Observations of putative pollinators of *Hemipilia flabellata* Bur. et Franch. (Orchidaceae) in north-west Yunnan Province, China

YI-BO LUO* AND SING-CHI CHEN

Laboratory of Systematic and Evolutionary Botany & Herbarium (PE), Institute of Botany, Chinese Academy of Sciences, Xiangshan, Beijing 100093, China

Interactions between *Hemipilia flabellata* and anthophilous insects were studied in north-western Yunnan, China during June–July of 1996 and 1997. Twenty-seven species of insects were seen visiting the flowers of this species, although only male and female individuals of the bee *Anthophora mangkamensis* Wu were observed carrying the pollinaria. The frequency of visits by *A. mangkamensis* to this orchid was rather low. Pollinating bees normally sampled only the lowermost flowers in an inflorescence, staying for 1–3 seconds. This species is also characterized by low pollination, low fruit set (1–22%) and a very striking decrease in fruit production from bottom to top of the inflorescence. All of these indicate that the orchid may rely on deception to attract visitors. *Ajuga forrestii* Diels (Labiatae) appears to be the main or exclusive subsidiary nectar source for *A. mangkamensis* during the flowering period of *H. flabellata*. It appears that *H. flabellata* utilizes a food deceit pollination strategy for pollination. The orchid benefits from the great variation of flowers in colour and shape. Phenological, functional-morphometric and distribution data indicate that *A. mangkamensis* is an optimal pollinator of *H. flabellata* and thus the anthecological adaptation of the orchid to the pollen vector may be unique.

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ADDITIONAL KEY WORDS:—*Anthophora mangkamensis* – Apoidea – food deception – Hengduan Mts – pollination system.

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* Corresponding author.

INTRODUCTION

Hemipilia Lindl. is a genus of about 13 species distributed in south-western China and the Himalayas, with a few species extending to Burma and Thailand. It is treated as a member of the subtribe Orchidinae under tribe Orchideae (Dressler, 1993) and considered to be related to *Ophrys*, *Orchis* and *Dactylorhiza*. The genus *Hemipilia* differs from other genera of subtribe Orchidinae by having a large rostellum and separate caudicles in the base of the anther.

There is an extensive literature dealing with the various aspects of pollination ecology in Orchidaceae (see Dafni, 1987; Nilsson, 1992; Johnson, Steiner & White-head, 1998). Dafni (1987) recognized two deception patterns—sexual and food—in the subtribe Orchidinae, and suggested that the transition from reward to deception is the main evolutionary trend in this tribe. Cropper and Calder (1990) summarized three types of food deception in Orchidaceae according to the relationship of mimics and models. Nilsson (1992) reviewed the pollination biology of Orchidaceae and estimated that 8000–10 000 species of orchids provide no reward for pollinators. His discussion focused on two deceit pollination systems: food deception and pseudocopulation. In the former he distinguished a group of plants which mimic co-blooming flowers which provide rewards. In China, no work on pollination observation in either Orchideae or Orchidaceae has hitherto been undertaken despite the presence of about 250 species of the former and more than 1000 of the latter.

The purpose of our study was to attempt to understand the floral biology and pollination mechanism in *Hemipilia flabellata*, a species endemic to the Hengduan Mountains of south-western Sichuan and north-western Yunnan. Since it is a little-studied species, some vegetative features and morphological data, especially the gynostemium structure, are provided.

Hemipilia flabellata is an erect, 10–15 cm tall, perennial terrestrial herb with one leaf above the soil surface and a terminal loosely spicate inflorescence of between one and 16 flowers. The flowers are highly zygomorphic and have six free perianth segments. The dorsal sepal is erect, concave and cucullate, while the lateral sepals are spreading and deflexed at the upper part. The petals are erect and ovate-lanceolate in shape. The lip is broadly ovate in outline with an ascending short claw at the base. *H. flabellata* usually grows in well-drained, stony or calcareous soils at the margin of forest or shrub, or in woodlands and amongst scattered shrubs, in habitat typical of terrestrial orchids where there is low competition and disturbance.

MATERIAL AND METHODS

This study was conducted at Tunguancun, about 129 km north of Lijiang, north-western Yunnan Province, during June–July of 1996 and 1997. The nine populations (A–I) of *H. flabellata* investigated were located on the verges of a road built about 40 years ago, along the bottom of a long valley running from west to east between Lijiang and Zhongdian. When we began working, two populations (G, H) at about 2600 m altitude were in full bloom. Our main observations were made on the other seven populations (A–F, I) at an elevation of about 2860 m.

The plants in populations A–F grow on step sites on south-facing slopes with

various biotypes. The woody vegetation is dominated by some pioneer species such as the seedlings of *Pinus yunnanensis* Franch. (Pinaceae), *Salix* sp. (Saliaceae), *Coriaria nepalensis* Wall. (Coriariaceae) and *Lespedeza* sp. (Leguminosae). Representative herbaceous species are *Pedicularis* spp. (Scrophulariaceae), *Anemone hupehensis* Lem. (Ranunculaceae), *Epipactis mairei* Schltr. and *E. helleborine* (L.) Crantz. (both Orchidaceae). More or less concurrently blooming plants within or near the studying areas are *Rosa multiflora* Thunb. (Rosaceae), *Wikstroemia* sp. (Themelaeaceae), *Pedicularis* spp., *Cynoglossum amabile* Stapf et Drum (Boraginaceae), *Vicia amoena* Fisch. ex Ser. (Leguminosae) and *Silene* sp. (Caryophyllaceae). Near to populations D and E, there were two clusters of *Orchis chusua* D. Don (Orchidaceae), blooming somewhat later than *H. flabellata*. Population C was on very shady rocks under *Pinus armandii* Franch. and *P. yunnanensis* Franch. Population G was found on a flat area almost completely covered with fragmentary stones beside the Chongdian River. The dominant plants were *Coriaria nepalensis* and *Oxyria sinensis* Hemsl. (Polygonaceae), while the concurrent blooming species, *Epipactis mairei*, was scattered throughout the whole population. Along the river bank, some *Rosa multiflora* and *Myricaria squamosa* Desv. (Tamaricaceae) were in flower. Population H grew on the margin of a woodland that consisted of *Pinus yunnanensis* and *C. nepalensis*, and partly extended into the forest.

Observation periods for pollinator activity were spread throughout the day to maximize the probability of observing floral pollinators and visitors. Pollinators were defined as insects with pollinaria attached to their bodies, while visitors included those insects visiting flowers and probing for nectar or alighting on flowers but without pollinaria on their bodies. The number of times and hours (in parenthesis) spent in observation at the study sites were: A 3 (12), B 2 (4), C 3 (2.5), D 3 (6.5), F 5 (19), G 2 (11) and I 3 (25). Behavioural data of pollinators recorded included the number of inflorescences visited, number of flowers visited per inflorescence, the time spent on each flower and inflorescence, and the number of pollinaria deposited on each insect specimen. Pollen vectors were captured after they had visited *H. flabellata*, or when they alighted on other plants. Also, the insects, especially the bees, that appeared on the other plants near the localities of *H. flabellata* were collected to check whether or not they carried pollinaria. Under natural conditions, the percentage of pollinaria removed per spike was assessed towards the end of anthesis using a magnifying glass. Insects collected during the study period were identified by entomologists from the Entomology Department, Institute of Zoology, Chinese Academy of Sciences; vouchers of the visits are lodged there. The plant vouchers were kept in Herbarium (PE), Institute of Botany, Chinese Academy of Sciences.

For breeding systems, the cross- and self-pollination experiments were carried out using isolated flowers or spikes. In addition to the hand-pollination of this species, the cross-pollination between *H. flabellata* and *O. chusua* were carried out in D and E. About 2 or 3 weeks after pollination the ovaries obviously swelled and they were counted as fruit sets. Unfortunately, the isolated but untouched flowers in C were unintentionally destroyed by road maintenance workers.

FLOWERING PHENOLOGY

The flowering season of *H. flabellata* varies greatly with the elevation and the degree of shadiness. Generally, the populations at the low altitudes (about 2600 m)

TABLE 1. Relative freqency of spikes of *Hemipilia flabellata* in different stages of anthesis at different locations

Locations	Date	No. spikes examined	Percent flowers open in spikes (mean)			
			0	>0 and ≤50	>50 and <100	100
G, H	June 30	349	5	22	49	24
A, D, E	July 1	83	75	17	1	7
B, C		46	98	2	0	0
F		—	—	—	—	—
A, D, E	July 3	—	—	—	—	—
B, C		47	96	4	0	0
F		171	17	44	19	20
A, D, E	July 7	123	29	29	22	20
B, C		133	63	26	9	2
F		181	9	14	23	54
A, D, E	July 11	155	18	27	25	30
B, C		161	44	30	17	9
F		227	1	12	16	71
A, D, E	July 15	166	9	16	22	53
B, C		169	21	26	29	24
F		213	0	3	9	88
A, D, E	July 19	206	5	9	22	64
B, C		206	9	9	22	60
F		223	0	1	3	96
A, D, E	July 23	202	3	3	13	81
B, C		224	4	8	11	77
F		190	0	1	0	99
A, D, E	July 29	133	0	1	2	97
B, C		208	0	1	3	96
F		—	—	—	—	—

flower 2–3 weeks earlier than those at higher altitudes (2800–2900 m) and those at the exposed sites about 1 week earlier than those at the shady ones (Table 1). At populations G and H, the flowering periods started around 10 June and extended to the first week of July. Among populations A–F at almost the same elevation, the earliest flowering took place at the exposed sites (e.g. F), where the flowering began around 20 June and lasted about 3 weeks. In the woods or in areas which receive less sunshine (e.g. C and B), flowering commenced around the 1 July and ended around 23 July. The maximum number of open flowers varied between habitats: 7–15 July in populations A, D, E, 11–19 July in B and C and 3–11 July in F (Table 1).

On an average, it took 13 days ($N=6$, range 7–17) for all flowers to open in an inflorescence. The flowers opened acropetally. About 2 weeks after the flowers opened, the unpollinated flowers began withering with the spur first and then the lip becoming yellow-white in colour. Pollinated flowers withered about 1 week earlier than unpollinated ones.

FLOWER VISITORS AND POLLINATORS

The visitors comprised Hymenoptera [Apoidea (6 spp.), Formicidae (1 sp.)], Diptera (7 spp.), Lepidoptera (10 spp.), Coleoptera (more than 2 spp.) and Thysanoptera (1 sp.) (Table 2). All visiting insects were directly captured on the flowers

TABLE 2. Visitors to the flowers of *Hemipilia flabellata*

Visitors	Localities	No. seen on H. flabella	No. captured on H. flabella	No. captured on other flowers	Total no. recorded	No. on which pollinaria were counted	No. of pollinaria recorded
Hymenoptera							
Apidae							
Anthophora manglamensis Wu f, m	B, F	27	3	6	9	3	8
Apis cerana Fabricius w	B, F, G	2	4	40	46	—	—
Bombus festivus Smith w	E, G, H	—	1	17	18	—	—
B. parthenius Richards w, m	G, F, E, B, A	—	1	36	37	—	—
B. grahami (Frison) w	F, G	2	1	5	8	—	—
Anthidium rubopunctatum Wu m, f	F	—	1	6	7	—	—
Lasioglossum upinensis Moravizm m, f	B	1	1	9	11	—	—
Diptera							
Cyrtidae							
Philopota nigroaenea Motschulsky m	C	—	1	1	2	—	—
Dexiidae							
Billaea carinifrons Fallen m	F	often	5	1	—	—	—
Syrphidae							
Rhingia binotata Brunetti m	F, G	often	5	—	1	—	—
Volucella plumatoides Herve-Bazin m	F	—	1	—	3	—	—
Eristalus paria (Bigot) m	G	2	1	—	3	—	—
Episyphus balteatus (Macquart) m	H	—	2	—	2	—	—
Melanostoma sp. m	F	7	1	—	8	—	—
Lepidoptera							
Pieridae							
Anthocharis bieti Oberthur m	A, F	7	2	—	9	—	—
Hesperiidae							
Ochlodes sixataarsa Evans m, f	B, G	19	4	—	28	—	—
Boro cinnara (Wallace) f	F	—	1	—	1	—	—
Melitaea jezabel Oberthur m	F	—	1	—	1	—	—
Satyridae							
Erebia nero Styr. m	F	—	1	—	1	—	—
Ypthima imitans Elwes et Edwards m	F	—	1	—	1	—	—
Callerebia suroia Tytler f	G	—	1	—	1	—	—
Drepnidae							
Nordstroemia niva Chu et Wang f	B	2	1	—	3	—	—
Nymphalidae							
Euthalia perlella Chou et Wang m	B	—	1	—	1	—	—
Apatura bietii Oberthur m	B	—	1	—	1	—	—

TABLE 3. Previous collections of *Anthophora mangkamensis*

Localities	Elevation (m)	The collected time
Xizang: Markan	2700	2 June 1976
Yunnan: Dêqên	3180	21 July 1982
Yunnan: Weixi	2500–2900	22–26 July 1981
Yunnan: Lijian	2800	11 July 1984
Yunnan: Dali	?	15 April 1957
Yunnan: Kuming	1900	18 March 1955
Yunnan: Kuming	1900	31 April 1982
Sichuan: Xiangcheng	2900	28 June 1982
Sichuan: Kangding	2600	29 May 1983
Sichuan: Emei	550–750	31 March 1957

of *H. flabellata* at different localities. The insects most frequently observed on the flowers were the members of Diptera, including *Billaea carinifrons* Fallen (Dexiidae), *Rhingia binotata* Brunetti and *Melanostoma* sp. (both Syrphidae). *Ochlodes sivataarsa* Evans and *Anthocharis bieti* Oberthur of Lepidoptera, and coleopteran species were also often found on the flowers. However, the most important visitor was *Anthophora mangkamensis* Wu; this was found to be the only pollen vector of *H. flabellata*. One species of ant was occasionally observed transporting the pollinaria at population F, but the pollinaria appeared to have been taken from the flower by other insects.

Both males and females of *A. mangkamensis* visited *H. flabellata* and carried the pollinaria (Fig. 1B, C). Actually, only one of four visitors was male, to which three pollinaria of *H. flabellata* were attached; the three females carried a total of seven pollinaria. Individuals of *A. mangkamensis* were also observed on the flowers of *Ajuga forrestii* Diels (Labiatae); one female carrying a single pollinarium was captured. Occasionally, males and females of *Lasioglossum upinense* Morawitz and *Anthidium rubopunctatum* Wu appeared on the flowers of *H. flabellata*. All bumble bees and honeybees captured on the flowers of *H. flabellata* were workers.

PHENOLOGY OF POLLINATOR AND FOOD PLANTS

A. mangkamensis, the only pollen vector of *H. flabellata*, is a recently described species (Wu Yan-Ru, 1982); it is endemic to south-western Sichuan and north-western and central Yunnan, and the details of its phenology are still unknown. However, the specimens kept in the Institute of Zoology in Beijing indicate that the periods when this solitary bee is most abundant at different locations vary greatly with elevation (Table 3). Above 2500 m, it is abundant during June–July. Based on present data, at least in our study area, the flowering season of *H. flabellata* generally coincides with the season when *A. mangkamensis* appears. Of 65 specimens of *A. mangkamensis* in the Institute of Zoology, only five are male; in our collection of 10 specimens, only one is male. Therefore, female individuals of *A. mangkamensis* visiting *H. flabellata* predominate over the males during the season of activity.

Wu (1982) mentioned that *A. mangkamensis* visits the flowers of Ranunculaceae. However, according to our observation, it was mainly observed on the flowers of *Ajuga forrestii*, despite there being more than 13 species of plant, including at least one species of Ranunculaceae, in flower during our study period. Like *H. flabellata*

TABLE 4. Frequency of visits of *Anthophora mangkamensis* to *Hemipilia flabellata* at different periods

Time	8.00–10.00	10.00–12.00	12.00–14.00	14.00–16.00	16.00–18.00
Frequency	3	11	4	7	5

and *A. mangkamensis*, *Ajuga forrestii* is an endemic species of the Hengduan Mts, although it has a wider distribution (Chen Gheih, 1977). Its flowering season is from April to August (Chen Gheih, 1977), which is longer than that of *H. flabellata* and is also concurrent with the mainly active periods of *A. mangkamensis*. However, *Ajuga forrestii* has not been found growing within the populations of *H. flabellata* and their surroundings, for the former is confined to wet habitats, according to Chen Gheih (1977). In fact, only two populations of *Ajuga forrestii*, both of which were far away from our study areas, were found. Therefore, *A. mangkamensis* must travel a relatively long distance before arriving at the plants of *H. flabellata*.

Usually, the species of *Anthophora* make nests on walls and have relatively stable nesting sites (Wu Yan-Ru, 1965). Typically, *H. flabellata* grows on calcareous soils. Some of our study populations (e.g. B and F) were located on very steep slopes or near vertical cliffs and it is possible that some nests of *A. mangkamensis* are close to the populations of *H. flabellata*, although no direct observations have been made. After emergence, the bees seem to lack foraging routines (Wu Yan-Ru, pers. comm.), and thus their appearance is perhaps restricted by their food resource.

BEHAVIOUR OR FLOWER POLLINATORS AND VISITORS

No individuals of *A. mangkamensis* appeared in rain or overcast weather, and observations were made mainly on sunny days. Their visits peaked twice throughout the day: between 10.00 and 12.00 h and 14.00 and 16.00 h (Table 4). This may be related to the air temperature, since the places we observed are all above 2500 m altitude where the annual mean difference between the lowest and highest daily temperature according to the local weather station records, exceeds 12–13°C.

The visiting frequency of *A. mangkamensis* was very low; in over 75 hours of observations of different populations, only 30 visitations were recorded. The maximum number of bees reached three per hour during the most favourable period. Hoverflies (Syrphidae) have a somewhat similar appearance while in flight, so the real number of visits may have been lower. In the course of 30 visitations, 73% involved a single spike, 20% two spikes and 7% three spikes. The bees usually landed on an inflorescence for only a few seconds and never for more than 20 seconds. They mainly visited the flowers in the lower part of spike and only in two cases did the bees directly approach the flowers in the upper part of spike. According to our observations, 74% of bees visited only one flower, 13% two flowers and 13% three or more flowers. The bees usually completed a visitation to a flower within 1–3 seconds and never stayed for more than 10 seconds. The whole process from landing on one flower to departing from the population usually lasted less than 30 seconds. Occasionally we saw the bee visiting an inflorescence on which the two lowermost flowers had clearly swollen ovaries. Moreover, the bees were seen to visit flowers at early, middle or late flowering stage of *H. flabellata*. It appears that the

pollination system of *H. flabellata* worked fully throughout the whole anthesis period.

The process of a bee moving from other plants to *H. flabellata* was not observed during our fieldwork. The bees approached swiftly and directly landed on the flowers; they visited one or few flowers and then departed from the population at speed. In only one case was a bee seen to rest on the leaf of *Quarcus pannosa* Hand.-Mazz. (Fagaceae) for about 4 seconds after visiting three flowers of *H. flabellata*. The general behaviour pattern of *A. mangkamensis*, such as visiting only one spike, one flower per spike, the short duration (one to 20 seconds) of visitation, and less than three pollinaria being taken by a bee, suggest that it is being subjected to deception.

When alighting on *A. forrestii*, the bee usually grasps the lower lip with its front claws and supports itself on adjacent flowers by the back claws. When inserting its proboscis into the corolla-tube to absorb nectar along the upper lip, its head presses against the upper lip so that the extended anthers and style are positioned below its body (Fig. 1F). The mean corolla-tube length of *Ajuga forrestii* is 7.4 mm (SD = 0.70, $n = 11$) and the mean proboscis length (mentum + glossa) of *A. mangkamensis* is 9.5 mm (SD = 1.31, $n = 8$). Therefore, the bee may be able to reach the bottom of the corolla tube and probe for nectar without pressing its head into the flowers. The morphometric relation between tube length and proboscis length indicates that this bee could be one of the legitimate pollen vectors.

In all, 70 workers of four species and four queens of two species of bumble-bee, 36 workers of *Apis ceranan* Fabricius and 18 female and male individuals of *Anthidium rubopunctatum* Wu and *Lasioglassum upinense* Morawitz, were checked for pollinaria of *H. flabellata*, but no pollinaria were carried by them (Table 2). Obviously, they are not the pollinators of *H. flabellata*, although they occasionally visited the plant.

On the other hand, some flies also often visited flowers of *H. flabellata*. At least five species of Syrphidae, one species of Cyrtidae and one species of Dexiidae were seen to probe the sepals or labellum before entering the spur mouth (Table 2). *Melanostoma* (Syrphidae) were often observed to enter the spur; sometimes half the body would enter. In one case, at population G, we found one fly which had completely entered and could not escape. Lepidoptera, especially *Ochlodes sivataarsa* and *Anthocharis bietti*, often inserted their proboscides into the spur. However, none of them carried away the pollinaria.

Small Coleoptera appeared on the flowers of *H. flabellata*, and sometimes gnawed the petals and rostellum. However, in most cases they used the flower as a shelter. Thysanopthera seem to do the same. Sometimes, spiders were seen on the flowers and on one flower at population G at least four pollinaria hung on the ovary by the thread of a spider while two ants were seen gnawing the massulae on the pollinaria. Those pollinaria were probably taken out by small beetles after gnawing the rostellum.

Figure 1. *Hemipilia flabellata*, variation in floral coloration, and attachment of pollinaria on pollen vector. A, Species on stony soils, two individuals with two different colour flowers. B & C, *Anthophora mangkamensis* with pollinaria attached on heads. B, male bee. C, female bee. D & E, *Hemipilia flabellata*, flowers of different individuals. H, *Anthophora mangkamensis* on the flower of *Ajuga forestii*.

FUNCTIONAL MORPHOLOGY

The colour of the flower varies from deep purple to snow-white (Fig. 1A, D, E). All parts are rigid and able to support the weight of visiting bees. The dorsal sepal and petals are incurved to form a hood over the column. The lateral sepals spread nearly horizontally with a slightly incurved twist along the upper margins. (Fig. 1D, E) The lip has an ascending short claw and a downward-pointing labellum. The lip base is extended backward into a spur which spreads horizontally or slightly upwards and forms an obtuse angle with the lip (Fig. 2F). The spur is slightly expanded at its upper 2–3 mm and then abruptly tapers downards. Seen from the front, the entrance to the spur is located at the lip base and in the front of the stigma (Fig. 2A); its outline is almost circular with a ventral furrow (Fig. 2B).

The column laterally expands and forms two incurved wings; the front margins of wings are obviously thickened and thus form two horn-like protuberances (Fig. 2A, B). There is a semi-circular cavity in front of, and a semi-square cavity above, the spur mouth (Fig. 2A). The anther is nearly erect with two parallel thecae (Fig. 2C, D). The thecae taper towards the base and extend forwards, thus forming two anther channels. Each theca encloses a long-caudicled pollinarium. The caudicle is twisted, narrowed downwards and terminates in a viscidium (Fig. 2E). The outline of viscidia is suborbicular when completely spread with both sides bent upwards to form a canoe-like structure (Fig. 2G). The outer surface of one side is converted into viscid matter, while the outer surface of the other connects to the long caudicale of the pollinia at the posterior position (Fig. 2G). The rostellum is well developed and three-lobed. The median lobe is tongue-shaped and lies obliquely erect between two anthers (Fig. 2A, E). The two lateral lobes are extended forwards along the interior walls of the column, and thus form two shadow channels where the anther channels are situated (Fig. 2B). The end of the lateral rostellar lobe bears one canoe-shaped viscidium. One side of the viscidium that its outer surface converted into the viscid matter is positioned on the outside of the lateral rostellar lobe (Fig. 2C, D). The viscidia are situated directly above the entrance to the spur, facing each other obliquely (Fig. 2C). The stigma is located below the rostellum and behind the entrance to the spur (Fig. 2B). The stigmatic surface is slightly convex, and the front part projects forward. The outline of the stigma is thus a chair-like structure.

Mean length of the spur is 15.56 mm (SD = 1.78, $n = 63$); mean width between the two front basal margins of the column wings is 1.78 mm (SD = 0.23, $n = 103$) and between the two horn-like protuberances, 1.07 mm (SD = 0.11, $n = 63$). Since the two viscidia hold nearly the same position as the horn-like protuberances, the width between two viscidia also is about 1.07 mm. The mean length of proboscis (mentum + glossa) of *A. mangkamensis* is (our specimens) 8 mm ($n = 1$) or (Institute of Zoology specimens) 9.5 mm (SD = 1.31, $n = 8$) while the mean width of the face across the base of the labrum is 1.6 mm (SD = 0.22, $n = 9$) or 1.79 mm (SD = 0.42, $n = 19$). This result reveals that the spurs are much longer than the depth which can be reached by the mouthparts of the bees, thus ensuring that the latter will penetrate into the spur as deeply as possible when they probe for supposed nectar. On the other hand, the relatively small width between the viscidia makes it possible that they could be deposited on the sides of clypeus (Fig. 1B, C).

The viscidia instantly adhere to the surface of the labrum so effectively that the pollinaria can be removed from the thecae when the bee retracts. After leaving the flower, no movement of the pollinia has been observed. In most cases the pollinaria
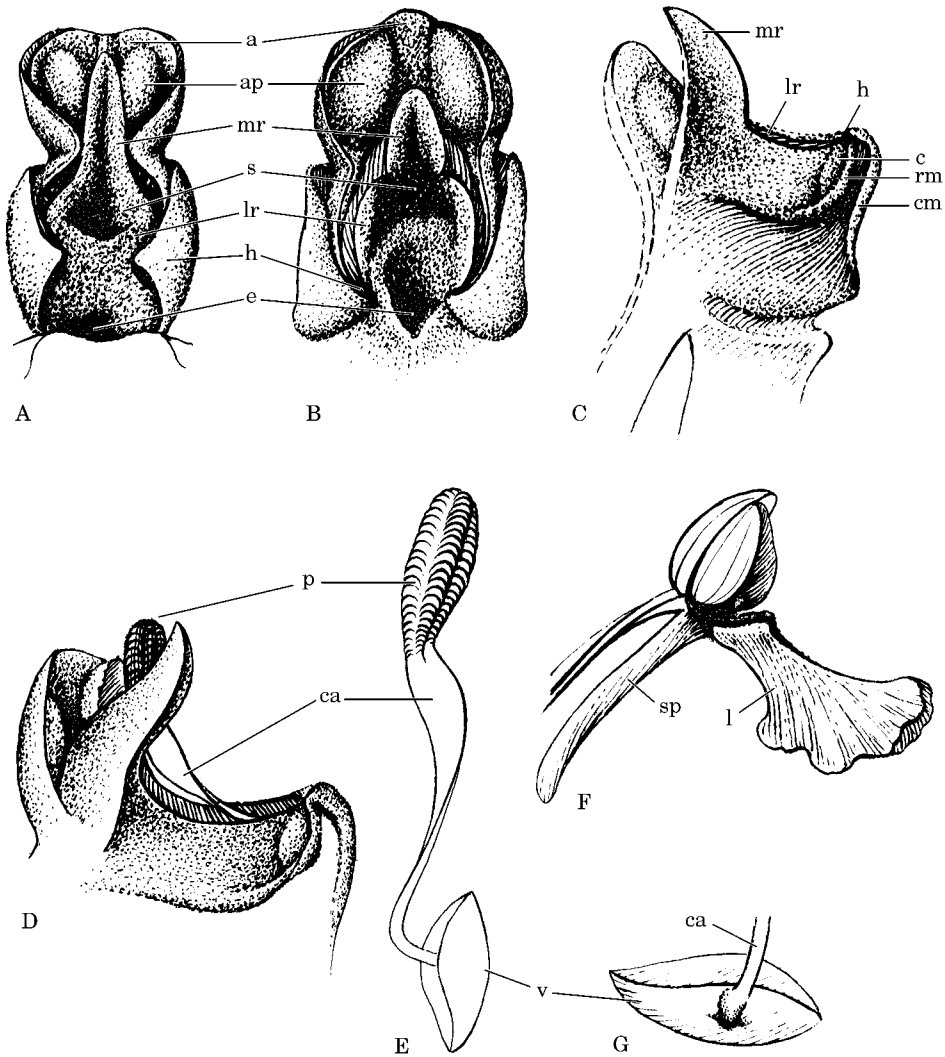
Figure 2. Floral morphology in *Hemipilia flabellata*, drawn from specimen Luo Yi-bo 65 and preserved flowers by Mrs Chai Shu-qin. A, column, front view (10 ×). B, column, vertical view (10 ×). C & D, longitudinal section of column, C (10 ×), D (15 ×). E, pollinaria, vertical view (26.6 ×). F, lateral view of flower (2 ×). G, viscidium (40 ×). Abbreviations: a, anther; ap, anther-pockets with enclosed pollinia; mr, medial rostellum lobe; lr, lateral rostellum lobe; s, stigma; e, entrance of spur; h, horn-like protuberance; c, viscidium cell; cm, column margin; rm, lateral rostellum lobe margin; p, pollinia; ca, caudicle; v, viscidium; sp, spur; l, labellum or lip.

lie parallel to the proboscis and directed forwards (Fig. 1B, C). The width of the entrance to the spur is much narrower than that between two viscidia (Fig. 2B), so the forward-directed pollinia cannot be taken into the spur when the proboscis is inserted into it. The chair-like stigma is located below the rostellum and behind the entrance to the spur. So when a pollen vector penetrates into the spur, the long

caudicles, about 1.5 mm in length ($n = 7$), hold the pollinia in front of the vector (Fig. 1B, C), and the chair-like stigma causes the massulae to adhere to it.

Therefore, from the view of morphology, *A. mangkamensis* is an 'optimal' pollinator species for *H. flabellata*. On the other hand, the flora structure of *H. flabellata* is obviously different from that of *Orchis* and *Dactylorhiza* species. For example, the two viscidia of *H. flabellata* are widely separated and positioned on the sides above the entrance to the spur. All these features determine that the pollination process and mechanism of this species are different from that of the *Orchis* and *Dactylorhiza* species which have been extensively studied by many authors (Nilsson, 1980, 1981, 1983a, 1984; Fritz, 1990). However, the detailed pollination process and mechanism of *H. flabellata* still requires further study due to the lack of direct observation data in this study.

FRUIT SET

The succession of pollination was low with only 12% of the flowers (654/5477) developing into fruits (Table 5). On the other hand, only about 30% of individuals in all the studied sites went on to set fruit (Table 5). The natural fruit set was constantly at a low level at eight populations, between less than 1% and 22% (Table 5). Among the fruiting plants ($n = 384$), about 56.7%, 26.4% and 11.3% of individuals produced respectively one, two and three fruits, and 5.6% individuals had more than four fruits (Table 6). Of 384 plants, only one with five flowers all set fruits on a spike. The highest number of fruits on a single spike was seven, and this spike bore eight flowers. Based on our observation, the proportion of bees visiting more than two flowers during one visitation was 26%; sometimes they continually visited two or more flowers in the same spike. Thus it is possible to set two or more fruits in a single spike. Occasionally, bees visited the flowers higher up a spike where the lower flowers had obviously swollen ovaries. Thus the unusually high number of fruits may be the result of two or more visits by bees.

A very striking decrease in fruit production existed from the bottom to the top of the inflorescence (Table 7). The lowermost three flowers were pollinated to a much higher degree than those at higher positions on the spike. Among the fruiting individuals, 76% of the lowermost three flowers set fruit (Table 7). Compared with the flowers in the lowermost position (position 1), the flowers in position 4 were about three times less likely to set fruit, in position 5 about five times and in position 6 or 7 more than ten times (Table 7). The rather dramatic decrease in fruit set is a result of behavioural response of bees because they receive no reward from the flowers. The bees usually begin at the bottom flowers and visit only one or a few of the next flowers up before departing.

The number of flowers on a single spike varied from 1 to 16. At the population level, the number of fruits per plant bore little relation to spike size (the number of flowers). For example, the mean number of flowers per spike at G was highest (5.38) but fruit set only reached 19.31%. In contrast, population F had the highest fruit set (22.32%) but also the lowest mean number of flowers per spike (2.96) (Table 5). However, within the same population the multiflowered spikes revealed an advantage in sexual reproductive capacity. In population G, 635 plants in total were examined for fruit set. There were 209 individuals with seven or more flowers per spike and

TABLE 5. Natural fruit set in *Hemipilia flabellata* at different locations

Locations	No. of plants	No. of flowers	No. flowers per plant	Plants with fruits (%)	No. fruits	No. fruits per individual	Fruit set (%) Mean	SD	Range
A	68	238	3.50	16.2	16	0.23	4.41	12.17	0–75
B	147	413	2.81	2.0	3	0.02	0.54	3.82	0–33
C	63	226	3.59	17.5	19	0.30	5.23	12.03	0–50
D	68	221	3.25	11.6	23	0.34	10.59	24.97	0–100
E	36	115	3.19	22.2	10	0.28	8.06	16.36	0–50
F	233	689	2.96	44.6	169	0.73	22.32	30.23	0–100
G	635	3417	5.38	32.3	352	0.55	19.31	15.07	0–100
H	35	158	4.52	25.7	18	0.51	12.00	28.57	0–100
Total	1285	5477	4.26	29.9	654	0.51	9.06		

TABLE 6. Relative frequency of the number of fruits produced in spikes of *Hemipilia flabellata*

Localities	No. of plants	No. of fruits in spikes (%)							
		0	1	2	3	4	5	6	7
A	68	83.82	11.76	1.47	2.94	—	—	—	—
B	147	97.96	2.04	—	—	—	—	—	—
C	63	82.54	4.76	12.70	—	—	—	—	—
D	68	82.35	8.82	4.41	2.94	—	1.47	—	—
E	36	77.78	19.44	—	2.78	—	—	—	—
F	233	55.36	27.04	10.30	5.58	1.29	—	—	0.43
G	635	67.72	17.48	9.29	3.31	1.73	0.31	0.16	—
H	35	74.29	14.28	2.86	5.71	—	2.86	—	—
Total	1285	71.75	16.03	7.47	3.19	1.09	0.31	0.08	0.08

426 plants with six or fewer flowers. Among the former about 50% individuals went on to fruit while among the latter only 24.4% did so. Apparently, smaller or fewer-flowered individuals are more easily neglected by bees. This may be, as noted by Nilsson (1983a), a simple consequence of a large floral display having a higher attractive power for pollinators.

Generally, the populations situated near the road (A–E) had lower fruit set (5.76%) than those located further away (F–H) (17.87%) (Table 6). Proctor and Yeo (1979) noted that the movements of *Anthophora resta* and *A. acervorum* are extremely quick and they are more easily frightened by the human presence than the bumble-bee. In our observations, *A. mangkamensis* also moved very quickly and is very sensitive to the human presence or the noise and fumes produced by vehicles; this may have caused the decrease of fruit set. On the other hand, this result also indicates that *H. flabellata* is pollinated by *A. mangkamensis* rather than bumble-bees, for the latter are less sensitive to disturbance by human activities compared with *A. mangkamensis*.

Hand-pollution experiments showed that after being pollinated with the pollen of the same species or with that of *O. chusua* fruit set is almost complete (Table 8). Under natural conditions, as mentioned above, it is very much lower. This indicates that auto-pollution does not occur in this species since relatively high fecundity is a particularly useful character for detecting auto-pollution (Catling, 1990).

On 16–17 July, we checked the number of pollinaria removed and the lumps of pollen deposited on the stigma in 178 flowers from 43 plants at three locations (Table 9). We found 68% of flowers had one or both pollinaria removed and 45.5% had more or less pollinia deposited on the surface of stigma. During the flowering period, we did not observe spontaneous self-pollination by the rupture of pollinia on to the stigma of the same flower. The higher the position of a flower on the spike, the less opportunity its pollinaria have of being removed and its stigma of being pollinated (Table 9). This condition coincides with the distribution of fruits on the spike. The pollination rate (the ratio of the number of flowers from which a pollinium had been removed to the number in which a pollinium, or part of a pollinium had been deposited) was 1.39 (Table 9). This result indicates some wastage of pollinia. We saw some pollinia which had adhered to the petals of ovaries. From the view of the pollination mechanism, it is possible that both the depositing and removing of the pollinia could occur in a single visitation by a bee. Therefore, the wastage of pollinia may be caused by other insects, such as beetles and the larvae of moths and butterflies; sometimes the latter destroyed the whole flower.

TABLE 7. Distribution of fruits in spikes of *Hemipilia flabellata* under natural conditions

Locations	No. flowers examined	Distribution of fruits in spikes (%)									
		1	2	3	4	5	6	7	8	9	10
A	238	2.52	1.68	0.42	1.68	0.42	—	—	—	—	—
B	413	0.48	0.24	—	—	—	—	—	—	—	—
C	226	2.21	2.21	2.65	0.44	0.88	—	—	—	—	—
D	221	4.52	3.17	1.81	0.45	0.45	—	—	—	—	—
E	115	3.48	3.48	1.74	—	—	—	—	—	—	—
F	689	11.32	7.40	3.05	1.31	0.72	0.43	0.14	0.14	—	—
G	3417	2.40	2.51	2.08	1.37	0.88	0.41	0.41	0.06	0.15	0.03
H	158	3.16	2.53	3.16	0.87	1.74	—	—	—	—	—
Total	5477	3.50	2.96	2.01	1.13	0.75	0.31	0.29	0.05	0.11	0.02

TABLE 8. Results of experiments on pollination in *Hemipilia flabellata*

Conditions	No. of plants	No. of flowers	Ovaries swollen (%)		
			Mean	SD	Range
Cross-pollinated	22	64	87.5	0.125	67–100
Self-pollinated	20	71	87.5	0.125	75–100
With *Orchis chusua* pollen	12	38	75.0	0.250	50–100

DISCUSSION

The low pollination and fruit set of *H. flabellata* under natural conditions indicates that auto-pollination in this species does not occur. The same conclusion could also be drawn from the floral structure, in which the pollinia and the stigma are completely separated by the rostellum and there is little possibility for the pollinia to fall directly onto the stigma.

Generally, the low pollination and fruit set are considered to be a typical character of food deception orchids (Calvo, 1990). In addition, the strong decline in fruit set from the bottom to the top of a spike of *H. flabellata* resembles that of *Orchis* and *Dactylorhiza*, which have typical food-deception systems (Nilsson, 1980, 1981, 1983a, 1984; Fritz, 1990). On the other hand, the general behavioural pattern of *A. mangkamensis* when visiting the flowers of *H. flabellata* also indicates that the bee is deceived. Our collection of both male and female individuals carrying pollinaria shows that no sexual deceit occurred in *H. flabellata*, indicating that the pollination of *H. flabellata* occurs as the result of food deception.

There are two types of food deception in the family Orchidaceae. The first consists of a tripartite association of model, mimic and dupe (Little, 1983; Dafni, 1984, 1987; Cropper & Calder, 1990; Nilsson, 1992). A low population density of the mimic in relation to the model, with the supply of compensating rewards by the more common model subsidizing system, are a prerequisite for the success of this system (Daafni & Calder, 1987; Cropper & Calder, 1990; Nilsson, 1992). The dependence of the mimic on the model leads to some constraints on the mimic's phenology, distribution and morphology variation (Dafni, 1987; Nilsson, 1992). Typical examples of this system in the subtribe Orchidinae include *Orchis israelitica* (Dafni & Ivri, 1981), *O. caspia* (Dafni, 1983) and *Cephalanthera rubra* (Nilsson, 1983b). The second consists of a bipartite association, mimic and dupe, whereby the relationship between the mimic and the model is very loose or even absent (Cropper & Calder, 1990; Nilsson, 1992). This system mainly makes use of the 'naiveté' principle to deceive unaware nectar-seeking pollinators. Generally, the orchids in this system bloom gregariously and exhibit display polymorphisms; they include *D. sambucina*, *O. mascula*, *O. morio* and *O. spitzelii* (Nilsson, 1980, 1983a, 1984; Fritz, 1990).

According to our study, the food deception pollination system of *H. flabellata* revealed the following features: (1) the species usually grows gregariously, with many individuals, sometimes forming groups of many hundreds of individuals. (2) The colour of flowers varies greatly, from deep purple to snow white, and the length and shape of the spur shows some degrees of variation. (3) The fitness (fruit set) shows no relationship to the models, while the fruit sets differ between populations,

TABLE 9. Information on pollinaria in spikes of *Hemipilia flabellata* (*n* = 43). *Swollen ovaries counted as pollinia deposited

Pollinia condition	The positions of flowers in spikes												Total	% pollinia removed	% pollinia deposited*
	1	2	3	4	5	6	7	8	9	10	11	12			
One pollinarium removed	17	7	8	9	2	1	2	0	1	0	1	0	48	27	
Two pollinaria removed	20	18	14	8	7	4	2	0	0	0	0	0	73	41	
Untouched	6	15	6	7	7	7	4	3	0	1	0	1	57	32	
Total	43	40	28	24	16	12	8	3	1	1	1	1	178	—	
Pollinia deposited	24	23	12	11	6	3	2	0	0	0	0	0	81		

this appears to be caused by human activity rather than by the presence or lack of a food model. (4) The number of *H. flabellata* greatly exceeds that of *Ajuga forrestii*, which is very rare in the areas under study. We therefore suggest that *H. flabellata* should be considered to have a bipartite (food deception) rather than tripartite (mimicry) system.

Identical phenology, interactive morphology and insect behaviour suggest strongly that *A. mangkamensis* is the major pollinator of *H. flabellata*. It would appear that neither bumble-bees nor honeybees act as pollinators of *H. flabellata*, although they frequently appear on its flowers or on other plants in the vicinity. Our observations show that visitation by *A. mangkmanesis* occurs throughout the whole flowering season of *H. flabellata*; the bee will also visit flowers higher up a spike when the lower flowers have obviously swollen ovaries. This seems to indicate the food deception pollination system of *H. flabellata* is functional throughout the whole anthesis, and appears not to use the 'naiveté' principle seen in *D. sambucina*, *O. mascula*, *O. morio* and *O. spitzelii* (Nilsson, 1980; 1983a, 1984; Fritz, 1990).

Up until this study, we had observed *A. mangkamensis* visiting *Ajuga forrestii* in addition to *H. flabellata*, concluding that *A. forrestii* was its only food resource. However, it would appear that *Anthophora* sp. is polyphagous (Wu, 1965; Proctor & Yeo, 1975; Dafni, 1983). Moreover, Wu (1982) recorded *A. mangkamensis* visiting species of Ranunculaceae. Therefore, it is possible that we overlooked the fact that *A. mangkamensis* may visit other plants besides *Ajuga forrestii*. Cropper & Calder (1990) argued that pollinator naiveté may not necessarily be a prerequisite for food deception, as most species of bee visit unfamiliar species to monitor food availability during normal foraging activity. They believed that *Thelymitra epipactoides* is a typical food deception (without models) pollination system and that this species increases the likelihood of bee visitation by having a highly variable visual display rather than by making use of the naiveté principle. Similarly, the flowers of *H. flabellata* also showed great variation in colour and shape, perhaps making use of same mechanism to achieve optimal pollination rates.

Based largely on the mismatch between the morphometrics of the flower and pollinator, Nilsson (1983a, 1984) and Fritz (1990) suggested that the adaptation to bumble-bee queens in *O. mascula*, *O. morio* and *O. spitzelli* of northern and central Europe may be derived and is likely evolved from adaptation to long-tongued solitary bees in early-flowering Mediterranean population complexes. The reason behind the adaptation to bumble-bee pollination is perhaps the relative sparseness of solitary bees in more northerly latitudes compared to the Mediterranean area (Nilsson, 1983a, 1984). Assessing the morphology both of the spur and of bee fauna in the Mediterranean area, Nilsson (1983a, 1984) suggested that some types of solitary bee are the chief pollinating agents. The studies by Dafni (1983) and Dafni & Ivri (1981) on *O. israelitica* and *O. caspia* in Israel seem to support the suggestions of Nilsson and Fritz. Both of those species are pollinated by solitary bees and the bee fly (*Bombylius* sp.). Since *A. mangkamensis* appears to be the 'optimal' pollinator of *H. flabellata* based on their morphometric relations, the latter's adaptation to the former appears to represent an original phenomenon.

From the view of floral structure, especially in view of the fact that the thecae are completely separated, *Hemipilia* is closer to *Platanthera* or the subtribe Habenariinae than *Orchis* or *Dactylorhiza*. However, *H. flabellata* is similar to most species of *Orchis* and *Dactylorhiza* in its pollination model because no deceit pollination model has so far been found in *Platanthera* and other genera of Haberiinae. Considering the

available evidence from *Orchis* and some related genera such as *Ophrys* and *Serapias*, Dafni (1987) suggested two main evolutionary lines, one of which involved the transition from reward to food mimicry and shelter imitation in which optical cues are the primary stimuli. Nilsson (1992) noted that in the family Orchidaceae deception has arisen many times in distantly related groups. Therefore, we suggest that the food deception of *H. flabellata* represents a parallel evolution line to *Orchis* or *Dactylorhiza* with respect to rewarding flowers and deception.

Although the reproductive success of *H. flabellata* is low, the high number of seeds per fruit is sufficient to maintain or expand the size of the population and even to develop new populations. This conclusion is supported by the following fact. The sites where populations of *H. flabellata* grow can be regarded as the result of interference by humans since all of them occur along the road built about forty years ago. That the accompanying plants are all pioneering species, such as *Pinus yunnanensis*, *Salix* sp., *Coriaria nepalensis*, *Oxyria sinensis*, etc., is further confirmation that these populations have developed recently. The rapid development of these populations, some of more than one thousand individuals, demonstrates the very high efficiency of sexual reproduction in *H. flabellata*, there being no vegetable propagation nor auto-pollination known from this orchid.

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