similar to that of *Calypso* (Mosquin, 1970; Ackerman, 1981) that is considered having a close relationship to *Changnienia*, which also indirectly supports our conclusion. Stoutamire (1971) suggested that if pollinators learn to avoid the non-rewarding flowers after a few of visits, pollination efficiency and plant fecundity might be lower in large population than in small one. In present study, the sizes of natural populations (populations 3 - 6) with totally 108 individuals are much smaller than those of other orchids with deceptive strategy for pollination. For example, *H. flabellate* has 635 individuals in a population (Luo and Chen, 1999). However, fruit set of *C. amoena* (26.98% in average in four natural populations) is not low in comparison with that of *H. flabellate* with 12%. Our result seems to support Stoutamire's idea. The number of pollinia removal is far higher than that of fruit set (Table 2), indicating that there are some degrees of pollinia wasting in *C. amoena*.

Most orchid species are self-compatible (Gill, 1989), but under natural conditions fruit set in the Orchidaceae is predominantly pollinator dependent. Based on observation in the field, we speculate that the possible reasons that Wang *et al.* (1994) did not observe pollinators and fruits in their study might be that (1) the pollen vectors in Tiantangzhai was different from ones in Shennongjia; (2) observation time may be not long enough, since bumblebees is very astute, and visits infrequently to *C. amoena*; and (3) the best favorable observation time might be missed since the pollinators had learned the deceptive flower so that they hardly appeared in the late flowering period of *C. amoena*.

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# 濒危植物独花兰的传粉生物学初步观察

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**摘要：**　独花兰(*Changnienia amoena* Chien)为我国特有的单种属植物，近年因生境破碎化和过度采挖，其野生资源日渐减少。迄今对独花兰极为有限的研究表明，其结实率很低或根本不结实，其传粉媒介也一直未被发现。2002年3~4月，我们对神农架2个移植居群和5个天然居群进行了传粉生物学的定点观察，发现雌性三条熊蜂(*Bombus* (*Diversobombus*) *trifasciatus* Smith)、仿熊蜂(*Bombus* (*Tricornibombus*) *imitator* Pittion)和蜜蜂均访问独花兰，但只有三条熊蜂身体粘有花粉块，是独花兰的有效传粉者。三条熊蜂的访问频率很低，在113 h的观察中只有9次访问，但在一个天然居群 (population 3) 中曾观察到一天4次的最高访问频率；访问主要在12:00~15:00出现，但在花上停留时间很短，不超过10 s。在未被授粉的情况下，独花兰花朵大约3周后自然枯萎，但受粉后3、4 d内即出现一系列形态和颜色的变化，包括花梗逐渐伸长，子房在花梗逐渐停止伸长后开始膨大等，表明花梗伸长可作为结实(授粉成功)的指标。人工授粉实验表明，自花、异花受粉后花梗均伸长，而套袋隔离花的则花梗不伸长，说明独花兰是自交亲和的异交种，需要昆虫传粉。根据传粉者的访问频率、居群中果实的分布，尤其是花距内无花蜜等特征，我们认为独花兰是一种欺骗性传粉的兰花。相对于其他欺骗性传粉的兰花，独花兰的自然结实率并不很低(26.98%)，这与居群规模小会提高欺骗性传粉兰花结实率这一观点吻合。花粉块的输出数远高于结实数，这说明独花兰存在一定的花粉浪费。

**关键词：**　独花兰；三条熊蜂；欺骗性传粉；长梗伸长

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