RESEARCH PAPER

Pollination by deceit in *Paphiopedilum barbigerum* (Orchidaceae): a staminode exploits the innate colour preferences of hoverflies (Syrphidae)

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ABSTRACT

Paphiopedilum barbigerum T. Tang et F. T. Wang, a slipper orchid native to southwest China and northern Vietnam, produces deceptive flowers that are self-compatible but incapable of mechanical self-pollination (autogamy). The flowers are visited by females of *Allograpta javana* and *Episyrphus balteatus* (Syrphidae) that disperse the orchid's massulate pollen onto the receptive stigmas. Measurements of insect bodies and floral architecture show that the physical dimensions of these two fly species correlate with the relative positions of the receptive stigma and dehiscent anthers of *P. barbigerum*. These hoverflies land on the slippery centralised wart located on the shiny yellow staminode and then fall backwards through the labellum entrance. They are temporarily trapped in the inflated chamber composed of the interconnected labellum and column. The attractive staminode of *P. barbigerum* strongly reflects the colour yellow (500–560 nm), a colour preferred innately by most pollen-eating members of the Syrphidae. No scent molecules were detected using GC mass spectrometry analysis, showing that the primary attractant in this system is visual, not olfactory. Pollination-by-deceit in *P. barbigerum* is contrasted with its congener, *P. dianthum*, a brood site mimic that is pollinated by ovipositing females of *E. balteatus*. As the natural rate of fruit set in *P. barbigerum* (mean 26.3% pooled over three seasons) is lower than that of *P. dianthum* (mean 58.5% over two seasons), the evolution of false brood sites in some *Paphiopedilum* spp. should be selectively advantageous as they may provide an increase in the attention and return rates of dependable pollinators to flowers that always lack a reward.

INTRODUCTION

Pollination-by-deceit (*sensu* Faegri & van der Pijl 1971) has evolved repeatedly and independently in several lineages of insect-pollinated plants, and it is estimated that a third of all orchid species (Orchidaceae) are pollinated-by-deceit (van der Pijl & Dodson 1966; Nilsson 1992; Schiestl 2005; Tremblay *et al.* 2005). Different orchid species lure different insect pollinators with a variety of visual and/or olfactory cues (Faegri & van der Pijl 1971;

Dafni 1984; Wilson & Ågren 1989; Renner 2006). Anthophilous members of the Coleoptera, Diptera, Hymenoptera and Lepidoptera are the obligatory and often specific pollinators of various orchid species with modes of floral presentation that mimic edible rewards, brood sites and/or the bodies of receptive females (Jersáková *et al.* 2006; Renner 2006).

Hoverflies (Syrphidae) are particularly important pollinators of flowering plants throughout the world (Larson *et al.* 2001; Committee on the Status of Pollinators in

North America 2007), as the winged adults of many of their species usually feed exclusively on nectar and pollen (Irvin *et al.* 1999). Behavioural experiments show that hoverflies use colour as their primary cue for locating food sources. Most hoverfly species tested thus far show an innate preference for colours in wavelengths from 460 to 600 nm (Lunau & Wacht 1994; Wacht *et al.* 1996; Sutherland *et al.* 1999), the common colours of anther and pollen grain coats. Besides colour, appropriate odours also enhance floral foraging by hoverflies (Laubertie *et al.* 2006).

As the larvae of most hoverfly species are predaceous, gravid females must oviposit near prey species such as aphids (Christens 1994), which they locate by a combination of visual and olfactory signals. These signals also stimulate egg-laying behaviours (Budenberg & Powell 1992; Scholz & Poehling 2000; Sutherland *et al.* 2001). Therefore, some orchid species with deceptive flowers that are pollinated primarily by hoverflies may express generalised food deception, brood site deception, or a combination of both deceptive modes with intergrated visual and olfactory cues (Irvin & Dafni 1977; Atwood 1985; Dafni & Calder 1986; Christens 1994; Bänziger 1996; Bower 2001).

Within the slipper orchids (subfamily Cypripedioideae; Dressler 1993), several species placed in subgenus *Paphiopedilum* (genus *Paphiopedilum*) are pollinated by hoverflies, and recent interpretations of their modes of floral presentation suggest that this lineage subdivides into species that employ either brood site or food deception to exploit their pollinators (Atwood 1985; Bänziger 1994, 1996, 2002). Specifically, combinations of visual cues located on the staminode, lateral petals and sepals in association with scent cues are considered critical in determining which mode of pollination-by-deceit is employed by a *Paphiopedilum* species. The question is, therefore, whether floral evolution and speciation in this subgenus is driven primarily by shifts in visual or in olfactory cues.

This question can be answered only by continued *in situ* experimentation and observations of the pollination biology of many species within subgenus *Paphiopedilum* (*sensu* Cribb 1998). Currently, only eight out of 60 species in this lineage have been examined, and the presentation of their visual and olfactory cues has not been analysed. In this study, we have not only observed the behaviour of pollen vectors of *P. barbigerum* T. Tang et F. T. Wang *in situ*, but also measured its floral architecture and analysed its colour, odour and breeding system (based on experimental hand-pollinations).

Four questions are addressed. First, are hoverflies the obligate pollinators of this species or can it self-pollinate in the absence of pollen vectors? Second, if the flowers are pollinated by hoverflies, are these insects attracted primarily by visual or olfactory cues? Particular attention is paid to the colour pattern and epidermal sculpting of the staminode because past observations on *Paphiopedilum* spp. have shown that hoverflies appear to prefer to land

on discrete portions of this organ before they tumble into the inflated labellum trap (Bänziger 1996; Cribb 1998). Third, how does reproductive success (the conversion of pistils into viable, seed-filled fruits) in *P. barbigerum* compare to other species in the same subgenus? Fruit set in most cross-pollinated deceptive orchids is notoriously low and current interpretations of orchid evolution emphasise the importance of infrequent/inadequate pollinator visitation in diversification within the family (see Tremblay *et al.* 2005).

MATERIALS AND METHODS

The study species

Paphiopedilum barbigerum is a lithophytic–subepiphytic perennial herb restricted to southwestern China and northern Vietnam (Cribb 1998; Averyanov *et al.* 2003). It grows on cliffs and in rock crevices in open forests. Cribb (1998) found it on the bases of tree trunks, but this mode of establishment was not observed in our populations. In some parts of its distribution, *P. barbigerum* forms large clumps containing up to 30 rhizomatous stems.

According to Cribb (1998) and Chen *et al.* (1999), flowering occurs in late summer. A mature plant produces one or more peduncles and each peduncle terminates in a solitary flower. Most flowers have a white dorsal sepal with a basal green mark or, less frequently, a central brown vein. The lateral petals are ochre in colour with greenish tips. The labellum is reddish brown and there is a yellow staminode bearing a centralised warty protuberance. Purple hairs extend from the base of the labellum chamber up through the rear exit orifices located under each anther.

Study sites

Observations were made on plants growing in the Maolan National Nature Reserve, in southeastern Guizhou Province, southwest China (29°09′20″–25°20′50″N, 107°52′10″–108°05′40″E) from 2004 to 2006. Morning temperatures were from 15 to 20 °C and afternoon temperatures ranged from 21 to 30 °C over three flowering seasons. Annual precipitation within the reserve was 1750–1950 mm. *Cyclobalanopsis glauca* (Thunb.) Oerst. and *Platycarya longipes* Wu are the dominant tree species in the shallow soils of the limestone outcrops (Ran *et al.* 2003). *Anemone hupehensis* Lemoine, *Lysionotus denticulosus* W. T. Wang, *Hemiboea cavaleriei* Lévle. var. *paucinervis* W. T. Wang et Z. Y. Li, *Hygrophila salicifolia* (Vahl) Nees., *Isodon amethystoides* Bentham, *Kalimeris indica* (L.) Sch.-Bip., *Lycoris aurea* (L'Héritier) Herbert, *Pimpinella candolleana* Wight et Arn., *Phryma leptostachya* L. var *asiatica* Hara, *Ranunculus japonicus* Thunb. and *Torenia biniflora* Chin et Hong were sympatric with *P. barbigerum* and had overlapping flowering periods.

We studied seven populations at least 800 m apart on the slopes of different limestone hills at elevations from
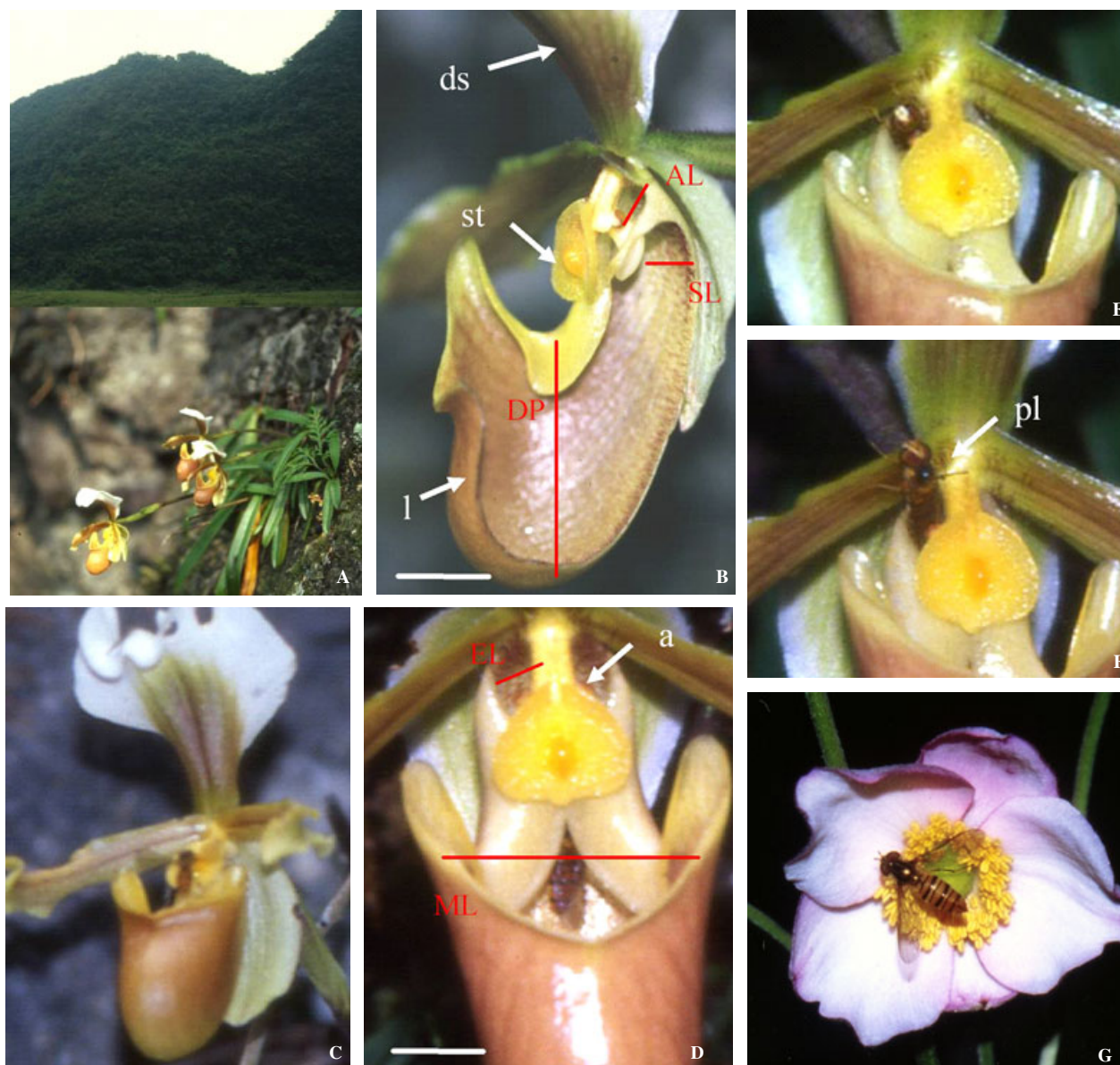
Fig. 1. A: Lithophytic and flowering habitat of *Paphiopedilum barbigerum*. B: Longitudinal section of the flower of *P. barbigerum*. ds, dorsal sepal; l, labellum; st, staminode; AL, height between the anther and the bottom of the labellum; SL, height between the stigma and interior surface of the labellum; DP, labellum depth from the entrance rim to the bottom surface. Bar = 5 mm. C: *Episyrphus balteatus* trying to grasp the wart on the staminode. D: *E. balteatus* crawling towards the elevated rear of the labellum sac towards the nodding stigma. a, anther; ML, entrance diameter of the labellum; EL, rear exit width of the labellum. Bar = 6 mm. E: *E. balteatus* exiting the flower of *P. barbigerum* through a rear escape aperture. F: *E. balteatus* with dorsally deposited pollen on its thorax. pl, pollen. G: *E. balteatus* visiting a flower of *Anemone hupehensis*.

600 to 700 m (Fig. 1A). The number of individual rhizomatous stems counted in each population varied from 100 to 810. The number of flowering stems (peduncles) produced annually by each population varied from 8 to 313 over a 3-year period (Table 1). Population F produced the greatest number of flowers over the three study seasons.

Floral lifespan

In 2005, we used a 10×10 m plot in population F to record the floral lifespan. All the unopened buds were tagged in this plot. A flower was recorded as open when the dorsal sepal separated from the labellum and the labellum slit expanded. The same flower was recorded as

Table 1. Latitude and demographics of seven populations of *Paphiopedilum barbigerum*.

population	latitude	number of plant stems	year	number of flowers
A	700	448	2004–2006	15–39
B	600	324	2004–2006	27–124
C	660	276	2004–2006	15–32
D	650	100	2004–2006	2–50
E	680	294	2004–2006	17–88
F	670	810	2004–2006	74–313
G	650	205	2005–2006	8–16
H	680	180	2006	41

dead when its dorsal sepal developed brown spots and/or the sepal withered and collapsed.

Floral colours

Spectral reflectance was measured in 2007 for seven flowers from seven individuals using a spectroradiometer (NF333; Nippon Denshoku, Japan) following the method described by Fukaya *et al.* (2004) and Zhang *et al.* (2007). The flowers analysed came from the greenhouse of the Beijing Botanical Gardens, and their flowering times paralleled wild plants in the Maolan area. Previous studies conducted by Lunau & Wacht (1994), Wacht *et al.* (1996) and Sutherland *et al.* (1999) showed that hoverflies preferred green and yellow colours with wavelengths from 460 to 600 nm. We examined the spectral reflectance of the floral organs (staminode, labellum, petal and basal part of dorsal sepal) within a 400–700 nm spectral range. We also assayed the spectral reflectance of the green foliage leaves on the same plants for comparison.

Floral fragrance

We collected the fragrance of one flower from population F and one air control sample on September 7, 2005. A second flower and air control sample was taken on September 14, 2006. Each flower was enclosed in an inertia bag (Reynolds Co., USA) for 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 glass tubes. As we observed that insect visitation to flowers of *P. barbigerum* peaked between 10:00 and 14:00 h (see below), floral fragrance samples were taken between 11:00 and 13:00 h. The air was passed through the glass tube for 1 h at a flow rate of approximately 100 ml·min^{-1}. Fragrance component analyses were undertaken in November 2005 and October 2006. Volatiles were desorbed from the 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 in 1 min to transfer the volatile compounds into a GC-MS (Trace2000-Voyager, Finnigan, Thermo-Quest). Compounds were identified by searching the NIST library in the Xcalibur data system (Finnigan), and were comparing with any compounds identified in the air control.

Flower visitors

We recorded flower–insect interactions for a total of 225 h from September to October 2004–2005. Daily observations were made between 9:00 and 17:00 h from the day when the first flower in a population opened until the last flower in each population withered. Flower visitors were photographed using a Nikon D70 (Japan) with a Nikkor macro lens (105 mm) and also videotaped (Sony 43E, Japan). We recorded the behaviour of insects in relation to the orchid flower. This included the insect's aerial approach to the flower, where it landed on the flower and when and how it entered the labellum (see Nilsson 1979).

We recorded the genders of visiting hoverflies. In male flies, the two compound eyes are so close together that they contact each other, but the compound eyes of female flies do not converge (Irvin *et al.* 1999). We also timed insect visits with a stopwatch to determine how long it took them to escape from the inflated labellum trap from the time they actually entered the labellum chamber until they exited *via* the rear basal aperture located under each anther. We also observed whether the insects visiting flowers of *P. barbigerum* visited other co-blooming species within the seven sites.

Floral foragers were captured in a net or with a plastic box placed directly over a rear floral aperture exit as the insects emerged. Once collected, these specimens were euthanised with ethyl acetate and examined for depositions of pollen of *P. barbigerum*. Pinned and labelled specimens were identified by entomologists from the China Agricultural University and deposited in the State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences.

Some insects collected on flowers of *P. barbigerum* were also examined for the presence of pollen of co-blooming species. Whole insects or individual limbs were glued to aluminium stubs, coated with 40–50 nm gold and viewed under a Hitachi S-800 scanning electron microscope at 30 kV. Pollen grains found adhering to insect hairs and/or cuticles were identified by comparing pollen wall apertures and exine sculpting with a library of pollen grains made from dehiscent anthers of co-blooming species collected at sites A–F.

Functional morphological parameters

Flower and insect dimensions were measured using the same set of vernier calipers (SM and CTW 0–125 mm, Shanghai, China) during the 2005–2006 field seasons. We measured the thorax height, body length and body width (across the broadest portion of the thorax) of euthanised insects collected only after they were observed exiting the rear apertures of the flowers (see Li *et al.* 2006).

Seventy flowers were collected at random from the seven populations. We performed six measurements on each specimen using the same digital calipers. These measurements followed those made by Li *et al.* (2006), with the addition of measurement six (see below). The first three measurements were made using the entire flower (Fig. 1B and D): (i) DP = the depth of the labellum from the rim margin to the base (floor) of the labellum, (ii) ML = the entrance diameter of the labellum rim, (iii) EL = the rear exit width of the labellum. The last three measurements were made following a longitudinal bisection of the labellum sac of the same flower (Fig. 1B), (iv) AL = the length of the rear exit aperture as measured from the dehiscent surface of the anther to the base of the labellum, (v) SL = the height between the inverted receptive surface of the stigma to the opposite bottom surface of the labellum and (vi) the length of purple hairs lining the interior of the labellum sac.

Breeding system and natural rates of fruit set

The breeding system was assessed by hand-pollination of individual buds isolated in separate bags made of sulphur paper: we waited until the flower bud opened and then subjected the stigma to one of three treatments are

1 *Cross-pollination.* The bag was removed and the stigma was hand-pollinated with pollen derived from a second plant in bloom a minimum of 5 m away. Pollen was applied until it was visible to the naked eye and the bag was replaced. The bag was not removed until 2 weeks after the flower withered.

2 *Self-pollination.* The stigma was hand-pollinated with pollen from the same flower. The physical application of pollen and the replacement and final removal of the bag followed treatment (1).

3 *Mechanical self-pollination (controls).* The bag was not removed after the flower opened and no pollen was applied by hand for the duration of flowering. Final removal of the bag followed the protocol in treatment (1).

All treatments were assessed 14 days after the labellum and perianth segments withered, to see whether the ovaries had expanded and changed from reddish-purple to dark green.

To determine rates of natural insect-pollinated flowers in 2004, 2005 and 2006, we counted all flowers in bloom in the seven populations in each year. We returned to the same sites 14 days after withering of the last flower in each population to record the number of peduncles terminating in developing fruits.

RESULTS

Flowering phenology

The flowering period of *P. barbigerum* started towards the end of August and proceeded for 5–6 weeks. Peak flowering within populations occurred from September 6 to September 16 in 2005 (Fig. 2). The floral lifespan of an individual flower was 20.5 ± 2 days (n = 45) if it was not pollinated. Pollinated flowers (n = 16) usually withered about 7 days earlier.

Floral colours

The reflectance spectra of floral organs of *P. barbigerum* flowers are shown in Fig. 3. While the wart and its staminode reflected strongly at intermediate wavelengths (500–560 nm; green and yellow), the wart had a stronger reflection (540–560 nm) than surrounding portions of the same staminode (Fig. 3). The labellum, petals and basal portion of the dorsal sepal reflected strongly at longer
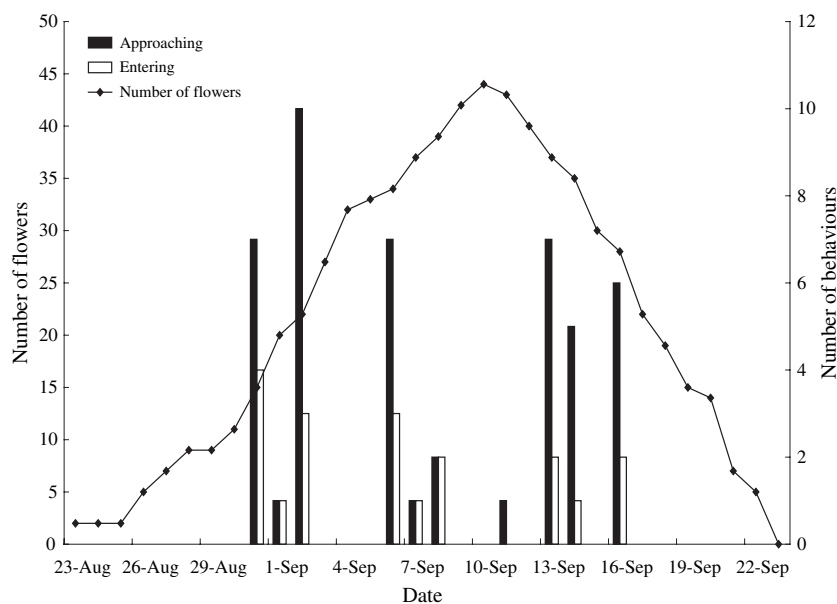
Fig. 2. Flowering peak phenology in *Paphiopedilum barbigerum* and visitation rates of hoverflies over the flowering season.
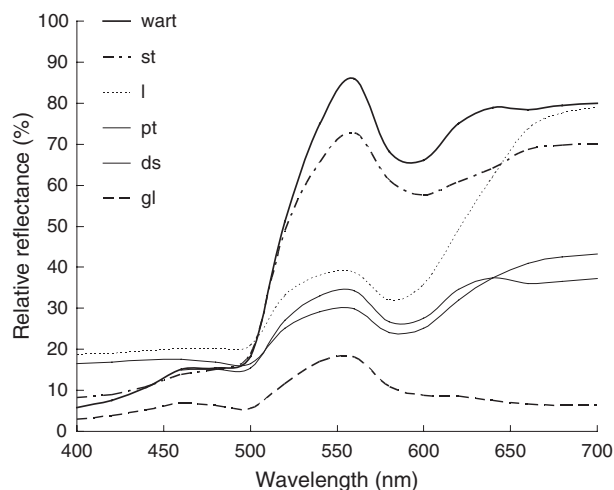
Fig. 3. Spectral reflection of *Paphiopedilum barbigerum* flowers. st, Staminode; ds, base of dorsal sepal; l, labellum; pt, petals; gl, green leaf.

wavelengths (620–700 nm, red). The green leaves showed only limited reflectance (Fig. 3).

Floral fragrance

Our noses were unable to detect any fragrance in *P. barbigerum* flowers at our field site or from botanic garden specimens. Analyses of the flower samples using GC-MS failed to find aromatic molecules. Sample components of the flower were the same as those of the air control sample.

Flower visitors and their behaviours

Five species of arthropod, belonging to the Araneida, Hymenoptera and Diptera, were found on *P. barbigerum*. However, the majority of species observed and collected were hoverflies (Syrphidae; Table 2). Miner bees in the genus *Lasioglossum* (Halictidae) landed on the yellow

staminode and crawled down into the labellum, but then flew out of the labellum using the same entrance. Five spiders were found in the labellum but all escaped *via* the same entrance. Both miner bees and spiders failed to carry orchid pollen.

Three species of hoverfly (Syrphidae) visited *P. barbigerum*. Representatives of all three species first landed on the staminode (Table 2) but only some representatives of two species fell into the labellum and then escaped through the rear exit of the flower carrying pieces of the massulate pollinia. Orchid pollen dispersal by *Allograpta javana* (Wiedemann) was noted in only one of the 3 years. Orchid pollen dispersal by *E. balteatus* (DeGeer) occurred in all 3 years. The visitations of pollinators usually began by 09:40 h, peaking between 10:00 and 14:00 h (Fig. 4).

The majority of hoverfly visitors were females. Only three males of *E. balteatus* visited *P. barbigerum* during the 3-year period. These male flies did not exit *via* the rear of the flower and did not carry orchid pollen. They escaped *via* the entrance, as in the case of the miner bees and spiders. Of the observed hoverfly visitations to *P. barbigerum*, 95% (118/124) of captured specimens were identified as *E. balteatus*. In addition, 97% (29/30) of the captured hoverflies carrying the orchid's pollen were identified as *E. balteatus*.

Although the visitation and pollen carrying capacities of the two hoverfly species varied greatly, their behaviour on and in the orchid flowers was identical. Each hoverfly usually hovered approximately 5 cm in front of the orchid flower before attempting to land on the centralised wart on the staminode. Contact between the wart and the fly lasted <1 s per visit as the insect could not cling to the wart's slippery surface (Fig. 1C). The insect fell into the labellum through the broad entrance rim. The fly remained in the labellum chamber from 20 to >3600 s (Fig. 1D and E), until they crawled out through one of the rear apertures under each anther located on either side of the staminode. The fly contacted the dehiscent anther as it crawled out of a rear aperture. The anther

Table 2. Insect interactions on and within flowers of *Paphiopedilum barbigerum*.

visitor species	approaching	touching staminode	entering	pollen dispersal
Araneida				
Unidentified spp.	–	0	5	0
Diptera				
Episyrphus balteatus (female)	118	102	68	29
Episyrphus balteatus (male)	3	3	2	0
Allograpta javana	3	1	1	1
Allobaccha apicalis	3	0	0	0
Hymenoptera				
Lasioglossum sp.	3	2	2	0
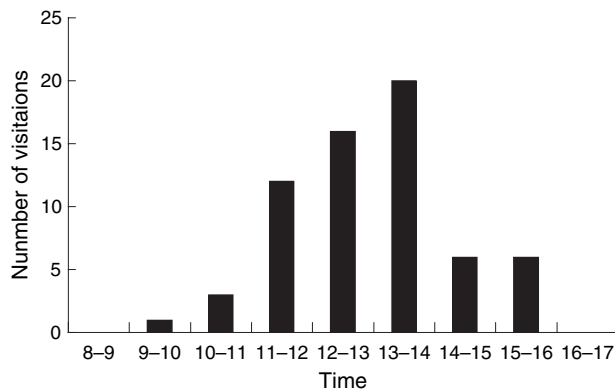
Fig. 4. Insects visits to flowers of *Paphiopedilum barbigerum*.

deposited portions of its pollinia on the dorsum of the insect's thorax (Fig. 1F) in irregular amorphous lumps.

Upon escape, a few flies were observed to rest for brief periods by perching on the dorsal sepal or lateral petals. The majority of hoverflies flew away from the site following the resting period. There were only two occasions, over the three observation seasons, in which we observed a hoverfly exiting the flower and then the same insect immediately visiting a second flower of *P. barbigerum* in the same site. We did not observe hoverflies attempting to oviposit on or within the flowers of *P. barbigerum*. No eggs were found on or in the orchid flowers over the three seasons of observation.

We recorded seven observations of males (seven times) and 10 observations of females (10 times) of *E. balteatus* visiting flowers of co-blooming plants of *Anemone hupehensis* (a nectarless flower) and *Kalimeris indica* (with nectariferous florets). When hoverflies visited these flowers, they first landed directly on the yellow anthers to probe for pollen (Fig. 1G). We never observed a hoverfly flying back and forth between flowers of *P. barbigerum* and flowers of any other sympatric species. However, analyses of pollen loads of hoverflies caught emerging from *P. barbigerum* showed that they also carried pollen grains of *Anemone hupehensis* and *Kalimeris indica*, regardless of whether or not they carried orchid pollen.

Floral dimensions and functional morphology

No nectar, starch bodies, liquid oil secretions, wax plates or resin secretions were found in or on any floral organs of *P. barbigerum*. We did not find blackish papillae warts or glandular hairs on staminodes, sepals or petals that are indicative of brood site imitation in other *Paphiopedilum* spp. (*sensu* Atwood 1985).

Table 3 represents the comparative measurements of flower and insect dimensions. Labellum depth (DP) is far greater than the body lengths of either hoverfly species. The height between the stigma and the bottom surface of

Table 3. Floral functional morphology of *Paphiopedilum barbigerum* and body size of hoverflies.

floral traits	size (mm)	*Episyrphus balteatus*	*Allograpta javana*	hoverflies (mm)
DP	19.9 ± 3	2.1 ± 0.3	1.7 ± 0.3	Thorax height
ML	21.0 ± 3.6	9.5 ± 0.8	8.0 ± 1.0	Body length
EL	3.3 ± 0.4			
SL	3.4 ± 0.4			
AL	3.3 ± 0.4			

DP, labellum depth from the entrance to the bottom; ML, mouth diameter of the labellum; EL, rear exit width of the labellum; SL, height between the stigma and bottom of the labellum; AL, height between the anther and the bottom of the labellum (n = 70 flowers measured).
Measurements of *E. balteatus* (n = 20 specimens) and *A. javana* (n = 3 specimens) all taken after the insect exited via the rear anther aperture.

the labellum and the height between the anther and the bottom of the labellum (AL) are also greater than the thorax height of the two hoverfly species. The erect purple hairs, extending from the bottom of the labellum to the two rear apertures were up to 2 mm in length. These stiff, overlapping floral hairs probably functioned as a supportive comparatively rigid mat that elevated the pollinator sufficiently to allow the stigma to press against the insect's dorsum as it crawled underneath the column.

Breeding system and natural rates of fruit set

All pistils self-pollinated (n = 12) or cross-pollinated (n = 12) by hand produced fruit, indicating self-compatibility. None of the control flowers (bagged without hand-pollination; n = 12) set fruit, indicating that spatial isolation and orientation of the receptive stigma surface prevented mechanical self-pollination (autogamy), and no agamospermy occurred.

We pooled the results of fruit set in open (never bagged, naturally insect-pollinated) flowers for all populations over 3 years. In 2004, 22% of all flowers developed fruits; while in 2005 and 2006, the conversion ratio of flowers into fruits was 35% and 21%, respectively.

DISCUSSION

The exploitation of *E. balteatus* by *P. barbigerum*

Our results show that, as in all members of subgenus *Paphiopedilum* studied previously (Atwood 1985; Bänziger 1994, 1996, 2002; Shi *et al.* 2007), *P. barbigerum* was pollinated exclusively by hoverflies (Table 4). Specifically, females of *E. balteatus* were the primary pollinators as 97% of flies of this species carried this orchid's pollen as they emerged from the rear floral apertures and contacted the dehiscent anthers. The natural distribution of *P. barbigerum* in China is currently restricted to two southern provinces (Chen *et al.* 1999). It is possible that the hoverfly, *A. javana*, and other hoverfly species are more important as pollinators of this orchid at other latitudes and elevations.

Variation in pollination-by-deceit mechanisms in *Paphiopedilum* spp. (subgenus *Paphiopedilum*) based on epidermal sculpture and colour patterns

We note that this is the second Chinese *Paphiopedilum* species pollinated primarily by females of *E. balteatus*. The pollination of *P. dianthum* T. Tang et F. T. Wang is also dependent on this hoverfly (Shi *et al.* 2007). Although *P. barbigerum* and *P. dianthum* share the same pollinator, they do not exploit the same insect behaviour. *Paphiopedilum dianthum* is a brood site mimic orchid (*sensu* Dafni 1984), with 'dummy aphids' found on its lateral petals. The females of *E. balteatus* often oviposit on the flower prior to falling into the labellum trap (Shi *et al.* 2007; Table 4). As hoverfly larvae eat aphids (Chris-

Table 4. Comparative floral presentation in *Paphiopedilum* species pollinated by hoverflies.

| species | hoverfly gender | odour discerned by human nose | dummy aphids | staminode | | | references |
				false honeydew	colour	centralised wart	
Paphiopedilum barbigerum	♀	–	–	–	Y	+	See text
Paphiopedilum bellatulum	♀	Sf	–	–	Y	–	Bänziger 2002
Paphiopedilum callosum	♀	A	+ on labellum	–	G	–	Bänziger 2002
Paphiopedilum charlesworthii (no detailed work)	?	–	–	?	W staminode Y wart	+	Bänziger 1994; Cribb 1998
Paphiopedilum dianthum	♀	–	+ on petals	–	G	–	Shi *et al.* 2007
Paphiopedilum parishii	♀, ♂	H	–	–	G	–	Bänziger 2002
Paphiopedilum rothschildianum	♀	PA	+ petals and staminode	–	GY	–	Atwood 1985
Paphiopedilum villosum	♀	U	–	+	Y	+	Bänziger 1996

A, smells like aphids; H, smells like aphid honeydew; PA, peppery and aphid odour mixed; Sf, general sweet-floral; U, urine; G, green; Y, yellow; GY, pale yellow with green; W, white.

tens 1994), the very presence of 'decoy aphids' may stimulate the act of egg laying in adult females (Budenberg & Powell 1992; Scholz & Poehling 2000). Hoverfly eggs are also reported on the small black, often hairy, sculptures on the staminodes, lateral petals and/or labella of *P. rothschildianum* (Rchb.f.) Stein and *P. callosum* (Rchb.f.) Pfitz. (Atwood 1985; Bänziger 1994, 2002). The number of fly eggs found on the staminodes of *Paphiopedilum* species bearing hairy dummy aphids is extremely variable. One may find as many as 76 hoverfly eggs on one staminode of *P. rothschildianum* (Atwood 1985) and as many as 10 eggs on one staminode of *P. callosum* (Bänziger 2002). However, only one hoverfly egg has been found, to date, on a staminode of *P. dianthum* (Shi *et al.* 2007). The gravid female hoverflies land directly on the staminodes of *P. rothschildianum* and *P. callosum* before falling into the labellum traps (Atwood 1985; Bänziger 2002). In contrast, female hoverflies generally land and lay eggs on the lateral petals of *P. dianthum*, and then fly towards the staminode (Shi *et al.* 2007). Consequently, the behaviours of female hoverflies on and in flowers of *P. rothschildianum*, *P. callosum* and *P. dianthum* appear to converge with the behaviours of other true flies known to pollinate the flowers of some stapeliads (Asclepiadaceae) and *Aristolochia* sp. (Aristolochiaceae). These flies also lay their eggs on the flowers before they are trapped by the floral mechanisms (see Faegri & van der Pijl 1971).

In contrast, dummy aphids and other floral modifications that exploit the behaviours of egg-laying flies are not present on *P. barbigerum*, and the behaviour of their hoverflies fails to suggest a typical trend towards brood site mimicry (see Dafni & Bernhardt 1990). In *P. barbigerum*, the staminode was so attractive that nearly all potential pollinators attempted to land on its wart, ignoring the other floral organs. The hoverflies 'lost their grip' on the wart almost instantly (<1 s per visit) before tumbling into the labellum. This mode of hoverfly pollination parallels systems described previously for *P. bellatulum*

(Rchb.f.) Stein, *P. charlesworthii* (Rolfe) Pfitz, *P. parishii* (Rchb.f.) Stein and *P. villosum* (Lindl.) Stein. Hoverflies do not lay eggs on these flowers either, instead, each flower lacks aphid decoys, and presents a yellow, non-secretory, but often papillose and shiny, staminode (Bänziger 1996, 2002). Bänziger *et al.* (2005) suggested that the pollination syndrome of these four *Paphiopedilum* spp. was based on 'opportunistic' food deception.

Why are hoverflies attracted to the yellow staminode and wart of some *Paphiopedilum* species? Multiple choice experiments with artificial flowers showed that syrphids known as drone flies (*Eristalis tenax*) also prefer to land on yellow artificial flowers (Ilse 1949; Kugler 1956). Similar colour choice experiments produce similar results with *E. balteatus* (Sutherland *et al.* 1999). The colour yellow also stimulates proboscis extension in syrphid flies (Lunau & Wacht 1994). In particular, the response of these hoverflies to yellow models varies with gender and physical age (Sutherland *et al.* 1999). While both male and female hoverflies prefer colours with wavelengths between 460 and 520 nm (green and yellow), females show a significantly stronger preference for these wavelengths compared to males. Furthermore, younger winged adults have stronger preferences for yellow compared to older hoverflies. As the insect ages, its preference shifts from yellow to blue (440 nm) and green (475–500 nm).

Our measurements show that the yellow staminode of *P. barbigerum* reflects between 500 and 560 nm. Other organs in the same flower reflect between 620 and 700 nm and these colour ranges do not appear to attract hoverflies (Sutherland *et al.* 1999). While all our measurements were conducted on flowers of greenhouse specimens, we must note that the yellow staminode is self-consistent in this species, both at our study sites and throughout its natural distribution (Cribb 1998; Chen *et al.* 1999). We conclude that, although flowers of *P. barbigerum* always lack edible rewards, females of *E. balteatus* must visit them because such a large, broad

yellow, staminode presents them with a colour stimulus. That is, the hoverfly is innately attracted to this inedible but yellow stimulus, similar to the way in which some agonistic male fishes are attracted to experimental red models and some female birds prefer to brood giant artificial eggs (see review in Manning 1967).

More important, if this yellow stimulus is so compelling, why did it fail to attract more male hoverflies over the three seasons of observation? We found that female hoverflies appear more attracted to yellow compared to males (see above). In general, most males in the Diptera, Hymenoptera and Lepidoptera emerge from their pupa hours or days before the larger egg-filled females (Charles D. Michener, personal communication). By the time *P. barbigerum* is in peak bloom, male hoverflies may be too old to respond consistently to the yellow lure and have already shifted foraging bouts to co-blooming species that combine typical yellow androecia with perianths of different and contrasting pigments. For example, at our sites male hoverflies visited both *Anemone hupehensis* (pink calyx) and the light purple inflorescences of *K. indica*. Male hoverflies are of less value to this orchid anyway as they fail to carry depositions of pollinia because they can escape from the labellum chamber without ever contacting the dehiscent anthers.

The adaptive significance of scentless flowers of *P. barbigerum*

What was not anticipated was the total absence of floral odour in *P. barbigerum*. In general, odour is a primary attractant in the pollination mechanisms of orchids and other plants pollinated-by-deceit (van der Pijl & Dodson 1966; Nilsson 1992; Dressler 1993; Kunze & Gumbert 2001). Flower-visiting flies representing several families within the Diptera have different olfactory preferences. Floral odours enhance the attractiveness of flowers to food-seeking hoverflies (Laubertie *et al.* 2006). Many hoverfly-pollinated flowers are strongly scented and offer pollen as their only edible reward (Bernhardt 1989; Bernhardt *et al.* 2003; Vance *et al.* 2004). A review of the literature shows that fly-pollinated orchids produce fragrances ranging from sweet to unpleasant, as discerned by the human nose (van der Pijl & Dodson 1966; Dafni & Calder 1986; Christens 1994).

Dixon (1959) suggested that hoverfly oviposition is normally stimulated by a combination of olfactory and visual signals. More recent authors argue that brood site mimics must smell like their putative prey or host models to attract gravid hoverflies (Irvin & Dafni 1977; Atwood 1985; Bänziger 2002). A discernible floral fragrance is supposed to enhance the attraction of female hoverflies to the aphid-like decoys on flowers of *P. rothschildianum* (Rchb.f.) Stein (Atwood 1985). However, *P. dianthum* also lacks a discernible fragrance to the human nose when sampled *in situ* yet it continues to lure gravid hoverflies that lay eggs on or near the flower's black warts (Shi *et al.* 2007).

Orchid species that lack edible rewards and are pollinated, at least in part, by hoverflies often release sweet perfumes (Dafni & Calder 1986; Burns-Balogh & Bernhardt 1988; Christens 1994). Such odours are reported in some hoverfly-pollinated members of subgenus *Paphiopedilum* with food deceptive modes of floral presentation, including *P. villosum*, *P. parishii* and *P. bellatulum* (Bänziger 2002). However, the human nose also fails to detect scent in *P. charlesworthii in situ*, a putative food deceptive species (Table 4). In this study the GC-MS analysis repeatedly failed to detect scent components in *P. barbigerum*, although the same equipment and protocol recorded and identified scents in the deceptive flowers of the allied orchid genus, *Cypripedium* (Li *et al.* 2006, 2008). We conclude that the unusually broad visual signal of the staminode of *P. barbigerum* is sufficient to attract and deceive hoverflies in the absence of scent. This could be selectively advantageous, as the visual cue may save the plant additional resources that would normally be spent on the genesis of odour molecules. This condition emphasising visual over olfactory cues may have evolved repeatedly in the subgenus. Some putatively odourless *Paphiopedilum* spp. (*e.g. P. charlesworthii*) appear to increase their visual cue by offering an enlarged dorsal sepal with a vivid colour that contrasts with the yellow staminode (see Bänziger 1994). Beside colour and odour, it is suggested that non-volatile chemical cues are also important in both oviposition (Budenberg & Powell 1992; Scholz & Poehling 2000; Sutherland *et al.* 2001) and food foraging behaviours of hoverflies (Wacht *et al.* 1996; Sutherland *et al.* 1999). Studying the potential influence of non-volatile chemical stimuli of *P. barbigerum* and *P. dianthum* on the egg-laying or food foraging behaviours of hoverflies should be considered in future.

Relationship between *P. barbigerum* and other co-blooming hoverfly-pollinated species

In general, adult hoverflies are polytrophic insects, taking pollen and nectar from a wide variety of flowering plants in the habitat (Bernhardt & Burns-Balogh 1986; Bernhardt 1989; Bernhardt *et al.* 2003; Vance *et al.* 2004). Over three seasons of observation, we continuously found that male and female hoverflies of *E. balteatus* landed directly on the yellow anthers of the flowers of other co-blooming species, whether the flower secreted nectar or not. However, we never observed a hoverfly flying directly back and forth between the flowers of *P. barbigerum* and the flowers of co-blooming species. Obviously, the staminode of *P. barbigerum* is neither a specific mimic nor guild mimic (see Dafni & Bernhardt 1990) of the androecia presented by other co-blooming species in its habitat. Rather, *P. barbigerum* competes with other species that are also dependent on the limited resource of generalist foraging hoverflies. This system is most often called non-mimic deception and has evolved independently in many other orchid lineages pollinated by other polytrophic flies,

wasps and/or polylectic bees (see review in Dafni & Bernhardt 1990).

Pollination-by-deceit, reproductive efficiency and its implications for diversity

Insect–flower observations and comparative measurements show that female hoverflies are of a sufficient size and strength to both contact receptive stigmas and carry deposits of pollinia of *P. barbigerum* at the Maolan sites. However, this orchid shows much the same drop in fecundity attributed to the majority of cross-pollinated orchids that offer no rewards (Tremblay *et al.* 2005). While these flowers are also self-compatible, there is no mechanical 'fail safe' self-pollination mechanism over the floral life span. Fertilisation will not occur if female hoverflies learn to avoid the mimic after their first negative experience and never deposit viable pollen on the receptive stigma of a second flower. The rate of fruit set *in situ* for open (insect-mediated) flowers of *P. barbigerum* is only 26.3% when results are pooled over three seasons. This is lower than the average fruit set (58.5%) for the brood site mimic, *P. dianthum*, pooled over two seasons (Shi *et al.* 2007), although both species share the exact same pollinator and produce odourless flowers (see above). Similar results are found in two other *Paphiopedilum* species that also exploit the same hoverfly species. *Paphiopedilum callosum* is another brood site mimic and the conversion of its flowers into fruit is 90%, while 8% is the conversion ratio for the food-deceptive *P. villosum* (Bänziger 2002).

While it is dangerous to speculate on reproductive efficiency within a lineage based on only four out of 60 species, what if this current trend is not coincidental? As no edible rewards have ever been detected in any species in subfamily *Cypripedioideae* (Dressler 1993), different modes of pollination-by-deceit must dominate floral evolution within the subgenus *Paphiopedilum*. Therefore, stochastic and ecological events resulting in speciation within this lineage appear to reflect, in part, selective responses towards higher and more dependable rates of plant fertility over time. Thus far, floral evolution in subgenus *Paphiopedilum* obviously reflects the exploitation of the same group of insects in two different ways. Consequently, species with brood site mimicry appear to evolve from ancestors with food deceptive mechanisms.

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