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),
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 Kozopoljanski (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 heteroclitra*, and in some areas the species are sympatric, especially in southern and southwestern 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 $36 \pm 4 (N = 40)$ 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

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

Table 1. The occurrence of red and yellow male flowers on individual plants of *Kadsura longipedunculata* in 14 populations in Xinning County.
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-
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 °C for 10 min, and were then cryofocused in a cold trap at −100 °C. The cold trap was then quickly heated to 200 °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 ±0.1 °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
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 reference 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 × yellow, yellow × yellow, red × red, yellow × 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 monoeccious, 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

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

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 Megomamma 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
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

<table>
<thead>
<tr>
<th>Source</th>
<th>Yellow male flower</th>
<th>Red male flower</th>
<th>Female flower</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling time</td>
<td>21:00–22:00 non-active</td>
<td>23:30–03:00 active</td>
<td>08:00–09:00 non-active</td>
<td>21:00–22:00 non-active</td>
</tr>
<tr>
<td>Flower–visitor activity</td>
<td>21:00–22:00 non-active</td>
<td>23:30–03:00 active</td>
<td>08:00–09:00 non-active</td>
<td>21:00–22:00 non-active</td>
</tr>
</tbody>
</table>

- **Fomic acid**: +
- **Acetic acid**: +< 0.1 + < 0.1 +< 0.1 +< 0.1 +
- **Propionic acid**: + + + + + +
- **Pentanoic acid**: –< 0.1 – 0.1 –< 0.1 – 0.1 –
- **Acetic acid, ethyl ester**: –< 0.1 – < 0.1 – < 0.1 – < 0.1 –
- **Butanoic acid, methyl ester**: – 96.8 – 98.9 – 97.1 –
- **Acetic acid, isobutyl ester**: + + + + + + + +
- **Hexanal**: – + + – + + – + + +
- **2-Butenal, 3-methyl**: + + + + + + + + +
- **Pyrusic acid**: + – + – – – – – –
- **2-pentanone, 3-methyl**: – – – – – – – – –
- **Benzen, ethyl**: –< 0.1 + –< 0.1 + –< 0.1 + –< 0.1 +
- **Acetic acid, anhydride**: + – + – + – + + +
- **with fomic 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)

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

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

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by microscope slides hung 1 m or more from the source plant. Moreover, the female flowers are pendulous, and the gynoecium 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 gynoecium 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-

cious 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; 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-
rellia 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-Karlsen 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-

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

<table>
<thead>
<tr>
<th>Pollination treatments</th>
<th>flowers (n)</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural pollination</td>
<td>20</td>
<td>0.08</td>
<td>0.92</td>
<td>0.58</td>
<td>0.19</td>
</tr>
<tr>
<td>Yellow (♂) × yellow (♀)</td>
<td>20</td>
<td>0.03</td>
<td>1</td>
<td>0.64</td>
<td>0.33</td>
</tr>
<tr>
<td>Red (♂) × yellow (♀)</td>
<td>20</td>
<td>0.12</td>
<td>1</td>
<td>0.63</td>
<td>0.30</td>
</tr>
<tr>
<td>Yellow (♂) × red (♀)</td>
<td>20</td>
<td>0.07</td>
<td>0.93</td>
<td>0.63</td>
<td>0.25</td>
</tr>
<tr>
<td>Red (♂) × red (♀)</td>
<td>20</td>
<td>0.05</td>
<td>1</td>
<td>0.63</td>
<td>0.28</td>
</tr>
<tr>
<td>Yellow self</td>
<td>20</td>
<td>0</td>
<td>0.32</td>
<td>0.14</td>
<td>0.09</td>
</tr>
<tr>
<td>Red self</td>
<td>20</td>
<td>0</td>
<td>0.24</td>
<td>0.14</td>
<td>0.06</td>
</tr>
</tbody>
</table>
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; Williams & Friedman, 2004). Members of the Illiciaceae (Thien, White & Yats, 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 protrusable 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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