

Pollination of *Kadsura longipedunculata* (Schisandraceae), a monoecious basal angiosperm, by female, pollen-eating *Megommata* sp. (Cecidomyiidae: Diptera) in China

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The plants of *Kadsura longipedunculata* (Schisandraceae) are monoecious and possess either red or yellow male flowers (the androecium), with yellow tepals, and yellow female flowers. All flower types simultaneously produce heat and floral odours (dominated by methyl butyrate) throughout a 4–5-h nocturnal period. The flowers are pollinated only by female, pollen-eating *Megommata* sp. (Cecidomyiidae). Pollen is the only reward, and female flowers use the same attractants as male flowers but offer no food (pollination by deceit). Open pollinated flowers in nature varied in fruit set from 8 to 92%. *Megommata* (subfamily Cecidomyiinae, supertribe Cecidomyiidi), consists of six described species, which feed on Coccoidea (scale insects) and are distributed worldwide. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, **93**, 523–536.

ADDITIONAL KEYWORDS: basal angiosperms – floral thermogenesis – methyl butyrate – pollination by deceit.

INTRODUCTION

The Austrobaileyales contains four families of basal flowering plants (Austrobaileyaceae, Trimeniaceae, Illiciaceae, and Schisandraceae), members of the ANITA grade, forming the base of the angiosperm phylogenetic tree (Qiu *et al.*, 1999, 2000; Renner, 1999; Barkman *et al.*, 2000; Graham & Olmstead, 2000; Soltis *et al.*, 2000; Zanis *et al.*, 2002; Borsch *et al.*, 2003; Qiu *et al.*, 2005). The systematic position of the Austrobaileyales in the phylogenetic tree is crucial for understanding the early evolution of flowering plants (Williams & Friedman, 2002, 2004).

Based on the shape of the torus in pistillate flowers, and its subsequent fruit type, Schisandraceae contains two genera of scandent shrubs and vines, *Kadsura* (22 species) and *Schisandra* (25 species), which are distributed in tropical and warm temperate regions of east and south-eastern Asia (Smith, 1947; Law, 1996; Saunders, 1998, 2000), except for one species, *Schisandra glabra*, found in the south-eastern United States (Smith, 1947) and the Sierra Madre of Mexico (Panero & Aranda, 1998). Recent phylogenetic analysis using nucleic acid data, however, indicates that part of the genus *Schisandra* is nested in *Kadsura* (Hao, Chye & Saunders, 2001; Liu *et al.*, 2006). Members of Schisandraceae produce unisexual flowers (plants dioecious or monoecious),

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Table 1. The occurrence of red and yellow male flowers on individual plants of *Kadsura longipedunculata* in 14 populations in Xinning County

Population	Location	Altitude (m)	Total numbers of individuals	Number of yellow plants	Number of red plants	Ratio of yellow to red plants
1	Yujia Bridge I	300	12	8	4	2
2	Yujia Bridge II	300	4	3	1	3
3	Guanpu Bridge I	300	6	4	2	2
4	Xianlongshi	350	10	8	2	4
5	Lajiao Peak	350	12	8	4	2
6	Guanpu Bridge II	400	4	1	3	0.33
7	Yumidi	400	3	3	0	8
8	Caiyuan	400	8	5	3	1.67
9	Langshan Shitian	400	46	39	7	5.57
10	Xiaojiachong	450	2	0	2	0
11	Naitou Mountain	500	4	1	3	0.33
12	Chashantou	500	7	4	3	1.33
13	Zihua ping	700	15	7	8	0.86
14	Shunhuang Mountain	700	4	3	1	3

with individuals of some species changing sex from year to year (Okada, 1971; Ueda, 1988). The morphology of male flowers (androecium) is highly variable, with several different androecial types, whereas the gynoecial structure is more stable (Smith, 1947; Saunders, 1998).

The reproductive biology of some species of Schisandraceae (mostly cultivated) is discussed by Kozoljanski (1946), Smith (1947), Okada (1971), Ueda (1988), Willemstein (1987) and Saunders (1998, 2000). In a recent study of *S. glabra*, it was shown that the male and female flowers (dioecious plants) are thermogenic, and function as a host site for Diptera and small Coleoptera that pollinate in the process of ovipositing (Liu *et al.*, 2006). On the other hand, *Schisandra henryi* (also dioecious) is not thermogenic and is pollinated by female, pollen-eating *Megommata* sp. (Cecidomyiidae, Diptera), based on visual floral cues in which only male flowers produce food (pollination by deceit; Yuan *et al.*, 2007).

This paper presents a pollination study of *Kadsura longipedunculata* Finet & Gagnepain, a monoecious species of Schisandraceae distributed in eastern and south-central China at 100–1200 m a.s.l., in various habitats including rocky slopes, along streams, and hillsides of forests in Hunan Province, China. As noted by Saunders (1998), this species is common in China and is used medicinally. It is closely related to *Kadsura heteroclita*, and in some areas the species are sympatric, especially in southern and south-western China, and may hybridize (Saunders, 1998). Individual plants of *K. longipedunculata* have male flowers with exclusively red or exclusively yellow androecia, whereas the tepals of both sexes are pale

yellow, and the gynoecium is green (Saunders, 1998). The pollination study focused on the following questions. (1) Are male and female flowers insect- or wind-pollinated (or both)? (2) Which insects visit, and does the pollination system involve a variety of insects? (3) What role does the colour polymorphism play in male flowers? (4) What are the chemical components of the floral fragrance of male and female flowers, as determined by gas chromatography and mass spectrometry? (5) What are the floral rewards for insect visitors to male and female flowers?

MATERIAL AND METHODS

Field studies were conducted in 14 populations of *K. longipedunculata* distributed in Xinning County, Hunan Province, China in 2004 and 2005 (Table 1). The Langshan Shitian population, on an open slope at 400 m a.s.l., is located 15 km north-east of Jingshi (a town). This site was used primarily to record floral temperatures, and to analyse floral odours with gas chromatography–mass spectrometry (GC-MS) and the phenology of flowering.

The plants flower from early May to mid-October, peaking in August. The solitary flowers are borne on slender pedicels in the axils of leaves (young growth; Figs 1A–C, 2A–C). The male flowers are 1.5–2.6 cm in diameter, with about ten yellow tepals (7.5–13-mm long; Figs 1, 2B, C). The androecium contains 36 ± 4 ($N = 40$) stamens tightly aggregated into a subglobose head, and may be yellow or red (Fig. 1A, B). The wide stamen connectives are yellow or red and trapezoidal (Fig. 1D, E). Thecae are lateral and contiguous to those of adjacent stamens. The gynoecium of female

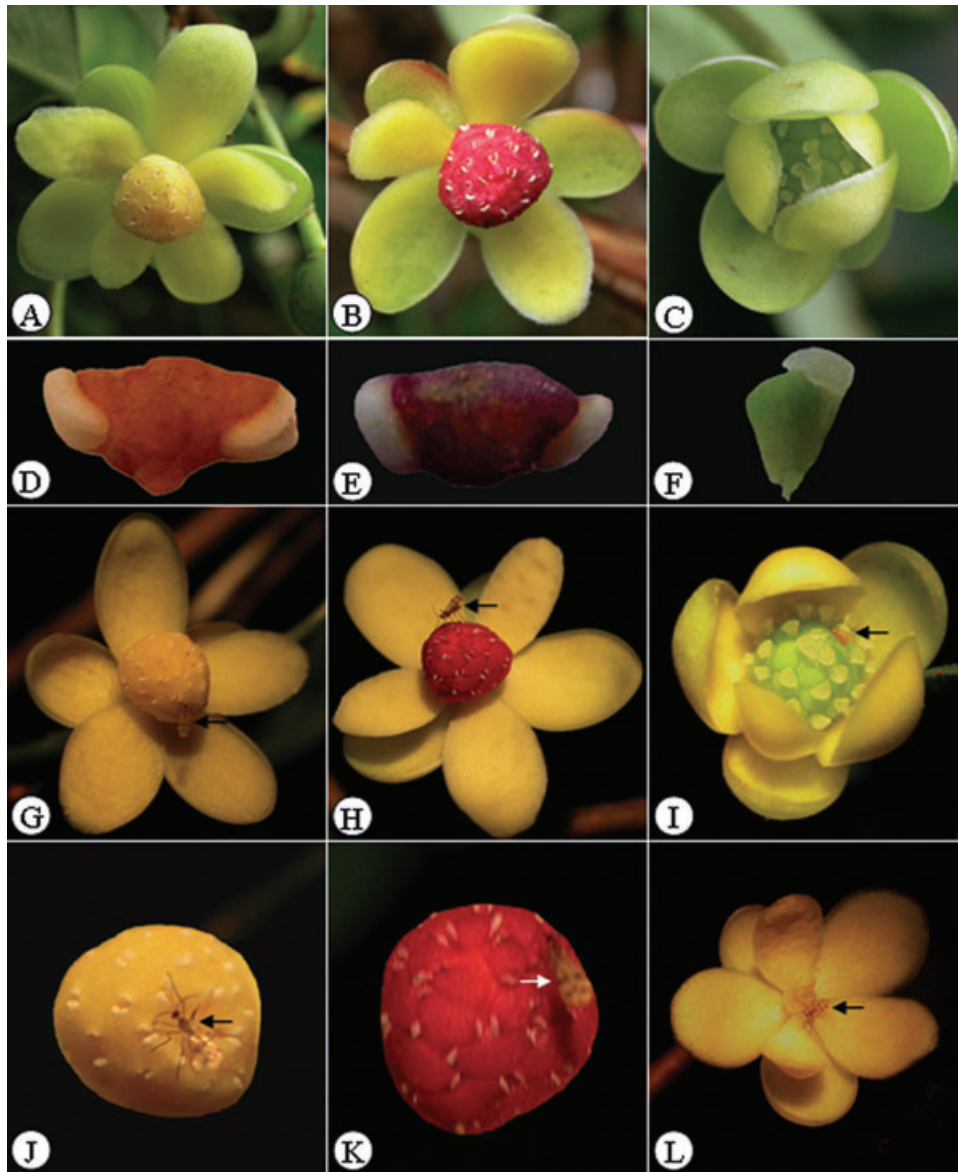


Figure 1. *Kadsura longipedunculata* (A–L). A, an open male flower with a yellow androecium (×3). B, an open male flower with a red androecium (×3). C, female flower (×3). D, a stamen from a yellow male flower (×15). E, a stamen from a red male flower (×15). F, a carpel (×10). G–I, *Megommata* sp. on male and female reproductive structures (×3). J, a *Megommata* sp. on a yellow male androecium (tepals removed) (×3). K, *Megommata* sp. on a red androecium (tepals removed) (×3). L, *Megommata* sp. (centre) on tepals with androecium and torus removed (red flower) (×3).

flowers contains 20–60 apocarpous carpels (Fig. 1C, I). The crested stigmas are dry; no nectar was observed in both male and female flowers. The tepals of male flowers are reflexed when open (Fig. 1A, B); however, in female flowers they form a ‘drum-shaped’ chamber with an orifice at the top (Fig. 1C).

PHENOLOGY

The flowering period of *K. longipedunculata* was recorded using 100 flowers on ten plants randomly

selected in the Langshan Shitian population ($N = 46$ plants). The flowers were observed with a 10× hand lens, and the following traits were recorded: tepal movement, presence or absence of floral odour, time of stigma and stamen secretions (if present), and anther dehiscence. Stigmas were considered to be receptive if they displayed a white, glassy, and moist appearance. The number of flowers opening daily on 15 plants selected at random in the population was recorded throughout the flowering season. The ratio of male to female flowers in Lang-

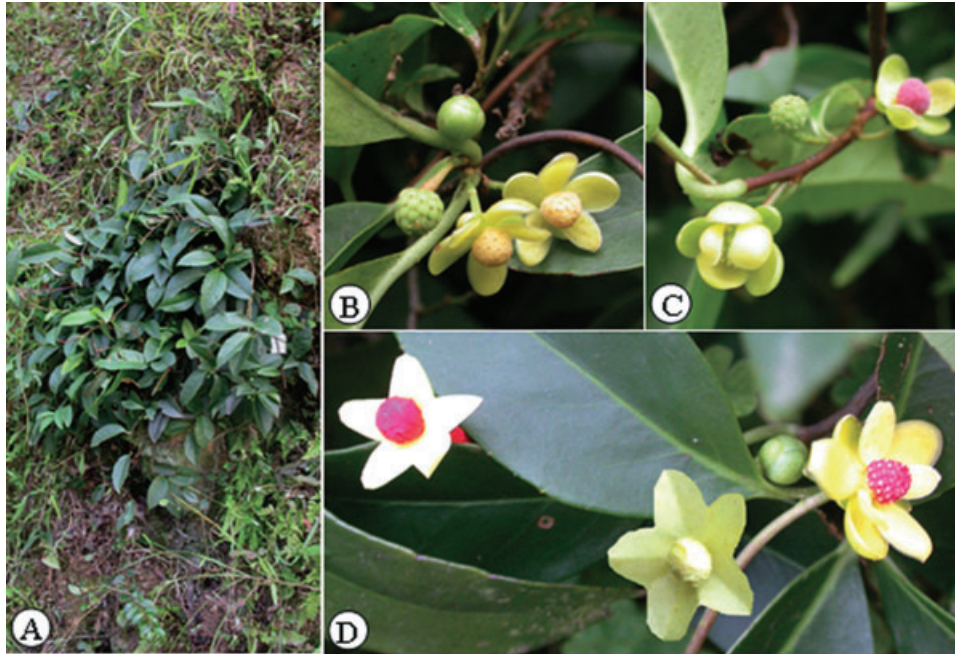


Figure 2. *Kadsura longipedunculata*. A, an individual plant ($\times 0.1$). B, inflorescence of a yellow male plant ($\times 1$). C, inflorescence of a red male plant ($\times 1$). D, two artificial flowers (yellow and red), with one red male flower (on the right) ($\times 1$).

shan Shitian was calculated and compared with two other populations.

Artificial paper flowers with a red or yellow androecium were tied to the plant branches at 22:00 h every night for 4 days (July 15–18 2005) to simulate the colour polymorphism of the male flowers (Fig. 2D). The 100 paper flowers were mixed with natural male flowers, and added on a daily basis to a given plant to match the number of natural male flowers. Observations of artificial and natural male flowers were noted throughout the day and night (40 h). In addition, the tepals of 18 yellow and 11 red opening flowers were removed by hand on July 25 and 26 2005 (Fig. 1J, K). The torus (receptacle) of 12 yellow and nine red male flowers was cut out with scissors (Fig. 1L). These flowers were observed for insect visits for approximately 20 h.

The male and female flowers emit the odour of fresh pineapple (22:00–02:00 h), as detected by smell. Samples of floral odours were taken at 21:00–22:00 h, 23:00–00:30 h, and at 08:00–09:00 h. Individual flowers were enclosed in a polyethylene bag for 1 h prior to sampling. Controls (blanks), polyethylene bags with no flowers, were simultaneously collected at the above times. The fragrance-laden air was pumped through a glass cartridge containing the adsorbent Tenax TA (50 mg) with a battery-operated pump for 1 h at a flow rate of 100 mL min^{-1} . The volatiles were removed from the adsorbent by heating in a CP-4010

TCT thermal device (Chrompack) at $250 \text{ }^\circ\text{C}$ for 10 min, and were then cryofocused in a cold trap at $-100 \text{ }^\circ\text{C}$. The cold trap was then quickly heated to $200 \text{ }^\circ\text{C}$ for 1 min to transfer the volatile compounds into a GC-MS (Trace 2000-Voyager). Identifications of floral volatiles were determined by comparing their mass spectra with those in the NIST computer library, using the Finnigan XCALIBUR data system.

Floral and ambient temperatures were measured using a Teflon-coated contact sensor (1.2 mm in diameter, 0.6 m in length), connected to a portable battery-powered TR-52 Thermo Recorder (T & D Corporation, accurate to $\pm 0.1 \text{ }^\circ\text{C}$). The flower temperature was taken by inserting the sensor between the inner tepals and the base of the torus, and the ambient temperature was measured by placing the sensor in the air, about 1 cm from the flower. Floral and ambient temperatures were recorded every 30 min for the three types of flowers, from flowers opening until 18:00 h the next day. Excess temperature for each flower was calculated as the difference between flower and ambient temperature. Ten flowers from different plants for each type were sampled. Mean excess flower temperature during this period was calculated and graphed.

INSECT POLLINATION

Insects visiting the flowers of *K. longipedunculata* and their behaviour were recorded throughout the

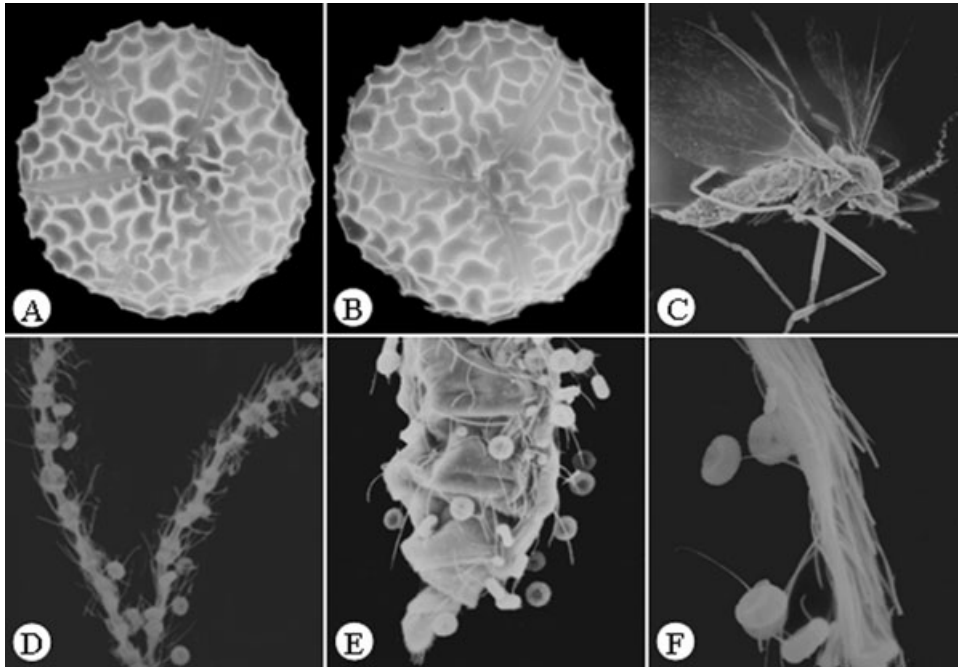


Figure 3. *Kadsura longipedunculata*. A and B, pollen ($\times 2100$). C, *Megommata* sp., captured in a flower of *K. longipedunculata* ($\times 35$). D–F, body parts of *Megommata* sp. covered with pollen of *K. longipedunculata*: antennae (D), abdomen (E), and leg (F) (all $\times 300$).

day and night (100 h of observation) in the Langshan Shitian population. In addition, two plants of each floral type in the population (red or yellow male flowers plus female flowers) were selected for continuous observation of flowers for 2 days. The frequency of insect visits, as well as the length of time they remained in the flower, was recorded. In addition, some insects were collected using jars containing ethyl acetate. Some insects were observed with a Hitachi S-800 scanning electron microscope (SEM), and the pollen grains attached to the insect bodies were compared with pollen collected from the anthers of *K. longipedunculata* for identification (Fig. 3). Vouchers were deposited at China Agricultural University, Beijing.

WIND POLLINATION

To test whether the pollen of *K. longipedunculata* is dispersed by wind, one individual plant was chosen and microscope slides covered with petroleum jelly were placed around this plant at 0.5-m intervals, 1–3 m above the ground, for 20 m. The slides were removed after 2 days, and the pollen grains were counted using a compound light microscope.

HAND POLLINATIONS AND FRUIT SET

Fruit set in plants of *K. longipedunculata* was analysed in the Langshan Shitian population, with refer-

ence to cross- and self-pollinations between the three forms of flowers. The experiments were conducted from July 20 to August 20 2005. Nylon mesh bags were used to cover 140 female flower buds ($N = 11$ plants), 70 male flowers with a red androecium ($N = 5$ plants), and 70 male flowers with a yellow androecium ($N = 6$ plants). Please note that all plants produce male and female flowers; however, a given plant will produce male flowers that are all yellow or all red in regard to their androecium. When the flowers opened, seven types of hand-pollination crosses were conducted: cross-pollinations, red \times yellow, yellow \times yellow, red \times red, yellow \times red; self-pollinations, in red plants and in yellow plants. As a control, open-pollinated female flowers were also marked. Pollen used for cross-pollination was collected from plants 100 m from the recipient experimental plants. Fruits were examined in early November and the number of fruiting carpels per flower was recorded. The average percentage fruit set of the various crosses and the control were calculated.

RESULTS

FLORAL PHENOLOGY

All plants are monoecious, and the red- or yellow-coloured male flowers are randomly distributed in the 14 populations (Table 1). The male flowers open at approximately 21:30 h, 1 h earlier than the female

Table 2. Ratio of male to female flowers on 15 plants of *Kadsura longipedunculata* selected at random in the Langshan Shitian population

Plant	Colour of male flowers	Numbers of male flowers	Numbers of female flowers	Ratio of male to female flowers
1	Yellow	72	2	36
2	Red	30	1	30
3	Red	56	2	28
4	Red	40	2	20
5	Yellow	148	8	18.5
6	Yellow	29	2	14.5
7	Yellow	159	18	8.8
8	Yellow	50	6	8.3
9	Red	198	28	7.1
10	Yellow	57	9	6.3
11	Yellow	41	14	2.9
12	Yellow	32	15	2.1
13	Red	21	12	1.8
14	Yellow	13	16	0.81
15	Yellow	3	21	0.14

flowers. The anthers dehisce via longitudinal slits 30 min after the tepals unfold. The yellow pollen grains accumulate on the edge of the slits, but by 08:00 h the following day they are devoid of pollen, and the flower falls as a unit from the torus in the evening (19:00 h). The anthesis of a male flower averages 22 h ($N = 100$ flowers, $N = 18$ plants). No differences were observed in floral phenology between red and yellow male flowers. The stigmas of female flowers are receptive for an average of 2.6 days ($N = 50$ flowers, $N = 8$ plants). The tepals fall about 2 days after the flower opens.

The ratio of individual plants with either red or yellow male flowers varies in the 14 populations (Table 1). In addition, the ratio of male flowers to female flowers on a given plant is always male biased (Langshan Shitian, Table 2). In the Langshan Shitian, Yujia Bridge, and Zihuaping populations, the ratios varied, but were also always male biased (Fig. 4). During August 2004, in the Langshan Shitian population, the number of open male flowers was always greater than female flowers, and the ratio of daily opening of male to female flowers varied from 3.8 to 11 (Fig. 5). These results suggest that male flowers were always more abundant than female flowers, so as to increase the pollination efficiency of the breeding system based on deceit.

FRAGRANCE

The male and female flowers of *K. longipedunculata* produce a faint fragrance shortly after the flower

opens (at approximately 21:30 h–22:00 h). The intensity of the floral fragrance gradually increases, and is very strong about 1 h after the flower opens, remains strong for about 4 h (until 02:00 h), and then declines, and no odour could be detected at sunrise. The male red- and yellow-colour forms and the female flowers have a similar chemical profile dominated by butanoic acid, methyl ester (methyl butyrate, *c.* 97% of the floral fragrance; Table 3). Trace chemical compounds (0.1%) include acetic acid, pentanoic acid, ethyl benzene, and 1,2-dimethyl benzene, as well as other trace chemicals (less than 0.1%; Table 3). The tepals and the torus strongly emit the fragrance.

FLORAL HEAT PRODUCTION

The closed flowers of *K. longipedunculata* are not thermogenic; however, shortly after opening the flowers begin to produce heat, and reach a peak temperature (2.2 °C above ambient) after 1.5–2 h (Fig. 6). The flower temperature then gradually decreases back to ambient temperature by about 4.5 h after anthesis (Fig. 6). If the ambient temperature exceeds 28 °C during the day, heat production declines below ambient temperature (at 38.9 °C the flower may cool as much as 4.4 °C lower than the ambient temperature; Fig. 6). The male red, yellow, and female flowers exhibit similar temperature patterns (Fig. 6). The female flowers are only thermogenic for 1 day, although the stigmas are receptive for 2.6 days.

INSECT POLLINATION

Insect visitors observed and collected on flowers and vegetative parts of *K. longipedunculata* in the Langshan Shitian population are listed in Table 4. All insects except *Megommata* sp. (Cecidomyiidae, Diptera) visited only male flowers and leaves, but not female flowers. Adult female, pollen-eating *Megommata* sp. are small (1.1 ± 2 mm, $N = 20$), and this unidentified species is the sole pollinator of *K. longipedunculata*. The females visit male and female flowers at night, commencing between 21:30 h and 22:00 h until around 02:00 h. After 03:00 h and during the day, few *Megommata* sp. visit the flowers of *K. longipedunculata* (Fig. 7). Floral visits correlate with peak heat (Fig. 6) and fragrance production (Table 3).

The time that adult female *Megommata* sp. remain on a flower varies from a few seconds to 30 min, and no difference was detected in visitations among the red and yellow male flowers of *K. longipedunculata*. When visiting male flowers, the insects usually land on the tepals and then quickly crawl onto the androecium (Fig. 1G, H), and probe between the stamens and eat pollen. In the process, pollen grains around

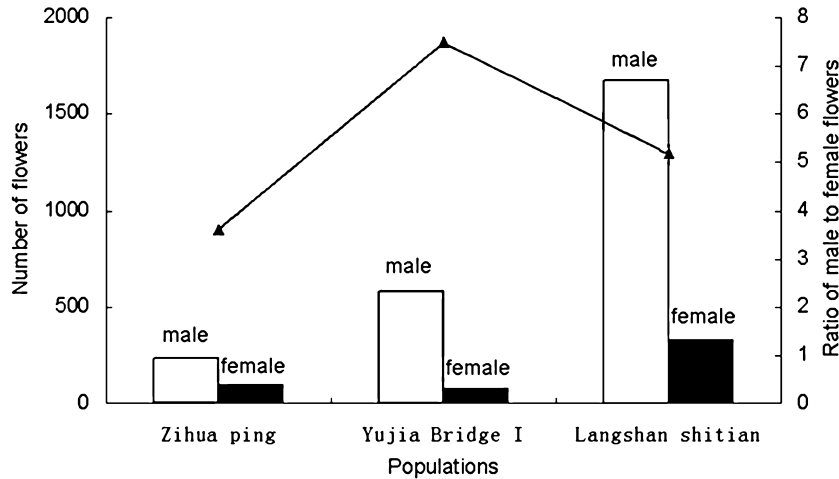


Figure 4. The number of flowers and ratio of male to female flowers in three populations of *Kadsura longipedunculata*. The black line denotes the ratio of male to female flowers.

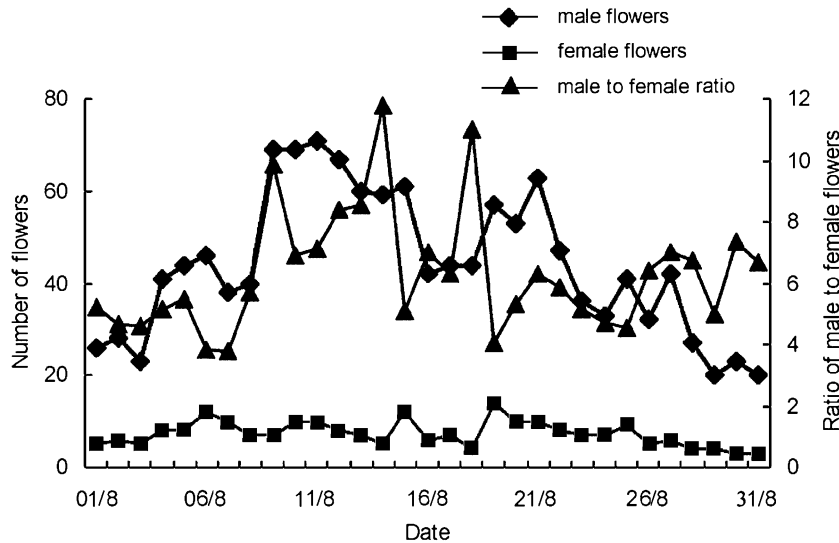


Figure 5. The number of male (◆) and female (■) flowers opening each day, and the ratio of male to female flowers (▲) on 15 individual plants of *Kadsura longipedunculata* in August 2004, in the Langshan Shitian population.

the anther slits adhere to all parts of the insects (Fig. 3C–F). Similar visits to female flowers transferred pollen grains to the stigmas (Fig. 1I).

No adult male *Megommata* sp. were observed or captured on the male or female flowers of *K. longipedunculata*. Adult male and female *Megommata* sp. were found in the populations of *K. longipedunculata*, hanging on abandoned spider webs, and were also observed mating. The males die shortly after mating. It is not known how closely the life cycles of *Megommata* sp. and *K. longipedunculata* are meshed in the mutualistic interaction.

Pollen grains from flowers of *K. longipedunculata* observed with SEM (Fig. 3A, B), and compared with

those attached to the bodies and in the gut of female *Megommata* sp. (Fig. 3C–F), were found to be the same.

No insects visited the artificial flowers (Fig. 2D); however, *Megommata* sp. visited natural flowers with no tepals (Fig. 1J, K), and were active on flowers with tepals but with no torus (Fig. 1L).

WIND POLLINATION

Pollen traps, glass microscope slides (14 cm²) coated with petroleum jelly, captured an average of 1.5 pollen grains of *K. longipedunculata* at a distance of 0.5 m from the source. No pollen grains were captured

Table 3. Chemical composition (%) of the *Kadsura longipedunculata* floral scent

Source	Yellow male flower			Red male flower			Female flower			Control
	21:00–22:00 non-active	23:30–03:00 active	08:00–09:00 non-active	21:00–22:00 non-active	23:30–03:00 active	08:00–09:00 non-active	21:00–22:00 non-active	23:30–03:00 active	08:00–09:00 non-active	
Flower-visitor activity										
Fornic acid	+	–	+	+	–	+	+	–	+	+
Acetic acid	+	< 0.1	+	+	< 0.1	+	+	< 0.1	+	–
Propionic acid	+	+	+	+	+	+	+	+	+	–
Pentanoic acid	–	< 0.1	–	–	0.1	–	–	< 0.1	–	–
Acetic acid, ethyl ester	–	< 0.1	–	–	< 0.1	–	–	0.1	–	–
Butanoic acid, methyl ester	–	96.8	–	–	96.9	–	–	97.1	–	–
Acetic acid, isobutyl ester	+	+	+	+	+	+	+	+	+	+
Hexanal	–	+	+	–	+	+	–	+	+	+
2-Butenal, 3-methyl	+	+	+	+	+	+	+	+	+	+
Pyruvic acid	+	–	+	+	–	+	+	–	–	–
2-pentanone, 3-methyl-	–	–	+	–	–	+	–	–	+	–
Benzene, ethyl	–	< 0.1	+	–	< 0.1	+	–	< 0.1	+	–
Benzene, 1,2-dimethyl-	–	< 0.1	+	–	< 0.1	+	–	< 0.1	+	–
Acetic acid, anhydride with formic acid	+	–	–	+	–	–	+	–	–	+
Ethyl acetate	+	+	–	+	+	–	+	+	–	+
1R- α -Pinene	+	–	–	+	–	–	+	–	–	+
Dodecane	+	+	+	+	+	+	+	+	+	+
6-methyl, octadecane	+	–	–	+	–	–	+	–	–	+

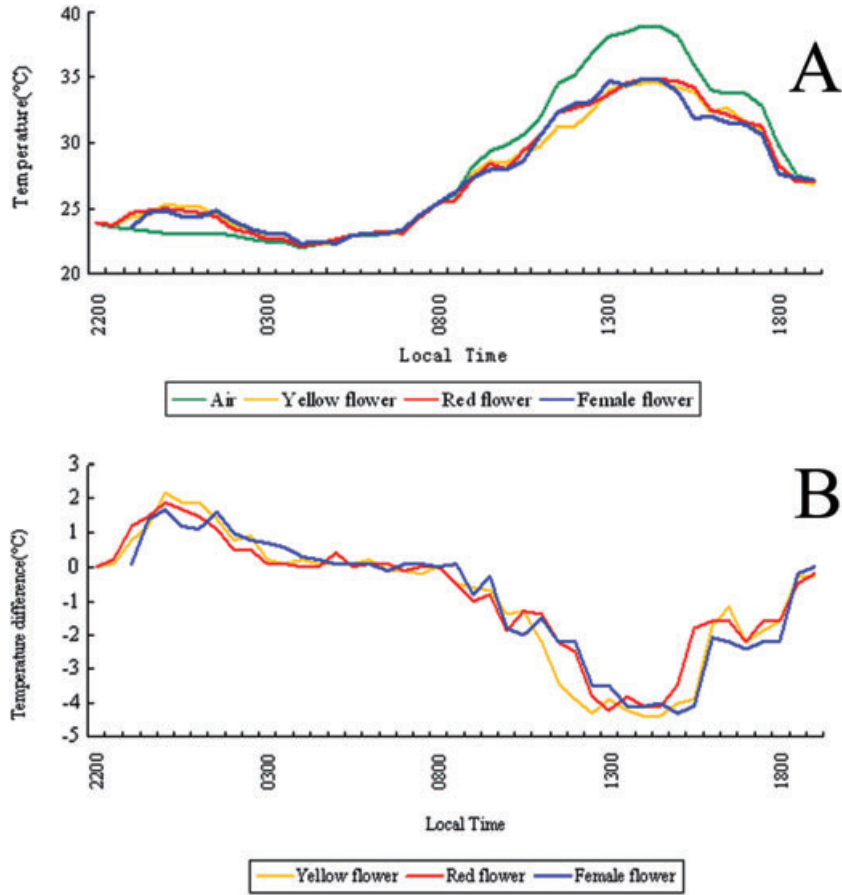


Figure 6. Temperature curves (°C) throughout the first day of male and female flowers of *Kadsura longipedunculata*. A, temperature curves for red male flowers, yellow male flowers, and female flowers. B, temperature difference between flower temperature and the ambient temperature. Note the peak in the temperature difference between 22:00 h.

Table 4. Insect visitors captured or observed on the flowers of *Kadsura longipedunculata* in the Langshan Shitian population (approximately 100 h of observation over 32 days)

Taxon	Mean body length (mm)	Number	Site	Time
Diptera				
Cecidomyiidae <i>Megommata</i> sp.	1.1 ± 0.2 (N = 10)	> 200	R, Y, F, L	N
Syrphidae	12.3 (N = 3)	3	Y	D
Tendipedidae	2.1	1	R	D
Hymenoptera				
Formicidae	2.4 ± 0.1 (N = 3)	5	R, Y, L	D,N
Orthoptera				
Gryllidae	17	1	Y	N
Homoptera				
Cicadellidae	2.4	1	R	D
Collembola	2.8 ± 0.2 (N = 6)	12	Y, R, L	D,N

D, day; F, female flower; L, leaves; N, night; R, red flower; Y, yellow flower.

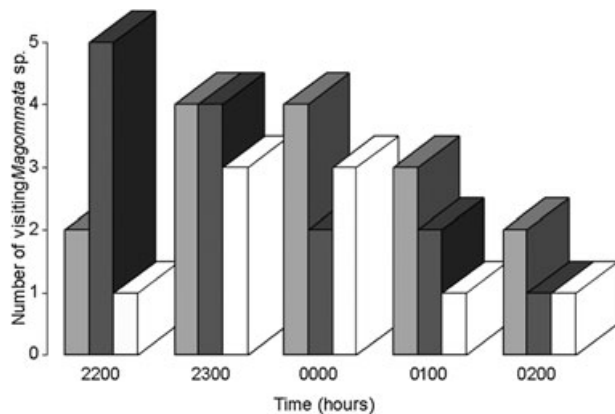


Figure 7. Visits (number and time) of *Megommata* sp. to the red and yellow male flowers (black and grey bars, respectively) and female flowers (white bars) of *Kadsura longipedunculata*.

by microscope slides hung 1 m or more from the source plant. Moreover, the female flowers are pendulous, and the gynoeceum is shaped by tepals that form a 'drum' with a small orifice, and therefore airborne pollen would have difficulty reaching the stigmas. Wind pollination plays no role in the pollination of *K. longipedunculata*.

CROSSING EXPERIMENTS

The gynoeceum of *K. longipedunculata* bears numerous carpels, spirally arranged around a subglobose or ellipsoidal receptacle, with each fruit bearing 20–60 fruitlets (each with one or two seeds). The fruits are red, purple, or sometimes black when ripe, and are dispersed by birds (Saunders, 1998). Female flowers covered with nylon bags set no fruit, indicating pollination is necessary for fruit set (Table 5).

A few self-pollinated flowers fell in a few days, whereas others developed into fruits (Table 5). Average fruit set for plants with yellow or red male flowers were similar (15%, Table 5). Open pollinated flowers in nature varied in fruit set from 8 to 92% (Table 5), and hand cross-pollinated plants averaged 64% (Table 5).

DISCUSSION

POLLINATION INSECTS

The flowers of *K. longipedunculata* are pollinated only by female, pollen-eating *Megommata* sp., primarily in a 4–5-h nocturnal period. Our study shows that male and female flowers simultaneously produce heat and floral odour to attract female *Megommata* sp. Pollen is the only reward and the female flowers use the same attractants as male flowers (floral odour and heat), but offer no food rewards (deceit). Some preda-

ceous adult Diptera require nectar or pollen for additional nutrition for energy and reproduction (Schneider, 1948; Chambers, 1988; Irwin *et al.*, 2003). It is reasonable therefore to conclude that the pollen of *K. longipedunculata* and *S. henryi* benefits female *Megommata* sp. Deceit is also used in the pollination mechanism of *S. glabra*, involving floral heat and odours in which Diptera and small Coleoptera use male and female flowers as sites to oviposit (Liu *et al.*, 2006).

Harris (1967) lists 19 genera of Cecidomyiidae (gall midges) that are associated with Coccoidea (scale insects), including the six described species of *Megommata* (subfamily Cecidomyiinae, Supertribe Cecidomyiidi; Harris, 1967). These *Megommata* are predaceous on Coccoidea, and are distributed in the Philippines, West Africa, Malaysia, and the Seychelles (Harris, 1967). Members of Cecidomyiidae include gall-inducing mycophagous, predaceous, and parasitoid species, which occur worldwide and comprise more than 5000 extant species (Gagné, 2004; Grimaldi & Engel, 2005). Fossils of Cecidomyiidae are found in the Cretaceous (Gagné, 2004; Grimaldi & Engel, 2005).

The Cecidomyiidae are known to visit flowers for nectar, and to lay eggs in flowers of many species (Proctor & Yeo, 1973; Roskam, 1992; Proctor, Yeo & Lack, 1996). Feil (1992) found that gall midges pollinated members of *Siparuna* (Monimiaceae), in which eggs are laid in the flowers. In another example of pollination by Cecidomyiidae, female gall midges oviposit and feed on mycelia of the fungus *Choanephora*, produced on male flowers of the monoecious tree *Artocarpus integer* (Moraceae). The female flowers are not infected with the fungus, and the gall midges are apparently attracted to male and female flowers by floral odour (Sakai, Kato & Nagamasu, 2000).

Diptera first appeared in the Triassic (Rohdendorf, 1974; Downes & Dahlem, 1987), long before the evolution of angiosperms in the early Cretaceous (Doyle & Hickey, 1976; Crane, 1993; Sun *et al.*, 2002; Friis *et al.*, 2003). The lineages of insect pollinators of extant basal angiosperms were established by the Late Jurassic (Rohdendorf, 1974; Crowson, 1981; Labandeira & Sepkoski, 1993; Farrell, 1998; Ren, 1998; Grimaldi, 1999), and many basal angiosperms are pollinated by Diptera (Bernhardt & Thien, 1987; Thien, Azuma & Kawano, 2000; Endress, 2001; Tosaki, Renner & Takahashi, 2001).

Flowers of extant basal angiosperms tend to be bisexual, protogynous, fragrant, thermogenic, and pollinated by a wide variety of insects (Bernhardt & Thien, 1987; Endress, 1990; Labandeira, 1998a, b; Grimaldi, 1999; Bernhardt, 2000; Thien *et al.*, 2000). Thien *et al.* (2003) reported a wind and general insect pollination system based on floral deception in *Ambo-*

Table 5. Percentage fruit set in natural and manipulated crosses of *Kadsura longipedunculata*

Pollination treatments	flowers (<i>n</i>)	Percentage fruit set			
		Minimum	Maximum	Mean	SD
Natural pollination	20	0.08	0.92	0.58	0.19
Yellow (♂) × yellow (♀)	20	0.03	1	0.64	0.33
Red (♂) × yellow (♀)	20	0.12	1	0.63	0.30
Yellow (♂) × red (♀)	20	0.07	0.93	0.63	0.25
Red (♂) × red (♀)	20	0.05	1	0.63	0.28
Yellow self	20	0	0.32	0.14	0.09
Red self	20	0	0.24	0.14	0.06

rella trichopoda (dioecious). Plants (andromonoecious) of *Trimenia moorei* (Trimeniaceae; Austrobaileyales) also exhibit a wind–insect pollination system, with strongly scented flowers and pollen acting as a resource for insect visitors (Bernhardt *et al.*, 2003).

FLOWER COLOUR

In monoecious *K. longipedunculata*, however, no significant differences were found between the colour morphs for pollinator visitation rates under natural conditions. In addition, *Megommata* sp. did not visit yellow or red artificial flowers, although they were attracted to real flowers consisting only of tepals. We conclude that colour may play a role in attracting a few insects in daylight hours; however, most insect visits correlated with nocturnal production of heat and fragrance. The flower colour polymorphism in *K. longipedunculata* may be the product of random genetic drift, or a remnant from a previous pollination syndrome based on diurnal pollinator(s) before switching to a specialized nocturnal system.

FLORAL ODOUR

Floral odour is a primary attractant in a wide variety of insect–flower interactions (Knudsen, Tollsten & Bergström, 1993), and is hypothesized to be an archaic insect-attraction mechanism that preceded colour (van der Pijl, 1960). In *K. longipedunculata*, the dominant compound (96.8%) of floral odours is butanoic acid methyl ester (methyl butyrate, Table 3; Knudsen *et al.*, 1993). This compound has been found in the floral odours of *Gardenia* (Tsuneya *et al.*, 1979), cones of *Picea abies*, *Pinus sylvestris*, and *Larix sibirica* (Gymnosperm, Borg-Karlson *et al.*, 1985), and *Victoria* (Nymphaeaceae: Kite, Reynolds & Prance, 1991).

HEAT PRODUCTION

Floral thermogenesis and emission of floral odours are correlated in *K. longipedunculata*. The tempera-

tures of male and female flowers peak at 2.2 °C above ambient temperature, and if the diurnal ambient temperature exceeds 28 °C the flowers can be cooler than air temperature by up to 4.4 °C. The daily range of flower temperatures is between 22.2 and 34.5 °C, whereas ambient temperatures range from 22 to 38.9 °C. Floral thermogenesis occurs in both clades of Schisandraceae, as found in *S. glabra* (subgenus *Schisandra*, section *Schisandra*) and *K. longipedunculata* (subgenus *Kadsura*, section *Kadsura*) (Liu *et al.*, 2006). In the family floral heat enhances floral odour, and also plays a role in deceit pollination involving floral brood sites for insects (*S. glabra*).

Floral thermogenesis has been reported in only 11 of approximately 377 extant families of flowering plants, as follows (Ervik & Barfod, 1999; Patino, Grace & Bänziger, 2000; Thien *et al.*, 2000; Thien, 2006): Nelumbonaceae and Rafflesiaceae (Eudicots); Araceae, Cyclanthaceae, and Arecaceae (Monocots); Annonaceae, Magnoliaceae, and Aristolochiaceae (Eumagnoliids); Nymphaeaceae (Nymphaeales); Schisandraceae and Illiciaceae (Austrobaileyales). Only two families with thermogenic species are known in the eudicots. The other thermogenic families are found primarily in the ANITA grade, monocots, and eumagnoliids. Thermogenic flowers tend to be large and fleshy, with strong fruity odours, and chambers adapted for pollination by beetles. Silberbauer-Gottsberger *et al.* (2001) concluded that thermogenesis, large flowers, and other characters associated with beetle pollination in Central Amazonian Annonaceae, Araceae, Arecaceae, and Cyclanthaceae, are the results of convergent evolution. Indeed, many scientists associate thermogenic plants with large flowers, strong odours, and beetle pollination.

The flowers of *Schisandra* and *Kadsura*, however, are small (1.5–3 cm in diameter), and the reproductive parts (heat-producing structures) are only 3–8 mm in diameter, and are pollinated primarily by Diptera. In early plant reproductive systems involving small flowers, heat is a resource that could be provided without the evolution of elaborate morpho-

logical features (Thien *et al.*, 2000). The Austrobaileyales is sister to the clade of eumagnoliids, monocots, and eudicots (99.9% of all extant angiosperm species; Williams & Friedman, 2004). The Austrobaileyales is therefore a pivotal group, as members possess not only thermogenic flowers, but also other basal characters including a four-celled embryo sac (female gametophyte) and diploid endosperm (Endress, 2001; Bernhardt *et al.*, 2003; Williams & Friedman, 2004).

Members of the Illiciaceae (Thien, White & Yatsu, 1983; White & Thien, 1985), Schisandraceae (Liu *et al.*, 2006; Yuan *et al.*, 2007), Trimeniaceae (Bernhardt *et al.*, 2003), and Austrobaileyaceae (Endress, 1980) are pollinated primarily by Diptera (Labandeira, 1998b; Endress, 2001). The pollination modes usually involve small bisexual or unisexual flowers, deceit mechanisms, floral odours, floral thermogenesis, and self-incompatibility mechanisms.

Labandeira (2005) hypothesized a 'fungus gnat pollination syndrome' consisting of fungus gnats (Mycetophilidae), gall midges (Cecidomyiidae), and other nematoceran dipterans. In this syndrome, small sized (lightly built) dipterans, with similar modestly protrusible labellate mouthparts, are nectarivores or pollinivores of small, radially symmetrical, odourless ANITA grade flowers (Labandeira, 2005). The appearance of this pollination syndrome occurred early in angiosperm evolution in mid-late Early Cretaceous (Labandeira, 2005). The pollination syndrome should be modified to indicate that many species in the Austrobaileyales and Nymphaeales produce floral odours and heat (Thien *et al.*, 1983; Bernhardt *et al.*, 2003; Liu *et al.*, 2006).

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REFERENCES

- Barkman TJ, Chenery G, McNeal JR, Lyons-Weiler J, Ellisens WJ, Moore G, Wolfe AD, dePamphilis CW. 2000. Independent and combined analyses of sequences from all three genomic compartments converge on the root of flowering plant phylogeny. *Proceedings of the National Academy of Sciences of the USA* **97**: 13166–13171.
- Bernhardt P. 2000. Convergent evolution and adaptive radiation of beetle-pollinated angiosperms. *Plant Systematics and Evolution* **222**: 293–320.
- Bernhardt P, Thien LB. 1987. Self-isolation and insect pollination in the primitive angiosperms: new evaluations of older hypotheses. *Plant Systematics and Evolution* **156**: 159–176.
- Bernhardt P, Sage T, Weston P, Azuma H, Lam M, Thien LB, Bruhl J. 2003. The pollination of *Trimenia moorei* (Trimeniaceae): Floral volatiles, insect/wind pollen vectors and stigmatic self-incompatibility in a basal angiosperm. *Annals of Botany* **92**: 445–458.
- Borg-Karlson AK, Eidmann HH, Lindstrom M, Norin T, Wierma N. 1985. Odoriferous compounds from the flowers of the conifers *Picea abies*, *Pinus sylvestris*, and *Larix sibirica*. *Phytochemistry* **24**: 455–456.
- Borsch T, Hilu KW, Quandt D, Wilde V, Neinhuis C, Barthlott W. 2003. Noncoding plastid trnT-trnF sequences reveal a well resolved phylogeny of basal angiosperms. *Journal of Evolutionary Biology* **16**: 558–576.
- Chambers RJ. 1988. Syrphidae. In: Minks AK, Harrewijn P, eds. *World crop pests: aphids, their biology, natural enemies and control 2B*. Amsterdam, Netherlands: Elsevier, 259–267.
- Crane PR. 1993. Time for the angiosperms. *Nature* **366**: 631–632.
- Crowson RA. 1981. *The biology of the coleoptera*. New York: Academic Press.
- Downes WL Jr, Dahlem GA. 1987. Keys to the evolution of Diptera: role of Homoptera. *Environmental Entomology* **16**: 847–854.
- Doyle JA, Hickey LJ. 1976. Pollen and leaves from the mid-Cretaceous Potomac Group and their bearing on early angiosperm evolution. In: Beck CB, ed. *Origin and early evolution of Angiosperms*. New York: Columbia University Press, 139–206.
- Endress PK. 1980. The reproductive structures and systematic position of the Austrobaileyaceae. *Botanische Jahrbücher für Systematik* **101**: 393–433.
- Endress PK. 1990. Evolution of reproductive structures and functions in primitive angiosperms (Magnoliidae). *Memoirs of the New York Botanical Garden* **55**: 5–34.
- Endress PK. 2001. The flowers in extant basal angiosperms and inferences on ancestral flowers. *International Journal of Plant Sciences* **162**: 105–115.
- Ervik F, Barfod A. 1999. Thermogenesis in palm inflorescences and its ecological significance. *Acta Botánica Venezuelica* **22**: 195–212.
- Farrell BD. 1998. 'Inordinate fondness' explained: why are there so many beetles? *Science* **281**: 555–559.
- Feil JP. 1992. Reproductive ecology of dioecious Siparuna (Monimiaceae) in Ecuador – A case of gall midge pollination. *Botanical Journal of the Linnean Society* **110**: 171–203.
- Friis EM, Doyle JA, Endress PK, Leng Q. 2003. Archaeofructus – angiosperm precursor or specialized early angiosperm? *Trends in Plant Science* **8**: 369–373.
- Gagné RJ. 2004. A Catalog of the Cecidomyiidae (Diptera) of the World. *Memoirs of the Entomological Society of Washington* **25**: 5–350.
- Graham SW, Olmstead RG. 2000. Utility of 17 chloroplast genes for inferring the phylogeny of the basal angiosperms. *American Journal of Botany* **7**: 1712–1730.

- Grimaldi D.** 1999. The co-radiations of pollinating insects and angiosperms in the Cretaceous. *Annals of the Missouri Botanical Garden* **86**: 373–406.
- Grimaldi D, Engel MS.** 2005. *Evolution of the insects*. New York: Cambridge University Press.
- Hao G, Chye M-L, Saunders RMK.** 2001. A phylogenetic analysis of the Schisandraceae based on morphology and nuclear ribosomal ITS sequences. *Botanical Journal of the Linnean Society* **135**: 401–411.
- Harris KM.** 1967. A systematic revision and biological review of the cecidomyiid predators (Diptera: Cecidomyiidae) on world Coccoidea (Hemiptera: Homoptera). *Transactions of the Entomological Society of London* **119**: 401–494.
- Irwin RE, Strauss SY, Emerson A, Guibert G.** 2003. The role of herbivores in the maintenance of a flower-color polymorphism in wild radish. *Ecology* **84**: 1733–1743.
- Kite G, Reynolds T, Prance GT.** 1991. Potential pollinator-attracting chemicals from *Victoria* (Nymphaeaceae). *Biochemical Systematics and Ecology* **19**: 535–540.
- Knudsen JT, Tollsten L, Bergström G.** 1993. Floral scents—a checklist of volatile compounds isolated by head-space techniques. *Phytochemistry* **332**: 253–280.
- Kozo-Poljanski BM.** 1946. The floral mechanism of *Woo-we-dzy Schizandra chinensis* (Turcz.) Baill. *Comptes Rendus (Doklady) de l'Academie Des Sciences de l'URSS* **53**: 749–751.
- Labandeira CC.** 1998a. Early history of arthropod and vascular plant associations. *Annual Review of Ecology and Planetary Sciences* **26**: 329–377.
- Labandeira CC.** 1998b. How old is the flower and the fly? *Science* **280**: 57–59.
- Labandeira CC.** 2005. Fossil history and evolutionary ecology of Diptera and their associations with plants. In: Yeates DK, Wiegmann BM, eds. *The evolutionary biology of flies*. New York: Columbia University Press, 217–272.
- Labandeira CC, Sepkoski JJ Jr.** 1993. Insect diversity in the fossil record. *Science* **261**: 310–315.
- Law YW.** 1996. Magnoliaceae Trib. Schisandreae DC. In: Law YW, ed. *Flora reipublicae popularis sinicae*, 30 (1). Beijing: Science Press, 232–269. (in Chinese.)
- Liu Z, Hao G, Luo YB, Thien LB, Rosso SW, Lu AM, Chen ZD.** 2006. Phylogeny and androecial evolution in Schisandraceae, inferred from sequences of nuclear ribosomal DNA ITS and chloroplast DNA TRNL-F regions. *International Journal of Plant Sciences* **167**: 539–550.
- Okada H.** 1971. On the monoecism of *Kadsura japonica* (Thunb.) Dunal. *Journal of Japanese Botany* **46**: 29–33.
- Panero JL, Aranda PD.** 1998. The family Schisandraceae: a new record for the flora of Mexico. *Brittonia* **50**: 87–90.
- Patino S, Grace J, Bänziger H.** 2000. Endothermy by flowers of *Rhizanthus lowii* (Rafflesiaceae). *Oecologia* **124**: 149–155.
- van der Pijl L.** 1960. Ecological aspects of flower evolution. II. Zoophilous flower classes. *Evolution* **15**: 44–59.
- Proctor M, Yeo PF.** 1973. *The pollination of flowers*. London, UK: Collins.
- Proctor M, Yeo P, Lack A.** 1996. *The natural history of pollination*. London, UK: Harper Collins.
- Qiu YL, Lee J, Bernasconi-Quadroni R, Soltis DR, Soltis PS, Zanis M, Zimmer EA, Chen Z, Savolainen V, Chase MW.** 1999. The earliest angiosperms: evidence from mitochondrial, plastid, and nuclear genomes. *Nature* **402**: 404–407.
- Qiu YL, Lee J, Bernasconi-Quadroni R, Soltis DR, Soltis PS, Zanis M, Zimmer EA, Chen Z, Savolainen V, Chase MW.** 2000. Phylogeny of basal angiosperms: analyses of five genes from three genomes. *International Journal of Plant Sciences* **161**: S3–S27.
- Qiu YL, Dombrowska O, Lee J, Li L, Whitlock BA, Bernasconi-Quadroni F, Rest JS, Davis CC, Borsch T, Hilu KW.** 2005. Phylogenetic analyses of basal angiosperms based on nine plastid, mitochondrial, and nuclear genes. *International Journal of Plant Sciences* **166**: 815–842.
- Ren D.** 1998. Flower-associated brachycera flies as fossil evidence for Jurassic angiosperm origins. *Science* **280**: 85–88.
- Renner SS.** 1999. Circumscription and phylogeny of Laurales: evidence from molecular and morphological data. *American Journal of Botany* **86**: 1301–1315.
- Rohdendorf B.** 1974. *The historical development of diptera*. Edmonton: University of Alberta Press.
- Roskam JC.** 1992. Evolution of the gall-inducing guild. In: Shorthouse JD, Rohfritsch O, eds. *Biology of insect-induced galls*. New York: Oxford University Press, 34–49.
- Sakai S, Kato M, Nagamasu H.** 2000. *Artocarpus* (Moraceae) -gall midge pollination mutualism mediated by male-flower parasitic fungus. *American Journal of Botany* **87**: 440–445.
- Saunders RMK.** 1998. Monograph of *Kadsura* (Schisandraceae). *Systematic Botany Monographs* **54**: 1–106.
- Saunders RMK.** 2000. Monograph of *Schizandra* (Schisandraceae). *Systematic Botany Monographs* **58**: 1–146.
- Schneider F.** 1948. Beitrag zur kenntnis der generationsverhältnisse und diapause räuberischer schwebfliegen. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* **21**: 249–285.
- Silberbauer-Gottsberger I, Webber AC, Kuchmeister H, Gottsberger G.** 2001. Convergence in beetle-pollinated Central Amazonian Annonaceae, Araceae, Arecaceae, and Cyclanthaceae. In: Gottsberger G, Liede S, eds. *Life forms and dynamics in tropical forests dissertationes botanicae*, vol. 346. Berlin-Stuttgart, Germany: J. Cramer in der Gebrüder Borntraeger Verlagsbuchhandlung, 165–183.
- Smith AC.** 1947. The families Illiciaceae and Schisandraceae. *Sargentia* **7**: 1–224.
- Soltis PS, Soltis DE, Zanis MJ, Kim S.** 2000. Basal lineages of angiosperms: relationships and implications for floral evolution. *International Journal of Plant Sciences* **161**: S97–S107.
- Sun G, Ji Q, Dilcher DL, Zheng S, Nixon KC, Wang X.** 2002. Archaeofractaceae, a new basal angiosperm family. *Science* **296**: 899–904.
- Thien LB.** 2006. Heat at the base of the tree. 17th International Symposium of the German Botanical Society, Biodiversity and Evolutionary Biology, p. 24. Bonn, Germany.
- Thien LB, White DA, Yatsu LY.** 1983. The reproductive

- biology of a relict – *Illicium floridanum* Ellis. *American Journal of Botany* **70**: 719–727.
- Thien LB, Azuma H, Kawano S. 2000.** New perspectives on the pollination biology of basal angiosperms. *International Journal of Plant Sciences* **161**: S225–S235.
- Thien LB, Sage TL, Jaffre T, Bernhardt P, Pontieri V, Weston PH, Malloch D, Azuma H, Graham SW, McPherson MA, Rai HS, Sage RF, Dupre JL. 2003.** The population structure and floral biology of *Amborella trichopoda* (Amborellaceae). *Annals of the Missouri Botanical Garden* **90**: 466–490.
- Tosaki Y, Renner SS, Takahashi H. 2001.** Pollination of *Sarcandra glabra* (Chloranthaceae) in natural populations in Japan. *Journal of Plant Research* **114**: 423–427.
- Tsuneya T, Ikeda N, Shiga M, Ichikawa N. 1979.** GC-MS analysis of *Gardenia* flower volatiles. In: *Proceedings of the VII international congress of essential oils*. Kyoto, 454–457.
- Ueda K. 1988.** Sex change in a woody vine species, *Schisandra chinensis*, a preliminary note. *Journal of Japanese Botany* **63**: 319–321.
- White DA, Thien LB. 1985.** The pollination of *Illicium parviflorum* (Illiciaceae). *Journal of the Elisha Mitchell Scientific Society* **101**: 15–18.
- Willemstein SC. 1987.** *An evolutionary basis for pollination ecology*. Leiden: Brill/Leiden University Press.
- Williams JH, Friedman WE. 2002.** Identification of diploid endosperm in an early angiosperm lineage. *Nature* **415**: 522–526.
- Williams JH, Friedman WE. 2004.** The four-celled female gametophyte of *Illicium* (Illiciaceae; Austrobaileyales): implications for understanding the origin and early evolution of monocots, eumagnoliids, and eudicots. *American Journal of Botany* **91**: 332–351.
- Yuan LC, Luo YB, Thien LB, Fan JH, Xu HL, Chen ZD. 2007.** Pollination of *Schisandra henryi* (Schisandraceae) by Female, Pollen-eating *Megommata* Species (Cecidomyiidae, Diptera) in South-central China. *Annals of Botany* **99**: 451–460.
- Zanis MJ, Soltis DE, Soltis PS, Mathews S, Donoghue MJ. 2002.** The root of angiosperms revisited. *Proceedings of the National Academy of Sciences of the USA* **99**: 6848–6853.