ANNALS OF
BOTANY
Founded 1887

The pollination of a self-incompatible, food-mimic orchid, *Coelogyne fimbriata* (Orchidaceae), by female *Vespula* wasps

Jin Cheng[1,2], Jun Shi[1,2], Fa-Zhi Shangguan[3], Amots Dafni[4], Zhen-Hai Deng[5] and Yi-Bo Luo[1,*]

[1]*State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, People's Republic of China,* [2]*Graduate School, Chinese Academy of Sciences, Beijing 100049, People's Republic of China,* [3]*College of Biology, Guizhou University, Guiyang 500251, People's Republic of China,* [4]*Laboratory of Pollination Ecology, Institute of Evolution, Haifa University, Haifa, Israel and* [5]*Yachang Orchids Nature Reserve, Leye 533209, People's Republic of China*

• *Background and Aims* The study of specialized interactions between species is crucial to our understanding of processes in evolutionary ecology due to their profound effect on life cycles and diversification. Obligate pollination by a single wasp species is rare in Orchidaceae except in species with sexually deceptive flowers that are pollinated exclusively by male insects. The object of this study was to document pollination of the food-deceptive flowers of *Coelogyne fimbriata*, a species pollinated exclusively by female wasps.
• *Methods* Field observations and experiments were conducted in two populations of *C. fimbriata*. Floral phenology was recorded, and functional floral architecture was measured. Insect visitors to flowers were observed from 2005 to 2007. Bioassay experiments were conducted to check whether the floral odour attracted pollinators. Natural (insect-mediated) rates of pollinarium removal, pollinium deposition on stigmas, and fruit set were recorded. To determine the importance of cross-pollination, the breeding system was assessed via controlled, hand-pollination experiments.
• *Key Results* Two populations of *C. fimbriata* with fragrant, nectarless flowers are pollinated by females of the same *Vespula* species (Vespidae, Hymenoptera). Experiments on wasps show that they crawl towards the source of the odour. The flowering period appears to coincide with an annual peak in *Vespula* colony expansion when additional workers forage for carbohydrates. Rates of pollinarium removal (0·069–0·918) and pollinium deposition on stigmas (0·025–0·695) are extremely variable. However, fruit set in *C. fimbriata* is always low (0·014–0·069) and appears to be based on self-incompatibility coupled with intraclonal (geitonogamous) deposition of pollinia.
• *Conclusions* *Coelogyne fimbriata* and *Steveniella satyrioides* are now the only orchid species known to have food-deceptive flowers that are pollinated exclusively by eusocial, worker wasps. In *C. fimbriata*, floral odour appears to be the major attractant. Sub-populations may go through flowering seasons when pollinators are abundant or infrequent, but fruit set always remains low because the obligate pollinator does not often appear to transfer pollinaria between intercompatible genets.

Key words: *Coelogyne fimbriata*, *Vespula* wasps, food deception, floral odour, pollinarium removal, pollinium deposition, self-incompatibility.

INTRODUCTION

Knowledge of the causes and consequences of specialization in species interactions is crucial for understanding both ecological and evolutionary processes because they have such a profound effect on speciation, interspecific isolation and relationships between taxa sharing the same habitats (Brown, 1984; Waser *et al.*, 1996). In particular, many members of Orchidaceae are well known for their canalized pollination systems in which only one or a few animal species pollinate each plant species (Johnson and Steiner, 2000). It is estimated that approx. 60 % of all orchid species have only one pollinator species or a discrete lineage of pollinators (Tremblay, 1992). In particular, documentation of orchid species pollinated exclusively by aculeate wasps remains skewed. A few species pollinated exclusively by wasps offer edible rewards (Chase, 1986; Jakubska *et al.*, 2005; Johnson, 2005;

Johnson *et al.*, 2007). Far more often, though, orchid species produce flowers modified for sexual deception and are pollinated exclusively by male wasps that belong to solitary (asocial) species representing such families as Ichneumonidae, Scoliidae and Tiphiidae (Schiestl *et al.*, 1999, 2003; Jersáková *et al.*, 2006). Currently, there is only one example of an orchid species, *Steveniella satyrioides*, that lacks edible rewards, has food-mimic flowers and is pollinated exclusively by eusocial wasps (Nazarov, 1995).

Orchids pollinated in part or exclusively by eusocial wasps are known to secrete abundant nectar accessible in shallow floral depressions or short spurs (Chase, 1986; Jakubska *et al.*, 2005; Johnson, 2005; Johnson *et al.*, 2007). These rewarding, wasp orchids have dull coloured but strongly scented flowers that are usually avoided by other anthophilous insects (Chase, 1986; Johnson, 2005; Johnson *et al.*, 2007).

It is estimated that one-third of all orchid species have food-deceptive flowers, whereas an additional 400 species are

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* For correspondence. E-mail luoyb@ibcas.ac.cn

sexually deceptive and pseudocopulatory (Cozzolino and Widmer, 2005). Bees, flies, moths, beetles and butterflies are the dominant pollinators of food-deceptive species, whereas male wasps, bees and flies are the most common pollinators of sexually deceptive orchids (Schiestl, 2005). Although *S. satyrioides* exploits eusocial wasps as pollinators, it offers no nectar and has a pigmentation pattern indicative of flowers pollinated specifically by wasps. It has olive or yellowish-green flowers with a central reddish-brown pattern that may mimic a piece of flesh favoured by foraging workers (Nazarov, 1995). It remains unclear whether this is a rare pollination syndrome within Orchidaceae. It is possibly more difficult to deceive eusocial, foraging wasps with false food cues than anthophilous (syrphid) flies, moths, beetles and bees. As eusocial wasps are often opportunistic but predatory foragers, it is also possible that genetic constraints within Orchidaceae may preclude the repeated and independent evolution of dummy prey or carrion lures specific to wasp sensory systems. Consequently, finding and interpreting additional examples of wasp-pollinated orchid species that are pollinated by deceit, but lack sexually attractive characters, becomes a key point in understanding the evolutionary ecology of wasp–orchid flower interactions.

Floral colours in species of *Coelogyne* are dominated by greenish yellow (Chen, 1999; Clayton, 2002), and such pigmentation is associated with wasp-pollinated orchids (Nazarov, 1995; Johnson, 2005). So far, nectar secretion has not been detected in any species in this genus, but Clayton (2002) noted that about 21 species produce a discernible floral odour. Van der Cingel (2001) referred to *Coelogyne fragrans* as an odorous, wasp-pollinated orchid that lacks a reward. *Coelogyne fimbriata* is similar to *C. fragrans* in its floral morphology except for the fimbriate margins on its labellum (Clayton, 2002). The aim of this study is to confirm whether *C. fimbriata* is a sexually deceptive or a food-deceptive species dependent on wasp pollinators. We focus on the following questions. (*a*) Is *C. fimbriata* pollinated exclusively by eusocial wasps? (*b*) Does the floral fragrance of *C. fimbriata* attract and canalize the behaviour of the primary pollinator(s)? (*c*) Is *C. fimbriata* another example of a pollinator-limited (*sensu* Tremblay, 1992) orchid species and does its level of fruit set reflect differing levels of low fecundity indicative of a sexual-mimic or a food-mimic orchid?

MATERIALS AND METHODS

Study species

Coelogyne fimbriata is a phalanx-type clonal plant with creeping and slender rhizomes (Fig. 1B). These herbs grow on rocks and tree trunks within forests or at forest margins. In China, the species is distributed through the provinces of Fujian, Guangdong, Guangxi, Hainan, southern Jiangxi, southeastern Xizang and Yunnan (Chen, 1999). It is also found in Cambodia, north-eastern Indonesia, Laos, Malaysia, Thailand and Vietnam (Clayton, 2002). This species usually blooms at the end of summer, producing one or two flowers on a scape. When there are two flowers per scape, only one opens at a time. The sepals and lateral petals are pale yellow-green. The labellum has fimbriate margins and is pale yellow but
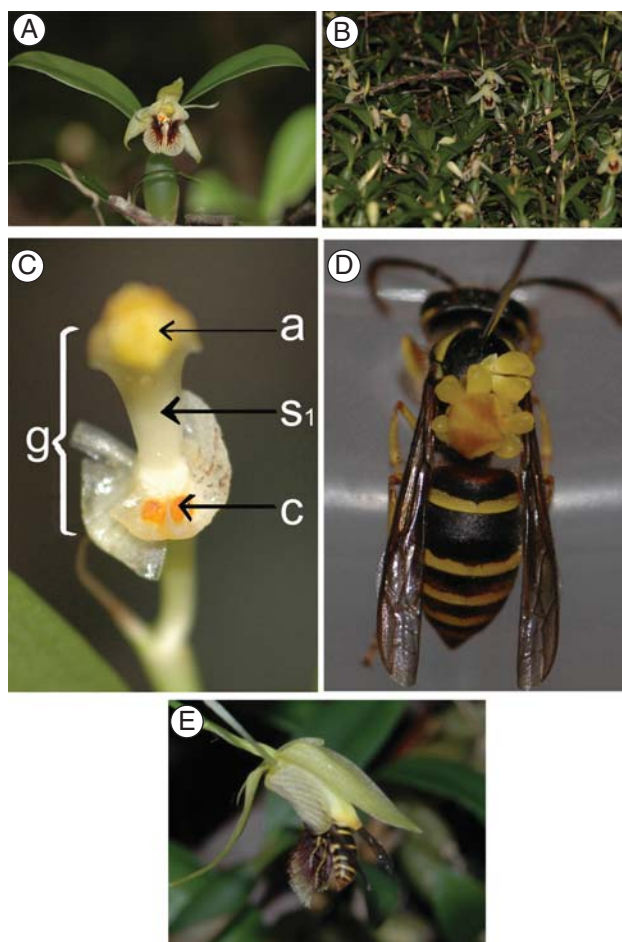

Fig. 1. *Coelogyne fimbriata* and its pollinating *Vespula* species. (A) A flower of *C. fimbriata*. (B) A clone of *C. fimbriata*. (C) Two prominent orange calli at the end of the passage: a, anther; s_1, style; c, calli; g, gynostemium. (D) A specimen of the *Vespula* wasp with pollinaria on its thorax. (E) The *Vespula* species that pollinates *C. fimbriata*.

also has dark brown stripes separated by a central white ridge. The ridge, in turn, is flanked by two indulate–crenate lamellae that are yellowish orange in colour (Chen, 1999; Clayton, 2002). The pollinarium consists of a cellular viscidium attached to a large and irregularly shaped caudicle. Four hard waxy pollinia, arranged in two pairs, terminate the caudicle (Clayton, 2002).

Study sites

The study site is located in the Yachang Orchid Nature Reserve in north-western Guangxi Province, SW China (24°37′–25°00′N, 106°08′–106°23′E). The elevation ranges from 400 to 1950 m. The annual average temperature is 16·3 °C (minimum, −3 °C; maximum, 38 °C). Mean annual rainfall is 1058 mm (Guangxi Forest Inventory and Planning Institute, 2004). The vegetation is sub-tropical evergreen forest consisting primarily of *Cyclobalanopsis glaucoides* and *Platycarya longipes*. The flowering period of *C. fimbriata* at these sites overlaps with sympatric populations of *Paphiopedilum dianthum*, *Cymbidium lancifolium*,

Cymbidium cyperifolium, *Anemone hupehensis*, *Aster* spp. and *Alyxia schlechteri*. Our observations and experiments were conducted in two populations 14 km apart named Fengyandong and Huangjingdong. These two populations were observed over 3 years (2005–2007). In 2006 and 2007, two clumped, clonal patches in each population were selected at random and named Feng1, Feng2 and Huang1, Huang2. They were used to record comparative rates of pollination success in multiflowering clones (see below).

Floral phenology

A flower was recorded as open when its dorsal sepal was separated from the expanded labellum. The functional floral life span was regarded as being over when the labellum withered and collapsed. The flowering time of individual flowers and the flowering period of each population were monitored from opening of the first flower to the collapse and withering of the last flower in both the Fengyandong and Huangjingdong subpopulations.

Floral morphology

Ten fully opened flowers were chosen at random in Fengyandong and measured with a ruler to an accuracy of 0·1 cm in 2005 and 2006. As this is a zygomorphic flower, an entrance and floral passage (or throat) incorporates the terminus of the column (top), labellum mid-lobe (bottom) and lateral margins of the labellum (sides). This entrance or passage is regarded as a key floral trait that could restrict the ability of prospective pollinators to enter and exit. Three measurements of the passage were made: (1) height, defined as the distance between the rostellum and surface of the mid-lobe of the labellum; (2) width, defined as the distance between the two lateral lobes of the labellum at the entrance of the passage; and (3) depth, defined as the full length of the floral passage (landing rim and floral tube). This passage is formed by the lateral lobes of the labellum so passage depth equals the full length of the lateral lobes of the labellum.

In addition to these physical measurements, a Nikon D70 digital camera was used to record changes in floral presentation (organ orientation) before and after pollinia were deposited on receptive stigmas.

Pollinators and their behaviour

Observations of pollinators and their respective movements on and within flowers were made at the Fengyandong site by J.C. from 29 August to 6 September 2005 and from 7 to 26 September 2006. Observations at the Huangjingdong site were made by F.-Z.S. from 7 to 26 September 2006. Flowers were observed at each site from 0900 h to 1800 h each day (440 h total observation time).

Visiting insects were collected and killed in jars containing fumes of ethyl acetate. The number of whole pollinaria and individual pollinia attached to each insect was recorded. Insect specimens were identified by Dr Huan-Li Xu (China Agricultural University). Dead specimens were measured using a ruler to an accuracy of 0·1 cm prior to pinning. The full body length of the specimen (head to tip of abdomen)
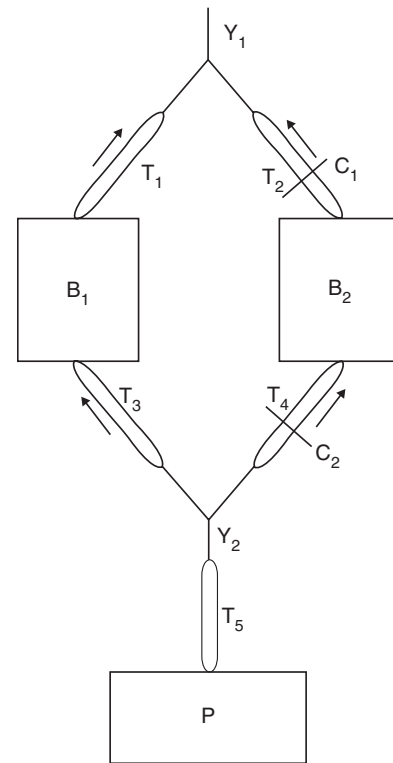

Fig. 2. The Y-tube equipment of the bioassay. Y_1 and Y_2, hard plastic tubes; B_1 and B_2, polyester oven bags (Toppits, Germany); T_1–T_5, soft plastic tubes; C_1 and C_2, clamps; P, portable battery-powered sampling pump.

was recorded, but the height and length of each thorax were also measured because this is where pollinia were most likely to be deposited.

Bioassay

'Y-tube' equipment was used following the method of Ayasse (2006; Fig. 2) to test whether floral fragrance determines the movement of pollinators. Ten flowers were put in B_2 (see Fig. 2), and T_2 and T_4 were closed using C_1 and C_2 to prevent floral odour from moving to the left side. The flowers were enclosed in B_2 for an hour. Then the air was moved through the tubes with a portable battery-powered sampling pump with a flow rate of approx. 20 mL min^{-1} while opening C_1 and C_2. The direction of flow is shown in Fig. 2. One wasp was put at the entrance of Y_1 to give it the option of crawling into one of the two arms of the T-tube. Each wasp was allowed 10 min to select an arm. When a wasp moved into an arm within 10 min and stayed in that arm for a minimum of 30 s, we considered the insect to have selected an option. When a wasp did not select either arm within the given time, we recorded that the wasp made no response (Tomoko *et al.*, 2007). Twelve wasps were used in this bioassay, performed on 16 September 2007.

Pollination success

In 2006 and 2007, the following counts were made: total number of flowers in two selected patches in each population,

total number of open flowers in each selected patch in which pollinaria were removed, and total number of open flowers in each selected patch in which stigmas contained one or more pollinia.

Breeding system

Preliminary hand-pollination experiments in 2005 indicated that fruits failed to form if a stigma received pollinia from the same flower (autogamy) or a flower on the same plant (geitonogamy). Because pre-zygotic self-incompatibility is rare in Orchidaceae (Tremblay *et al.*, 2005), experiments were performed in 2006 using four clones in two populations. We randomly selected 18 flowers within each clone and divided them into three treatments with six flowers in each treatment. Each flower was enclosed in a paper bag while it was in full bud. Twenty-four hours after flower opening, the stigma in each flower was subjected to one of the following three treatments. (1) Self-pollination (autogamy). The stigma was hand-pollinated with the whole pollinarium found in that flower. (2) Self-pollination (geitonogamy). The stigma received a whole pollinarium from a second flower from the same plant. (3) Cross-pollination. Each stigma received a whole pollinarium from a flower of another plant located a minimum of 200 m away.

The bag was then replaced, and the flower was checked every 2 d until the perianth segments withered. At that time the bag was removed permanently, and it was noted whether the ovary was swelling. Fruit set was counted at the end of the flowering period. An ovary was recorded as in fruit when it showed expansion and changed in colour from light to dark green. In 2007 only the self- and cross-pollination treatments were repeated, but each treatment had 20 flowers per clone. To reduce the influence of resource limitation, four clones were selected, with 36, 24, 16 and 42 flowers, and half of the flowers on each clone were treated by cross-pollination (interclonal) and half by self-pollination (intraclonal). In addition, two more clones with the same number (34) of flowers were selected. All flowers on one were cross-pollinated and all flowers on the other were self-pollinated.

Statistical analysis

All analyses were performed in SPSS 13·0 for Windows. Data were analysed by SPSS using descriptive statistics. Variation between two populations over 2 years, including the rate of pollinarium removal from flowers and deposition of pollinia on stigmas (see above), was analysed by one-way analysis of variance (ANOVA).

RESULTS

Floral phenology

The flowering period of *C. fimbriata* starts at the end of August and proceeds for 5–6 weeks until the beginning of October. The life span of an individual flower is $7·90 \pm 1·45$ d ($n = 10$).

Flower morphology

The mean height and width of the entrance of the floral passage are $0·54 \pm 0·06$ and $0·65 \pm 0·07$ cm, respectively

TABLE 1. *Measurements (in cm) of the floral architecture of* Coelogyne fimbriata *and the body size of* Vespula *sp.*

	n	Minimum	Maximum	Mean	s.d.
Floral entrance height of *C. fimbriata*	20	0·45	0·65	0·54	0·06
Floral entrance width of *C. fimbriata*	20	0·55	0·75	0·65	0·07
Floral passage depth of *C. fimbriata*	20	0·70	1·00	0·81	0·09
Length of *Vespula* sp.	16	1·20	1·70	1·43	0·13
Thorax height of *Vespula* sp.	16	0·30	0·45	0·40	0·04
Thorax width of *Vespula* sp.	16	0·30	0·40	0·36	0·03

($n = 20$). The depth of the passage is $0·81 \pm 0·09$ cm ($n = 20$; Table 1). Floral pigmentation patterns follow the descriptions by Clayton (2002; Fig. 1A), but two prominent orange calli were also found at the end of the passage (Fig. 1C).

The rostellum is large and projects beyond and between the stigma and the anther forming a flap over the stigmatic depression (*sensu* Burns-Balogh and Bernhardt, 1985; Fig. 3A). This flap bends and covers the stigmatic cavity within 24 h of pollinium deposition (Fig. 3B). Flowers of *C. fimbriata* that had been visited by insects did not have scratches or scarification on the labellum as noted previously in wasp-pollinated *S. satyrioides* (Nazarov, 1995).

In fresh flowers of *C. fimbriata*, the human nose detects a distinctive odour that is reminiscent of a combination of ripe pears and freshly cut grass. No secretion of nectar was detected.

Pollinators and their behaviour

The only insect species observed on these flowers over three seasons was identified as a species of *Vespula* (Vespidae; Fig. 1D). All 18 wasp specimens captured and pinned were females. Of the 18 specimens captured, 16 carried pollinaria of *C. fimbriata* on their thoraces. The mean number of individual pollinia on *Vespula* specimens was $8·75 \pm 8·20$ ($n = 16$). The maximum number of pollinia on a specimen was 28.
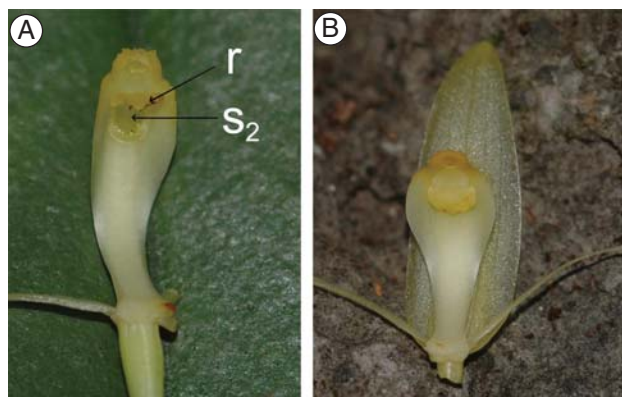

FIG. 3. (A) The rostellum projects beyond and between the stigma and anther. It blocks pollinaria on the thorax of the wasp, forcing individual pollinia into the stigmatic cavity as the insect exits the flower. r, rostellum; s_2, stigma. (B) The rostellum closes over the stigma within 24 h following pollinia deposition.

The mean height and width of thoraces carrying pollinaria is 0.40 ± 0.04 and 0.36 ± 0.03 cm ($n = 16$), with a mean body length of 1.43 ± 0.13 cm ($n = 16$; Table 1).

Other insects visit co-blooming species at these study sites. Hoverflies (Syrphidae) are observed on *P. dianthum* (with pollen) and *A. hupehensis* (Shi *et al.*, 2007). Non-domesticated honeybees (*Apis cerana*) carry pollen of *A. hupehensis* and the pollinaria of *Cymbidium lancifolium* (Cheng *et al.*, 2007). Syrphids and honeybees have not been observed on flowers of *C. fimbriata* at either population.

We recorded 29 visits to flowers of *C. fimbriata*. In only one instance did the wasp fail to remove or deposit pollinia. The average number of orchid flowers visited by a wasp during a flying and visiting bout is 1.31 ± 0.93 ($n = 29$) with a maximum of five flowers visited within a site during the same bout, dispersing pollinia from flower to flower.

Individual wasps approach flowers of *C. fimbriata* in a zigzag manner. With three exceptions we noted that all wasps first land directly on the labellum. The single exception observed in 2005 and the two exceptions observed in 2006 landed first on the lateral sepals. After landing on the labellum, a wasp crawls toward the orange calli located at the base of the passage (Fig. 1E). When it fails to find food at the base of the passage, it retreats backwards allowing its thorax to come into contact with the viscidium on the overhanging column, removing the pollinarium. *Vespula* wasps remain in each flower for only 6.80 ± 3.29 s ($n = 20$). During the process of visitation, a wasp never clasps any part of the labellum as has been described in the pollination of sexually mimetic orchids pollinated by male wasps (Schiestl, 2005). If a female wasp carrying orchid pollinaria on its thorax enters a second flower of *C. fimbriata* that lacks pollinia on its receptive stigma, the large, flap-shaped rostellum forces individual pollinia into the stigmatic cavity as the insect leaves the flower. Once these pollinia are deposited in the stigmatic cavity, the rostellum covers the stigmatic depression within 24 h. If a visiting wasp brings more pollinaria to a previously pollinated flower, the flap covering the stigma prevents additional deposition as the wasp backs out of the floral passage.

Result of the bioassay

Eleven wasps (0.917) selected the side Y bar containing flowers of *C. fimbriata* as soon as they faced the alternative channels. These wasps stayed at B_2 until the test finished. Only one wasp crawled up approximately one-third the length of T_2 and then backed out, remaining at the centre of Y_1 until the end of the experiment. This behaviour was recorded as non-responsive.

Pollination success

Rates of pollinarium removal, pollinium deposition and fruit set of four subpopulations in two seasons are presented in Table 2. There were significant differences in the percentage of pollinaria removal ($F = 74.152$, $P = 0.000$) and pollinia deposition ($F = 16.290$, $P = 0.007$) between the two populations (Table 2), but the actual rates of pollinarium removal ($F = 3.978$, $P = 0.184$ in Fengyandong; $F = 0.524$, $P = 0.544$ in Huangjingdong) and pollinium deposition ($F = 2.081$,

TABLE 2. *Rates of pollinaria removal, pollinia deposition and fruit set in four subpopulations of* C. fimbriata *over two seasons*

	Year	Feng1	Feng2	Huang1	Huang2
Number of flowers	2006	274	87	136	122
	2007	395	86	170	115
Ratio of flowers with pollinaria removed	2006	0.069	0.114	0.727	0.918
	2007	0.131	0.162	0.582	0.834
Ratio of flowers bearing pollinia on stigmas	2006	0.025	0.057	0.382	0.541
	2007	0.058	0.093	0.235	0.695
Fruit set	2006	0.014	0.034	0.044	0.041
	2007	0.015	0.034	0.035	0.069

$P = 0.286$ in Fengyandong; $F = 0.000$, $P = 0.989$ in Huangjingdong) were not significantly different between years. The difference in fruit set was not significant between populations ($F = 5.708$, $P = 0.054$) and between years ($F = 0.001$, $P = 0.975$ in Fengyandong; $F = 0.331$, $P = 0.623$ in Huangjingdong). The grand mean for fruit set in this species was only 3.62%.

Breeding system

No fruit was set by either the hand-mediated autogamous ($n = 24$, in 2006; $n = 80$, in 2007) or geitonogamous treatments ($n = 24$, in 2006). Fruit set based on hand-mediated cross-pollination (intraclonal) varied among clones in 2006, with $n = 6$ flowers for each clone (66.67, 83.33, 100 and 100 % in four clones). When these cross-pollination experiments were repeated in 2007 using $n = 20$ flowers for each of four clones, fruit sets of 80, 90, 95 and 100 % were obtained.

When half the flowers on the same clone were self-pollinated (autogamous) and half were cross-pollinated by hand, the self-pollinated flowers never set fruit. The rate of fruit set on the cross-pollinated flowers varied between clones from 88.89 to 100 %. Fruit set on the clone with all flowers cross-pollinated was 94.12 %. All flowers on the last clone were self-pollinated and there was no fruit set.

DISCUSSION

Exploitation of Vespula *parameters, foraging behaviour and the comparative roles of floral phenology, fragrance and visual cues*

Populations of *C. fimbriata* at the Yachang Orchid Reserve appear to be pollinated exclusively by a single species of *Vespula*, although other orchid-pollinating insects (honeybees and syrphid flies) are present. Although flower and wasp measurements show that the mean height and width of the entrance of *C. fimbriata* are slightly greater than the thorax dimensions of the wasp, only one out of 29 wasps failed to contact the rostellum, preventing either removal or deposition of pollinia. We conclude that the physical dimensions of the solitary pollinator species 'fit' the functional architecture in the majority of flowers produced by *C. fimbriata*.

Flowers of *C. fimbriata* lack edible rewards (nectar or food bodies). Both field observations and experiments indicate a trend towards a form of edible reward mimesis attractive only to female wasps collecting food for the developing colony. Odour appears to be the primary attractant for three, inter-related reasons. First, the zigzag flight approach of wasps to flowers suggests that these insects are attracted initially by floral odour (Johnson, 2005). Secondly, bioassay experiments also show that captive wasps continue to crawl towards the same odour source. Thirdly, the wasps should not be able to see the orange calli until they crawl down the labellum. Because all the wasps observed and captured were worker females, we discount the possibility that this is a pseudocopulatory flower as is common in other wasp-pollinated lineages of Orchidaceae (Schiestl, 2005; Ayasse, 2006).

Like most social wasps, *Vespula* females are known to forage for protein (arthropods and/or carrion), but they also forage extensively for carbohydrate-rich foods such as nectar and fruit juices. These foods are important energy resources shared by the colony (Richter, 2000). The availability of carbohydrates in the habitat is regarded as a limiting factor in wasp development, and ultimately in the regulation of population size within the colony (Akre *et al.*, 1980; Stringer, 1989; Vail and Skinner, 2000). Yellow jacket (*Vespula* and *Dolichovespula*) colonies are known to grow most rapidly and produce the majority of their males and gynes in late summer to early autumn (Akre *et al.*, 1980; Vail and Skinner, 2000). J. Cheng (unpubl. res.) collected seven nests of the unidentified *Vespula* species within our study sites and reported that all contained emergent, winged males as well as many new larvae at various phases of development during the flowering period of *C. fimbriata*. We also note that this orchid blooms at the time of year when the local people harvest *Vespula* larvae as a traditional food (*sensu* Feng *et al.*, 2001). Therefore, *C. fimbriata*, flowering from the end of August to early October, appears to exploit a critical period in colony expansion and reproductive effort in at least one *Vespula* species. We suggest that *C. fimbriata* employs a floral fragrance that mimics the odour of carbohydrate resources (such as fruits and the flowers of co-blooming species), attracting only the female workers of this *Vespula* species during the critical period of colony expansion. The odour of *C. fimbriata* flowers at our sites may attract *Vespula* individuals that would otherwise forage on fruits or such nectar-secreting herbs as *A. schelechtei* and various species of *Aster*.

Generally, a combination of odour and visual pattern is required for social wasps to locate their food sources (Richter, 2000), especially when these insects hunt for arthropods (Free, 1970; Reid *et al.*, 1995). This explains pollination of *S. satyrioides* by female wasps. Nazarov (1995) interpreted the reddish-brown centre of this flower as dummy prey. However, as scent is regarded as the main cue for yellow jackets searching for carbohydrates (Ross *et al.*, 1984; Hendrichs *et al.*, 1994), we suggest that floral odour, not visual pattern, is the main cue for *Vespula* wasps visiting flowers of *C. fimbriata*. Specifically, the *Vespula* species pollinating *C. fimbriata* behaves differently from *Paravespula vulgaris* pollinating *S. satyrioides*. Females of *P. vulgaris* plunge their mandibles into the spur on the labellum of *S. satyrioides*

after these insects inspect the papillae at the labellum base. These aggressive insects attempt to tear the labellum, leaving scars and scratches on 78 % of pollinated flowers of *S. satyrioides*. To date, this behaviour has not been recorded for the *Vespula* species visiting *C. fimbriata*.

Pollinator efficiency vs. self-incompatibility and their evolutionary implications

The combined conversion rate of flowers into fruit for our populations is <0·04. This low rate of fecundity is often recorded in orchids with food-mimetic flowers (Neiland and Wilcock, 1998). Low fruit set is associated with infrequent and insufficient visits by primary pollinators, as preferred pollen vectors learn to avoid flowers that offer primary attractants but no rewards. Therefore, mimetic orchid species with low levels of fecundity are commonly classified as pollinator limited (Neiland and Wilcock, 1998; Tremblay *et al.*, 2005). This explanation is insufficient to explain low fruit set in *C. fimbriata* because wasp-mediated visits to *C. fimbriata* show a high degree of variation between sub-populations; rates of pollinarium removal by wasps vary from 0·069 to 0·918 according to sub-population. More importantly, the rate of natural deposition of individual pollinia in stigmatic cavities varies from 0·025 to 0·695. Consequently, although the rate of fruit set in all sub-populations, regardless of season, was always <0·10, the rate of stigmas bearing pollinia in the Huang sub-populations ranged from 0·235 to 0·695. This rate is as high as or higher than in some widely separated populations of the mimetic flowers of *Cypripedium fasciculatum* (Lipow *et al.*, 2002).

Hand pollination experiments in 2006 and 2007 show that although individual clones have a high capacity for fruit set, ovary maturation depends exclusively on cross-pollination. Furthermore, our field experiments show that a clone cannot be 'deceived' by cross-pollinating some flowers and self-pollinating others. We conclude that, whereas wasps visiting flowers within the Huang subpopulations often transfer pollinia to receptive stigmas, the majority of these depositions are geitonogamous and/or between different genets that share one or both of the same S alleles (*sensu* Richards, 1986).

Therefore, whereas some sub-populations and individual clones of *C. fimbriata* are pollinator limited, over a 2-year period virtually all populations and clones in this study should also be regarded as compatible pollen limited (*sensu* Vance *et al.*, 2004). This species of *Vespula*, even when common within sub-populations, is an inferior agent for cross-pollination based on current plant demography. The rostellum always blocks access to the stigmatic cavity within 24 h following pollinium deposition, regardless of pollen source, which ultimately prevents any future prospect of the same flower receiving additional cross-compatible pollinia. We suggest that this is directly comparable with polyad blockage occurring in some self-incompatible Australian *Acacia* spp. If the receptive stigma of an *Acacia* is self-pollinated, the single polyad usually covers the circumference of the stigmatic surface, preventing the deposition of a second cross-compatible polyad, and fruit set cannot occur (Knox and Kenrick, 1983).

The pollination biology and breeding system of *C. fimbriata* may help explain why self-compatibility occurs repeatedly in Orchidaceae (Tremblay 1992; Tremblay *et al.*, 2005), but mechanical self-pollination (autogamy) in the absence of pollen vectors is often restricted to discrete lineages distributed in seasonally stressful habitats (Burns-Balogh and Bernhardt, 1985). In Orchidaceae, species with mimetic flowers outnumber species offering rewards, but food-mimic species, in particular, often suffer the lowest rates of fecundity (Neiland and Wilcock, 1998).

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