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Pollination of the lady's slipper *Cypripedium henryi* Rolfe (Orchidaceae)

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The pollination ecology of *Cypripedium henryi* Rolfe, a slipper orchid endemic to west China, was investigated, and its floral shape, size, colour, and scent were analysed. Examination of the breeding system suggests that the flowers are self-compatible, but need pollen vectors for successful reproduction. The flower is rewardless; over 15 insects belonging to Araneida, Hymenoptera, Diptera, Lepidoptera, and Coleoptera were recorded as flower visitors, but most only alighted or rested on the flower. In the total 32 h of observations over 2 years, female *Lasioglossum* bees were found to be the most frequent visitors and the only pollinators. They showed a high visitation frequency and, surprisingly, re-visited the same flowers frequently. *Cypripedium henryi* probably attracts pollinators visiting the flowers through general food deception (odour components, colour, false nectar guides), as well as special structures (slippery labellum, slippery staminode). Although three *Lasioglossum* species visited the flowers, only *L. sauterum* Fan et Ebmer was found with pollen. *Lasioglossum flavohirtum* Ebmer was large and climbed out from the entrance. Morphologically, *L. sichuanense* Fan et Ebmer could be considered as a potential pollinator, but the collected specimens were found to have no pollen of *C. henryi* on their bodies. It was speculated that the particular floral scent of *C. henryi* discouraged the entrance of *L. sichuanense* bees. *Lasioglossum sauterum* was matched morphologically to the flower, but not all of the visitations resulted in effective pollinations, as some flowers of *C. henryi* were frequently re-visited and the pollen mass had been taken away by bees on previous visitations. © 2008 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2008, **156**, 491–499.

ADDITIONAL KEYWORDS: breeding system – deceptive pollination – floral functional morphology – *Lasioglossum* – re-visitation.

INTRODUCTION

The lady's slippers (Cypripedioideae) are well-known orchids of the north temperate and certain tropical zones (Dressler, 1993). This subfamily comprises five genera with 158 species and has flowers that do not offer a reward to pollinators (Pridgeon *et al*., 1999). The slipper orchid flower is a one-way trap flower, and the pollinator enters into the labellum from the front entrance, and exits through one of the two basal orifices that are formed by the staminode blocking the opening at the base of the labellum. When the pollinator exits through a basal orifice, it first passes the stigma and picks up a portion of a pollinium. Thus, one pollination case is completed if the pollinator has already been smeared with pollen during a previous visitation. Although all the slipper orchids have this fixed pollination route, their pollinators and pollination mechanisms show a high degree of diversity (Stoutamire, 1967; Nilsson, 1979; Sugiura *et al*., 2001; Bänziger, Sun & Luo, 2005).

One group of lady's slippers is *Cypripedium*, a genus of about 45 species widespread in the north temperate zones (Cribb, 1997). Mainly based on the

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labellum morphology, five distinctive types can be recognized in the genus: 'arietinum', 'guttatum', 'calceolus', 'japonicum', and 'margaritaceum' (Cribb, 1997). To date, most *Cypripedium* species have been found to be pollinated by various bees (Cribb, 1997; Van der Cingel, 2001), and fly pollination has only been reported in *C. reginae* Walt (Vogt, 1990) and hypothesized in *C. debile* Rchb., *C. margaritaceum* Franch., *C. lichiangense* Cribb & S.C. Chen, and their allies (Cribb, 1997). The path of the pollinators through the slipper-shaped flower is probably controlled by a number of features of floral morphology (for example, inflexed margins, lines of hairs leading to the rear exits, false nectar guides, etc.) (Catling & Catling, 1991). Stoutamire (1967) suggested that the sizes of the labellum mouth and basal orifices and of the escape route under the stigma and anthers determine the possible pollinators. However, it seems that a similar floral morphology does not always mean similar pollinators. For example, the European *C. calceolus* L. and the North American *C. parviflorum* Salisb. are similar in floral morphology and only differ with regard to staminode shape and colour (Cribb, 1997), but have different pollinators. The most frequent and regular vectors of the former are female *Andrena haemorrhoa* (F.) (Nilsson, 1979), whereas those of the latter are male *Ceratina* bees (Stoutamire, 1967). By contrast, the East Asian *C. plectrochilum* Franch. and the North American *C. arietinum* R. Br. are a vicarious species pair with similar floral morphology, and are distinguished from each other by the features of the dorsal sepal and staminode (Chen, 1983). Both have similar pollinators: species of *Lasioglossum* (Stoutamire, 1967; Li *et al*., in press).

The species *C. henryi* Rolfe is endemic to west China. It resembles superficially *C. calceolus*, but differs by its usually two- or three-flowered inflorescence, smaller green or yellow–green flowers, small glossy labellum, and anther filaments that are not covered by the staminode (Cribb, 1997). It is hard to predict whether *C. henryi* will have pollinators similar to or different from those of *C. calceolus* on the basis of the floral morphological differences. In this study, we investigated the pollination ecology of *C. henryi* to determine whether or not it has similar pollinators to *C. calceolus*. In addition, we investigated the pollination mechanism of *C. henryi* through an analysis of its floral shape, size, colour, and scent.

MATERIAL

This study was carried out during the anthesis of *C. henryi* from May 15th to 25th, 2004 and from May 13th to 25th, 2005. A population of about 100 individuals of *C. henryi* was found in Danyun Gorge,

Huanglong Nature Reserve, Sichuan, China. The altitude of the locality is 2000 m and *C. henryi* grows along a path under sparse low deciduous trees or amongst grasses. The main co-blooming species are *Rhododendron* sp., *Fragaria orientalis* A. Los., *Ranunculus* sp., *Taraxacum* sp., and *Lysimachia* sp.. A few other orchids, *C. plectrochilum*, *C. tibeticum* King ex Rolfe, *C. sichuanense* Perner, *C. fasciolatum* Franch., and *Calanthe tricarinata* Lindl., are also found in this community, but only *C. plectrochilum* was flowering during our study.

Cypripedium henryi is 30–60 cm tall with one to four flowers (usually two or three). The pedicel and ovary length is 30–60 cm with dense glandular hairs. The flowers are entirely greenish yellow or green with a glossy labellum. The dorsal sepal is similar to the united lateral sepals, 3–4.5 cm long and 1–1.5 cm wide. The petals are 3–5 cm long, 1–2 cm wide, and not twisted. The labellum is ellipsoidal, 1.5–2.5 cm long, 1–2 cm wide, and 1–1.5 cm deep, with hairs and red veins in the bottom. The staminode is oblong, 6–7 mm long, 3–4 mm wide, and with or without red spots on the surface (Fig. 1A). The stigma is oblong, and its surface is retuse groove with papillose (Fig. 1C). The pollen is aggregated into two yellow or yellow–green masses of sticky, friable grains (Fig. 1C).

METHODS

FLORAL VISITORS AND THEIR BEHAVIOUR

Pollination observations of *C. henryi* were made for a total of 32 h over 15 days. The behaviour of visitors was recorded from the moment when they entered the vicinity of the flowers to the moment when they left. Every insect activity, including approaching, alighting, entering, creeping, and climbing, was recorded with a tape recorder. Some of the visiting insects were impossible to identify to the species' level with certainty in the field. In such cases, samples were collected in order to cover the diversity of the visiting insects. In practice, insects were often only identified to a particular group of species or type in the field. For instance, '*Lasioglossum* type' means the bees agreed in all perceptible characters with this genus.

FLORAL FUNCTIONAL MORPHOLOGY AND INSECT MEASUREMENT

To assess the relationship between floral structure and pollinators, 20 flowers were randomly chosen, and flower traits, especially those which are considered to be related to the success of pollination, including the entrance diameter of the labellum (ML), the height between the stigma and bottom of the
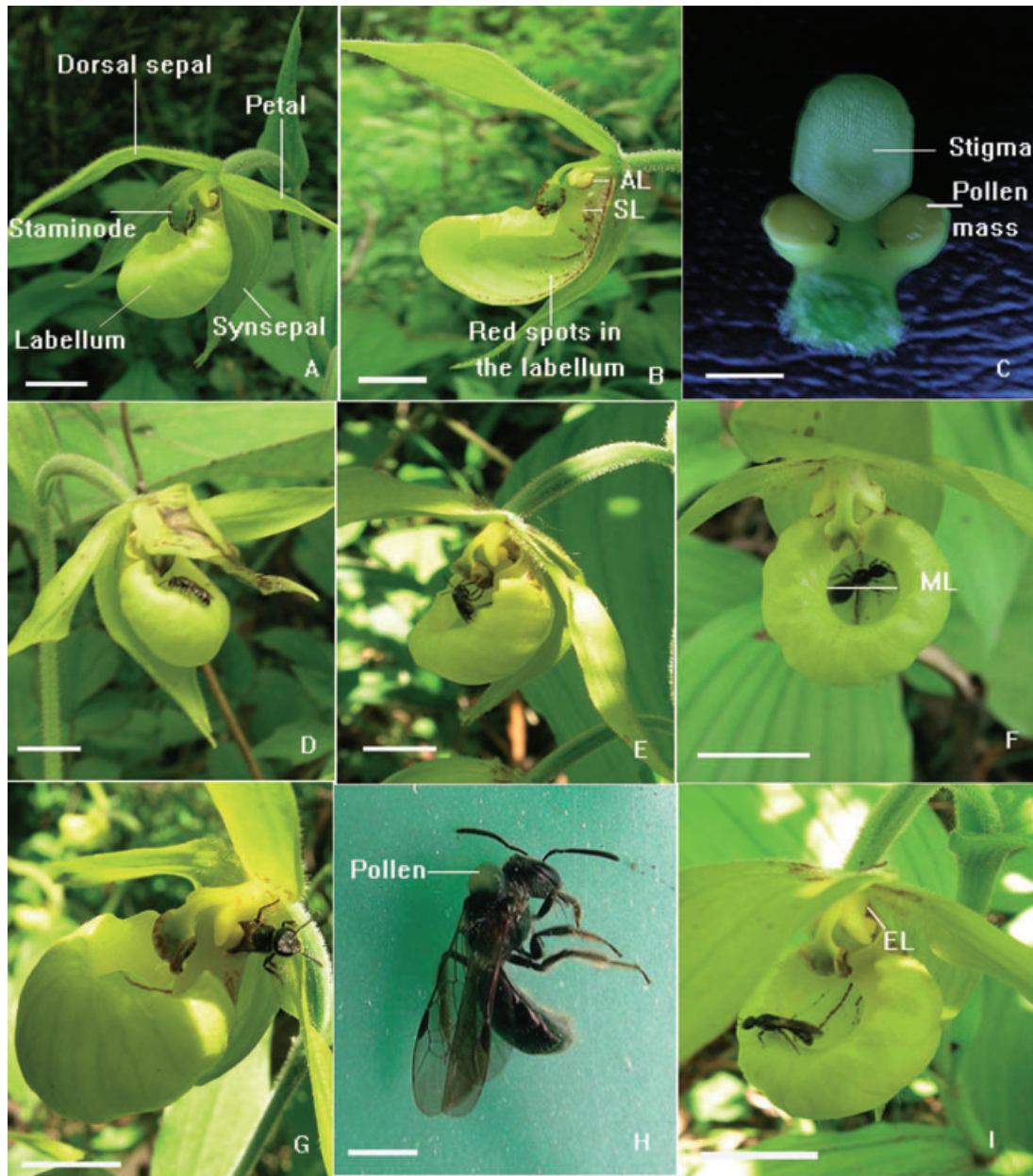
Figure 1. Flowers of *Cypripedium henryi* and its pollinators. All photographs were taken under natural conditions. Scale bar, 1 cm. A, Close view of an individual flower of *C. henryi*. B, Longitudinal section of the labellum of *C. henryi* showing the internal path of the pollinator (AL, height between the anther and the bottom of the labellum; SL, height between the stigma and the bottom of the labellum). C, Stigma and anther of *C. henryi*. D, *Lasioglossum* sp. entering the labellum of *C. henryi* from the entrance. E, *Lasioglossum* sp. scratching the staminode and sliding into the labellum of *C. henryi*. F, *Lasioglossum* sp. in the labellum of *C. henryi* (ML, mouth diameter of the labellum). G, *Lasioglossum* sp. escaping from the exit of the labellum of *C. henryi*. H, *Lasioglossum sauterum* with a large pollen mass of *C. henryi* on its thorax. I, *Lasioglossum* sp. escaping from the entrance of the labellum of *C. henryi* (EL, exit width of the labellum).

labellum (SL), the height between the anther and the bottom of the labellum (AL) and the exit width of the labellum (EL) (Stoutamire, 1967; Nilsson, 1979), were measured with digital callipers to the closest 0.1 mm. ML and EL were measured in whole flowers (Fig. 1F,

I). SL and AL were measured in the longitudinal cross-section of the flower through the column (Fig. 1B). The body length, body width, and thorax height of the pollinators were also measured with digital callipers to the closest 0.1 mm.

Table 1. Activity of different visitors to the flowers of *Cypripedium henryi*

Species	No. alighting	No. entering the labellum	No. with pollen mass
Araneida			
Arachnida sp.	1	0	0
Coleoptera			
Chrysomela sp.	1	0	0
Diptera			
Rhingia laevigata Loew	8	0	0
Episyrphus balteatus De Geer	11	1	0
Platycheirus sp.	9	0	0
Scathophaga sp.	4	0	0
Calliphora vomitoria (L.)	37	0	0
Delia unguitigris Xue	8	0	0
Polietes orientalis Pont	10	1	0
Hymenoptera			
Lasioglossum flavohirtum Ebmer, *Lasioglossum sichuanense* Fan et Ebmer, and *Lasioglossum sauterum* Fan et Ebmer	124	87	45
Formica sp.	9	1	0
Lepidoptera			
Pieris rapae L.	5	0	0
Unidentified spp.	38	4	0

BREEDING SYSTEM

Three treatments were conducted to test the mating system of *C. henryi*. In each treatment, ten plants were randomly chosen, and the lowest flower of the inflorescence was chosen for hand pollination. The labellum of each flower was picked off after the flower had been treated as follows: (1) cross-pollination from the pollen mass of another flower at least 1 m away from the experimental plant; (2) self-pollination from the pollen mass of the same flower; (3) no pollination after the labellum had been removed. The fruit set of treated flowers and natural flowers was recorded in July every year.

FLORAL FRAGRANCE COLLECTION AND ANALYSIS

Floral fragrance collection was carried out on May 17th and May 18th, 2004. Two floral fragrance samples and one air control were collected. For field sampling studies, one inflorescence with two flowers was enclosed in an inertia bag (Reynolds Company, USA) for about 1 h prior to sampling. The fragrance-laden air was drawn through a sorbent tube with a portable battery-powered sampling pump. Air samples were collected using Tenax TA packed in a glass tube. The air was passed through the glass tube for 1 h between 11:00 and 12:00 h at a flow rate of approximately 100 mL min⁻¹ on a sunny day.

The analysis of fragrance components was carried out on June 16th, 2004. Volatiles were desorbed from Tenax TA by heating in a CP-4010 TCT thermal desorption device (Chrompack, the Netherlands) at 250 °C for 10 min, and then cryo-focused in a cold trap at –100 °C. The cold trap was then quickly heated to 200 °C for 1 min to transfer the volatile compounds into the gas chromatograph-mass spectrometer (Trace 2000-Voyager, Finnigan, Thermo-Quest). The compounds were identified by searching the NIST library in the Xcalibur data system (Finnigan), and by comparing with the compositions in the air control.

RESULTS

FLORAL VISITORS AND THEIR BEHAVIOUR

Over 15 different species of insect, belonging to Araneida, Hymenoptera, Diptera, Lepidoptera, and Coleoptera, were recorded as flower visitors, but most only alighted or rested on the flower (Table 1). The only insects carrying pollen of *C. henryi* were female *Lasioglossum* bees. In the total 32 h of observations over 2 years, *Lasioglossum* bees were also the most frequent visitors, with a total of 124 visits alighting on floral parts. The percentage of *Lasioglossum* bees entering the labellum was 70.16% (87/124) (Table 1). *Lasioglossum* bees were observed 45 times with a pollen mass on their thorax when they escaped from

Table 2. Visitation numbers of *Lasioglossum* bees to the individual flowers of *Cypripedium henryi*

Flower	Flower*										
Bees	1-1	1-2	1-3	2-1	2-2	2-3	3-1	3-2	3-3	4-1	4-2
No. alighting	14	12	9	12	12	9	8	2	7	3	1
No. entering	10	8	5	7	9	6	5	0	4	1	0
No. with pollen	2	3	3	3	4	3	3	0	3	1	

*The observations were carried out on four inflorescences with 11 flowers. The first number is the position of the plant, and the second number is the position of the flower in the inflorescence: '1' is the lowest, '2' is the middle and '3' is the highest flower.

the exit, which accounts for 51.72% of the bees entering the labellum. Five pollen-bearing *Lasioglossum* bees were observed entering five flowers. After these bees had exited the flowers, three flowers were picked and their labella were removed; it was found that the stigma of each flower retained a pollen mass.

Three *Lasioglossum* species (all females) were recorded as visitors: *L. sichuanense* Fan et Ebmer, *L. flavohirtum* Ebmer, and *L. sauterum* Fan et Ebmer. However, in the collected *Lasioglossum* specimens ($N = 18$), only five *L. sauterum* ($N = 10$) were found with pollen of *C. henryi* on the thorax. No pollen of *C. henryi* was found on the bodies of *L. flavohirtum* ($N = 3$) and *L. sichuanense* ($N = 5$). In addition to *C. henryi*, *Lasioglossum* bees were also found to be frequent visitors of the concurrently rewarding flowers of *Fragaria orientalis* and *Ranunculus* sp.

When *Lasioglossum* bees approached the flowers of *C. henryi*, they usually performed an undulating flight, indicating nearby chemical stimulation. *Lasioglossum* bees landed on almost all floral parts of *C. henryi*, including the labellum, dorsal sepal, staminode, and petals. The part they landed on most frequently (72 times) was the labellum, followed by the staminode (15 times). After *Lasioglossum* bees had escaped from the flower, they usually rested on the dorsal sepal, bract, or labellum, and then flew away.

Although the mouth of the labellum was the only entrance used by *Lasioglossum* bees, two different processes were recognized. In one process, *Lasioglossum* bees entered the labellum largely under control, with little or no involuntary element. The bees crawled into the labellum as if they wanted to explore the structures concealed inside (Fig. 1D). In total, bees were recorded entering the labellum in this manner 53 times, accounting for 60.92% of the entering bees. In the other process, the bees slid or tumbled into the labellum from the slippery margins of the labellum and staminode. Within this process, there were three different situations according to the different floral parts from which the bees entered. In the first, the bees slid into the labellum from the slippery and pendant staminode after they had landed directly on the staminode. In the second, bees occasionally slid into the labellum from its lateral slippery margin when they wanted to explore something interesting within. In the third, the bees landed on the labellum initially and were then attracted by the staminode; they tumbled into the labellum when they attempted to climb onto the staminode (Fig. 1F).

After *Lasioglossum* bees had entered the labellum, they stretched out towards the lateral margins or staminode and attempted to gain a foothold with their claws. Because of the slippery inflected rim of the entrance and the slippery staminode, most failed to escape through the entrance. Only 14 times did we observe *Lasioglossum* bees escaping from the entrance (Fig. 1I). *Lasioglossum* bees (73 times) that failed to climb out through the entrance tumbled to the bottom of the labellum, and then walked around the labellum (Fig. 1F), sometimes with a buzzing noise.

Lasioglossum bees that did not escape from the entrance exited along the route from the bottom of the labellum to the stigma, anther and, finally, through the basal orifice of the labellum (Fig. 1G). Bees usually squeezed under the stigma and anther because their thorax height was greater than the height between the stigma and the bottom of the labellum (SL) or between the anther and the bottom of the labellum (AL). After they had passed the stigma and anther, a pollen mass would be present on their thorax (Fig. 1H); if they had been smeared previously with a pollen mass, the pollen would stick on the stigma. The time spent by *Lasioglossum* bees in the labellum varied greatly from 3 s to 4 h and 20 min, with an average of 3 min and 46 s. No bees were found dead in the labellum.

In addition, many *Lasioglossum* bees re-visited the same flowers frequently. The visitation numbers were recorded of *Lasioglossum* bees entering the labellum of every flower in a cluster of four plants and 11 flowers from 10:00 to 16:00 h on May 21st, 2004 (Table 2). *Lasioglossum* bees entered the labellum of nine flowers 55 times, but took pollen away only 25

Table 3. Size measurement of floral functional morphology of *Cypripedium henryi* and of *Lasioglossum* bees

Floral trait	Size (cm)	Thorax height (cm)	Body width (cm)	Body length (cm)	*Lasioglossum* bee
ML	0.53 ± 0.091	0.262 ± 0.019	0.298 ± 0.014	1.037 ± 0.016	*L. flavohirtum*
SL	0.205 ± 0.028	0.203 ± 0.025	0.227 ± 0.018	0.747 ± 0.135	*L. sauterum*
AL	0.153 ± 0.029	0.189 ± 0.012	0.198 ± 0.022	0.673 ± 0.057	*L. sichuanense*
EL	0.326 ± 0.06				

AL, height between the anther and the bottom of the labellum; EL, exit width of the labellum; ML, mouth diameter of the labellum; SL, height between the stigma and the bottom of the labellum.
The results for the floral trait size were obtained from 20 individuals; the results for *L. sauterum* were obtained from ten individuals, for *L. sichuanense* from five individuals, and for *L. flavohirtum* from three individuals.

times when they escaped through the exit. Only two flowers had no visits from *Lasioglossum* bees, and one flower had only one bee entering its labellum; the other eight flowers were frequently re-entered by *Lasioglossum* bees between four and ten times.

FLORAL FUNCTIONAL MORPHOLOGY

The measurements of the floral functional morphology and thorax height, body length, and body width of the three *Lasioglossum* bee species are listed in Table 3. From Table 3, the following conclusions can be drawn.

1. The body widths of the three *Lasioglossum* bees are smaller than the widths of the entrance (ML) and exit (EL), and they can enter into the entrance and escape from the exit easily.
2. The body length of *L. flavohirtum* (1.037 ± 0.016 cm, $N = 3$) is nearly as long as the depth of the labellum (1.309 ± 0.114 cm, $N = 20$), so that these bees can climb out from the entrance. By contrast, the body lengths of *L. sauterum* (0.747 ± 0.135 cm, $N = 10$) and *L. sichuanense* (0.673 ± 0.057 cm, $N = 10$) are apparently shorter than the depth of the labellum, and they cannot escape easily from the entrance.
3. The thorax height of both *L. sauterum* (0.203 ± 0.025 cm, $N = 10$) and *L. sichuanense* (0.189 ± 0.012 cm, $N = 10$) is greater than the height between the anther and the bottom of the labellum (AL) (0.153 ± 0.029 cm, $N = 20$). They must squeeze past the anther, such that a portion of the pollen will be smeared on the thorax.
4. The thorax height of *L. sauterum* (0.203 ± 0.025 cm, $N = 10$) is almost the same as the height between the stigma and the bottom of the labellum (SL) (0.205 ± 0.028 cm, $N = 20$). The thorax height of *L. sichuanense* (0.189 ± 0.012 cm, $N = 10$) is slightly smaller than SL. Both bees can pass the stigma without much resistance, but the smeared pollen on their bodies can be swept off by the stigma.

Table 4. Breeding system of *Cypripedium henryi*

| Treatment	No. of flowers	No. of capsules	Fruit set (%)
Cross-pollinated	10	6	60
Self-pollinated	10	4	40
Labellum-deleted only	10	0	0
Natural pollinated (2004)	100	17	17
Natural pollinated (2005)	61	6	19.4

BREEDING SYSTEM

Control flowers that were labellum-deleted and not pollinated ($N = 10$) set no fruit. Hand self-pollination ($N = 10$) produced 40% fruit set, and cross-pollination ($N = 10$) 60% fruit set (Table 4). Natural fruit set was 17% ($N = 100$) in 2004 and 19.4% ($N = 61$) in 2005.

FLORAL FRAGRANCE

Flowering spikes of *C. henryi* emit a weak honey and spice fragrance, pleasant to the human nose. A typical gas chromatogram is presented in Figure 2. By comparison with airborne pollutants and background air analysis, only four chemical compounds were found to be the volatile constituents of the floral fragrance of *C. henryi* (Table 5). The relative abundance of dodecanal (69.34%) is much higher than that of undecane (19.3%), decanal (2.01%), and nonanal (9.35%).

DISCUSSION

The results of the breeding system experiment indicate that the flowers of *C. henryi* are self-compatible, but need pollen vectors for successful reproduction (Table 4). Although not all flowers set fruit in hand self-pollination and cross-pollination samples, the fruit set is obviously higher than that of natural pollination. This result suggests that the fruit set of *C. henryi* is mainly limited by pollinators. The finding of pollen-bearing *L. sauterum* and the high visitation
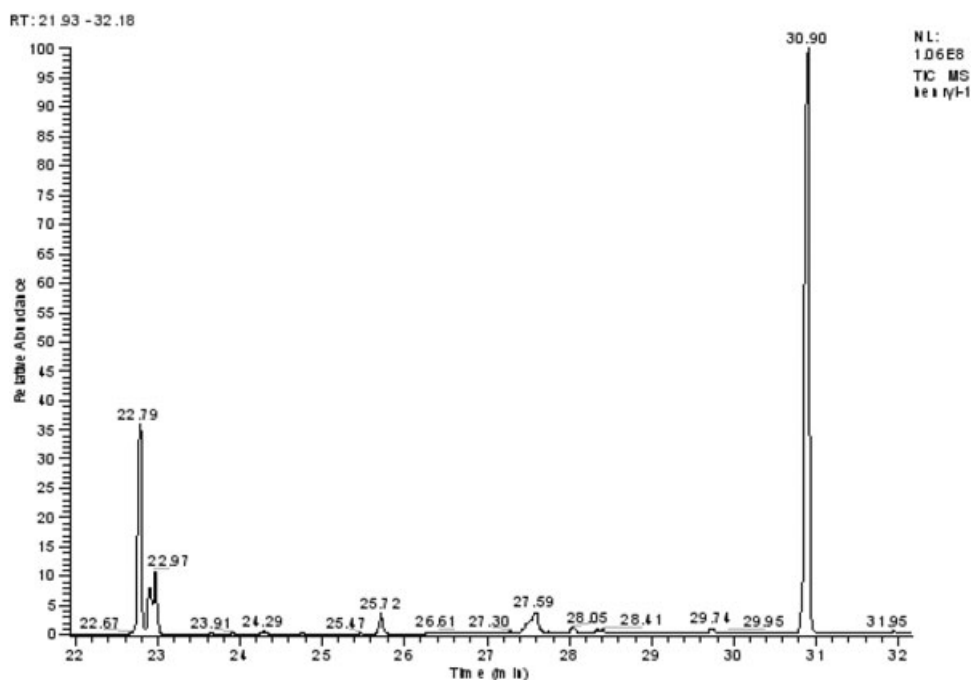
Figure 2. Part of the gas chromatogram of the floral scent of *Cypripedium henryi*.

Table 5. Volatile compounds and their relative abundance from the flower fragrance of *Cypripedium henryi*

Retention time	Volatile compound	Skeletal type	Relative abundance (%)
22.79	Undecane	T	19.30
25.72	Decanal	B	2.01
27.59	Nonanal	T	9.35
30.90	Dodecanal	T	69.34

Compounds classified according to general skeletal type: B, benzenoid; T, terpenoid.

frequency of female *Lasioglossum* bees (Table 1) suggest that *C. henryi* is a halictid bee-pollinated flower. The main pollinators of *C. henryi* are different from those of the allied *C. calceolus*, which is pollinated largely by female *Andrena haemorrhoa* and other *Andrena* spp., with species of *Lasioglossum* and *Halictus* also acting as pollen vectors (Nilsson, 1979). Female Halictidae (sweat bees) also pollinate other slipper orchids (see review by Bänziger *et al.*, 2005).

MECHANISM OF ATTRACTION

Pollination by deceit is one of the keys to orchid floral and species diversity (Cozzolino & Widmer, 2005; Schiestl, 2005). Most *Cypripedium* species attract insects through a generalized food deception system (Stoutamire, 1967; Nilsson, 1979; Vogt, 1990; Bän-

ziger *et al.*, 2005). Only in *C. macranthos* var. *rebunense* did Sugiura *et al.* (2001, 2002) consider that it mimics the nectar-producing flowers of *Pedicularis schistostegia* to attract bumblebees. In our study, *Fragaria orientalis* and *Ranunculus* sp. were the main co-blooming flowers with *C. henryi*, and were frequently visited by *Lasioglossum* bees. However, the flowers of *Fragaria orientalis* are white and those of *Ranunculus* sp. are yellow, and are quite different in coloration and shape from those of *C. henryi*. Thus, there may be no particular model plant that promotes *Lasioglossum* bees to visit the nectarless *C. henryi* flowers.

The labellum is a common landing place for pollinators in Orchidaceae (Van der Pijl & Dodson, 1966). In addition, Nilsson (1981) considered that, in *Cypripedium*, the inflected rim of the entrance, particularly that close to the staminode, may function partly as a sliding zone to promote the entrance of bees passively in *C. calceolus* and several other species. However, in certain other species, for example *C. acaule* and *C. arietinum*, the pollinators enter voluntarily because of the bee's motivation to explore the concealed structures inside the labellum (Stoutamire, 1967). The labellum of *C. henryi* is not only slippery, but also has red spots inside (Fig. 1B). Thus, some *Lasioglossum* bees were observed to enter voluntarily, as if to explore the inside of the labellum, and some entered passively as a result of a loss of balance. Therefore, the labellum of *C. henryi* may promote *Lasioglossum* bees to land on and enter the labellum

by trapping the pollinators both voluntarily and passively.

In addition to the labellum, the slippery staminode provides an alternative landing site for *Lasioglossum* bees, and sometimes the bees may stretch towards the staminode after they have landed on the labellum (Fig. 1F). These results indicate that the bees are apparently attracted by the staminode. In *C. calceolus*, the crimson-spotted floral structures are considered to be false nectar guides, which are important for the attraction and entering of the bees (Nilsson, 1979). In *C. guttatum*, the staminode is shiny and slippery, and may function as a sliding zone to promote the entrance of bees when they attempt to bridge the hollow part of the pouch by trying to climb onto the staminode (Bänziger *et al*., 2005). As it is both slippery and possesses red spots (Fig. 1A, B), the staminode of *C. henryi* seems to function as both a false nectar guide and sliding zone. Undoubtedly, the staminode of *C. henryi* plays an important role in promoting *Lasioglossum* bees to land on and enter the labellum.

POLLINATORS

Although three *Lasioglossum* species visited the flowers of *C. henryi* (Table 1), they displayed various degrees of association with this orchid. *Lasioglossum flavohirtum* is apparently larger in size than *L. sichuanense* and *L. sauterum* (Table 3), and its body length is almost equal to the depth of the labellum. Therefore, it can climb out from the labellum entrance of *C. henryi* and cannot be regarded as a pollinator of *C. henryi*. Similarly, in *C. calceolus*, Nilsson (1981) stated that the depth of the labellum should be at least 3–4 mm longer than the body length of the visiting bees to prevent the visiting bees from escaping easily via the entrance.

Lasioglossum sauterum matches morphologically the floral structures of *C. henryi* (Table 3). However, only five of the ten individuals collected as they escaped from the exit of the flower were found with a pollen mass of *C. henryi* on the thorax. These results suggest that not all *L. sauterum* bees are effective pollinators. It is noteworthy that some flowers of *C. henryi* were frequently re-visited by *Lasioglossum* bees (Table 2). Although the flower of *Cypripedium* has two lateral fertile anthers, one pollinator will take most of the pollen mass away from one anther (Fig. 1H), and there will be no pollen for removal for later visiting bees. Thus, some bees did not carry pollen on their bodies because of the limited pollen in the anther.

Lasioglossum sichuanense is similar to *L. sauterum* in body size and, morphologically, can be considered as a potential pollinator of *C. henryi* (Table 3).

However, five *L. sichuanense* specimens collected after visiting the flowers of *C. henryi* had no pollen of *C. henryi* on their bodies. The only explanation for this is that *L. sichuanense* visits the flowers of *C. henryi*, but does not enter the labellum. It is interesting to note that another *Cypripedium* species, *C. plectrochilum*, co-blooms with *C. henryi* at the same site, and both species can set fruit by hand hybridization. Moreover, *L. sichuanense* is the main pollinator of *C. plectrochilum* (Li *et al*., in press). However, no intermediate forms between these two slipper orchids were found in our study area. Considering the compatibility of artificial cross-pollination between *C. henryi* and *C. plectrochilum*, the absence of intermediate forms suggests that no pollen is transferred between *C. plectrochilum* and *C. henryi*. In other words, *L. sichuanense* bees probably do not enter the labellum of *C. henryi* although they land on this site. Red spots are present on the labellum and staminode of both *C. henryi* and *C. plectrochilum*. Thus, these red spots cannot discourage *L. sichuanense* bees from entering the labellum of *C. henryi*. Nilsson (1979) suggested that floral scent is important in enticing visitors to enter the labellum of *C. calceolus*. Our floral scent analysis shows that the dominating compound in the floral scent of *C. henryi* is dodecanal (69.34%), whereas that of *C. plectrochilum* is 3-methyl-3-decen-2-one (79.54%) (Li *et al*., in press). It is reasonable to hypothesize that the floral scent of *C. henryi* discourages *L. sichuanense* from entering into its labellum. Whether the floral scent is responsible for the reproductive isolation between these two sympatric co-blooming *Cypripedium* species that share at least some visitor species is not known, and requires further direct experiments.

floral fragrance, and Mr Tang Siyuan, Mr Dong Li, Mr Tai Yundong, and Mr Yang Baolin (Huanglong Administration of National Scenic Spots) for their help with the fieldwork.

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