Pollination of wild lady slipper orchids Cypripedium yunnanense and C. flavum (Orchidaceae) in south-west China: why are there no hybrids?

HANS BÄNZIGER1,2, HAIQIN SUN1 and YI-BO LUO1*

1State Key Laboratory of Systematic and Evolutionary Botany, Chinese Academy of Sciences, 20 Nanxincun, Xianshang, Beijing 100093, People’s Republic of China
2Department of Entomology, Faculty of Agriculture, Chiang Mai University, 239 Huay Kaeo Road, Chiang Mai 50202, Thailand

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Rewardless kettle-trap flowers Cypripedium yunnanense and C. flavum were watched for pollinators during 73 and 101 man-hours, respectively, in north-west Yunnan at 3490–3590 m a.s.l. They differ from typical Cypripedium, such as C. calceolus, in having a broad infolded flap of the lip extending all around the entrance of the pouch (instead of being restricted to the vicinity of the staminode) and in the flap not being slippery. Cypripedium yunnanense is pollinated by Lasioglossum zonulum euronotum (Halictidae), and C. flavum is pollinated by Andrena orchidea and Andrena sp. (Andrenidae, two of nine new hymenopterans discovered at the sites). The bees do not inadvertently fall into the trap by slipping (as often occurs in other slipper orchids), but enter it by crawling down the flap in full control of their movements. No natural hybrids between the two orchids are known, although they occur in close-by or mixed stands, are co-flowering and size compatible with regard to their pollinators, and produce fruits following manual cross-pollination in situ. Analyses of the (non-Cypripedium) pollen carried indicate that: (1) the two andrenids are probably oligolectic; (2) the andrenids and the halictid do not share the same flower species; and (3) the halictid is polylectic. Points (1) and (2) are probable reasons for the lack of hybrids. The other reason why Lasioglossum zonulum euronotum does not visit C. flavum despite being polylectic may be the flower’s odour; cases of discrimination of closely related flowers by polylectic Lasioglossum have been reported elsewhere. Blow flies Calliphora vomitoria and Calliphora pattoni (Calliphoridae) also enter the orchids, some smearing themselves with pollen, yet they are not pollinators. They are too large to leave by the exit and die imprisoned. However, they may be accidental pollinators of the rather larger C. tibeticum present at the sites. Such accidental pollinators probably play an important role in the evolution of new pollination syndromes. © 2008 The Linnean Society of London, Botanical Journal of the Linnean Society, 2008, 156, 51–64.


INTRODUCTION

The state of knowledge about the pollination of lady slipper orchids has been reviewed mainly by Stoutamire (1967), Nilsson (1979), and Bänziger, Sun & Luo (2005) for temperate species (Cypripedium), and by Bänziger (1996) for tropical species (Paphiopedilum). Only a fraction of the known species have so far been researched, and results are often preliminary because of the difficulty of such studies. This is unfortunate because a correct understanding of reproduction is fundamental for the conservation of these increasingly scarce or endangered plants.

Pollinators are known in nine of the c. 45 known species of Cypripedium (Müller, 1868, 1869; Guignard, 1886; Stoutamire, 1967; Daumann, 1969; Kipping, 1971; Nilsson, 1979; Catling & Knerer, 1980; Davis, 1986; Sugiiura et al., 2002; Bänziger et al., 2005), in six of the 70 species of Paphiopedilum (Atwood, 1985; Bänziger, 1994, 1996, 2002), in one of

*Corresponding author. E-mail: luoyb@ibcas.ac.cn
the 15 species of *Phragmipedium* (Dodson, 1966), and in none of the monotypic *Mexipedium* and *Selenipedium*. Although Hymenoptera (Andrenidae, Apidae, Halictidae, Megachilidae) are involved in the transfer of pollen in *Cypripedium*, Diptera (Syrphidae) are the vectors in *Paphiopedilum* and, curiously, both insect orders in *Phragmipedium*. Lady slippers are rewardless kettle-traps that cheat by one or a combination of tricks, such as food deception, brood-site deception, and floral mimicry (Atwood, 1985; Bänziger, 1996, 2002; Sugiuara et al., 2002).

In the first study of the natural pollination of a lady slipper orchid in China, home to some 30 species of *Cypripedium* (Cribb, 1997; Chen, 1999), it was shown that *Cypripedium guttatum* Sw. had unusual features for a *Cypripedium*. Aspects of the flower morphology and the slippery means of trapping the pollinators were more akin to *Paphiopedilum* than to *Cypripedium* (Bänziger et al., 2005). Yet, the pollinators were not Diptera but Hymenoptera: at least three species of sweat bees (Halictidae), namely *Lasiusoglossum virideglauccum* Ebmer & Sakagami, *L. clypeinitens* Ebmer, and *L. sauterum* Fan & Ebmer; *L. mystaphium* Ebmer was an almost certain pollinator and *L. alloclatum* Ebmer & Sakagami was a probable pollinator.

In the current pollination study, two slipper orchids with more classical *Cypripedium* features were investigated, namely *C. yunnanense* Franchet and *C. flavum* P. F. Hunt & Sumh. Our first objective was to establish the type and behaviour of the pollinators to check whether the pollination mechanisms of these two species were indeed orthodox for *Cypripedium* orchids. Secondly, there was a need to shed light on the intriguing enigma of why no hybrids occur between these two species. They grow in mixed or near-by stands, are co-flowering and, although they look very different, are mutually size and function compatible with regard to their pollinators. Thus, they are unlike two other co-flowering slipper orchids at the same sites: *C. guttatum* and *C. tibeticum* King ex Rolfe. It was shown that the former is and can only be pollinated by much smaller pollinators, whereas the pollinators of the latter, although not yet known, must be much larger than those of the other three species present, otherwise they would not be able to acquire and deposit pollen.

Natural hybrids have been described from several species of *Cypripedium*, for example between *C. calceolus* L. and *C. macranthos* Sw. (= *ventricosum* Sw.), and between *C. parviflorum* Salisb. and *C. candidum* Muhl. ex Wild. (= *andrewsii* Fuller) (Stoutamire, 1967; Klier, Loeschke & Wendel, 1991). Many more natural hybrids are known in tropical slipper orchids, for example between *Paphiopedilum tonsum* (Rchb. f) Stein and *P. superbiens* (Rchb. f) Stein (= *frankeanum* Rolfe) (Rolfe, 1908), and between *P. venustum* (Wall.) Pfitzer and *P. insigne* (Wall. ex Lindl.) Pfitzer (= *venusto-insigne* U. C. Pradhan) (Pradhan, 1976). Hundreds of different hybrid combinations have been produced by artificial crossing, a standard practice in the multimillion US$ orchid agroindustry and amongst orchid enthusiasts. Incompatibility amongst *Cypripedium* species is unknown. Yet, there have been no reports on natural hybrids between *C. yunnanense* and *C. flavum*.

The distribution of *C. yunnanense* and *C. flavum* encompasses north-west Yunnan, south-west Sichuan, and south-east Tibet, at 2700–3700 m a.s.l., but *C. flavum*, which is far more common than *C. yunnanense*, is also present in west Hubei and south Gansu (Cribb, 1997; Chen, 1999; Chen, Ti & Luo, 1999).

**MATERIAL, METHODS, STUDY SITES, AND HABITAT**

These were essentially the same as in the study of *C. guttatum* growing at the same sites (for details, see Bänziger et al., 2005). However, *C. guttatum* was studied mainly at Na Pa Hai (site a, at 3490 m a.s.l.), whereas *C. yunnanense* and *C. flavum* were researched also at Tian Sheng Qiao 1 (site b) and 2 (site c), which were 0.5 km from each other, at 3590 m a.s.l., some 20 km east of Zhongdian town, north-west Yunnan, south-west China.

Flower watching sessions of *C. yunnanense* and *C. flavum* were carried out between 5 and 21 June 2002 for a total of 73 and 101 man-hours, respectively. In order to better understand the interrelationship between the guilds of pollinating, visiting, and non-visiting sympatric insects of the slipper orchids at the study sites, a fourth species, *C. tibeticum* King ex Rolfe, was also watched (4 man-hours) at site a.

The rate of natural pollination was assessed by marking 127 *C. yunnanense* and 484 *C. flavum*, and checking ovary swelling 2 weeks later and fruit development after 3 months (by H.S.). To assess possible incompatibility between *C. yunnanense* and *C. flavum*, six pairs were manually cross-pollinated *in situ*. Pollinator access was prevented by cutting off the orchid’s lip.

For the study of polylectic versus oligolectic, pollen was removed from the bees with a pin soaked in Canada balsam, mounted in this medium on a microscope slide, and examined under a compound light microscope.

For the slipper orchids, the systematics of Cribb (1997) were followed. For the flower visiting bees, Scheuchl (2005), who described three of the five new species of Andrenidae caught, Ebmer (2002) and
A. Ebmer (Puchenau, Austria, pers. comm.), who described all three new species of Halictidae, Dathe (2005), who described one of the two new species of Colletinae, and B. Tkalcu (Prague, Czech Republic, unpubl. data), who identified the two new Megachilidae and the Apidae, were followed.

**POPULATION STRUCTURE AND FLOWERING**

The slipper orchids tended to grow on slopes facing north-east, in small clearings, where they generally were in full sunlight for only 2–5 h per day and in the shade of the surrounding vegetation for the rest of the day. *Cypripedium yunnanense* grew in clumps of a few flowers to up to nearly 20 flowers. At the three sites, some 20 clumps with a total of about 130 flowers were found. The largest concentration of *C. yunnanense* was 51 flowers over an area of 5 × 15 m (site b). *Cypripedium flavum* can have large populations: at site c alone, over 1000 flowers were estimated, with little evidence of clump formation. At sites b and c, flowers or clumps of *C. yunnanense* and *C. flavum* grew intermingled, adjacent, or only a couple of metres apart. This allowed for contemporaneous watching of both species for pollinators.

Flowering started around the last week of May and ended in mid-July. From our observations, flowering in *C. yunnanense* peaked early in the second week of June, and about a week later in *C. flavum*. Flower longevity was about 3 weeks; therefore, the flowering periods of the two species overlapped.

**FUNCTIONAL MORPHOLOGY (FIGS 1, 2, 5–8)**

*C. YUNNANENSE*

The flower stands 0.3–0.4 m high, and hence is well above the grass carpet of its habitat. Basally, the petals are densely covered with hairs up to 2.9 mm long, part of the mechanism to keep the pollinator adequately pressed against the anthers for pollen acquisition (cf. Fig. 3). The ellipsoid to reniform lip is 3.5–5 cm long, 2.5–3.2 cm wide, and 2 cm high. The outer surface is rugulose, pink–purple, with more or less evident deep purple stripes radiating from the mouth of the pouch-like lip. This system of stripes, present even more clearly on the sepal and petals, converging as it does towards the centre of the flower, i.e. the entrance of the pouch, is interpreted as a false nectar guide. The entrance is further enhanced by its often conspicuously white rim, darkish mouth, and the downward-pointing staminode.

Along the rim the pouch is infolded, although in a different manner than in other *Cypripedium*, such as *C. calceolus*. In this, the rim is rounded (not ridge-like) and very narrowly infolded (about 1 mm) all along the pouch entrance, except near the staminode where the rim extends into a long concave flap reaching down for 3–4 mm. In *C. yunnanense* (and *C. flavum*), the fold is ridge-like (Figs 1, 2, 5–8) and the flap extends all around the mouth (Figs 6, 8) of the lip and down for 5–10 mm. The flap’s lowest rim is only about 10–12 mm from the pouch bottom. This distance is narrow enough for the pollinators to bridge without necessarily needing to jump down to the bottom. Thus, the pouch actually has two entrances: an outer one at the white rim, and an inner, narrower entrance at the lower edge of the flap (Figs 2, 6, 8). The entrances tend to be elliptical rather than circular (0.9–1.3 × 0.6–1.0 cm for the outer and 0.5–0.9 × 0.7–1.0 cm for the inner entrance), and (unlike in *C. flavum*) are set across each other, i.e. the outer entrance’s span is greater crosswise than lengthwise, the opposite of the inner entrance. On the outside the flap is not slippery, as bees were seen to freely crawl down and up without slipping off. Therefore, the pouch does not function as a pit trap, as the victims do not inadvertently fall into it. It is more similar to a fish trap with a funnel-like entrance, easy to enter but difficult to leave. Additional devices making an escape by the entrance difficult are the overhanging lip walls and lack of hairs to climb them. Hairs are found mainly on the hind wall leading up to the stigma, anthers, and the legitimate exit, where the longest are up to 2.9 mm. The pouch has ‘windows’ near the exit, but they are much less evident than in *C. calceolus*, and covered partly by the petals.

The papillose stigma is tongue-shaped, 5 mm wide and 8 mm long. The anthers are 2.9 × 3.5 mm and the exit gap is 3.0–3.5 mm wide. The three mechanisms keeping the exiting pollinator adequately pressed (elastically in order to allow a certain size variation of the pollinator) against the anthers in order to smear off pollen are essentially the same as detailed in *C. guttatum*. The cordate to triangular, 10 mm wide and long staminode is basally and laterally whitish to pale pink–purple, centrally and distally more or less dark pink–purple.

The scent of the flower is a sweetish fragrance, curiously reminiscent of Milo powder (Nestle’s health beverage).

*C. FLAVUM*

Except for the colour and shape of some structures, this slipper orchid is otherwise functionally quite similar to *C. yunnanense*. The differences are provided here. The sepal and petals are mostly plain yellow, but may be reddish brown or with brown–red dots. The hairs at the base of the petals are only about 1 mm long. There are no lines which could
serve as nectar guides. The pouch is 3.5–4.5 cm long and 3–3.5 cm wide, yellow, occasionally with a green tinge, generally without or with a few brown–red dots, and the surface is not rugulose. The outer pouch entrance is 0.9–1.1 cm wide and 1.0–1.3 cm long, its rim concolorous with the pouch; the inner entrance is 0.5–0.9 cm wide and 0.8–1.3 cm long (the elliptical entrances being set in the same direction, not cross-wise). The flap is therefore slightly longer than in *C. yunnanense*. The staminode, 5 mm wide and 8 mm long, is tongue-shaped, yellow on its upper one-fifth to one-third, dark maroon on the lower two-thirds to four-fifths, sometimes yellow at its lowest rim. The exit gap is 3.0–3.5 mm wide. The stigma is quadrangle-like and 7–8 mm wide.

The odour is very different from that of the other three species in the study area, which are all fragrant. *Cypripedium flavum* has a sweetish odour mixed with a somewhat unpleasant acrid note, vaguely reminiscent of chloridric acid.

**THE FLOWER VISITORS**

**HALICHTIDAE**

The sweat bees present have already been listed, and their size and behaviour treated, in the study of *C. guttatum*. Of the ten species found, three were new to science, and described by Ebmer (2002). *Lasioglossum zonulum euronotum* Ebmer (Figs 3, 9, 10), the only pollinator of *C. yunnanense*, was the largest, with a thorax height of 2.5–3.1 mm.

**ANDRENNIDAE**

Eight species of solitary bees were present (Table 1). Only two pollinated *C. flavum*, namely *Andrena orchidea* Scheuchl (Figs 4, 11) and *Andrena* sp. nov.
Both were new taxa at the time of our study, but only the former was described because the latter is known only from a single female specimen not clearly distinguishable from *A. fucata* (Scheuchl, 2005). Of the other six species of Andrenidae, three species were also new discoveries, and described as *A. euphorbiacea* Scheuchl and *A. solutiscopa* Scheuchl, but *Andrena sp. nov. (bicolor* Fabricius group) remains untreated because it belongs to a group needing taxonomic revision (Scheuchl, 2005).

The genus *Andrena* is huge, with probably over 1300 species worldwide, but they are mainly holarctic and, unlike the Halictidae, only few species occur in the tropics of the Oriental region (Michener, 2000). Nests are in the ground, walls, wood, etc., mostly

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**Figures 5–8.** Morphology of the flowers of *Cypripedium yunnanense* and *C. flavum*. Figs 5, 6. *C. yunnanense*, frontal view and longitudinal cross-section of the flower through the column. Figs 7, 8. *C. flavum*, frontal view and longitudinal cross-section of the flower through the column. a, anther; f, flap; hw, climbable hind wall; l, lip; p, petal; rie, rim at the inner entrance; roe, rim at the outer entrance; s, staminode; se, sepal; st, stigma. Bar, 1 cm. Drawings by Mrs Li Ai-li.
individual, rarely communal. No eusocial species are known (Michener, 2000). Many species are polylectic, but there are also a number of broadly and even strictly oligolectic species (Wестрих, 1989; Michener, 2000; E. Scheuchl, Velden, Germany, pers. comm.).

**BEHAVIOUR OF THE POLLINATOR OF C. YUNNANENSE: LASIOGLOSSUM ZONULUM Euronotum** (Table 3, Figs 9, 10)

The pollinators generally flew quite straight to the flower, although sometimes they zig-zagged before landing on it, showing that at least sometimes the scent played a role in guiding the bees. The 16 landings seen were all onto the pouch, never the staminode. The pollinators then crawled to the outer entrance of the pouch. They entered it from the lateral side where the flap is least steep. Without slipping, the pollinators crawled down the flap to the inner entrance of the pouch. The next movements were less clearly seen because they occurred very rapidly. The bees bridged the space between the flap’s lower rim and the pouch bottom either by lowering themselves down with extended front legs to touch the pouch bottom, whilst keeping their grip on the rim with their extended hind legs, or by jumping down.

Once inside the pouch, there was no (or not much) thrashing to free themselves, possibly because they were not frightened by a sudden tumbling as occurs in other slipper orchids. The bees proceeded rapidly up the hind channel to the stigma and on to the anthers. Backing down from a very advanced position...
was not observed, although, in a number of cases, a backing down from a less forward position was seen, but this was not sufficiently advanced to result in the acquisition of pollen, with consequent selfing on climbing up again, as has been seen in *C. guttatum*.

Extrication from the exit gap occurred in the relatively short time of about 1 min if the anthers were still fully pollen-loaded, or briefer if less pollen remained to be smeared off. The whole imprisonment lasted some 0.5–5 min.

Pollen was acquired in eight cases. In two cases, no pollen was acquired as it was already exhausted by previous pollinators. Previously visited flowers were visited more frequently than ‘virgin’ ones, as also noted in *C. calceolus* and *C. guttatum* (Nilsson, 1979; Bänziger et al., 2005).

### Behaviour of the pollinators of *C. flavum*: *Andrena orchidea* and *Andrena sp.*

*Andrena orchidea* (cf. *fucata* Smith) (Table 3, Figs 4, 11–13)

The pollinators exhibited a rather rapid flight towards the orchid but, in two cases, they briefly hovered in front of it. The landings and subsequent behavioural phases by six *A. orchidea* and one *Andrena sp.* nov. were essentially the same as mentioned for *C. yunnanense* – no ‘unintended’ slipping off and tumbling, but rapid crawling down the flap into the pouch. In one case, a somewhat vehement thrashing first followed entering the pouch, but the bee then quickly climbed up the hind channel. In another case, one *A. orchidea* retreated three times from near the anther but no pollen was acquired. The time spent in the pouch was 1–2 min and pollen was acquired in seven cases. Two specimens had a smear already on arrival at the flower; one of them did not attempt to enter the flower, but the other managed – exceptionally – to leave by the entrance, and so no pollen deposition occurred.

### Behaviour of Other Bees Found at the Study Sites (Tables 2, 4; Tables 1–5 in Bänziger et al., 2005)

The sweat bees *L. allodalum*, *L. clypeinitens*, *L. flavohirtum* Ebmer, *L. mystaphium*, and *L. virideglaucum* visited a wide variety of flower species and occasionally entered the pouch of *C. yunnanense*; the first three also occasionally entered the pouch of *C. flavum*. However, they are not pollinators of *C. yunnanense* and *C. flavum* because they are too small to acquire pollen; they are pollinators of the much smaller *C. guttatum*, except *L. flavohirtum* which is slightly too large for it.

Three species of bumble bee and two species of plaster bee were the only Apidae present. Of the bumble bees, one *Pyrobombus lepidus yuennanicola* and one *Megabombus trifasciatus* entered *C. flavum*, but were too large to leave by the exit and, after many attempts, escaped by the entrance. The colletine *Hylaeus sinensis* entered *C. yunnanensis*, but left quickly and unhindered by the exit because it was too small to acquire a smear.

None of the six Andrenidae species or two Megachilidae species visited the slipper orchids, but were found on other flowers. They would have been too small (*A. solutiscopa* too large) to acquire pollen from the slipper orchids. The two megachilids and two colletines were new species. *Andrena euphorbiacea* and *A. nippon* are evidently polylectic (see below).

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**Table 1.** Andrenidae collected in the study area and their dimensions, listed in order of size

<table>
<thead>
<tr>
<th>Andrena species</th>
<th>Number of specimens</th>
<th>Thorax height (mm)</th>
<th>Total body length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Smallest</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Andrena</em> sp. <a href="Kirby">minutula</a> group</td>
<td>1</td>
<td>1.8</td>
<td>6.6</td>
</tr>
<tr>
<td><strong>Small</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Andrena euphorbiacea</em> Scheuchl</td>
<td>6</td>
<td>2.0–2.4</td>
<td>7.5–9.2</td>
</tr>
<tr>
<td><em>Andrena tsukubana</em> Hirashima</td>
<td>3</td>
<td>2.1–2.3</td>
<td>10.0–10.4</td>
</tr>
<tr>
<td><em>Andrena nippon</em> Tadauchi &amp; Hirashima</td>
<td>9</td>
<td>2.3–2.7</td>
<td>9.2–10.1</td>
</tr>
<tr>
<td><em>Andrena sp.</em> (bicolor Fabricius group)</td>
<td>1</td>
<td>2.5</td>
<td>10.4</td>
</tr>
<tr>
<td><strong>Medium</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Andrena orchidea</em> Scheuchl</td>
<td>3</td>
<td>3.1–3.3</td>
<td>11.7–12.7</td>
</tr>
<tr>
<td><em>Andrena sp.</em> (cf. <em>fucata</em> Smith)</td>
<td>1</td>
<td>3.2</td>
<td>11.5</td>
</tr>
<tr>
<td><strong>Large</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Andrena solutiscopa</em> Scheuchl</td>
<td>1</td>
<td>3.8</td>
<td>14.4</td>
</tr>
</tbody>
</table>

All are females except two *Andrena tsukubana*.

*New to science at the time of the study.*

Blow flies (Calliphoridae) as potential pollinators of *C. yunnanense* and *C. flavum*

Blow flies were examined in some detail because, as a result of their association with the two slipper orchids, they may be mistaken as pollinators. The species present included *Calliphora vomitoria* (L.), *Cal. pattoni* (Aubertin), *Cal. zaidanensis* (Fan), *Calliphora sp.*, *Lucilia caesar* (L.), *Lucilia sericata* (Meigen), *Chrysomya phaonis* (Séguy), and *Triceratopyga calliphoroides* (Rhodendorf). With some 40 sightings (not necessarily always different individuals), *Cal. vomitoria* was by far the most common, the other species having been seen/caught only a few times. The flies visited *Euphorbia stracheyi* Boiss., *Euphorbia yunnanensis* Radcliffe-Smith, and *Crataegus oresbia* W. W. Smith, and rested on various leaves and the pouch of the two slipper orchids (Figs 15, 16). They evidently sunbasked, as they did not move for long periods and did not probe the surface with their tongues. Three male and three female *Cal. vomitoria* and one male *Cal. pattoni* were found imprisoned in the pouch of *C. yunnanense* and *C. flavum*; several additional flies, including one female *L. caesar*, attempted to enter. Possibly, it was to seek shelter when it became too cold during heavy cloud cover, or too hot from solar irradiation. At 3500 m a.s.l., surface temperatures change widely and rapidly because of the very thin air and strong solar irradiation. However, some may have been tricked into seeking food.

Table 2. Additional Hymenoptera collected in the study area and their dimensions

<table>
<thead>
<tr>
<th>Hymenopteran taxa</th>
<th>Number of specimens</th>
<th>Thorax height (mm)</th>
<th>Total body length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apidae, bumble bees (Bombini)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Megabombus trifasciatus</em> (Smith)</td>
<td>1</td>
<td>6.2</td>
<td>17.8</td>
</tr>
<tr>
<td><em>Megabombus securus</em> (Frison)</td>
<td>1</td>
<td>8.1</td>
<td>23.0</td>
</tr>
<tr>
<td><em>Pyrobombus lepidus yuennanicola</em> (Bischoff)</td>
<td>5</td>
<td>3.9–4.5</td>
<td>10.7–11.5</td>
</tr>
<tr>
<td>Apidae, plaster bees (Colletiniae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Hylaeus sinensis</em> Dathe</td>
<td>1</td>
<td>1.4–1.5</td>
<td>7.3</td>
</tr>
<tr>
<td><em>Hylaeus sp.</em> (near <em>medialis</em> Morawitz)</td>
<td>1</td>
<td>1.4–1.5</td>
<td>7.3</td>
</tr>
<tr>
<td>Megachilidae, leaf-cutting bees</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Megachile sp.</em></td>
<td>1</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td><em>Hoplitis sp.</em></td>
<td>1</td>
<td>2</td>
<td>9</td>
</tr>
</tbody>
</table>

Further ten species of Halictidae found in the same study area have been listed in Bänziger et al. (2005: table 1), and are not listed here.

*New to science at the time of the study.

Table 3. Behaviour of pollinators of *Cypripedium yunnanense* and *Cypripedium flavum*

<table>
<thead>
<tr>
<th></th>
<th>Flying to orchid</th>
<th>Landing on pouch</th>
<th>Crawling down flap</th>
<th>Proceeding from rim of flap, down to pouch bottom</th>
<th>Time to exit (min)</th>
<th>Pollen acquisition</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cypripedium yunnanense</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lasioglossum zonulum euronotum</em></td>
<td>19</td>
<td>16</td>
<td>12</td>
<td>11</td>
<td>0.5; 1; 1; 2; 2.5; 5;</td>
<td>8*</td>
</tr>
<tr>
<td><em>Cypripedium flavum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Andrena orchidea</em></td>
<td>7</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>Less than 2; 1.3; 1.75; not noted in one</td>
<td>4</td>
</tr>
<tr>
<td><em>Andrena sp.</em> (cf. <em>fucata</em>)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Andrena orchidea</em> or <em>Andrena sp.</em></td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>Less than 2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Not noted</td>
<td>2</td>
</tr>
</tbody>
</table>

*Anther pollen exhausted in two cases; one specimen not known how it left the pouch.*
The thorax height of these blow flies is 4–5.4 mm, not a hindrance to sneaking through the outer and inner entrances, with only the largest flies requiring some pushing through the flexible lower flap rim. However, the thorax is far too big, and the flies are not strong enough, to force themselves through the maximum exit gap of 3.5 mm. Their weakness is readily evident when the legs of the flies and bees

Table 4. Andrenid bees on flowers other than Cypripedium yunnanense and Cypripedium flavum

<table>
<thead>
<tr>
<th></th>
<th>Heracleum candidans</th>
<th>Rosa ometensis</th>
<th>Anemone obtusifolia</th>
<th>Euphorbia stracheyi</th>
<th>Polygonum macrophyllum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Umbelliferae)</td>
<td>(Rosaceae)</td>
<td>(Ranunculaceae)</td>
<td>(Euphorbiaceae)</td>
<td>(Polygonaceae)</td>
</tr>
<tr>
<td>Andrena nippon</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Andrena euphorbiacea</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Andrena tsukubana</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Andrena sp. (bicolor group)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Andrena sp. (minutula group)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Andrena solutiscopa was not found on flowers, but was collected only once whilst sunbasking on a stone. Andrena orchidacea and Andrena sp. (cf. fucata) were only found on Cypripedium flavum (see Table 3).

Figures 14–17. Non-pollinating visitors of Cypripedium yunnanense and C. flavum. Fig. 14. Female hoverfly Episyrphus balteatus on lip of C. flavum. Fig. 15. Sunbasking female Calliphora vomitoria on lip of C. yunnanense. Fig. 16. Sunbasking male blow fly Calliphora vomitoria on lip of C. flavum. Fig. 17. Male Calliphora vomitoria stuck to death at exit of C. flavum; note the pollen smear on the blow fly head. a, anther; h, head of fly; p, pollen.
are compared: thin in the former, robust in the latter. Nevertheless, the flies often pushed themselves as far as the anther, smearing themselves with the sticky pollen and getting themselves glued to the narrow walls. Because of this, and because blow flies have very strong backward-directed bristles (unlike bees which have short, soft hairs), they were unable to back down and perished there (Fig. 17). Thus, no selfing occurred. Other specimens, without smears, died of starvation or desiccation at the pouch bottom because they were unable to escape by the entrance.

**Hoverflies (Syrphidae) as potential pollinators of C. yunnanense and C. flavum**

Four species [Episyrphus balteatus (De Geer), Melanostoma orientale (Wiedemann), Sphaerophoria sp. (near nigriris Brunetti) and gen. sp.] were attracted to and landed on the slipper orchids from a total of some ten species present in the area. The two hoverflies are so closely related that they were unable to back down and perished there (Fig. 17). Thus, no selfing occurred. Other specimens, without smears, died of starvation or desiccation at the pouch bottom because they were unable to escape by the entrance.

**DISCUSSION**

A remarkable result of our studies was that 12 of the 25 species of hymenopterans found were new to science (including three new species of Halictidae mentioned in our previous study at the same sites). Seven have already been described.

Given the low population density of bees (only 122 specimens caught – still remarkable at 3600 m elevation) and the typical low insect attendance of slipper orchids, it is not surprising that, despite a total of 174 crosses between C. yunnanense and C. flavum, the natural fructification in C. yunnanense and C. flavum, and the results of the manual crossing experiments between them, had to be assessed from ovary swelling rather than capsule formation, which is less reliable. Natural fructification occurred in 21% of C. yunnanense and 11% of C. flavum. Three of the six crosses between C. yunnanense and C. flavum produced a swollen ovary, two of which survived yak grazing, showing that these orchids are evidently compatible.
C. parviflorum (Guignard, 1886; Stoutamire, 1967), both with seven successful acquisitions or depositions. Better researched are only C. calceolus and C. guttatum, with more than 80 and 11 pollen uptakes observed (Nilsson, 1979; Bänziger et al., 2005), respectively. Only very few cases, without trapping details, have been reported in the remaining Cypripedium studied, namely the North American C. acaule, C. arietinum, C. californicum, C. candidum, C. reginae, and the Japanese C. macranthos (Guignard, 1886; Stoutamire, 1967; Kipping, 1971; Catling & Knerer, 1980; Davis, 1986; Sugiyama et al., 2001).

The fine-tuned devices of C. yunnanense and C. flavum for manipulating pollinators, and the bees’ behaviour during the various phases of pollination are, with one exception, essentially the same as in C. guttatum, where they have been described in detail (Bänziger et al., 2005). The difference lies in the way in which the pollinators are captured, which, in C. yunnanense and C. flavum, is closer to that in C. calceolus. In this species, Nilsson (1979) noted that most landings were on the margin of the entrance, but large species occasionally landed also on the staminode, medium-sized species frequently also on the infolded flap, and small species ‘alighted directly into the entrance’. Once landed, most bees entered the flower in a ‘striving to explore the inside and not as an involuntary act caused by the inflected rim’. However, some bees attempted to climb onto the staminode, slipped off and fell into the pouch (Nilsson, 1979). Daumann (1969) saw bees slipping from the staminode and the flap which he, following Knoll (1922), pointed out to be slippery in this species. Recent studies (H. Bänziger, A. Gigon and S. Panthi, unpubl. data) agree with the above authors: four bees fell from the staminode, and imprisoned bees attempting to climb out by the flap constantly slid off its slippery surface; another seven bees entered the flower more distally where there is only a very narrow, rounded flap, four bees slipping and three not. To sum up, in C. calceolus, both ‘intentional’ entering of, and ‘unintentional’ tumbling into, the pouch occur.

In C. yunnanense and C. flavum, the staminode did not trigger the capture of the bees. None of them alighted on, or attempted to crawl onto, the staminode. Nor did any of the bees inadvertently fall off any other part of the flower, which evidently does not work as a pit trap. They entered the lip ‘voluntarily’ by crawling down the non-slippery flap, swiftly and in full control of their movements. It was more similar to the entering of a tubular flower, such as Salvia (Labiatae) or Strobilanthus (Acanthaceae). The main difference in such tubular flowers is that the legs and body of the bees are in constant contact with the narrow walls, hence ensuring the retreat from the same entrance. Inside the slipper orchids, by contrast, the overhanging walls of the wide-bodied pouch offer no climbable connection to the entrance. The chances of exiting through the entrance are slim, except by flying, which is possible for very small non-pollinators or, in the case of large non-pollinators, by ‘standing up’ to grip the flap and climb out. Actual pollinators are too small for this, or too large to fly out.

In C. guttatum and all six Paphiopedilum species studied so far, the pollinators tumbled into the pouch by slipping off the staminode (Atwood, 1985; Bänziger, 1994, 1996, 2002; Bänziger et al., 2005). The pollinators are all hoverflies (Syrphidae) except, significantly, in the Paphiopedilum-like C. guttatum, where they are bees. In its morphological counterpart, the Cypripedium-like P. micanthum T. Tang & F. T. Wang, pollinator capture is again by slipping off the staminode but, surprisingly, the pollinators are not flies but hymenoptera, namely the bumble bee Pyrobombus flavescens (Smith) (Apidae, Bombini) and the digger bee Anthophora villosula Smith (Apidae, Anthophorini) (H. Bänziger & J. Shi, unpubl. observ.).

Nevertheless, the staminode of C. yunnanense and C. flavum may well be the main centre of attraction and guidance for bees prior to landing, owing to its colour, shape, and possibly as an important organ of fragrance production (Bergström et al., 1992; but see Nilsson, 1979).

In C. yunnanense and C. flavum, only one and two pollinator species were found, respectively, which is unusual. In C. calceolus, six species of Andrena (Andrenidae), as well as four species of Lasioglossum and one Halictus species (all Halictidae), were involved in southern Sweden (Nilsson, 1979). Recent additions are three Andrenidae [A. cineraria (L.), A. lapponica Zetterstedt, A. nitida (Müller)], three Halictidae [Halictus rubicundus (Christ)], L. fulvicorne (Kirby), L. laevigatum (Kirby)] and, for the first time, one Megachilidae (Osmia parietina Curtis), observed at a single site in southern Switzerland (H. Bänziger, A. Gigon and S. Panthi, unpubl. data). Of these, only A. lapponica did not acquire a smear, although it is of the correct size. Several more andrenid and halictid species have been reported from C. calceolus in Germany (Müller, 1868, 1869, 1873) and the Czech Republic (Daumann, 1969), but it was not always clearly stated which species actually smeared off pollen. In C. guttatum, three confirmed and two probable Lasioglossum pollinator species have been reported (Bänziger et al., 2005). The explanation for the parsimony of pollinator species in C. yunnanense and C. flavum is evidently the local lack of suitably sized bees, namely with a thorax height of some 3–3.5 mm. All other potential pollinators were either too small or too large (Tables 1, 2; table 1 in Bänziger et al., 2005). A partial exception may be the unusually small Pyrobombus lepidus yuenmanicola which, thanks
to the strength typical of bumble bees, might be able to forcefully push itself through the exit and thereby smear off pollen as an ‘accidental’ illegitimate pollinator (see below). It is almost certain that research in other regions will result in the identification of additional or vicariating pollinator species of the two slipper orchids.

Interestingly, *A. orchidea* and *Andrena* sp. nov. have not been seen on flowers other than *C. flavum*, although the former was the second most common andrenid. It is obvious that, for their survival, these bees must forage on at least one other flower species, as *Cypripedium* does not offer food. The analysis of pollen carried by *A. orchidea* and *Andrena* sp. nov. allowed two important conclusions to be drawn.

1. Only one or two types of pollen (besides that of *C. flavum*) were present in the two species, respectively, corroborating the observation that the former species, at least, is an oligolege. Oligolecty is known in several Andrenidae, e.g. *A. curvungula* Thomson on *Campanula glomerata* L. and nine other species of *Campanula* (Campanulaceae), *A. tarsata* Nylander on *Potentilla anserina* L. and three further *Potentilla* species (Rosaceae), *A. lapponica* on *Vaccinium myrtillus* L. and three further *Vaccinium* species (Ericaceae) (Westrich, 1989), and *A. vaga* Panzer on *Salix* species (Salicaceae) (Skov, 2000).

2. The three types of pollen on *A. orchidea* and *Andrena* sp. nov. were different from the eight types carried by *L. zonulum euronotum*, corroborating the observation that the two andrenids and the halictid do not share the same flowers.

Points 1 and 2 provide a partial explanation for the lack of natural hybrids between *C. flavum* and *C. yunnanense*. The other part of the explanation – why *L. zonulum euronotum* visits *C. yunnanense* but not *C. flavum* – is more complex as *Lasioglossum* is notoriously polylectic (Westrich, 1989; Skov, 2000; A. Ebmer, pers. comm.). Our observations on the foraging habits of *L. zonulum euronotum* (Bänziger et al., 2005: table 2) and the pollen analyses (eight types of pollen carried in this study) confirm this also for south-west China. One reason why this halictid did not visit *C. flavum* is possibly its peculiar odour, which is of a rather different type from that of the other slipper orchids present. Cases in which the scents of orchids cheat a particular group of pollinators, or even only a single species, and how this can lead to the evolution of new species of flowers, have been discussed elsewhere (for example, Bergström et al., 1992). It can be tentatively assumed that the combination of the odour and certain visual cues provided by *C. flavum* may not match the desirable palette of rewards required by *L. zonulum euronotum*. Although strong polyleges, such as this bee, may visit a wide spectrum of flowers, it seems probable that they are not indiscriminately attracted to all flower species, and may even avoid some. For instance, the flowers not visited for pollen by otherwise polylectic halictid bees include two species of *Ajuga* and three species of *Teucrium* (Labiatae), five species of *Ribes* (Rosaceae), two species of *Sisymbrium* (Cruciferae), seven species of *Lathyrus*, three species of *Ononis*, and nine species of *Vicia* (Leguminosae) (Westrich, 1989). There are even cases of discrimination within genera, e.g. *Trifolium alpestre* L., *T. arvense* L., and others are not visited by *Lasioglossum*, whereas *T. pratense* L. and *T. repens* L. are (Westrich, 1989).

The finding that blow flies can enter the lip of *C. yunnanense* and *C. flavum*, but are not pollinators even when they smear themselves with pollen, is important in two respects. First, like other illegitimate visitors, they might be mistakenly assessed as pollinators. Possibly, the hoverfly *Syrphus torvus* Osten & Sacken and the scarabaeid beetle *Trichiotinus assimilis* (Gory & Percheron), considered as pollinators of *C. reginae* by Vogt (1990) (the result of a mere 8 h of flower watching), may be cases similar to our blow flies, although two of the hoverflies and one of the beetles were able to leave the flower with a smear, whereas our blow flies died imprisoned. However, the blow flies would probably be able to negotiate the exit gap of *C. tibeticum*, which spans more than 5 mm. In any case, *Cypripedium*, including *C. reginae*, are typically melittophilous, and the actual pollinators of *C. tibeticum* are probably bumble bees and/or certain large anthophorids. Second, data on ‘accidental’ pollinators, such as the blow flies, are often dismissed as irrelevant, when in fact they could be significant for the understanding of co-evolution and the development of new species. They may foreshadow how taxonomically completely unrelated, illegitimate pollen vectors may, when environmental changes come to bear during the course of time, supplant the legitimate pollinators while the orchid adapts. This may lead to unexpected new pollination syndromes and could explain why, apparently incongruously, *C. guttatum* and *P. micranthum* are pollinated by the same rather than by different groups of insects (bees in both, instead of bees in one and hoverflies in the other). In *C. guttatum*, the shape suggests myiophily, although the genetic relationship correctly indicates melittophilous, whereas, in *P. micranthum*, the shape correctly indicates melittophilous, although the genetic relationship wrongly suggests myiophily.

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