

RESEARCH PAPER

Pollination of *Cypripedium plectrochilum* (Orchidaceae) by *Lasioglossum* spp. (Halictidae): the roles of generalist attractants versus restrictive floral architecture

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ABSTRACT

The pollination of *Cypripedium plectrochilum* Franch. was studied in the Huanglong Nature Reserve, Sichuan, China. Although large bees (*Bombus*, *Apis*), small bees (*Ceratina*, *Lasioglossum*), ants (*Formica* sp.), true flies (Diptera) and a butterfly were all found to visit the flowers, only small bees, including three *Lasioglossum* spp. (*L. viridiclaucum*, *L. sichuanense* and *L. sp.*; Halictidae) and one *Ceratina* sp., carried the flower's pollen and contacted the receptive stigma. Measurements of floral architecture showed that interior floral dimensions best fit the exterior dimensions of *Lasioglossum* spp., leading to the consistent deposition and stigmatic reception of dorsally-placed, pollen smears. The floral fragrance was dominated by one ketone, 3-methyl-Decen-2-one. The conversion rate of flowers into capsules in open (insect) pollinated flowers at the site was more than 38%. We conclude that, while pigmentation patterns and floral fragrance attracted a wide variety of insect foragers, canalization of interior floral dimensions ultimately determined the spectrum of potential pollinators in this generalist, food-mimic flower. A review of the literature showed that the specialised mode of pollination-by-deceit in *C. plectrochilum*, limiting pollinators to a narrow and closely related guild of 'dupes' is typical for other members of this genus.

INTRODUCTION

Debates focusing on the degree of specialisation in plant–pollinator relationships remain remarkably vigorous (Waser *et al.* 1996; Waser 2006; Johnson & Steiner 2000; Fenster *et al.* 2004). Much work suggests extensive generalisation in plant–pollinator interactions is the rule (*sensu* Olesen & Jordano 2002), while Kay & Schemske (2004) suggest it may be incorrect because it is based on datasets of flower visitors and not effective pollinators. It is necessary to distinguish effective pollen vectors from mere visitors, because the selective effects of different floral visitors on plants can vary significantly (Schemske & Horvitz 1984).

Two overlapping trends appear repeatedly within the flowers of the Orchidaceae. First, about one-third of species produce flowers devoid or deficient in edible rewards (van der Pijl & Dodson 1966; Dressler 1993; Tremblay *et al.* 2005; Jersakova *et al.* 2006). Second, most orchid species have specialised pollination systems (*sensu* Tremblay 1992; Waser *et al.* 1996) dependent on a relatively narrow range of closely-related animals. Specialisation can occur at the level of functional group, *e.g.* large bees, medium-sized bees, small bees, small flies and diurnal Lepidoptera, but many orchids are specialised to single pollinator species (van der Pijl & Dodson 1966; Tremblay 1992; Ollerton 1998; Johnson & Steiner 2000; Johnson *et al.* 2003; Fenster *et al.* 2004; Blanco & Barboza 2005).

It is obvious that both trends contribute to each other within the adaptive radiation of pollination mechanisms within this natural lineage, Orchidaceae (Cozzolino & Widmer 2005). For example, a large proportion of orchid species endemic to the Neotropics offer inedible floral scents as their only reward (Dodson *et al.* 1969) and are pollinated, almost exclusively by male bees within the tribe *Euglossini* (Dressler 1993). Johnson & Steiner (2000) reviewed pollinator specificity in plant pollination systems and concluded that the degree of specialisation evolved via a combination of modified characters, including the advertisement of specific scents and colours, restrictive (canalized) floral morphology, nectar and, in some cases, unusual rewards such as oils, fragrances, resins, *etc.* However, pollination by deceit within the Orchidaceae must also encourage specialisation, *e.g.* sexual deceit in *Ophrys* (Schiestl *et al.* 2003) and floral mimicry in *Disa* (Anderson *et al.* 2005). Consequently, the morphological and biochemical evolution of pseudopollen, empty spurs, false brood sites, dummy females, *etc.* must attract a relatively narrow range of prospective pollinators consistently visiting the same deceptive presentation (Tremblay 1992; Nilsson 1992; Johnson & Steiner 2000; Tremblay *et al.* 2005).

However, most orchid species with non-rewarding flowers mimic the presence of nectar and are regarded as generalist food mimics. That is, the orchid flower does not mimic the flowers of a specific (Batesian) model species blooming at the same time (Dafni 1984; Ackerman 1986; Johnson & Steiner 2000; Gumbert & Kunze 2001). Therefore, the majority of visitors to the generalist food mimic usually represent naïve foragers that may visit a wide variety of plants in bloom within the same habitat. One would presume then that these orchids would initially attract a wider variety of prospective pollinators compared to orchid species that mimic specific rewards such as brood sites, or edible oils, or the bodies of female insects.

Members of the genus, *Cypripedium*, are now regarded as model taxa for the generalist food mimic mode of floral mimesis (Nilsson 1979; Dressler 1993). While Darwin (1862) never changed his opinion that *Cypripedium* spp. offered edible rewards secreted by glandular hairs inside the inflated labellum, and this interpretation was supported by naturalists for decades (Keeler 1916), fieldwork completed during the twentieth century and early this century concluded that there are no edible rewards available in any *Cypripedium* spp. studied thus far (Stoutamire 1967; Nilsson 1979; Catling & Knerer 1980; Cribb 1997; Bänziger *et al.* 2005). Floral architecture functions as a one-way 'kettle trap'. The pollinator enters the labellum through an expanded, dorsal front slit or orifice. To escape, the insect must exit via the rear of the flower, forcing it to pass under the receptive stigma and then exit through one of two apertures located under each dehiscent anther (Dressler 1993; Cribb 1997). The diameter of the dorsal entrance to the labellum (DL), height between the stigma and bottom of the labellum (SL), height

between the anther and the bottom of the labellum (AL), and rear exit width of the labellum (EL) vary among *Cypripedium* species. DL and EL are interpreted as traits limiting insect access and escape, while SL and AL determine whether insects contact the stigma and anthers, effecting pollen deposition and removal (Stoutamire 1967; Nilsson 1979; Cribb 1997).

Most field studies conclude that female bees in the families *Andrenidae*, *Halictidae*, *Megachilidae* and *Apidae s.l.* (*Xylocopinae*, *Anthophorini*, *Bombini*) are the most commonly observed and collected pollinators of *Cypripedium* spp. (Guignard 1886; Stoutamire 1967; Nilsson 1979; Catling & Knerer 1980; Davis 1986; Sugiura *et al.* 2001; Bänziger *et al.* 2005). All *Cypripedium* spp. are presumed to be generalist food or nectar mimics, with the current exception of *C. macranthum* var. *rebunense* (Kudo) Miyabe et Kudo, reinterpreted recently as a Batesian mimic of a co-blooming nectar secreting model species (Sugiura *et al.* 2001, 2002).

However, the extent of specialisation between a *Cypripedium* species and a restricted or narrow lineage of bees remains unclear, with a few important exceptions. In particular, following 4 years of detailed observations and collections, Nilsson (1979) found that *C. calceolus* L. was visited by more than 50 insect species, but females of *Andrena haemorrhoea* Fabricius were the principal pollinators. Nilsson (1979) concluded that *A. haemorrhoea* was attracted to the flower colour pattern offering false nectar guides and to its floral fragrance that appeared to mimic pheromones. He also suggested that the efficiency of *A. haemorrhoea* as a pollinator was determined by a correlation between the distance between the labellum and the receptive stigma *versus* the height of the bee's thorax, as a bee's thorax received the majority of pollen smears from the dehiscent anthers.

In this study, we examined the pollination ecology of the Chinese endemic, *Cypripedium plectrochilum* Franch., for three interrelated reasons. First, we located an unusually large population (see below). It is often notoriously difficult to observe insects entering mimetic orchid flowers and carrying off pollinia as the true pollinators often learn quickly and soon avoid flowers that always lack rewards (Johnson *et al.* 2003). This appears to be particularly common in orchid species persisting in reduced populations (P. Bernhardt, personal observation). Witnessing pollinator activity is often easier in mimetic species with large populations, as monitoring a large population blooming over several weeks increases the opportunity of viewing brief and discrete bursts of novel activity by naïve floral foragers (Bernhardt & Burns-Balogh 1986). Second, we can test the hypothesis of Nilsson (1979), that pollinator efficiency depends primarily on measurable floral and insect dimensions, by using a *Cypripedium* labellum that belongs to a morphological type distinct from *C. calceolus*. Cribb (1997) classified the conical, keeled and hairy labellum of *C. plectrochilum* as one of only two examples of the *arietinum* type. Third, molecular analyses corroborates a morphological phylogeny that places *C. plectrochilum* as

the sister species of *C. arietinum* R. Br. (Chen 1983; Cribb 1997). The two species are isolated on different continents, with *C. arietinum* endemic to temperate, northeastern North America (Luer 1975; Chen 1983). If floral architecture ultimately limits the spectrum of efficient pollinators in *Cypripedium* species, then *C. plectrochilum* should be pollinated by small bees in the genus *Lasioglossum* (Halictidae), as observed in *C. arietinum* by Stoutamire (1967).

MATERIALS AND METHODS

Study site

A large population of approximately 2000 flowering plants of *Cypripedium plectrochilum* is found in the Danyun Gorge, Huanglong Nature Reserve, Sichuan, China. The site is at an altitude of 2020 m in a valley with a shrub layer dominated by *Rhododendron* spp. and *Populus cathayana* Rehd. *Cypripedium plectrochilum* thrives in clumps on the slopes, with each clump producing 1–50 flowering stems. The orchids grow in forest gaps and galleries, with roots established in soils with a pH of 6.73–6.85 (measured with 0.1 n CaCl₂) (H. Perner, personal detection).

Floral fragrance collection and analysis

Two floral fragrance samples (each sample contained three flowers in the same clump) and one air control were collected from fresh flowers on 8 May 2004 using headspace techniques. For field sampling studies, the flowers were enclosed in an inertia bag (Reynolds Co., USA) for about 1 h prior to sampling. The fragrance-laden air was drawn from the bag through a sorbent tube with a portable battery-powered sampling pump, and was collected using Tenax TA packed in a glass tube. The air was led through the glass tube for 1 h between 11:00 and 12:00 am, with a flow rate of approximately 100 ml·min⁻¹.

The fragrance samples were analysed by TCT-GC-MS on 16 July 2004. 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 cryofocused 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 the GC-MS (Trace 2000-Voyager, Finnigan, Thermo-Quest). Compounds were identified by searching the NIST library in the Xcalibur data system (Finnigan), and by comparing with components in the air control.

Floral visitors and their behaviour

Floral visitors to *C. plectrochilum* were observed from 27 April to 25 May 2004 and 13 May to 25 May 2005. Field observations totaled 60 h, mainly on sunny days. From each observation point, one to five flower patches could be seen. The behaviour of visitors was recorded from the moment they entered the vicinity of the flowers until they

left. All pollinator activities defined by Nilsson (1979), including approaching, alighting, entering, creeping and climbing, were recorded with a tape recorder in as much detail as possible. Some of the visiting insects were impossible to identify to the species level with certainty in the field. In such cases, insects were often only identified to a group of species or type in the field. For instance, 'small bee' means bees agreed in all perceptible characters with similar size, such as *Lasioglossum* spp. As much care as possible was taken not to disturb the pollinator–plant relations, the insects observed visiting *C. plectrochilum* were captured and killed in a jar using ethyl acetate fumes only when it was absolutely necessary for identification. Insect specimens were identified by H. Xu, Chinese Agriculture University. Voucher specimens are deposited at the State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences.

Floral functional structure and insect measurement

In order to assess contact between floral structure and insects, 50 flowers on 50 stems were chosen at random and floral architecture measured of DL, SL, AL and EL (see above and Fig. 1A, B) with digital calipers (Wuxi Xigong Measuring Corp.) to the closest 0.1 mm. We also measured body length, body width of the widest point and thorax height of the collected insect visitors with the same calipers to the closest 0.1 mm.

Breeding systems

In 2005, we conducted three experiments to determine if a pre-zygotic self-recognition and rejection mechanism is present in *C. plectrochilum* and whether mechanical self-pollination (autogamy) occurred. In each experiment, ten flowers on ten flowering stems were chosen at random on the first day that the labellum expanded and opened, but the labellum of each flower was excised before any insect activity occurred on that day. The stigma on the remaining column received one of the following treatments:

- 1 Hand-manipulated cross-pollination. Stigma received pollen from a second flower growing on a plant a minimum of 1 m away.
- 2 Hand-manipulated self-pollination. Stigma received the entire contents of one anther from the same column.
- 3 Unmanipulated control. No hand-pollination was attempted following removal of the labellum.

Fruit set of treated flowers and the majority of plants in the population that were allowed to retain their labella (natural rate of pollination) were recorded in July every year.

RESULTS

Floral presentation

Each flowering stem of *Cypripedium plectrochilum* produced two to four leaves. The inflorescence was terminal,



Fig. 1. Floral morphology, insect visitors and pollinators of *Cypripedium plectrochilum* studied in Danyun gorge, Huanglong, Sichuan, China in 2004 and 2005. Note: All photos are taken under natural conditions in the field. Bar = 1 cm. (A) Close view of an individual flower. (B) Longitudinal section of labellum showing the internal path of the pollinator; DL = mouth diameter of labellum, SL = height between stigma and bottom of labellum, AL = height between anther and bottom of labellum, EL = exit width of labellum. (C) Large bee visiting the flower. (D) Butterfly visiting the flower. (E) Fly detecting the labellum mouth. (F) Syrphid fly on the labellum; (G) Ant entering into the labellum. (H) *Lasioglossum sauterum* landing on the labellum. (I) *L. sauterum* escaping from the exit of the labellum. Note the pollen smear accumulated and deposited on the thorax. (J) *L. sauterum* resting on the labellum after escaping. Note pollen smear is on the thorax and head.

pubescent and single-flowered. The resupinate flower was subtended by an elliptic–lanceolate leaf-like bract 2–3 cm long and 7–8 mm wide. Sepals and lateral petals were greenish-brown yellow. The staminode and keel-shaped

labellum were white, spotted with pink on the apical rim, with some green on the tip of the labellum keel (Fig. 1A, B). The lifespan of a single flower varied from 6 to 16 days, average 10.9 days ($n = 10$, $SD = 3.59$). At

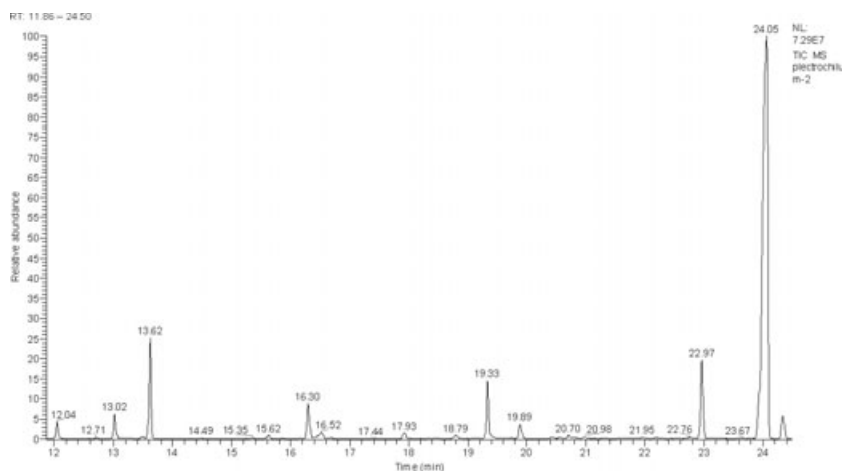


Fig. 2. Gas chromatogram of the floral fragrance of *Cypripedium plectrochilum* studied in Danyun gorge, Huanglong, Sichuan, China in 2004.

population level, the flowers of *C. plectrochilum* bloomed from late April to late May, early spring in the study site at more than 2000 m altitude. Only sympatric herbs *Fragaria orientalis* Lozinsk and scrub *Rhododendron* sp. overlapped with the flowering period of *C. plectrochilum*.

The flowers of *C. plectrochilum* generally send out a faint sweet scent for human olfaction. Five and six compounds were detected alternatively in the volatile constituents of two flower samples after contaminants were excluded (Fig. 2). Ethyl acetate and 2-propanone methylhydrazone were also detected in the control air samples, and their relative abundance was higher than in the flower samples. 3-Methyl-Decen-2-one then dominated the constituents in two flower samples (Table 1).

Floral visitors, pollinators and their behaviour

Representatives of three insect orders (Diptera, Lepidoptera and Hymenoptera) and 14 species visited flowers of *C. plectrochilum*. *Pieris rapae* L. was the only butterfly (Lepidoptera) recorded landing on the labellum. Flies landing on the labellum included *Delia unguigitris* Xue, *Polietes orientalis* Pont and four species in the Syrphidae, *Eristalis tenax* L., *Eupeodes corollae* Fabricius, *Platycheirus urakawensis* Matsumura and *Platycheirus peltatus* Meigen. Small wood ants (*Formica* sp.) were the dominant visitors

in the Hymenoptera. Large bees included workers of *Apis cerana* Fabricius and queens of *Bombus hypnorum* L. Small bees observed and collected were all female and *Ceratina* (*Ceratina* sp.; Apidae) and *Lasioglossum* (Halictidae); *L. sichuanense* Fan et Ebmer, *L. viridiclaucum* Ebmer and an unidentified *Lasioglossum* species.

Insect activity, including the frequency of approaching the flower, landing on floral organs, entering the labellum chamber and carrying off the flower pollen smears, is summarised in Table 2. Only ants, a solitary *Ceratina* sp. and *Lasioglossum* spp. crawled into the interior of the labellum. Ants were the most frequent visitors to flowers of *C. plectrochilum* (Fig. 1G) and could leave the flower by either crawling out of the labellum in the same way they entered or passed under the stigma and exited through the rear apertures under each anther. All ants exiting via the rear of the flower failed to carry pollen smears of *C. plectrochilum*.

Large bees (*Apis*, *Bombus*) (Fig. 1C) and the butterfly, *P. rapae* (Fig. 1D), found the labellum entrance but they could not move into the labellum chamber due to their large bodies and/or wide wings. Although flies (Fig. 1E, F) landed on the labellum and probed the entrance rim, they were not observed entering the labellum chamber.

Lasioglossum spp. were the only insects found to carry dorsally-deposited pollinia on their thoraces or on their

Table 1. Volatile compounds and their relative abundance from the flower fragrance of *Cypripedium plectrochilum*.

sample 1			sample 2			air control		
RT	VC	RA	RT	VC	RA	RT	VC	RA
7.81	Ethyl acetate	41.97	6.98	2-Propanone, methylhydrazone	24.26	7.44	Ethyl acetate	45.57
13.58	Pentanal 3-methyl	0.63	8.12	Ethyl acetate	33.3	8.74	Benzene	2.49
16.89	2-Propanoic acid, butyleste	1.17	13.02	Pentanal 3-methyl	1.36	16.32	2-Propanone, methylhydrazone	51.94
24.67	3-Decen-2-one, 3-methyl	56.24	16.30	2-Propanoic acid, butyleste	1.75	–	–	–
–	–	–	24.05	3-Decen-2-one, 3-methyl	39.33	–	–	–

RT = retention time, VC = volatile compounds, RA = relative abundance (%).

Table 2. Behaviour of different insect visitors towards flowers of *Cypripedium plectrochilum*.

insect group	no. approaching	no. alighting	no. entering	modes of escape		
				no. climbing	no. creeping	no. with pollen smear
ants	196	115	49	38	11	0
<i>Lasioglossum</i> bees	58	35	22	1	20	18
flies	71	33	0	0		0
syrphid flies	52	42	0	0		0
large bees	14	10	0	0		0
butterflies	6	3	0	0		0

heads and thoraces. A solitary female specimen of a small-bodied, *Ceratina* sp. (Apidae) was collected after it exited the rear aperture but it did not have a pollen smear. All collected *Lasioglossum* spp. were female. They were observed most often on warm sunny days, and the frequency of floral visitation was highest between 11:00 and 13:00. These bees usually performed an undulating flight as they approached the labellum, and 60% (n = 58 observations) of the bees that approached the labellum actually landed on its surface. We never observed a bee landing on a staminode; most landed on the edge of the white dorsum of the labellum, just in front of the entrance orifice (Fig. 1H). The bees then crawled to the centre and slowly entered the labellum chamber.

The percentage of *Lasioglossum* spp. entering the labellum was as high as 60% (n = 35 observations; Table 2). We did not observe the bees hesitating before entering the labellum chamber and we did not witness any flailing movements as if they were falling into the chamber by accident. *Lasioglossum* spp. remained inside the labellum from 20 s to 6 min. When they forced their way out through the rear aperture the dehiscent anther smeared their thoraces and occasionally their heads (Fig. 1I). As bees forced their way through the narrow exit canal between the labellum wall and the column, the entire labellum was observed to shake and vibrate, suggesting that these insects may employ some form of thoracic vibration.

On only one occasion did we observe a *Lasioglossum* specimen escaping by climbing out through the rim of the dorsal entrance. We only found one *Lasioglossum* specimen dead in a labellum chamber. Following a legitimate escape from the rear aperture, most *Lasioglossum* spp. rested briefly on the lateral petals, dorsal sepal, staminode or the labellum (Fig. 1J) before flying away and leaving the site. No bee was ever observed reentering the same flower following its first escape. No bee was ever observed to visit a second flower within the same field of observation after it flew away from the original flower.

Floral functional structure and insects measurement

Five insect groups (Table 3) were significantly different in body length (ANOVA, df = 4, F = 87.908, <0.001), body width (ANOVA, df = 4, F = 189.715, P < 0.001) and

Table 3. Size measurement of insect visitors of *Cypripedium plectrochilum*.

thorax height (cm)	body width (cm)	body length (cm)	visitors (n = 10)
0.465 ± 0.061	0.501 ± 0.073	1.446 ± 0.216	Large bees
0.195 ± 0.015	0.211 ± 0.026	0.59 ± 0.135	Flies
0.22 ± 0.024	0.269 ± 0.031	0.836 ± 0.144	Syrphid flies
0.189 ± 0.012	0.198 ± 0.022	0.673 ± 0.057	<i>Lasioglossum</i> bees
0.06 ± 0.012	0.065 ± 0.015	0.521 ± 0.131	Ants

thorax height (ANOVA, df = 4, F = 226.124, <0.001). In terms of body length, small bees were different from large bees (Tukey HSD, P < 0.001) and syrphid flies (Tukey HSD, P = 0.042), but the mean difference was not significant from flies (Tukey HSD, P = 0.613) and ants (Tukey HSD, P = 0.069). The body width of small bees was significantly different from large bees (Tukey HSD, P < 0.001), syrphid flies (Tukey HSD, P = 0.001) and ants (Tukey HSD, P < 0.001), but the mean difference was not significant compared with flies (Tukey HSD, P = 0.931). The thorax height of small bees was significantly different from that of large bees (Tukey HSD, P < 0.001), and ants (Tukey HSD, P < 0.001), but the mean difference was not significant compared with flies (Tukey HSD, P = 0.991) and syrphid flies (Tukey HSD, P = 0.162).

Apis and *Bombus* spp. (Fig. 1A, C) could not enter the labellum chamber as their body width was larger than the entrance diameter of the labellum (DL, 0.28 ± 0.028 cm, n = 50) (Independent samples *t*-test, *t* = 16.61, P < 0.001). In contrast, ant body length (Fig. 1G) was smaller than DL (*t* = -22.958, P < 0.001) and the exit width of the labellum (EL, 0.221 ± 0.025 cm, n = 50) (*t* = -18.981, P < 0.001), and their thorax height was also significantly smaller than the height between the stigma and bottom of the labellum (SL, 0.151 ± 0.018 cm, n = 50) (*t* = -14.782, P < 0.001) and the height between the anther and the bottom of the labellum (AL, 0.122 ± 0.014 cm, n = 50) (*t* = -12.208, P < 0.001). They entered the labellum chamber but were too short to contact the receptive stigma and too slender to contact the dehiscent anther when exiting.

Flies (Fig. 1F) had a body width smaller than DL (*t* = -6.692, P < 0.001) and EL (*t* = -1.155, P = 0.253).

They could have entered the labellum chamber and escaped from the exit. In contrast, syrphid flies (Fig. 1G) had a body width comparable to DL ($t = -0.994$, $P = 0.325$). They could have entered the labellum but blocked the exit as their body width were significantly larger than EL ($t = 4.921$, $P < 0.001$). It was fortunate for *C. plectrochilum* that flies and syrphid flies avoided entering as their thorax height was much larger than SL ($t = 7.318$, $P < 0.001$; $t = 10.13$, $P < 0.001$) and AL ($t = 14.278$, $P < 0.001$; $t = 16.583$, $P < 0.001$). It is presumed that these insects also lacked the physical strength or force of bees or ants. They do not have manipulative mandibles or forelegs.

All *Lasioglossum* spp. (Fig. 1H, J) could enter and exit the labellum chamber as their body width was smaller than DL ($t = -8.456$, $P < 0.001$) and smaller than EL ($t = -2.693$, $P = 0.01$). The height of their thoraces was larger than SL ($t = 6.613$, $P < 0.001$) and AL ($t = 13.182$, $P < 0.001$). Although the difference is significant, these bees physically contact the receptive stigmatic surface and the dehiscent anthers through a proposed combination of physical force and thoracic vibration, unlike the more common ants.

Breeding systems

In 2004, 45.9% of unmanipulated flowers observed ($n = 1317$) matured to become capsules. In 2005, 38.9% ($n = 1369$) set capsules. The difference in fruit set between the 2 years is significant (χ^2 , $P < 0.001$). Control flowers, with the labellum removed, but not hand-pollinated ($n = 10$) failed to set any fruit. Hand-self-pollinated ($n = 10$) flowers had 80% fruit set, and 90% of hand-cross-pollinated flowers ($n = 10$) became capsules (Table 4). The difference between hand-self-pollination and cross-pollination is significant (χ^2 , $P < 0.001$).

DISCUSSION

Our insect exclusion and hand-pollination experiments demonstrated that this large population of *C. plectrochilum* is self-compatible but incapable of mechanical self-pollination (Table 4). Field observations showed that, while a wide variety of native insects visited the flowers, small bees were the only effective dispersers of pollen. Over two seasons, fruit set in flowers indicated that *Lasioglossum* spp. were surprisingly effective agents of

cross-pollination in this generalist food mimic, as observations indicated that these bees did not return to the same flower at any recordable frequency. Bee-pollination dominates the genus *Cyripedium* (van der Pijl & Dodson 1966; Davis 1986; Cribb 1997; Sugiura *et al.* 2001, 2002) and pollination by small solitary bees has been shown in other species, indicating that the pollination system of *C. plectrochilum* is at least as specialised as that of *C. calceolus* s.s. and *C. arietinum* (Stoutamire 1967; Nilsson 1979).

However, our results are novel at two levels. First, while bee-pollination is common in this genus, the conversion rate of flowers into capsules is often very low in *Cyripedium* spp. (Cribb 1997; Sugiura *et al.* 2001, 2002). For example, fruit set in *C. acaule* is from 0 to 15% fruit set (Barrett & Helenum 1987; Primack & Stacy 1998), and fruit set in *C. calceolus* is usually <15% (Nilsson 1979; Kull 1998). The conversion rate of flowers into capsules in this population of *C. plectrochilum* was comparable with that of some populations of the diapiiid wasp-pollinated, *C. fasciculatum* Franch (Lipow *et al.* 2002; Ferguson *et al.* 2005). This means that, under certain conditions, the fecundity of some *Cyripedium* spp. is higher than other mimetic orchids, and competitive with other herbaceous perennials offering edible rewards. We attribute reproductive success in this case to the large population of *C. plectrochilum* in a relatively undisturbed habitat that is obviously rich in vernal, emerging flower-visiting insects. We must remember that *C. plectrochilum* is a non-model mimic that does not actively mimic anything else in bloom at the site. When individual plants form such a large population and bloom synchronously, the super stimuli of massed pigmentation patterns and floral odours may be unusually attractive to resident naïve pollinators with generalist foraging habits. In southern temperate Australia, for example, most female *Lasioglossum* spp. are generalist pollen and nectar foragers, often visiting nectar-rich species, pollen-rich but nectar-deficient species and mimetic species during the same foraging bout (Bernhardt & Burns-Balogh 1986; Bernhardt 1986, 1989, 1995). Specialised pollination in *C. plectrochilum* appears to depend on the exploitation of a common, congeneric and efficient lineage of generalist foragers.

It is not surprising, therefore, that *C. arietinum*, the only other species sharing the same labellum type (*sensu* Cribb 1997) as *C. plectrochilum*, is also pollinated by at least two *Lasioglossum* spp. (Stoutamire 1967). *Lasioglossum caeruleum* L., collected by Stoutamire, is regarded as an uncommon North American species (Michener, personal communication). As *Lasioglossum* s.s. is a pandemic genus consisting of 160 species (Michener 2000), it is most unlikely that a rare bee species is the dominant pollinator of *C. arietinum* throughout its natural distribution. We speculate that *Lasioglossum* taxa vary over the respective disjunctive distributions of these two 'sister' orchid species and the fidelity of any *Lasioglossum* sp. to either *Cyripedium* spp. fluctuates according to the geographic and/or seasonal demography of orchid populations in bloom. While it is tempting to regard the unique

Table 4. Results of fruit set of *Cyripedium plectrochilum*.

treatment	no. of flowers	no. of fruits	fruit set (%)
hand-cross-pollination	10	9	90
hand-self-pollination	10	8	80
unmanipulated control	10	0	0
natural pollination (2004)	1317	604	45.9
natural pollination (2005)	1369	532	38.9

keeled labellum shared by these two *Cypripedium* spp. as a specialised character for trapping and exploiting *Lasioglossum* spp., we must note that other similar-sized halictid bees are also the primary pollinators of *Cypripedium montanum* Douglas ex Lindl., which has a differently-shaped labellum (Bernhardt & Vance, unpublished data). In addition, the pollination character of *C. plectrochilum* overlaps strongly with the earlier work of Nilsson (1979) on the pollination of *C. calceolus*. *Cypripedium* spp. do not always suffer from infrequent insect visitation. *Cypripedium calceolus* was visited by over 50 insect species, although only one species of andrenid bee proved to be the primary pollinator. Similarly, *C. plectrochilum* was visited by at least 11 insect species (representing three orders) but the act of pollinia dispersal was relegated to three bee species in the same genus. It is unlikely that this broad range of non-pollinating visitors is confined to these two Eurasian orchids. Observations and collections of insects visiting the flowers of *C. montanum* suggest that, while six bee species transport pollinia, an estimated 20 species of bees, wasps, beetles and flies visit the flowers, land on the floral organs, and some even enter the labellum chamber without contacting dehiscent anthers and/or stigmatic surfaces (P. Bernhardt & N. Vance, in preparation). What does this tell us about the evolution of floral characters and genetic constraints within the genus and in *C. plectrochilum* in particular? Why does *C. plectrochilum* have only three potential pollinating insect species at the Huanglong Nature Reserve instead of 11?

With the exception of the inflated, keeled hairy labellum there is little that is unique about floral presentation in *C. plectrochilum* based on the behaviour of its generalist floral visitors. While we did not test for the presence of UV patterns, we may note that the white, pink and greenish pigmentation on the labellum is repeated in a number of vernal, shade-tolerant perennial herbs. The selectively adaptive trend towards light-coloured morphs flowering within shady habitats was observed by Keeler (1916), and the selective advantage of light-coloured flowers in certain habitats was addressed quantitatively by Mogford (1978). The insects that visited *C. plectrochilum* belong to lineages that also visit the light-coloured flowers of such temperate, vernal woodland herbs (Keeler 1916; Müller 1883; Proctor *et al.* 1996) as some species within such genera as *Claytonia*, *Erythronium*, *Fragaria*, *Hepatica*, *Trillium*, *Viola*.

Floral odour is a most important signal for chemical communication in insect-pollinated flowers (Pellmyr 1986; Knudsen *et al.* 1993; Schiestl *et al.* 2003; Raguso 2004; Schiestl 2005), and plays a significant role in both long- and short-distance attraction of insects (van der Pijl & Dodson 1966; Dobson *et al.* 1999). The dominant component of *C. plectrochilum*, 3-methyl-3-Decen-2-one, is a very uncommon scent compound (Knudsen *et al.* 2006), identified only in fresh flowers of fragrant *Edgeworthia chrysantha* Lindl., *Syringa obovata* Lindl. and steamed clam *Rangia cuneata* Gray (Chen *et al.* 1987; Tanchotikul & Hsieh 1991; Li *et al.* 2004). The short-distance undulating

flight of the three *Lasioglossum* spp. to *C. plectrochilum* was probably influenced by floral odour. However, the odour released by this orchid cannot be interpreted as specific for *Lasioglossum* spp., as ants, butterflies, *Ceratina* sp. and large bees attempted to enter the labellum. At best, the dominant component of this orchid, 3-methyl-3-Decen-2-one, can be speculated not to encourage true flies and syrphid flies to enter the labellum chamber. The literature on fly behaviour is replete with references to flies being attracted to amines and nitrogen-containing compounds, such as indole (Meeuse 1978; Willams 1983; Beehler *et al.* 1993; Kaiser 1993; Knudsen *et al.* 1993; Wall & Warnes 1994; Jürgens *et al.* 2006). Syrphid flies are the dominant pollinators of *C. reginae* L. (Vogt 1990), where the main component of the fragrance is benzoic acid methyl ester (Barkman *et al.* 1997). However, in some sexually deceptive orchids, the physiologically active scent compounds may not be detected from headspace sampling due to their relatively low volatility (Schiestl *et al.* 1999; Schiestl & Ayasse 2002). Therefore, it remains to be confirmed which scent compound of *C. plectrochilum* flowers actually matters for attraction of the particular *Lasioglossum* bees. In future studies, this question could be addressed using artificial array experiments, in which bees are given a direct choice among different scent compounds.

Floral architecture is best viewed as the primary character limiting the role of insect visitors to *C. plectrochilum* and, perhaps, to other small bee-pollinated *Cypripedium* spp. in general. Despite the specialised internal and external floral morphology associated with this genus, it is obvious that relatively small changes in floral dimensions cause pollinator shifts, limiting the physical size of the effective pollinators (Stoutamire 1967; Nilsson 1979; Catling & Knerer 1980; Bänziger *et al.* 2005). *C. acaule*, with its wide labellum entrance, accommodates large queen bumblebees as pollinators, while *C. plectrochilum* and *C. arietinum*, with their 1–2-mm wide entrance, depend exclusively on *Lasioglossum* spp. (Stoutamire 1967; Davis 1986; Barrett & Helenum 1987). In *C. calceolus*, the reduced labellum also prevents bumblebee queens from entering (Nilsson 1979). It is obvious that the narrow range of effective pollinators in most *Cypripedium* spp. studied thus far is dependent ultimately on a very specialised goodness of fit between pollinator body dimensions and DL, SL and AL floral dimensions. These ultimately determine whether the insect consistently contacts the stigmatic surface and whether it can escape *via* the rear of the flower and contact the dehiscent anther. Slender, non-pollinating ants wandering in and out of the flowers of *C. plectrochilum* are the price paid for closer canalization to the physical dimensions of *Lasioglossum* spp..

It may, therefore, be time for the community of floral biologists to reinterpret the function of the inflated labellum of the *Cypripedium* flower and its interconnecting diandrous column. The classical approach interprets the morphological arrangement of *Cypripedium* as a trap or kettle trap (van der Pijl & Dodson 1966; Dressler 1993).

If we continue to compare these flowers to hunting tools, the analogy must be expanded, as traps represent ancient and unusually sophisticated aspects of human technology (Balick & Cox 1996). If traps were not specialised devices we would catch leopards in lobster pots! Generalist presentation of colour and scent in *C. plectrochilum* guarantees a wide variety of insect visitors, but specialised architecture ultimately narrows the prospective pool of pollen vectors. Colour and scent components are extremely variable between and within *Cypripedium* spp. (Nilsson 1979, 1981; Bergström *et al.* 1992; Barkman *et al.* 1997) but exclusionary floral architecture appears to function as a mechanism prompting the specialised pollination throughout this genus and probably within the subfamily Cypripedioideae (*sensu* Dressler 1993; Stevens 2001).

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REFERENCES

- Ackerman J.D. (1986) Mechanisms and evolution of food deceptive pollination systems in orchids. *Lindleyana*, **1**(2), 108–113.
- Anderson B., Johnson S.D., Carbutt C. (2005) Exploitation of a specialized mutualism by a deceptive orchid. *American Journal of Botany*, **92**, 1342–1349.
- Balick M.J., Cox P.A. (1996) *Plants, People and Culture; The Science of Ethnobotany*. Scientific American Library, Hphlp, New York, USA.
- Bänziger H., Sun H., Luo Y. (2005) Pollination of a slippery lady slipper orchid in south-west China: *Cypripedium guttatum* (Orchidaceae). *Botanical Journal of the Linnean Society*, **148**, 251–264.
- Barkman T.J., Beaman J.H., Gage D.A. (1997) Floral fragrance variation in *Cypripedium*: implications for evolutionary and ecological studies. *Phytochemistry*, **44**(5), 875–882.
- Barrett S.C.H., Helenum K. (1987) The reproductive biology of boreal forest herbs. I. Breeding systems and pollination. *Canadian Journal of Botany*, **65**, 2036–2046.
- Beehler J.W., Millar J.G., Mulla M.S. (1993) Synergism between chemical attractants and visual cues influencing oviposition of the mosquito, *Culex quinquefasciatus* (Diptera: Culicidae). *Journal of Chemistry Ecology*, **19**, 635–644.
- Bergström G., Birgersson G., Groth I., Nilsson L.A. (1992) Floral fragrance disparity between three taxa of lady's slipper *Cypripedium calceolus* (Orchidaceae). *Phytochemistry*, **31**(7), 2315–2319.
- Bernhardt P. (1986) Bee-pollination of *Hibbertia fasciculata* (Dilleniaceae). *Plant Systematics and Evolution*, **152**, 231–241.
- Bernhardt P. (1989) The floral ecology of Australian *Acacia*. In: Stritton C.H., Zarucchi J.L. (Eds), *Advances in Legume Biology*. Monographs in Systematic Botany from the Missouri Botanical Garden, St Louis, MO: 127–155.
- Bernhardt P. (1995) The floral ecology of *Dianella caerulea* var. *assera* (Phormiaceae). *Cunninghamia*, **4**, 1–17.
- Bernhardt P., Burns-Balogh P. (1986) Floral mimicry of *Thelymitra nuda* (Orchidaceae). *Plant Systematics and Evolution*, **151**, 187–202.
- Blanco M.A., Barboza G. (2005) Pseudocopulatory pollination in *Lepanthes* (Orchidaceae: *Pleurothallidinae*) by fungus gnats. *Annals of Botany*, **95**, 763–772.
- Catling P.M., Knerer G. (1980) Pollination of the white lady's slipper (*Cypripedium candidum*) in Lambton County, southern Ontario. *The Canadian Field Naturalist*, **94**, 435–438.
- Chen S.C. (1983) Two pairs of vicarious species of *Cypripedium* (Orchidaceae) from eastern Asia and eastern North America. *Brittonia*, **35**, 159–163.
- Chen Y., Li Z., Li H. (1987) Analysis of fragrance volatiles of fresh flowers by pre-column absorption and GC/MS. *Chromatographia*, **23**(7), 502–506.
- Cozzolino S., Widmer A. (2005) Orchid diversity: an evolutionary consequence of deception? *Trends in Ecology and Evolution*, **20**, 487–494.
- Cribb P. (1997) *The Genus Cypripedium*. Timber Press, Portland, OR.
- Dafni A. (1984) Mimicry and deception in pollination. *Annual Review of Ecology and Systematics*, **15**, 259–278.
- Darwin C. (1862) *The Various Contrivances by which British and Foreign Orchids are Fertilized by Insects*. John Murray, London, UK.
- Davis R.W. (1986) The pollination ecology of *Cypripedium acaule* (Orchidaceae). *Rhodora*, **88**, 445–450.
- Dobson H.E.M., Danielson E.M., van Wesep I.D. (1999) Pollen odor chemicals as modulators of bumble bee foraging on *Rosa rugosa* Thunb (Rosaceae). *Plant Species Biology*, **14**, 153–166.
- Dodson C.H., Dressler R.L., Hilles H.G., Adams R.M., Williams N.H. (1969) Biologically active compounds in orchid fragrances. *Science*, **164**, 1234–1249.
- Dressler R.L. (1993) *Phylogeny and Classification of the Orchid Family*. Cambridge University Press, Cambridge, UK.
- Fenster C.B., Armbruster W.S., Wilson P., Dudash M.R., Thomson J.D. (2004) Pollination syndromes and floral specialization. *Annual Review of Ecology and Systematics*, **35**, 375–403.

- Ferguson C.S., Donham K., Brown J.L. (2005) *Cypripedium fasciculatum* (Orchidaceae) anthesis and fruit set in relationship to diapriid activity. *Selbyana*, **26**, 103–113.
- Guignard J.A. (1886) Insects and orchids. *Annual Reports of the Entomological Society of Ontario*, **16**, 39–48.
- Gumbert A., Kunze J. (2001) Colour similarity to rewarding model plants affects pollination in a food deceptive orchid, *Orchis boryi*. *Biological Journal of the Linnean Society*, **72**(3), 419–433.
- Jersakova J., Johnson S.D., Kindlmann P. (2006) Mechanism and evolution of deceptive pollination in orchids. *Biological Reviews*, **81**, 219–235.
- Johnson S.D., Steiner K.E. (2000) Generalization versus specialization in plant pollination systems. *Trends in Ecology and Evolution*, **15**, 140–143.
- Johnson S.D., Steiner K.E. (2003) Specialized pollination systems in southern Africa. *South African Journal of Science*, **99**, 345–348.
- Johnson S.D., Peter C.L., Nilsson L.A., Agren J. (2003) Pollination success in a deceptive orchid is enhanced by co-occurring rewarding magnet plants. *Ecology*, **84**, 2919–2927.
- Jürgens A., Dotterl S., Meve U. (2006) The chemical nature of fetid floral odors in stapeliads (Apocynaceae–Asclepiadoideae–Ceropegieae). *New Phytologist*, **172**, 452–468.
- Kaiser R. (1993) *The Scent of Orchids*. Roche, Basel, Switzerland.
- Kay K., Schemske D. (2004) Geographic patterns in plant–pollinator mutualistic networks: comment. *Ecology*, **85**, 875–878.
- Keeler H.L. (1916) *Our Early Wild Flowers*. Charles Scribner & Sons, New York, USA.
- Knudsen J.T., Tollsten L., Bergstrom G. (1993) Floral scents – a checklist of volatile compounds isolated by headspace techniques. *Phytochemistry*, **332**, 253–280.
- Knudsen J.T., Eriksson R., Gershenzon J., Stahl B. (2006) Diversity and distribution of floral scent. *The Botanical Review*, **72**(1), 1–20.
- Kull T. (1998) Fruit-set and recruitment in populations of *Cypripedium calceolus* L. in Estonia. *Botanical Journal of the Linnean Society*, **126**, 27–38.
- Li Z., Li X., Liu W., Gao Y. (2004) Study on chemical constituents of fragrance from fresh flowers of *Edgeworthia chrysantha*. *Chemistry and Industry of Forest Products*, **24**(1), 83–86.
- Lipow S.E., Bernhardt P., Vance N. (2002) Comparative rates of pollination and fruit set in widely separated populations of a rare orchid (*Cypripedium fasciculatum*). *International Journal of Plant Science*, **163**, 775–782.
- Luer C.A. (1975) *The Native Orchids of the United States and Canada Excluding Florida*. New York Botanical Garden, Bronx, NY.
- Meeuse B.J.D. (1978) The physiology of some sapromyophilous flowers. In: Richards A.J. (Ed.), *The Pollination of Flowers by Insects*. Academic Press, London, UK: 97–104.
- Michener C.D. (2000) *The Bees of the World*. The Johns Hopkins University Press, Baltimore, MD.
- Mogford D.J. (1978) Pollination and flower colour polymorphism with special reference to *Cirsium palustre*. In: Richards A.J. (Ed.), *The Pollination of Flowers by Insects*. Academic Press, London, UK: 191–199.
- Müller H. (1883) *The Fertilisation of Flowers*. Transl. D'Arcy W. Thompson. London, UK.
- Nilsson L.A. (1979) Anthecological studies of the lady's slipper, *Cypripedium calceolus* (Orchidaceae). *Botaniska Notiser*, **132**, 329–347.
- Nilsson L.A. (1981) Pollination ecology and evolutionary process in six species of orchids. *Acta Universitatis Upsaliensis*, **593**, 1–40.
- Nilsson L.A. (1992) Orchid pollination biology. *Trends in Ecology and Evolution*, **7**(8), 255–259.
- Olesen J.M., Jordano P. (2002) Geographic patterns in plant–pollinator mutualistic networks. *Ecology*, **83**, 2416–2424.
- Ollerton J. (1998) Sunbird surprise for syndromes. *Nature*, **394**, 726–727.
- Pellmyr O. (1986) Three pollination morphs in *Cimicifuga simplex*: incipient speciation due to inferiority in competition. *Oecologia*, **68**, 304–307.
- van der Pijl L., Dodson C.H. (1966) *Orchid Flowers: Their Pollination and Evolution*. University of Miami Press, Miami, FL.
- Primack R., Stacy E. (1998) Cost of reproduction in the pink lady's slipper orchid (*Cypripedium acaule*, Orchidaceae): an eleven-year experimental study of three populations. *American Journal of Botany*, **85**, 1672–1679.
- Proctor M., Yeo P., Lack A. (1996) *The Natural History of Pollination*. Timber Press, Portland, OR.
- Raguso R.A. (2004) Flowers as sensory billboards: progress towards an integrated understanding of floral advertisement. *Current Opinion in Plant Biology*, **7**, 434–440.
- Schemske D.W., Horvitz C.C. (1984) Variation among floral visitors in pollination ability: a precondition for mutualism specialization. *Science*, **225**, 519–521.
- Schiestl F.P. (2005) On the success of a swindle: pollination by deception in orchids. *Naturwissenschaften*, **92**, 255–264.
- Schiestl F.P., Ayasse M. (2002) Do changes in floral odor cause speciation in sexually deceptive orchids? *Plant Systematics and Evolution*, **234**, 111–119.
- Schiestl F.P., Ayasse M., Paulus H.F., Lofstedt C., Hansson B.S., Ibarra F., Francke W. (1999) Orchid pollination by sexual swindle. *Nature*, **399**, 421–422.
- Schiestl F.P., Peakall R., Mant J.G., Dolbarra F., Schulz C., Franke S., Francke W. (2003) The chemistry of sexual deception in an orchid–wasp pollination system. *Science*, **302**, 437–438.
- Stevens P.F. (2001 Onwards) Angiosperm phylogeny website, version 6, May 2005. <http://www.mobot.org/MOBOT/research/APweb/>. 28 September 2006.
- Stoutamire W.P. (1967) Flower biology of the lady's slippers (Orchidaceae: *Cypripedium*). *Michigan Botanist*, **3**, 107–119.
- Sugiura N., Taketoshi F., Ken I., Kenji K. (2001) Flowering phenology, pollination, and fruit set of *Cypripedium*

- macranthos* var. *rebunense*, a threatened lady's slipper (Orchidaceae). *Journal of Plant Research*, **114**, 171–178.
- Sugiura N., Goubara M., Kitamura K., Inoue K. (2002) Bumblebee pollination of *Cypripedium macranthos* var. *rebunense* (Orchidaceae): a possible case of floral mimicry of *Pedicularis schistostegia* (Orobanchiaceae). *Plant Systematics and Evolution*, **235**, 189–195.
- Tanchotikul U., Hsieh T.C.Y. (1991) Analysis of volatile flavor components in steamed Rangia clam by dynamic headspace sampling and simultaneous distillation and extraction. *Journal of Food Science*, **56**(2), 327–331.
- Tremblay R.L. (1992) Trends in the pollination ecology of the Orchidaceae: evolution and systematics. *Canadian Journal of Botany*, **70**, 642–650.
- Tremblay R.L., Ackerman J.D., Zimmerman J.K., Calvo R.N. (2005) Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. *Biological Journal of the Linnean Society*, **84**, 1–54.
- Vogt C.A. (1990) Pollination in *Cypripedium reginae* (Orchidaceae). *Lindleyana*, **5**(3), 145–150.
- Wall R., Warnes M. (1994) Responses of the sheep blowfly, *Lucilia sericata*, to carrion odour and carbon dioxide. *Entomologia Experimentalis et Applicata*, **73**, 239–246.
- Waser N.M. (2006) Specialization and generalization in plant–pollinator interactions: a historical perspective. In: Waser N.M., Ollerton J. (Eds), *Plant–Pollinator Interactions: from Specialization to Generalization*. The University of Chicago Press, Chicago, London: 4–17.
- Waser N.M., Chittak L., Price M.V., Williams N.M., Ollerton J. (1996) Generalization in pollination systems, and why it matters. *Ecology*, **77**, 1043–1060.
- Williams N.H. (1983) Floral fragrances as cues in animal behavior. In: Jones C.E., Little R.J. (Eds), *Handbook of Experimental Pollination Biology*. Van Nostrand Reinhold, New York, USA: 50–72.