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POLLINATION ECOLOGY AND BREEDING SYSTEM OF *XYLOPIA CHAMPIONII* (ANNONACEAE): CURCULIONID BEETLE POLLINATION, PROMOTED BY FLORAL SCENTS AND ELEVATED FLORAL TEMPERATURES

R. M. C. S. Ratnayake,* I. A. U. N. Gunatilleke,† D. S. A. Wijesundara,‡ and R. M. K. Saunders[1,]*

*Division of Ecology and Biodiversity, School of Biological Sciences, University of Hong Kong, Pokfulam Road, Hong Kong, China; †Department of Botany, University of Peradeniya, Peradeniya, Sri Lanka; and ‡Royal Botanic Gardens, Peradeniya, Sri Lanka

Data on the reproductive biology of the Annonaceae are rather fragmentary, particularly for paleotropical species. The pollination ecology and breeding system of the Sri Lankan endemic *Xylopia championii* (Annonaceae) are described in detail. The pollination ecology was investigated using a diverse range of approaches, including (1) observations of flower-level and population-level phenology, (2) assessments of floral visitors and effective pollinators, (3) monitoring of floral temperature *in situ* using a digital data logger, and (4) analysis of scent chemistry using solid-phase microextraction sampling and gas chromatography–mass spectrometry identification of volatiles. The breeding system was evaluated using pollen/ovule ratios and field-based controlled-pollination experiments. Intrafloral dichogamy (protogyny) occurs over a 2-d period, with a reproductively inactive phase between the pistillate and staminate phases, although there is no evidence of interfloral dichogamy. The inner petals close to form a pollination chamber during the reproductively active phases. The flowers are pollinated by a species of *Endaeus* weevil (Coleoptera: Curculionidae). Floral chamber temperatures are elevated to 8°C above ambient levels. The floral scent contains a combination of volatiles that have previously been observed in fruits and other flowers and that possibly mimic insect pheromones. *Xylopia championii* has an essentially xenogamous breeding system, promoted by protogyny. Although *X. championii* possesses numerous clear adaptations for cantharophily, there is no evidence for a species-specific interaction. The beetles are attracted to the flowers by strong scents; rewards offered to the beetles include heat energy and protection from predators. Low levels of fruit set in natural conditions suggest that pollinator availability may be a limiting factor.

Keywords: Annonaceae, breeding system, Coleoptera, Curculionidae, *Endaeus*, floral scent, floral temperature, pollination, Sri Lanka, *Xylopia championii*.

Introduction

Despite the accumulation of empirical data that suggests that the majority of angiosperm pollination systems are diversified and opportunistic (e.g., Waser et al. 1996), there is convincing evidence that the family Annonaceae possesses specialized pollination systems. Most Annonaceae species are beetle pollinated, with distinct small- and large-beetle pollination systems (e.g., Gottsberger 1999; Silberbauer-Gottsberger et al. 2003; Saunders, forthcoming), although a diverse array of other insect groups are reported to act as pollinators, including thrips (*Bocageopsis*, *Duguetia*, *Oxandra*, *Popowia*, and *Xylopia*: Gottsberger 1970; Webber and Gottsberger 1995; Küchmeister et al. 1998; Momose et al. 1998*a*, 1998*b*; Silberbauer-Gottsberger et al. 2003), flies (*Asimina*, *Monodora*, and *Pseuduvaria*: Gottsberger 1985; Norman et al. 1992; Su et al. 2005; Su and Saunders 2006), cockroaches (*Uvaria*: Nagamitsu and Inoue 1997), and bees (*Sapranthus*, *Unonopsis*, and *Uvaria*: Olesen 1992; Carvalho and Webber 2000; Silberbauer-Gottsberger et al. 2003). In general, however, relatively few Annonaceae species are restricted to a single species of pollinator.

Available data on the pollination ecology and breeding systems of the Annonaceae are rather fragmentary, particularly for paleotropical species. *Xylopia* is one of the largest genera in the family, with up to 160 species (Keßler 1993), and is the only pantropical genus in the family. Published data are only available for seven species, namely *Xylopia amazonica* (Webber 2002; Silberbauer-Gottsberger et al. 2003), *Xylopia aromatica* (Gottsberger 1970; Jürgens et al. 2000; Silberbauer-Gottsberger et al. 2003), *Xylopia benthamii* (Jürgens et al. 2000; Webber 2002), *Xylopia bocatorena* (Kress and Beach 1994), *Xylopia brasiliensis* (Andrade et al. 1996), *Xylopia crinita* (Küchmeister et al. 1998), and *Xylopia excellens* (Küchmeister et al. 1998; Silberbauer-Gottsberger et al. 2003). All these species are pollinated by small beetles, with the exception of *X. amazonica*, which is pollinated by thrips, and *X. aromatica*, which is pollinated by a combination of thrips (ca. 80% of visits) and small beetles (ca. 20%).

The reproductive biology of the Sri Lankan endemic *Xylopia championii* is described here in detail. This is the first such study for a paleotropical species of *Xylopia*. The flowers are typical of other species in the genus, with a whorl of three small sepals and two whorls of fleshy, yellowish-cream

[1] Author for correspondence; e-mail saunders@hkucc.hku.hk.

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petals (fig. 1*A*). During the reproductively active phases, the inner petals become compressed against each other, forming an enclosed pollination chamber over the reproductive organs (fig. 1*A*). The flowers are hermaphroditic, with numerous densely packed, spirally arranged stamens around an extended receptacle (fig. 1*B*). Each stamen has a tongue-shaped connective extension. A solitary carpel is located within a cavity formed by the extended receptacle so that the ovary is protected, and only the stigma is exposed above the top of the stamens.

The pollination ecology of *X. championii* is investigated in this article using a diverse range of approaches, including observations of flower-level and population-level phenology, floral temperature, scent chemistry, and assessments of floral visitors and effective pollinators. Corresponding data on the breeding system are also presented, using pollen/ovule ratios and field-based controlled-pollination experiments.

Material and Methods

Study Site

Field observations were conducted in the Sinharaja World Heritage Site in southwest Sri Lanka (fig. 2). Sinharaja Forest Reserve (06°21′–26′N, 80°21′–34′E) is located in southern Ratnapura District and northern Galle District and hence lies within the lowland wet vegetation zone. Sinharaja covers 11,187 ha, of which 8860 ha is designated as part of the reserve and is therefore comparatively undisturbed (Gunatilleke and Gunatilleke 1981). The mean annual temperature recorded for the area is 25°–27°C, and the mean annual rainfall is 4000–6000 mm (Ashton et al. 1995); the rainfall is largely derived from the southwest monsoon during May to July and the northeast monsoon during November to February. The vegetation is accordingly lowland and submontane tropical wet evergreen forest (Davis et al. 1995).

Fifty trees of *Xylopia championii* were permanently labeled with metal tags and used in all phenological and experimental studies. The tagged trees were 4–15 m ($x = 8.5$ m) in height and 27.5–84.5 cm ($x = 48.5$ cm) diameter at breast height (dbh). The size of reproductively mature individuals necessitated the construction of wooden platforms, which inevitably limited the extent of observations of pollinator activities and the number of individuals used in the controlled-pollination experiments. Voucher specimens (R. M. C. S. Ratnayake 03/03) have been deposited in HKU and PDA herbaria.

Floral Morphology and Phenology

Population-level phenological studies were conducted weekly over a 3-yr period (March 2002–March 2005) to determine the timing of the beginning and end of flowering, the peak flowering times, and whether anthesis is synchronized within and/or between individuals. Flower-level phenological changes were monitored by tagging 10 unopened flowers on five individuals of each species. Observations of floral morphology and sexual functioning were made daily before the onset of stigmatic receptivity and subsequently every hour. Changes in the following morphological characters were assessed: length and width of petals, relative position and shape of petals, color of petals and stamens, and wilting and abscission of floral organs.

The timing of the onset of stigmatic receptivity and its duration were also assessed. Receptivity was initially determined by immersing stigmas in 3% hydrogen peroxide (H_2O_2) solution and checking for bubble formation (Dafni 1992); bubbles form as a result of the activity of peroxidase enzymes and are

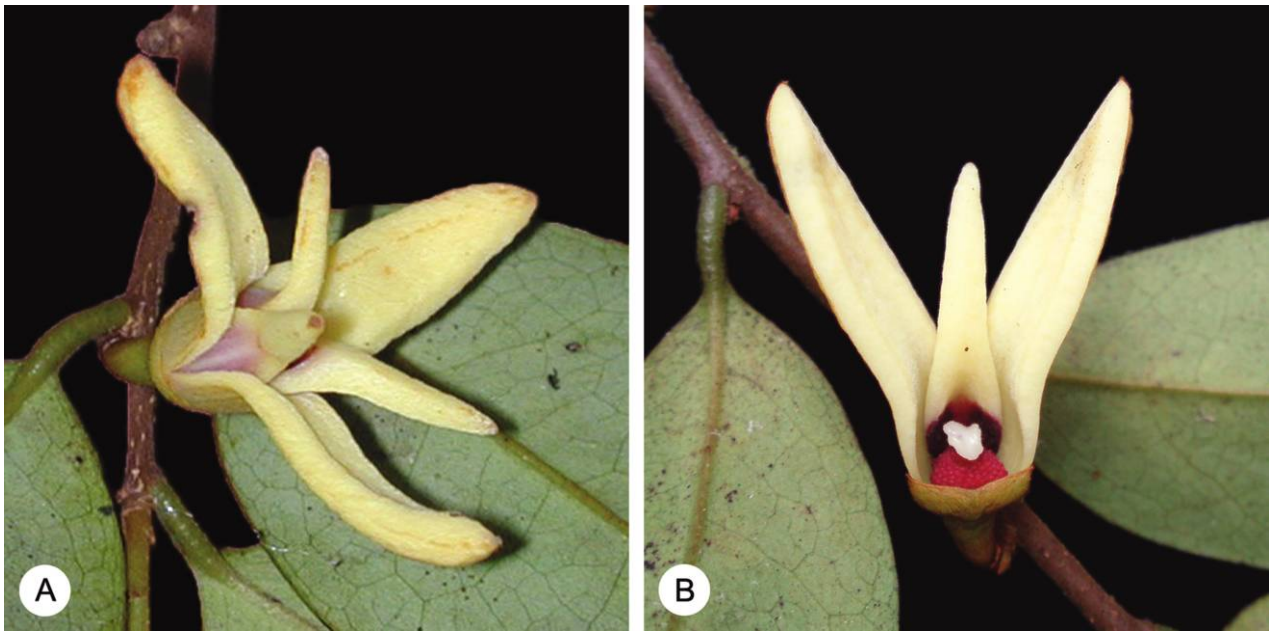

Fig. 1 Mature flowers of *Xylopia championii*. *A*, Entire flower, showing connivent inner petals. *B*, Dissected flower, with one outer petal and two inner petals removed to show numerous stamens (red) surrounding stigma (white); note also deep red pigmentation at base of inner petal.

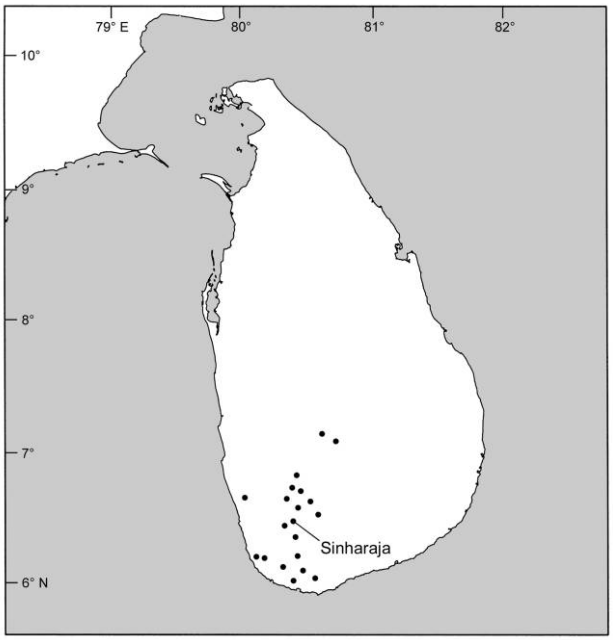

Fig. 2 Location of Sinharaja Forest and distribution of *Xylopia championii* in Sri Lanka (data abstracted from Huber 1985).

indicative of receptivity (Galen and Plowright 1987). Stigmatic receptivity was found to coincide with the appearance of a glistening stigmatic exudate, enabling a simple visual determination of receptivity. The onset of the staminate phase was readily apparent due to the mass release of pollen and color changes of the staminal connectives.

Floral Visitors and Pollinators

Extensive observations of the activities of floral visitors were undertaken during daylight hours (0600–1800 hours), supplemented with periodic nighttime observations to ensure that cumulative observations covered the entire anthesis period, from the onset of stigmatic receptivity to the end of the staminate phase. Hourly observations were based on a total of 15 flowers from five individuals; this data set was supplemented by numerous additional observations, recorded while monitoring flower-level phenology and floral temperature, and during the controlled-pollination experiments. The number and types of floral visitors were recorded by careful observation of the floral chamber without disturbance of visitors, with samples collected for subsequent identification. The arrival times and activities of the floral visitors were observed whenever possible.

Floral Temperature

Temperatures within the floral chambers (Seymour et al. 2003) were recorded using a digital data logger (DataTaker DT 800, DataTaker, Rowville, Australia), with type K thermocouples (welded tip, glass fiber insulated, 0.6 mm diameter, temperature resolution ± 0.0075°C). Using 15 flowers, measurements were made at 10-min intervals from immediately before the onset of the pistillate phase until petal abscission. Ambient air temperature data were collected simultaneously at 10-min intervals, using a Vaisala 50Y temperature sensor (Vaisala Oyj, Helsinki, Finland) attached to the same data logger. The temperature probes were fully cross-calibrated to ensure data consistency.

Floral Scent

Scent-producing flowers (pistillate and staminate phases) were collected with previously unused polypropylene bags (Azuma et al. 2001), which were immediately sealed to limit air movement. Solid-phase microextraction (SPME) samples were taken using fibers with a 65-μm divinylbenzene/polydimethylsiloxane coating; these have recently been shown to be preferable to those with a 100-μm coating (Goodrich et al. 2006). The fibers were held in a syringelike manual-sampling device (Supelco, Bellefonte, PA) and inserted into the bag for 2 h to enable adsorption of volatile compounds. The fibers were then transported to the laboratory for gas chromatography–mass spectrometry (GC-MS) analysis. Three replicate samples from different trees were analyzed. In order to detect volatiles that are merely experimental artifacts, controls were run using SPME fibers in empty polypropylene bags.

The GC-MS analysis was performed with an Agilent 5973 mass selective detector connected to an Agilent 6890N GC (Agilent Technologies, Palo Alto, CA) with a 30 m $\times$ 0.25 mm i.d. DB-WAX capillary column with a 0.25 μm film (J and W Scientific, Folsom, CA). Samples were injected into the gas chromatograph using helium as the carrier gas, and the following temperature combinations were applied: the oven was initially maintained at 50°C for the first 5 min and then increased at a rate of 5°C/min to a maximum of 230°C for 20 min. Electrical ionization mass spectrometry was used, with an acquisition range of 30–650 m/z. The NIST 02 MS library bundle (National Institute of Standards and Technology, Gaithersburg, MD) was screened for compounds with comparable mass spectra in order to identify volatile compounds, although the identities are necessarily rather equivocal in the absence of comparisons against authentic standards. Compounds with identity likelihood estimated at less than 80% in both pistillate- and staminate-phase flowers were treated as unknown compounds. Kovats indices (Kovats 1965) were calculated post hoc against n-alkane standards to provide confirmation of compound identity. The GC-MS analyses were not run under isothermal conditions, and therefore smaller alkanes separated during the first 5 min were not comparable to those separated subsequently; Kovats indices consequently could not be calculated for floral scent compounds with short retention times.

Plant Breeding System

The breeding system was assessed using two approaches: calculating the pollen/ovule (P/O) ratio and conducting a series of field-based controlled-pollination experiments. The ratio of the number of pollen grains and ovules produced by a flower can be used as an approximate indicator of breeding system, as follows (Cruden 1977): 2.7–5.4 suggests cleistogamy; 18.1–39.0 suggests obligate autogamy; 39.1–396.0 suggests facultative autogamy; 244.7–2588 suggests facultative xenogamy; and 2108–195,525 suggests obligate xenogamy. The numbers of pollen grains and ovules were counted using

standard techniques (Dafni 1992), based on 10 flowers from five individuals.

In order to determine whether viable pollen was available for controlled-pollination experiments, pollen germination rates were assessed *in vitro* using artificial sucrose solutions at ambient temperatures. Sucrose solutions of various concentrations (0%, 5%, 10%, 20%, and 25%) were prepared and mixed with 50% (w/v) H_3PO_3 and 50% $Ca(NO)_3$ (w/v) (Dafni 1992). Mature pollen was collected from 10 flowers from each of five individuals immediately after anther dehiscence and mixed with 10 μL of each sucrose solution on glass cavity slides. The slides were maintained in closed petri dishes with damp filter paper at ambient temperatures for 24 h. One hundred pollen grains were visually assessed to determine the proportion of pollen grains germinating, using six replicates of each treatment.

Controlled-pollination experiments were conducted at the beginning of the peak flowering season. The flowers were covered with chiffon cloth bags to exclude pollinators (Dafni 1992) before the onset of stigmatic receptivity. Pale green cloth was used as camouflage to protect the bagged flowers from attack by animals, monkeys in particular. The field experiments involved 132–260 flowers (varying between treatments) from eight individuals.

A total of four different controlled-pollination treatments were conducted (adapted from Dafni 1992) to determine breeding system: (1) control—flowers not bagged and left to freely pollinate; (2) test for geitonogamy—flowers bagged and artificially pollinated with pollen from another flower of the same individual that had previously been bagged; (3) test for xenogamy—flowers bagged and artificially pollinated with pollen from flowers of a different individual (10–100 m from recipient); (4) test for autogamy—flowers bagged but not artificially pollinated. Standard pollination treatments requiring emasculation of the flowers were not possible, since the flowers inevitably withered and abscised before the onset of stigmatic receptivity. The strong protogyny shown by flowers of both species (data presented in this article) precludes intraflower self-pollination (autogamy), and consequently pollination treatment 4 can also be regarded as a test for agamospermy. Since autogamous self-pollination is not possible, all references to "self-pollination" in this research refer to geitonogamous pollination (between different flowers of the same individual).

In the absence of clearly discernible external morphological changes associated with postpollination events, it was not possible to record with certainty the extent of pollination success until 2 wk after petal abscission. The number of fruits that developed after each treatment were assessed at 2-wk intervals until fruit maturity (after ca. 20 wk). The overall time between pollination and fruit maturity was recorded for each species. The percentage fruit set in relation to the number of the flowers pollinated in each treatment was also calculated. Some treated flowers were unavoidably destroyed by monkeys and were consequently excluded from the analyses. The statistical significance of comparisons between different pollination treatments and between different individuals were assessed by ANOVA on rank after testing for equal variance and discriminated using the Tukey test for unequal n (Spjotvoll/Stoline test). Comparison of the number of developing monocarps between different treatments required log transformation to satisfy statistical assumptions of normality. All statistical analyses were undertaken using Minitab software (Minitab 2001).

A quantitative evaluation of possible self-incompatibility was achieved using the index of self-incompatibility (ISI) developed by Zapata and Arroyo (1978). The ISI was calculated by dividing the percentage of fruits resulting from self-pollination by the percentage of fruits derived by cross-pollination. Resultant ISI values reflect the following possibilities: 0 = completely self-incompatible; 0–0.2 = mostly self-incompatible; 0.2–1 = partially self-incompatible; and >1 = self-compatible (Zapata and Arroyo 1978).

Results

Floral Morphology and Phenology

Although *Xylopia championii* flowers are typically hermaphroditic, a small proportion of the flowers examined in the field (ca. 5%) were found to be functionally staminate. These staminate flowers did not appear to be restricted to specific individuals. The receptacle was deeply concave, as in hermaphroditic flowers, although the central cavity contained an aborted, nonfunctional carpel (fig. 3). There was no apparent difference in the number of stamens in these flowers, and the pollen was viable.

At the population level, flowering was continuous in *X. championii*, although with a major flowering peak from December until April. Flowering of most of the individual trees was synchronized within the population during the peak flowering period, although there were some early- or late-flowering individuals. The flowering intensity of the trees was consistently low, with a maximum of only five reproductively active flowers borne concurrently on an individual tree, and there were days within the flowering period when a reproductively active tree did not bear any functional flowers. Casual observations suggest that there is no interfloral dichogamy: pistillate-phase flowers often occur alongside staminate-phase flowers within the same tree.

Xylopia championii flowers undergo several distinct developmental changes before, during, and after periods of sexual receptivity. For ease of discussion, we have categorized these changes into six different phases (referred to as stages I–VI: table 1; fig. 4). The flower buds and mature flowers were invariably pendent, with relatively little change of petal color throughout the six stages observed except for the intensification

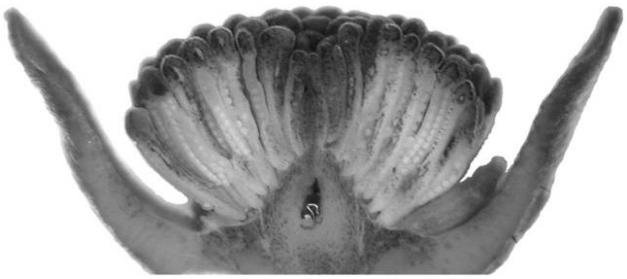

Fig. 3 Staminate flower of *Xylopia championii* (longitudinal section, after removal of corolla).

Table 1

Flower-Level Phenological Stages in *Xylopia championii*

Floral stage	Duration	Petal position	Inner and outer petal color (general)	Inner petal color (basal adaxial region)	Stamen color	Reproductive activity
I	25–30 d	Closed	Greenish yellow	Pale pink	Not visible	None
II	1–2 d	Gradually separate	Pale yellow	Pink	Not visible	None
III	6–8 h	Fully separated	Light yellow	Pink-purple	Pink	None
IV	15–17 h	Outer petals partially closed; inner petals form a tightly closed chamber	No change	Pink-purple	No change	Stigmas receptive
V	Ca. 6 h	Fully separated	No change	Red-purple	No change	None
VI	Ca. 17 h	Same as stage IV	No change	Dark red-purple	Dark maroon	Anther dehiscence

of the red pigmentation at the base of the petals adaxially (fig. 1*B*).

The unopen flower buds of *X. championii* were observed to develop over a prolonged period of 25–30 d (stage Ia–Ic; fig. 4), during which period the petals gradually turned yellow. The petals subsequently separated over 1–2 d (stage II): although the outer petals separated fully, the base of the inner petals initially remained in contact with each other immediately above the reproductive organs, forming an enclosed pollination chamber (stage IIa, IIb; fig. 4), only separating fully toward the end of stage II (stage IIc, IId; fig. 4). The petals subsequently reflexed outward over a period of 6–8 h (stage III; fig. 4) immediately before the flowers became sexually receptive.

The flowers were markedly protogynous, with the entire extent of reproductive activity restricted to a 2-d period. The pistillate phase (stage IV) lasted for ca. 15–17 h (fig. 5), from ca. 1500 hours on day 1 to 0600–0800 hours on day 2. During this phase, the petals closed so that the inner petals formed a pollination chamber over the reproductive organs (fig. 4), and the stigma was noticeably wet due to the secretion of a sticky exudate. This phase was clearly correlated with the emission of a strong fruity odor.

The pistillate phase was succeeded by an interim period (stage V; fig. 4) of 6–8 h (fig. 5), during which the flowers were not sexually functional and floral scent dissipated. The petals again separated during this phase, and the flowers superficially resembled those of stage III.

The interim period was followed by a staminate phase (stage VI), during which the petals closed once again to form a pollination chamber (fig. 4), the anthers dehisced, and there was an obvious increase in floral scent. This stage continued for ca. 17 h, from 1400 hours on day 2 to 0600–0800 hours the following morning (fig. 5). The freshly dehisced pollen tetrads were loosely connected to each other, presumably due to the presence of a sticky pollenkitt. At the end of stage VI, the petals and stamens abscised, irrespective of whether the ovules had been fertilized or not.

Floral Visitors and Pollinators

A small unidentified weevil species belonging to the genus *Endaeus* (Coleoptera: Curculionidae) (fig. 6) was the most common visitor to *X. championii* flowers, with each floral chamber containing up to two or three individuals concurrently. The weevils landed on the calyx tube or outer petals before entering the floral chamber. If the floral chamber was fully closed, they entered by crawling between the inner petals. The weevils were crepuscular and nocturnal, with their arrival at the flowers occurring around 1800 hours on day 1 (*3* in fig. 5). They remained overnight inside the floral chamber, before departing at around 0500–0800 hours the following morning (*5* in fig. 5). Similar arrival and departure times were recorded for the beetles during the staminate phase the following night (*8* and *9* in fig. 5). A correlation exists between the number of individuals per floral chamber and the

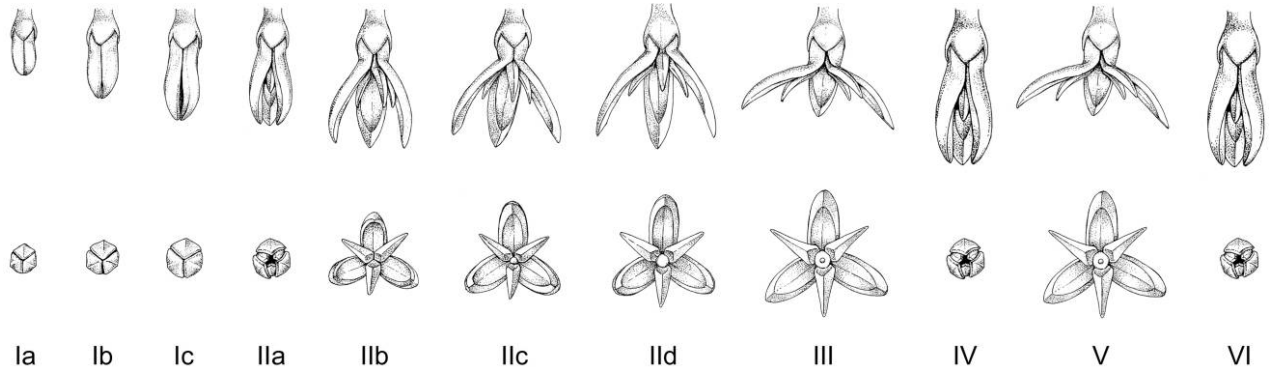

Fig. 4 Flower-level phenological changes in *Xylopia championii*, showing changes in size and position of petals. *Upper row*, lateral view; *lower row*, apical view. Roman numerals represent the six different phenological stages described in table 1. Drawings by Ngai Yuen Yi.

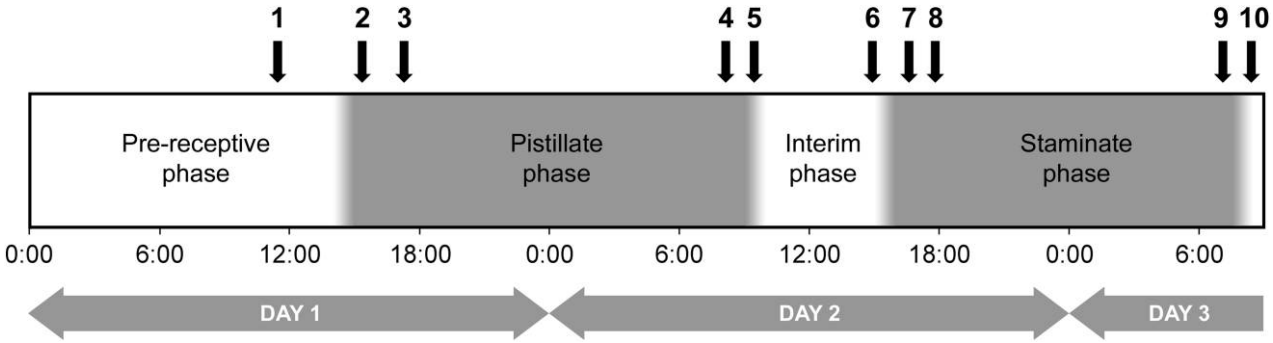

Fig. 5 Timing of phenological events during sexually functional phases of *Xylopia championii* flowers. Numerical codes: *1* = inner petals form pollination chamber over reproductive organs; *2* = initiation of stigmatic receptivity, elevated floral temperature, and scent production; *3* = arrival of pollinators; *4* = departure of pollinators; *5* = cessation of stigmatic receptivity and scent production and separation of petals; *6* = inner petals form pollination chamber over reproductive organs; *7* = initiation of anther dehiscence and scent production; *8* = arrival of pollinators; *9* = departure of pollinators; *10* = abscission of stamens and petals and cessation of scent production.

occurrence of the pistillate and staminate phases of the flower (fig. 7), with a notable absence of weevils during the interim phase and before stigmatic receptivity. The weevils were generally present in pairs and were often observed copulating, although neither eggs nor larvae were ever observed in older flowers. Brown spots on the petals of flowers that had been visited by weevil suggests that the weevils may feed on petals. Pollen grains of *X. championii* were observed on the dorsal surfaces and mouth parts of the weevils. It can be conjectured that hairs on the bodies of the beetles assist with pollen attachment.

The only other floral visitor commonly observed was an unidentified species of ant (Formicidae). In general, only one or two individual ants were ever observed directly associated with the flowers.

Floral Temperature

There was clear evidence of elevated floral temperatures in *X. championii* (fig. 8), beginning immediately before the onset of stigmatic receptivity. The temperature within the floral chamber reached 30.3°C (ca. 8°C above ambient levels) during the pistillate phase (peaking around 1800–2000 hours), but fell to only ca. 1.3°C above ambient levels during the interim phase. During the staminate phase the following day, temperatures rose to 30.9°C (ca. 8°C above ambient levels) by ca. 1900 hours, and then gradually declined.

Floral Scent

Floral scent production was coincident with floral heat, with a gradual intensification of the scent associated with increasing floral temperatures. The fruity scent persisted throughout the pistillate phase, diminished during the interim phase, and then intensified again during the staminate phase.

The GC-MS analysis of floral volatiles revealed the presence of 43 volatile compounds, of which 19 were common to both pistillate- and staminate-phase scent (table 2). Three compounds (1,4-dichlorobenzene, butyrolactone, and naphthalene) were found to be present in the blank controls and were accordingly ignored since they are likely to be of anthropogenic origin or experimental artifacts. The chemical identifications presented are tentative, given the absence of comparative studies against authentic standards.

Plant Breeding System

The pollen grains are released as loose tetrads when the anthers dehisce. The mean numbers of pollen grains and ovules per flower were $12,969 \pm 952$ and 5 ± 0.3 ($\pm$SD), respectively, and the resulting P/O ratio was accordingly 2625 ± 219.

The optimal sucrose concentration for pollen germination was 20%; under these conditions, *X. championii* showed 30% ($\pm$4.2 SD) pollen viability. These results therefore confirmed the feasibility of undertaking controlled-pollination experiments.

The overall time between pollination and fruit maturity was 115–130 d (mean = 122 d). The 20-wk monitoring of postpollination events therefore covered the entire developmental range up to the splitting of ripe fruits. Abscission of

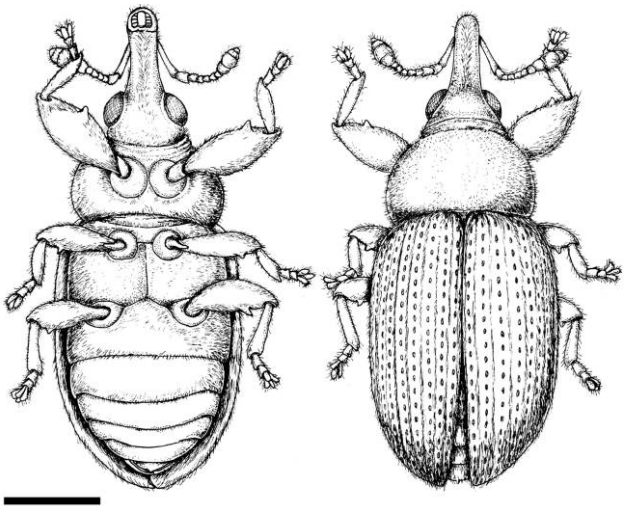

Fig. 6 Unidentified *Endaeus* species (Curculionidae), pollinator of *Xylopia championii*. Scale bar = 1 mm. Drawings by Ngai Yuen Yi.

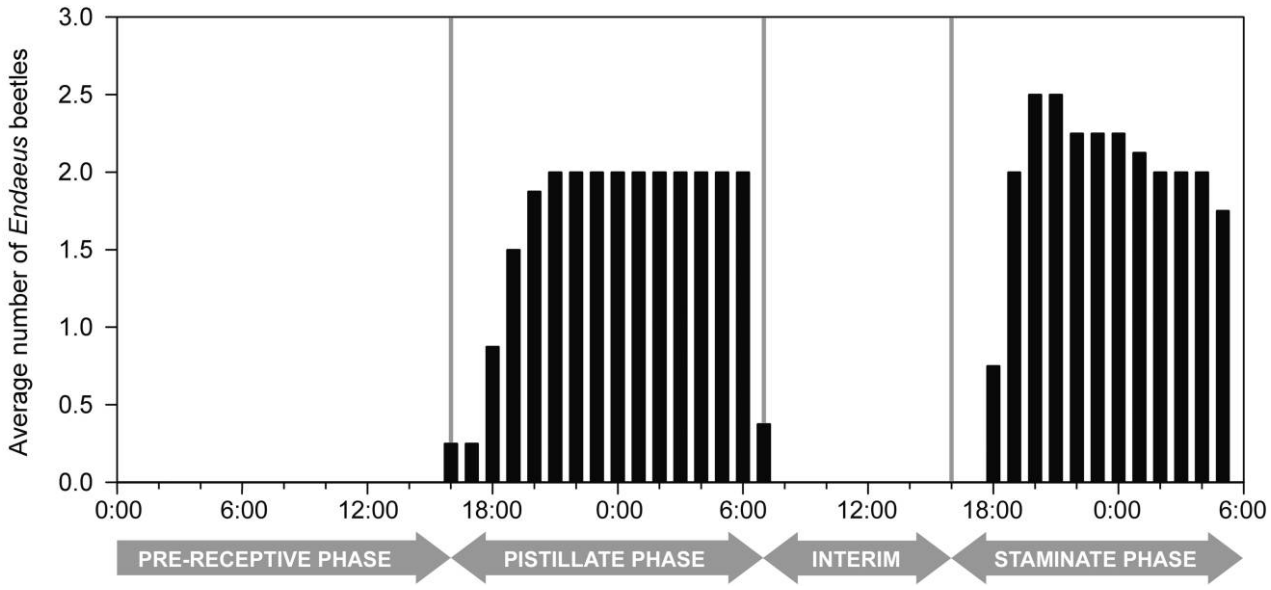

Fig. 7 Average number of *Endaeus* beetles per floral chamber in *Xylopia championii*.

entire flowers after 10–14 d was used as an indication of the failure of fertilization.

Flowers used as controls in the pollination experiments showed rapid abscission over the first few weeks, with 65%, 82%, and 88% of flowers dropping after 2, 4, and 6 wk, respectively (fig. 9). The remaining postfertilization flowers generally continued development, with relatively little subsequent abscission: after 20 wk, 5.0% (±3.4% SD) of flowers had resulted in fruit set (table 3). Flowers that had been bagged to exclude pollinators (without artificial pollination) showed a greater level of flower abscission, with 95%, 98%, and 100% of flowers abscising after 2, 4, and 6 wk, respectively (fig. 9), with none resulting in mature fruits (table 3). Flowers that had either been artificially self-pollinated or artificially cross-pollinated showed a gradual rate of abscission over the 20-wk monitoring period (fig. 9), although with significantly different final rates of fruit set: 3.1% (±1.7%) for those that had been self-pollinated and 29.4% (±12.3%) for those that had been cross-pollinated (table 3).

Pollination success in the eight individuals studied was not significantly different (assessed using one-way ANOVA), indicating that there was no individual plant effect on pollination treatments. The differences among treatments were significant, however (table 3): the selfing rate of *X. championii* was significantly lower than that resulting from cross-pollination, although there was no significant difference between the self-pollination treatment and the control. The ISI was calculated as 0.10.

Discussion

Floral Morphology and Phenology

Xylopia species (including *Xylopia championii*: Huber 1985) are invariably described as possessing hermaphroditic flowers. Our observation of staminate flowers in *X. championii* (fig. 3) appears to be unique in the genus, although the relatively small proportion of staminate flowers within populations (estimated at only 5%) suggests that it would have negligible effect on breeding system.

Several of the structural features of the flowers of *X. championii* are likely to be adaptations for beetle pollination. Most significantly, the ovary is protected within a strongly concave receptacle; similar conditions are observed in *Calycanthus* and *Eupomatia* (Gottsberger 1974). As with most Annonaceae species, the stamens possess protective staminal connectives and are tightly packed around the receptacle to prevent access by beetles, only separating during anther dehiscence.

Although the petals of *X. championii* separate early in floral development, subsequent changes in the position of the inner petals result in the formation of a floral pollination chamber during the sexually receptive phases (fig. 4). Pollination chambers are widespread in the Annonaceae, although there is a striking diversity in structure: the chamber can be derived from the union of the convex adaxial surfaces of the inner petals (e.g., *Polyalthia*), imbricate inner petals (e.g., some *Annona* species), apically connivent inner petals (e.g., *Goniothalamus* and *Orophea*), or apically connivent outer petals (e.g., *Dasymaschalon*). The diverse structural basis of these chambers is presumably a reflection of their independent evolutionary origin within the family. The pollination chamber has several possible functions, including protection of pollinators from predators, providing brood sites for pollinators, and the maintenance of optimal temperature and humidity levels.

Xylopia championii is markedly protogynous, with a 6–8-h interim period between the cessation of stigmatic receptivity and the onset of anther dehiscence (fig. 5). Protogyny has not only been widely reported in other *Xylopia* species (Andrade et al. 1996; Küchmeister et al. 1998; Webber 2002; Silberbauer-Gottsberger et al. 2003) but appears to be ubiquitous

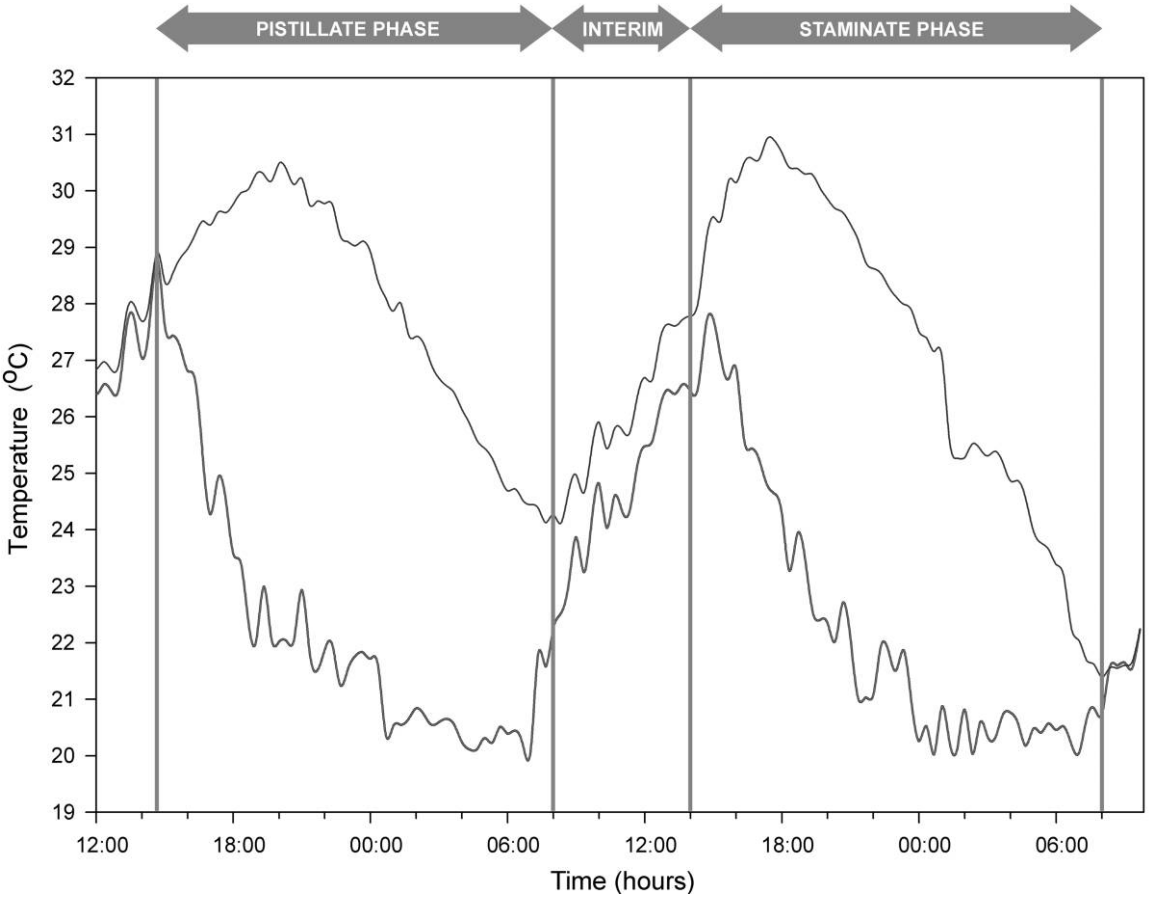

Fig. 8 Floral temperatures in *Xylopia championii* during pistillate, interim, and staminate phases. Upper line = internal flower temperature; lower line = ambient temperature.

among species with bisexual flowers in the family as an adaptation to minimize the chances of within-flower pollination. The concurrent presence of pistillate- and staminate-phase flowers on the same individual of *X. championii*, however, allows possible geitonogamous pollination.

The reproductive cycle of individual flowers of *Xylopia championii* extends over 2 d (fig. 5). This is again common throughout the family (and has also been observed in *Xylopia benthamii*: Webber 2002), although species with unisexual flowers (e.g., *Pseuduvaria*; Silberbauer-Gottsberger et al. 2003) inevitably show a 1-d cycle. Longer reproductive rhythms occasionally occur, as in *Asimina* (Willson and Schemske 1980; Norman and Clayton 1986; Norman et al. 1992; Rogstad 1993), *Deeringothamnus* (Norman 2003), and *Monodora* (Lamoureux 1975).

The timing of floral receptivity in *X. championii* is essentially crepuscular and nocturnal and inevitably coincides with periods of activity of the *Endaeus* floral visitors. This is typical of other beetle-pollinated *Xylopia* species (Andrade et al. 1996; Küchmeister et al. 1998; Jürgens et al. 2000; Webber 2002; Silberbauer-Gottsberger et al. 2003), whereas thrips-pollinated *Xylopia* species appear to be diurnal (*Xylopia brasiliensis*: Andrade et al. 1996; *Xylopia aromatica*: Jürgens et al. 2000).

Floral Visitors and Pollinators

The *Endaeus* weevil (Curculionidae) can be determined as an effective pollinator of *X. championii* on the basis of the following attributes: (1) their arrival time coincides with the onset of the functionally active phases of the flowers, with an increase in frequency during the pistillate and staminate phases and absence during the interim phase; (2) the body size (ca. 4 mm long, ca. 2 mm wide) is sufficiently small to enable them to be accommodated in the floral chamber; and (3) pollen grains of *X. championii* were observed attached to their bodies.

Although this is the first report of curculionid beetles as pollinators of *Xylopia*, other families of small beetles (Chrysomelidae, Nitidulidae, and Staphylinidae) have been reported previously for the genus (Andrade et al. 1996; Küchmeister et al. 1998; Jürgens et al. 2000; Webber 2002; Silberbauer-Gottsberger et al. 2003). Thrips (Thysanoptera) have also been shown to pollinate some species of *Xylopia* (Gottsberger 1970; Jürgens et al. 2000; Webber 2002; Silberbauer-Gottsberger et al. 2003). Pollination by small beetles (particularly Chrysomelidae, Curculionidae, Nitidulidae, and Staphylinidae) is extremely common in the Annonaceae (Gottsberger 1999; Silberbauer-Gottsberger et al. 2003) and is likely to represent the ancestral pollination system in the family.

Table 2

Putative Chemical Composition of Floral Volatiles Emitted by Pistillate- and Staminate-Phase Flowers of *Xylopia championii*

No.	IUPAC compound name[a]	Mean RT (min)	Identity likelihood		Kovats index	Percentage peak area	
			Pistillate phase (%)	Staminate phase (%)		Pistillate phase	Staminate phase
1	3,7,7-trimethylbicyclo[4.1.0]hept-3-ene [= 3-carene]	7.10	74	87	...	6.98	3.30
2	1-methyl-4-(1-methylethyl)-1,4-cyclohexadiene	8.46	...	87	...	...	1.25
3	Unknown	9.48	na	na	...	2.63	...
4	Hexanoic acid, ethyl ester	9.50	95	95	...	2.75	21.17
5	1-methyl-2-(1-methylethyl)-benzene	10.45	95	95	...	3.03	1.76
	1-methyl-4-(1-methylethyl)-benzene [= *p*-cymene]		95	97			
6	Unknown	15.48	na	na	507	...	2.19
7	1,5,5-trimethyl-6-methylene-cyclohexene	16.58	93	93	666	2.65	.69
8	(1R,2S,6S,7S,8S)-8-isopropyl-1,3-dimethyltricyclo[4.4.0.0(2,7)]dec-3-ene [= copaene]	16.82	99	...	700	.63	...
9	1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-one	17.35	91	74	772	2.93	1.59
10	3,7-dimethyl-1,6-octadien-3-ol [= R,S-linalool]	18.51	64	80	922	1.18	1.22
11	1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester [= acetic acid]	19.15	98	97	1001	.99	.59
	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate		98	98			
12	1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-1H-cyclopropa[a]naphthalene	19.35	93	...	1026	.77	...
13	(1R,2S,6S,7S,8S)-8-isopropyl-1,3-dimethyltricyclo[4.4.0.0(2,7)]dec-3-ene [= caryophyllene]	19.46	99	...	1039	1.13	...
14	4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol [= terpinen-4-ol]	19.78	96	96	1077	2.60	1.17
15	Decanoic acid, ethyl ester	20.67	...	95	1179	...	.68
16	Unknown	20.78	na	na	1191	.82	.79
17	1a,2,3,3a,4,5,6,7b-octahydro-1,1,3a,7-tetramethyl-1H-cyclopropa[a]naphthalene	21.06	94	...	1222	.91	...
18	Ethyl trans-4-decenoate	21.34	93	98	1253	1.47	9.22
19	Unknown	21.95	na	na	1319	...	.27
20	1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-1-H-cycloprop[e]azulene	21.99	99	...	1323	1.13	...
21	Unknown	22.06	na	na	1330	...	.56
22	1-methyl-5-methylene-8-(1-methylethyl)-1,6-cyclodecadiene [= germacrene D]	22.14	96	...	1339	6.24	...
23	Unknown	22.15	na	na	1340	...	2.30
24	1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene	23.29	97	96	1457	.80	.23
25	Unknown	23.44	na	na	1471	.53	.42
26	2,4-decadienoic acid, methyl ester	24.22	...	87	1548	...	.17
27	Unknown	24.95	na	na	1617	1.05	.89
28	Unknown	25.17	na	na	1637	...	.19
29	Ethyl 2,4-trans, cis-decadienoate	25.27	96	...	1646	23.81	...
30	Hexanoic acid [= caproic acid]	25.44	83	64	1662	4.69	39.01
31	Unknown	26.32	na	na	1741	.52	.41
32	2,6-bis(1,1-dimethylethyl)-4-methylphenol [= butylated hydroxytoluene]	26.67	96	...	1772	.48	...
33	Unknown	26.75	na	na	1779	.71	.36
34	Unknown	26.97	na	na	1798	.66	...
35	Unknown	28.35	na	na	1914	...	.16
36	Octanoic acid [= caprylic acid]	29.91	...	93	2038	...	1.36
37	Decahydro-1,1,7-trimethyl-4-methylene-1H-cycloprop[e]azulen-7-ol	30.87	97	81	2112	.88	.39
38	Unknown	31.81	na	na	2182	...	.31
39	Unknown	38.42	na	na	2621	...	.30
40	*n*-hexadecanoic acid [= palmitic acid]	44.92	...	97	2984	...	.71

Note. Compounds arranged according to retention time, excluding those identified from blank controls. Compounds with identity likelihood estimated as <80% listed as "Unknown." IUPAC = International Union of Pure and Applied Chemistry; na = not applicable; RT = retention time.

[a] Common names in brackets, if applicable.

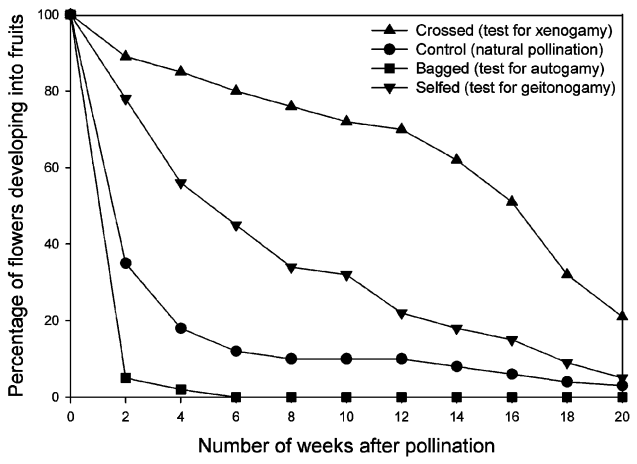

Fig. 9 Percentage of fruit survival after pollination treatments in *Xylopia championii*.

The unidentified ant species (Formicidae) is unlikely to be an effective pollinator, since individuals were observed to be generally diurnal in activity (and hence not active during the receptive phases of the flower) and were not observed to move actively between flowers. Ants are social insects and are unlikely to be efficient pollinators (Jolivet 1996).

Floral Temperature

The temperature within the floral chamber of *X. championii* is elevated to ca. 8°C above ambient levels (fig. 8). The coincidence of elevated temperatures with the onset of both stigmatic receptivity and anther dehiscence and the drop in temperature correlated with the interim phase suggests that the heat is generated by the flower rather than merely daytime heat that is retained into the night by the floral chamber. This is rather equivocal evidence of thermogenesis, however, because no evidence is presented for the existence of specific heat-producing tissues in the flower. The floral visitors nevertheless experience elevated temperatures within the floral chamber, irrespective of the origin of this heat. Elevated floral temperatures have previously been reported in several other *Xylopia* species (Küchmeister et al. 1998; Jürgens et al. 2000; Webber 2002; Silberbauer-Gottsberger et al. 2003), although it was noted as being absent in the thrips-pollinated species *X. aromatica* (Gottsberger 1970; Jürgens et al. 2000).

Elevated floral temperatures have also been reported for several other genera in the family, including *Anaxagorea* (Küchmeister et al. 1998; Jürgens et al. 2000), *Annona* (Gottsberger 1970, 1989*a*, 1989*b*, 1999; Gottsberger and Silberbauer-Gottsberger 1988; Maas-van de Kamer 1993; Silberbauer-Gottsberger et al. 1997), *Cymbopetalum* (Murray 1993; Webber and Gottsberger 1993), *Duguetia* (Küchmeister et al. 1998; Silberbauer-Gottsberger et al. 2001, 2003), *Enicosanthum* (Silberbauer-Gottsberger et al. 2003), and *Polyalthia* (Ratnayake et al. 2006*a*), all of which are beetle pollinated. It is likely that elevated floral temperature is more widespread in the Annonaceae than these data suggest, however, since it has not been investigated in most previous studies.

A possible role for elevated floral temperature is as a heat reward for floral visitors (Seymour and Schultze-Motel 1996; Seymour et al. 1998, 2003). By providing beetles with an energy reward, the flower allows them to conserve considerable levels of energy required for feeding, mating, and initiating flight. The temperatures maintained by thermogenic flowers are typically in the range favored by active beetles (Seymour and Schultze-Motel 1997); the flowers therefore assist with the maintenance of the body temperature of the beetles and stimulate their reproductive behavior, feeding, and digestion (Bernal and Ervik 1996; Patiño et al. 2000; Thien et al. 2000). Significantly, beetles require high thoracic temperatures (often above 30°C) to initiate flight (Seymour and Schultze-Motel 1996, 1997; Seymour et al. 2003).

Recent research has found that some beetles (including some Curculionidae; Hausmann et al. 2004) have infrared sensors known as IR sensilla or IR pit organs, which detect infrared radiation (Schmitz et al. 1997; Hammer et al. 2001; Sowards et al. 2001). Although there is no evidence that such sensors exist on the pollinators of *X. championii*, it is possible that the heat is generated in the flower as a direct attractant rather than simply an energy reward. Alternatively, it can also be speculated that floral heat may simply encourage the beetles to enter the floral chamber after they have been attracted to the flower by olfactory cues (cf. blowfly pollination of *Helicodiceros muscivorus*; Angioy et al. 2004).

Floral Scent

Many of the floral volatiles identified from *X. championii* are commonly produced by flowers of other angiosperms (Knudsen et al. 2006). Very few previous studies have focused on floral scent chemistry in the Annonaceae, although studies of *Cananga* (Ma et al. 1988); *Anaxagorea*, *Duguetia*,

Table 3

Percentage Fruit Set following Controlled-Pollination Experiments on *Xylopia championii*

	Pollination treatment			
	Control	Crossed	Selfed	Bagged
Number of flowers treated	238	152	260	132
Number of individuals treated	8	8	8	8
Percentage fruit set (mean ± SD)	5.0 ± 3.4^{A}	29.4 ± 12.3^{B}	3.1 ± 1.7^{A}	0

Note. Percentage fruit set was compared among treatments using ANOVA after log transformation and discriminated using the Tukey HSD test. Treatments with the same superscript letter do not differ significantly ($P < 0.05$). Results of flowers bagged before pistillate phase were excluded from the analysis due to 0 values.

Rollinia, and *Xylopia* (Jürgens et al. 2000); and *Asimina* (Goodrich et al. 2006) all reveal a broadly similar range of compounds to those found in *X. championii*.

The fruity odor of *X. championii* flowers suggests that pollinators may be attracted to the flowers by scents that mimic fruits. This hypothesis is reinforced by the common possession of several volatile compounds (or closely allied compounds) in both the floral scent of *X. championii* and in the fruits of other Annonaceae species. Germacrene D (compound 22; see table 2), for example, occurs in the fruits of *Anaxagorea dolichocarpa* (Fournier et al. 1994), *Annona atemoya* (Bartley 1987), and *Xylopia aromatica* (Stashenko et al. 2004); the related compound bicyclogermacrene occurs in the fruits of *A. atemoya* (Wyllie et al. 1987); and hexanoic acid (compound 30) occurs in the fruits of *Annona cherimola* (Idstein et al. 1984).

There is also equivocal evidence to suggest that some of the volatile components of the floral scent of *X. championii* may act as beetle attractants by mimicking insect pheromones. Although relatively little is known of the chemical attractants of curculionid beetles such as *Endaeus*, there is an extensive literature relating to the pheromones of other insect groups. The following floral volatiles isolated from *X. championii* have been identified as insect pheromones (El-Sayed 2006 and references therein): 3-carene (compound 1; see table 2); 1-methyl-4-(1-methylethyl)-benzene (= *p*-cymene, one of the possible identities of compound 5); 3,7-dimethyl-1,6-octadien-3-ol (= R,S-linalool; compound 10); acetic acid (one of the possible identities of compound 11); caryophyllene (compound 13); 4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol (= terpinen-4-ol; compound 14); 1-methyl-5-methylene-8-(1-methylethyl)-1,6-cyclodecadiene (= germacrene D; compound 22); ethyl 2,4-trans, cis-decadienoate (compound 29); hexanoic acid (= caproic acid; compound 30); octanoic acid (= caprylic acid; compound 36); and *n*-hexadecanoic acid (= palmitic acid; compound 40). Although these compounds elicit a variety of responses in insects, in most cases they act as aggregation pheromones; compounds that have specifically been highlighted as beetle attractants include 1, 10, 13, 30, and 40 (table 2). These conclusions are speculative, however, in the absence of empirical studies. The lack of strong visual cues nevertheless strongly suggests that the beetles are primarily attracted to the flowers in response to some form of olfactory cues.

Naphthalene was one of the volatiles observed in the floral scent of *X. championii*, although it was excluded from table 2 because it was detected in similar quantities in the blank controls. It has previously been isolated in significant quantities from the floral scents of *Rollinia*, *Xylopia* (Jürgens et al. 2000), and *Magnolia* (Azuma et al. 1996), although there is some disagreement whether it is likely to be of anthropogenic origin (as suggested by Jürgens et al. 2000) or whether it functions to protect flowers against herbivory (as suggested by Azuma et al. 1996). The data for *X. championii* suggests that in this case, at least, it appears to be an artifact.

Plant Breeding System

The P/O ratio value calculated for *X. championii* (2625 ± 219) suggests that it is likely to possess an obligately xenogamous breeding system, according to Cruden's (1977) scheme. Caution is required in the interpretation of these results, however, since P/O values are known to vary within and between populations and to be affected by various factors, including timing of flower formation in the flowering season (Cruden 2000 and references therein). Furthermore, Kress (1981) and Cruden (2000) found that the P/O ratio is lower in species in which pollen is dispersed in tetrads, as in *X. championii*. The P/O ratio is only imperfectly correlated with breeding system, however, and the field-based controlled-pollination experiments can be expected to provide a more accurate determination of breeding system.

None of the flowers that were bagged to exclude pollinators before the onset of the pistillate phase developed into fruits (fig. 9), indicating that autogamy is unlikely. This is presumably due to the marked protogyny observed, with the 6–8-h unreceptive interim phase separating the pistillate and staminate phases, but may also be due to a possible self-incompatibility mechanism.

All the other pollination treatments resulted in limited fruit set (fig. 9), although with a gradual decline in the number of fruits as a result of self-pollination, animal consumption (primarily by birds), weather conditions, inbreeding depression (for geitonogamous crosses; see discussion below), or overproduction (immature fruit abscission when the carrying capacity of an individual tree is exceeded). The relatively low flowering and fruiting intensities of individuals suggest that the latter explanation is less likely, however.

Levels of fruit set following artificial cross-pollination exceeded those following artificial geitonogamous self-pollination, suggesting that the population possesses a xenogamous breeding system. This breeding system is characteristic of woody species throughout the tropics (Bawa et al. 1985*b*). The lower fruit set following self-pollination is possibly due to a partial self-incompatibility mechanism: the selfing rate (3.1%) suggests that *X. championii* is intermediate between being self-incompatible and self-compatible (fide Dafni 1992); and the ISI was calculated as 0.10, indicating that individuals are likely to be mostly self-incompatible (fide Zapata and Arroyo 1978). Significantly, however, the gradual abscission of immature fruits after self-pollination (fig. 9) may be due to inbreeding within the population (Charlesworth and Charlesworth 1987; Stacy 2001); this is common in fragmented habitats (Harris and Johnson 2004). There are very few reports of self-incompatibility in the Annonaceae, possibly because of widespread protogyny in the family, which would limit the potential benefits of such a system. Partial self-incompatibility has previously been reported, however, in *Asimina* (Norman et al. 1992), *Polyalthia* (Rogstad 1994; Ratnayake et al. 2006*b*), *Sapranthus* (Bawa 1974), and *Uvaria* (Nagamitsu and Inoue 1997).

The levels of fruit set following artificial cross-pollination significantly exceeded those resulting from natural (open) pollination (table 3; fig. 9). This may be due to the scarcity of pollinators, at least during the assessment period, and it would imply that annual fluctuations in the population size of the pollinator may significantly affect the reproductive success of *X. championii*.

Conclusions

The effective pollinator of *X. championii* is shown to be a species of *Endaeus* weevil (Curculionidae). The beetles are

attracted to the flowers by a strong fruity scent and also possibly heat generated within the flower. The rewards provided by the flowers include heat energy (for the maintenance of body temperature), and the enclosed floral chamber may furthermore provide protection from predators and a site for meeting mates. It is also possible that the beetles are rewarded with pollen grains as food, although the evidence for this is circumstantial in the absence of gut contents analysis. Evidence of beetle damage to the petals was slight and is only weak evidence of feeding on petal tissue.

Although *X. championii* flowers show clear adaptations for small-beetle pollinations, the *Endaeus* beetles are generalist fruit eaters and are not specialized as pollinators. There is no evidence to suggest any specific one-to-one adaptation between the plant and beetle species, and it is likely that other fruit beetles may perform a role in pollination in different flowering periods or at different sites. There is a considerable body of data to indicate that the specificity of plant-pollinator interactions in the beetle-pollinated Annonaceae is with functional groups of beetles rather than with specific species of beetle (Saunders, forthcoming).

The comparatively low levels of fruit set in natural (open) pollination treatments suggest that pollinator availability may be a limiting factor and that annual fluctuations in the population size of the pollinator are likely to affect the reproductive success of the tree. Constraint of plant-pollinator interactions is prevalent among tropical lowland rainforest trees (Bawa et al. 1985*a*).

The flowers of *X. championii* are markedly protogynous, with a 6–12-h nonfunctional interim period between the pistillate and staminate phases. This clearly precludes autogamy, although geitonogamy (between different flowers belonging to the same individual) is possible because of the apparent lack of intraindividual synchrony in the sexual maturation of flowers. The co-occurrence of pistillate- and staminate-phase flowers on single individuals therefore suggests that self-pollination is possible despite the existence of complete intrafloral dichogamy. Significantly, the results of artificial controlled-pollination experiments indicate that *X. championii* possesses an essentially xenogamous breeding system. The low levels of fruit set following geitonogamous self-pollination are probably indicative of inbreeding depression.

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