Flora 207 (2012) 107–118

Contents lists available at SciVerse ScienceDirect

Flora

journal homepage: www.elsevier.de/flora

Pollination ecology of *Magnolia ovata* may explain the overall large flower size of the genus

Gerhard Gottsberger[a,*], Ilse Silberbauer-Gottsberger[a], Roger S. Seymour[b], Stefan Dötterl[c]

[a] Botanischer Garten und Herbarium, Universität Ulm, 89069 Ulm, Germany
[b] Ecology and Evolutionary Biology, The University of Adelaide, Adelaide, SA 5005, Australia
[c] Department of Plant Systematics, University of Bayreuth, 95440 Bayreuth, Germany

ARTICLE INFO

Article history:
Received 16 June 2011
Accepted 10 August 2011

Keywords:
Anthesis
Cantharophily
Dynastinae
Floral heating
Floral scent
Magnolia evolution

ABSTRACT

Flowering and fruiting biology of *Magnolia ovata* was studied in Atlantic forests in the interior of São Paulo State, Brazil. The large, bisexual flowers are protogynous, nocturnal, thermogenic and emit a strong scent in two consecutive evenings. In the first night of anthesis, the flowers are in the pistillate stage and thermogenesis starts at about sunset and lasts about 3 h. In the second night, the flowers enter the staminate stage and produce heat for 4 h. Heat is generated by the petals, gynoecium and anthers. Temperatures measured inside the petals reach 26.7 °C and 31.9 °C in the pistillate and staminate stages, 6.0 and 10.6 °C above ambient air, respectively. In the pistillate stage, the perianth opens after sunset and closes tightly a few hours later, and remains closed until the next evening. The initial opening and closing, however, is not synchronous for all flowers during the night. In the following evening, flowers in the staminate stage again open and remain so until the petals drop. Scent compounds, analyzed by GC–MS, contain C5-branched chain compounds, aliphatics, benzenoids and monoterpenoids. Emission of the most prominent compound, C5-branched methyl 2-methyl butyrate, commences before flower opening and continues throughout anthesis, but is accentuated in the thermogenic pistillate and staminate stages. Female and male individuals of only one beetle species, the dynastid scarab *Cyclocephala literata*, are attracted to the scented flowers in both pistillate and staminate stages. Once inside the flowers they feed on the petals and mate. Tests with synthetic methyl 2-methyl butyrate indicate that this compound is a strong attractant for the beetles. Because this scent compound is strongly emitted in both pistillate and staminate stages, the beetles fly indiscriminately between flowers of both stages. This behavior enhances pollen mixing and effective cross-pollination of the self-compatible species. The evolutionary history of *Magnolia* appears to be influenced by an ancestral condition of dynastid scarab beetle pollination. Large magnolia flowers are best explained as an archaic structure resulting from the initial association of tropical American species of section *Talauma* with large and voracious dynastid beetles.

Introduction

Magnolias are popular woody plants throughout the world. *Magnolia* (Magnoliaceae) are early-divergent flowering plants, and have large, solitary flowers with an elongated receptacle, on which numerous stamens and carpels are spirally arranged.

Extant members of Magnoliaceae exhibit disjunct tropical/subtropical/temperate distributions, with about one-third of the species in South America, Central America, the West Indies and Southeast North America; approximately two-thirds of the species are found in East and Southeast Asia, from the Himalayas eastwards to Japan and southwards through the Malayan Archipelago to New Guinea and New Britain. Fossil records from the Cretaceous and the Tertiary indicate the family formerly occurred across most of the northern hemisphere, i.e., in the northern parts of North America, Alaska, Greenland, Spitzbergen, and Europe, when in these regions suitable climate conditions prevailed (Frodin and Govaerts, 1996; Heywood et al., 2007; Nie et al., 2008; Nooteboom, 1993).

The family is divided into two subfamilies, Liriodendroideae (including only *Liriodendron* with two species) and Magnolioideae (ca. 220–240 species). Taxonomists have long debated the classification of Magnolioideae, and over the years the number of recognized genera has progressively been reduced. Nooteboom (1993) and Frodin and Govaerts (1996) recognized six genera. In recent classifications of Magnolioideae, based on chloroplast DNA phylogenetic analysis and morphological re-examinations (Figlar and Nooteboom, 2004; Figlar, 2006), only one genus *Magnolia* with three subgenera and 12 sections is recognized.

* Corresponding author.
E-mail address: gerhard.gottsberger@uni-ulm.de (G. Gottsberger).

0367-2530/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.flora.2011.11.003

The reproductive biology of temperate species of Magnolia has been studied in the USA, Japan and China (e.g., Heiser, 1962; Ishida, 1996; Thien, 1974; Yasukawa et al., 1992; Zhao and Sun, 2009). The temperate species of *Magnolia* and *Liriodendron* are visited and pollinated principally not only by several small beetles, but also by flies and bees. Their flowers are diurnal, protogynous and exhibit anthesis over several days.

The first study of the reproductive and pollination biology of the here investigated tropical Brazilian *Magnolia* ovata (A. St.-Hil.) Spreng. was done by Gibbs et al. (1977), under its former name *Talauma ovata* A. St.-Hil. Gibbs et al. (1977) indicated that *M. ovata* is self-compatible, with nocturnal, protogynous flowers that open and close in a two-night rhythm. Large dynastid scarab beetles are attracted in the evening hours and function as pollinators.

Gibbs et al. (1977) provided basic data on the reproductive biology of this species, however, they did not study thermogenicity of the flowers and also missed details of the floral rhythm in the pistillate and staminate stages. Also no analyses of floral scent were done to test for attraction of the beetles. Seymour et al. (2010) studied respiration and temperature patterns in the thermogenic flowers of *M. ovata*, but did not provide information on floral scent emission or describe details of its role in pollination biology. The aims of the present study is to describe the full complexity of the pollination biology of the tropical, dynastid scarab beetle-pollinated *Magnolia ovata* as follows: how closely are floral rhythm, thermogenesis and scent emission correlated and how do these phenomena work together to attract the beetles and make them effective pollinators? What features of the dynastid scarab beetle pollination system of Neotropical *Magnolia* species are important to understand the development of large flowers in *Magnolia*?

It is proposed that, although *Magnolia ovata* and other Neotropical magnolias are highly specialized in regard to their floral biology, they nevertheless can be considered to be basal members of the genus. In the light of this apparent contradiction, a new interpretation of the *Magnolia* flower is presented.

Materials and methods

Plant material

Magnolia ovata is distributed from São Paulo State in the South, to the northern Brazilian states of Minas Gerais, the Federal District and former Goiás (Frodin and Govaerts, 1996). The species was studied at three localities in the municipality of Botucatu (city coordinates 22°53′9″S, 48°26′42″W) and at one locality in the adjacent municipality of Pardinho (city coordinates 23°4′52″S, 48°22′25″W). The four localities are in the interior of São Paulo State, about 230 km (Botucatu) and 200 km (Pardinho) northwest of the city of São Paulo. Data were gathered during 15 flowering seasons between 1970 and 2010; in earlier years, observations were more sporadic, but intensified in the years 2007, 2008 and 2009. Voucher specimens were deposited in the herbaria of Brasília (UB), Botucatu (BOTU) and Ulm (ULM).

Study sites and their vegetation

Magnolia ovata grows in very wet, permanently waterlogged places along small streams and is one of the dominant large species in the community reaching a height of 20–25 m and a trunk circumference of 150 cm or more at breast height; buttresses and aerial knee roots probably function as pneumatophores. It is the only *Magnolia* species occurring in the eastern part of Brazil, growing mainly in southern forests of the Atlantic forest biome, which currently stretches over 27° of latitude along the Brazilian coastline. Today over 92% of its original area (1,363,000 km^2 in pre-Columbian times) has been cleared for agriculture, livestock farming and other uses, and the remaining forests are highly fragmented. The area also has the highest human population density in the country, with about 106 million people living in the region (Hirota, 2003). The natural vegetation in regions of Botucatu and Pardinho has been destroyed in many areas. Both municipalities occur on the top of Serra de Botucatu with irregular topography and under a cool climate, which latter apparently has slowed-down vegetation loss.

The three study sites in the surroundings of Botucatu were: (1) a gallery forest called "Mata Pinheirinho", named after the gymnosperm *Podocarpus sellowii* (Podocarpaceae), growing in this forest, 7 km west of Botucatu, close to the airport (altitude ca. 800 m); (2) a gallery forest near the Universidade Estadual Paulista, Campus de Botucatu, village of Rubiao Junior (ca. 950 m); (3) a gallery forest about 2 km NW of the campus (ca. 950 m). These evergreen gallery forests are separated by marshy vegetation with a dominant grassy layer from cerrado vegetation (semideciduous xeromorphic woodland; see, e.g., Gottsberger and Silberbauer-Gottsberger, 2006). A different site, used by Seymour et al. (2010), is close to the city of Pardinho at a private property called "Sitio Palmeiras" and its surroundings, altitude ca. 850 m. This wet forest, along a small stream, was formerly part of a continuous evergreen forest; it is now low secondary forest, bordered by marsh shrubs and pastures.

The climate of the Botucatu and Pardinho regions is characterized by an average yearly precipitation of about 1300 mm. A warm rainy season from October to March, alternates with a cooler dry season from April to September. The yearly average temperatures lie between 19 and 21 °C (Gottsberger and Silberbauer-Gottsberger, 2006).

Typical tall and low woody elements of the "Pardinho" and "Botucatu" forests, growing with *Magnolia ovata*, are *Drimys brasiliensis* (Winteraceae), *Vochysia tucanorum* (Vochysiaceae), *Syagrus romanzoffianum* (Arecaceae), *Hedyosmum brasiliense* (Chloranthaceae), representatives of the genera *Roupala* (Proteaceae), *Erythroxylum* (Erythroxylaceae), *Ilex* (Aquifoliaceae), *Bauhinia* (Caesalpiniaceae), *Esenbeckia* (Rutaceae), and also species of Myrtaceae, Lauraceae, Bignoniaceae, Fabaceae and Malpighiaceae. Typical epiphytes are *Philodendron selloum* (Araceae), *Tillandsia* spp. (Bromeliaceae), *Peperomia* spp. (Peperomiaceae) and several small orchids (e.g., *Phymatidium delicatulum*). Other woody elements in the "Mata Pinheirinho" in Botucatu are *Podocarpus sellowii* (Podocarpaceae), *Geonoma schottiana* (Arecaceae) and *Posoqueria longiflora* (Rubiaceae).

When we started our observations 40 years ago, the forest canopy at all sites had a height of ca. 20–25 m including *M. ovata*. Currently these forests have been partially cut and burned and the forest remnants are now mostly only 6–10 m tall (secondary forests), with *Magnolia* species not exceeding this height.

Flowering and fruiting of populations, characteristics of flowers and their anthesis

Duration and variation of flowering was noted over the years of study. Sizes, weight and morphology of buds and flowers were determined. The color chart of Kornerup and Wanscher (1961) was used to determine flower color. The position of flowers and young and old fruits, and the duration of fruit ripening were recorded. The opening and closing rhythm of flowers in the pistillate and staminate stages and the regularity or irregularity of these processes were observed. Functionality of pistils was assessed by observing the beginning and end of stigmatic exudate formation, and the effective function of the stamens was recorded based on the accessibility of pollen to insect visitors. Observations of the development of flowers and their anthesis are described by Gibbs et al. (1977) but are not totally congruent with our observations. For a better

understanding of floral biology and pollination, five different floral stages are distinguished, pre-pistillate, pistillate, pre-staminate, staminate, late-staminate stage.

Thermogenesis

Flower temperature measurements commenced in 2007 and more sophisticated methods were used in 2009. In 2007, 2008 and 2009 automatic temperature loggers connected with thermocouples were used to record the floral heat in relation to ambient temperature. The thermocouple was inserted into the tissue of the basal part of the inner petals, the region of the flowers with the highest temperatures; the air temperature probe hung freely at the same height. Flowers and equipment were protected from rain. In 2007 and 2008, a temperature logger (Bioblock Scientific 16200, Portugal) was used and measured the temperature every 15 min throughout the life of a flower. In 2009 another logger (Temperaturmessgerät PCE-T395, Paper-Consult Engineering Group, Germany) was used, and measurement intervals were 10 min. In 2009 additional thermocouples were inserted into buds of *M. ovata*, prior to opening. The probes were inserted into the base of the petals and into the gynoecium to measure the temperatures of central portions of the flower. Long-term respirometry and thermometry were performed and measurements were taken throughout the pistillate and staminate phases of anthesis in six flowers at 2 min intervals (for details of equipment, methods and results see Seymour et al., 2010).

Scent sampling and GC–MS analyses

Floral scent was collected from flowers using dynamic headspace methods, from five different developmental stages. Based on previous observations and experiments, the pre-pistillate stage commenced 1 or 2 days before flower opening (in case the buds produced scent) and ended early in the evening of the day the flowers opened (ca. 18:30 h). The pistillate stage commenced the first night, at ca. 19:00 to 24:00 h, and the pre-staminate stage at 24:00 h of the first night of flowering, until ca. 18:30 h of the evening of the second night. The staminate stage lasted from ca. 18:30 to 21:00 h of the second night and the late-staminate stage from ca. 21:00 h until midnight. These scent-collecting periods are in concordance with floral rhythm, thermogenesis and activity of pollinators.

Scent was collected from single flowers *in situ*, or for technical reasons from picked flowers placed in water (*ex situ*). Previous observations indicated that pollinators approached picked flowers in the same way as flowers on the trees. There was also no perceptible difference in scent intensity or quality of flowers on the trees versus picked flowers. A total of eight flowers were used for scent collection; one of these flowers (F3) was used for its scent production in three different flowering stages. The other flowers, all from different individuals, were sampled only once. Volatiles were trapped for 2 min in an adsorbent tube, which was inserted into the flower opening, using a membrane pump (G12/01 EB, ASF Thomas, Inc.) with a flow rate of 200 ml min^{-1}. Flowers were not bagged for scent accumulation, because the pistillate and staminate, and also other stages, were strongly scented. The adsorbent tube was filled with a mixture of 1.5 mg Tenax-TA (mesh 60–80; Supelco, Bellefonte, Pennsylvania, USA) and 1.5 mg Carbotrap (mesh 20–40; Supelco, Bellefonte, Pennsylvania, USA). To distinguish between plant volatiles and ambient contaminants, surrounding air was collected for comparison.

The scent samples were analyzed on a Varian Saturn 3800 gas chromatograph (GC) fitted with a 1079 injector and a ZB-5 column (5% phenyl polysiloxane, length 60 m, inner diameter 0.25 mm, film thickness 0.25 μm, Phenomenex), and a Varian Saturn 2000 mass spectrometer (MS). The adsorbent tubes were inserted via Varian's Chromatoprobe into the GC injector (Amirav and Dagan, 1997). The injector split vent was opened, and the injector was heated to 40 °C to flush any air from the system. After 2 min the split vent was closed and the injector heated 200 °C min^{-1} to 200 °C, then held at 200 °C for 1.7 min, after which the split vent was opened and the injector heated to 250 °C (to condition the adsorbent tubes for further scent collections) until the end of the run. Electronic flow control was used to maintain a constant helium carrier gas flow rate (1.0 ml min^{-1}). The GC oven temperature was held for 4.5 min at 40 °C, then increased by 6 °C min^{-1} to 260 °C and held for 3 min at this temperature. The mass spectra were taken at 70 eV with a scanning speed of 1 scan s^{-1} from m/z 30 to 350.

Analysis of data was performed using the Saturn Software package 5.2.1. For structure assignments of floral scent components the data bases NIST 08 and MassFinder 3 were used. Identifications were confirmed by comparison with published retention times (Adams, 2007). Identification of some compounds was also confirmed by comparison of mass spectra and retention times with those of authentic standards. We estimated the total absolute amount of scent trapped by injecting known amounts of monoterpenoids, benzenoids, and fatty acid derivatives. The mean response of these compounds (mean peak area) was used to determine the total amount of scent in the samples (Dötterl et al., 2005). For a visualization of scent-emitting tissues the neutral red test was conducted (Vogel, 1962).

Behavior of pollinating beetles and seed-dispersing birds

Beetles were observed with regard to their approach to flowers and their behavior inside pistillate and staminate stage flowers. In particular they were observed when licking or gnawing of floral parts and mating. Emphasis was given to their departure from flowers and movements between flowers of different stages. The behavior of seed-eating birds was observed on trees with opening fruits.

Biotests with beetles using synthetic methyl 2-methyl butyrate

For tests of scent-related beetle attraction, two paper model flowers with scent and a third dummy without scent were used. On October 30, 2009 two of these artificial flowers (white paper cones), that superficially imitated the form and size of a *M. ovata* flower, were installed below a small, not yet flowering tree. Each cone received one drop (ca. 0.1 ml) of methyl 2-methyl butyrate (Sigma–Aldrich, ≥98%). This substance is very volatile and disappears in a minute, so that new drops had to be added several times in order to have scent dummies emitting amounts of scent comparable to that of a few *M. ovata* flowers (as determined by the human nose). Occasionally, a mixture of methyl 2-methyl butyrate with glycerine was used, which somewhat delayed the dissipation of the synthetic scent. Experiments started at 19:15 h and lasted until ca. 21:00 h. In subsequent nights in November 2009, the tests were repeated, but under flowering trees.

Results

Flowering and fruiting of populations, flower characteristics and anthesis

Based on 15 years of observation at four localities, start of *Magnolia ovata* flowering usually occurs in the middle of October and it ends early in January. Flowering periods vary from year to year and at sites on slightly different altitudes. In addition, a preceding dry season, whether warmer or cooler and/or more humid or more

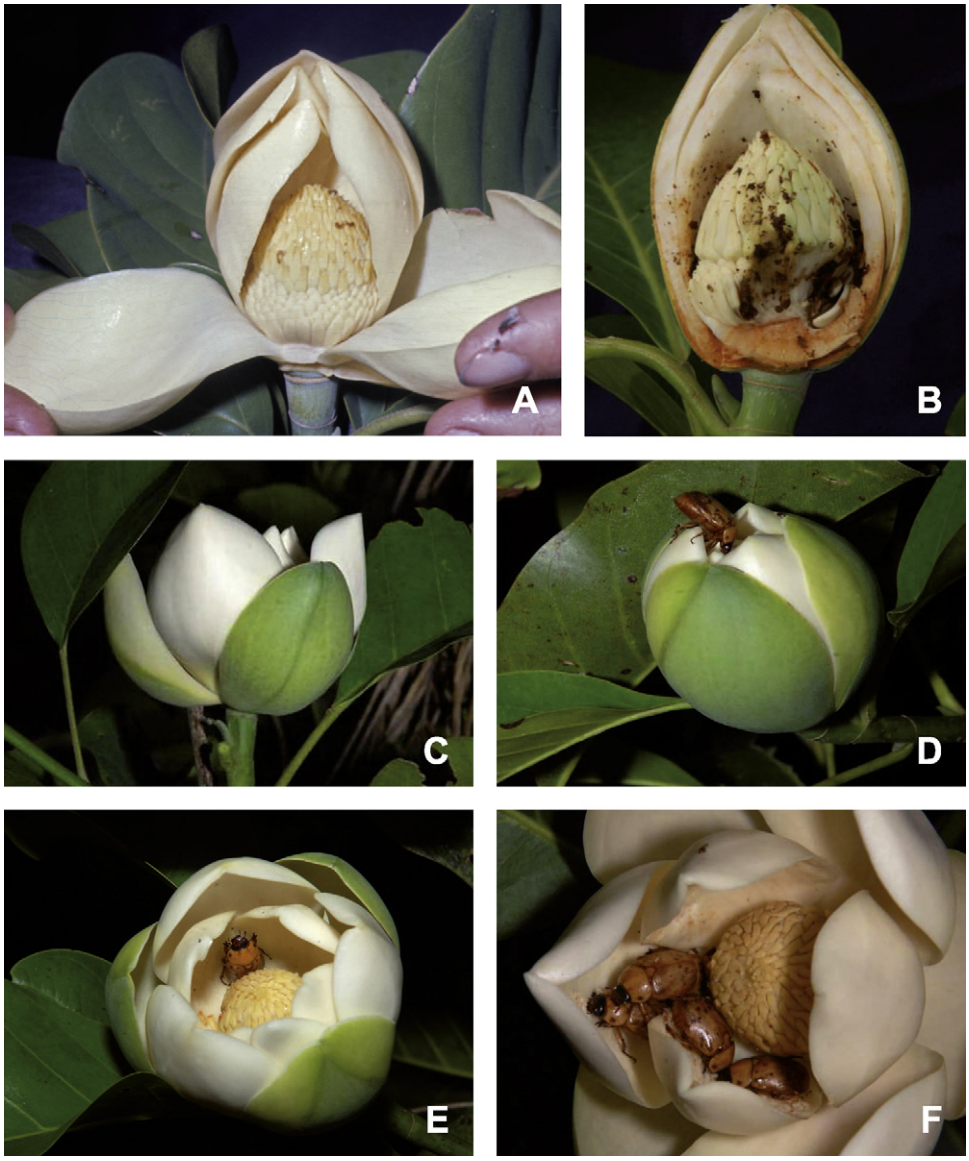

Fig. 1. *Magnolia ovata* flowers. Size (length) of flowers with peduncle ca. 6 cm and diameter of totally open flowers with expanded petals ca. 13 cm. (A) A flower at the beginning of the pistillate stage held open to show the reproductive organs with the receptive stigmas covered with transparent, viscous exudates. (B) Longitudinal cut of a large bud showing the relatively thin sepals and thick, fleshy petals. Interior partly gnawed by predatory insects. (C) Flower in the pistillate stage, already partly open, with green sepals and whitish petals. (D) An individual of *Cyclocephala literata* squeezing through the small opening of the petal tips into a pistillate stage flower. (E) A beetle inside the pollination chamber of an open pistillate stage flower gnawing on the inner side of the internal petals. (F) Several *C. literata* individuals in the floral chamber of a wide open pistillate stage flower gnawing on the petals. The two beetles at the left initiating to mate.

dry, might have influenced in different years earlier or later initiation and ending of flowering. In the year 2009, at the Pardinho site, the population of ca. 15 flowering individuals growing along a small stream (600 m length), started flowering on October 24 and finished already November 27. In most years the main flowering period was registered at all four sites in November. Flowering duration of an individual depended on the age and size of a tree. When forests were more intact, with thick-stemmed large trees of *M. ovata*, an individual would produce a total of 20–30 flowers that opened in successive waves.

Flower buds and open flowers are in a more or less upright position, however, always slightly inclined and occasionally nearly horizontally oriented. An opened flower with its peduncle is ca. 6 cm long and the length of the peduncle is ca. 1 cm. The perianth consists of three trimerous, alternating whorls (Fig. 1A–C). The outer whorl of sepals is green colored outside and the sepals have a length of ca. 5 cm and a width of ca. 4 cm. The outer petals when extended are larger (ca. 5.5–6 cm length) than the inner ones (ca. 5 cm). Following the color chart, the petals are close to pale yellow (1A3); other good descriptions of its color would be ivory white or creamy white. The petals are relatively thick and fleshy (Fig. 1B). Whole flowers in the pistillate ($n = 4$) and staminate ($n = 5$) stages were weighed, the whole flower and the stamens (mean 135 stamens per flower) separately, and also separately the receptacle and gynoecium. There were no significant differences between the masses of the whole flowers, their perianth, anthers and gynoecia between the pistillate and staminate stages, although the average weight of pistillate stage flowers was slightly higher (mean 48.5 g) than that of staminate stage ones (mean 46.4 g), principally based on a higher perianth weight in pistillate stage flowers.

Before the flowers open, several bracteoles detach. Then the green sepals separate slightly and the whitish-yellowish petal tips become visible. This is an indication that the petals will open soon and anthesis will be initiated. In warm weather this normally

Fig. 2. *Magnolia ovata* flowers. Length ca. 6 cm and diameter of totally open flowers with expanded petals ca. 13 cm. (A) Flower in staminate stage. Petals are partly open, the dehisced stamens are detached with pollen on the petals. A *Cyclocephala literata* individual feeds on pollen. Note white pollen sticking to the beetle's body. (B) A totally open flower in the late-staminate stage with expanded perianth and dehisced and fallen off stamens. One beetle is feeding on pollen. Note the gnaw marks of beetles principally on the inner side of the inner petals. (C) A post-staminate stage flower with the inner and outer petals and sepals gnawed and partly destroyed by the beetles. (D) Ripe woody fruit splitting and exposing its seeds with fleshy red sarcotesta (length of fruit ca. 15 cm).

occurred during the evening of the following day, however, during colder weather with relatively cold nights (a common phenomenon in the region of the Serra de Botucatu in October and November), flower opening could be delayed for another day. In the afternoon following initiation of flowering, the sepals spread and expose the still closed petals, which by continuous growth became larger and longer than the sepals. In the evening, the six petals became loose at their tips and open sometimes in 15 min, revealing the gynoecium (pistillate stage). The separation of petals from each other opens an entrance to the flower that varies from 1 to 4 cm in diameter. The opening in some flowers begins shortly after sunset (~19:30 h at the site in November 2009). Only a few flowers open later (23:00 h). In the first evening of anthesis, the white stigmas are brilliant with non-sweet, resin-like exudates (Fig. 1A); sticky exudates are also produced by the inner surface of the petals. In this pistillate stage the stamens are closed and enclosed by the bases of the petals. In the pistillate stage petals start closing approximately 2 h after opening in a staggered sequence. Petals close at the tip so tightly, that these re-closed flowers can be confused with pre-anthetic flowers. Later, during the second part of the first night, the pistillate stage ends, and no longer an exudate is produced, so that stigmas and petal surfaces are dry.

With re-opening at approximately the same time as in the first night flowers enter the staminate stage in the following evening (Fig. 2A–C). Petal opening in the staminate stage occurs slower than in the pistillate stage, and lasts ca. 1 h. After approximately 1–1½ h most staminate stage flowers have their petals totally open and horizontally oriented. During this opening process, the stamens detach from the receptacle and cling to the expanded petals, dehisce and expose their pollen. The late-staminate stage lasts from about 21:00 h until midnight. Flowers continue remaining in this stage during part of the next day until the sepals and petals finally drop off.

Fruits need 8–12 months to ripen. In November 2009 we observed opening of two ripe fruits that were produced in the previous flowering period (October–November 2008). The flowering

period of October and November 2009 resulted in ripe fruits in June and July 2010 (personal comm. Oswaldo Rodrigues), which is after 8–9 months, respectively. Fruits begin to develop in an erect gynoecium that becomes pendulous as it grows heavier. After 5–6 months, the fruits attain their final size, but are still green. When ripe, the receptacle and the fruit wall, consisting of concrescent biovulate carpels, become dry and woody and the fruit finally splits in an irregular star-like structure exposing the red seeds (Fig. 2D). Their brilliant color comes from a fleshy red sarcotesta. Upon opening, the seeds dangle from the open fruit, attached to it by the stretched and extended annular thickenings of protoxylem vessels.

Floral thermogenesis

Two methods of measuring thermogenesis were used and gave congruent results. Of all the floral parts, the highest temperatures recorded were in the inner petals. E.g., a flower measured in November 2, 2008, in the pistillate stage, reached peak petal temperatures of 25.3–26.7 °C between 19:20 and 20:20 h, with an ambient air temperature of 20.7 °C. The warming peak of the petals during the pistillate stage thus reached 6 °C. The same flower in the staminate stage reached peak petal temperatures of 28.4–31.9 °C (ambient air 21.3 °C) between 19:20 and 20:40 h the following evening. This is an even higher maximum heating of 10.6 °C (long-term thermometry is shown for one flower in Fig. 3).

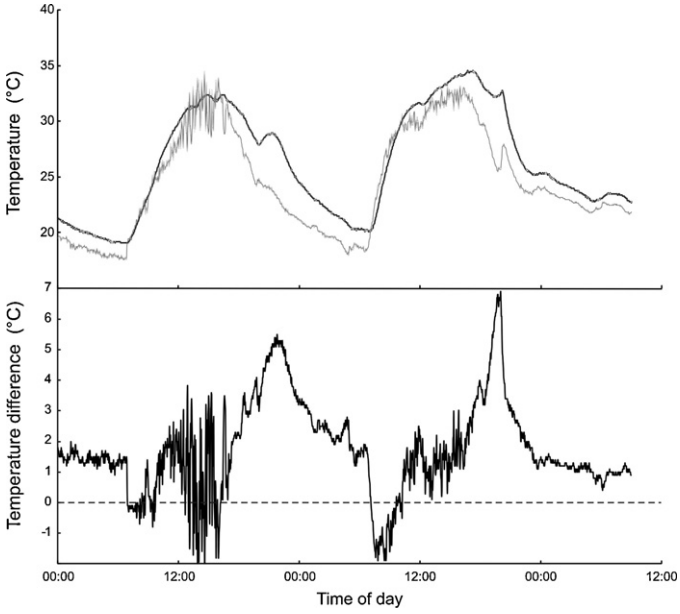

Fig. 3. Temperature of the receptacle (black), which represent the integrated interior of the flower, in relation to exterior temperature (grey) outside of the petals. The difference between these temperatures is shown below and indicates the timing of the two major episodes of thermogenesis in the pistillate stage on the first day and the staminate stage on the second day.

Flower scent

An aromatic and fruity scent is perceptible in buds, intensified strongly with the onset of anthesis, both in the pistillate and staminate stages. In the pistillate stage, the scent can be described as smelling fruit-like, reminiscent of apples and/or cherimoya (*Annona cherimoya*) and melon fruits. In the staminate stage the flowers also smell like fruits, but additionally like lemon grass (*Cymbopogon citratus*). A neutral red test indicated the inner surface of the inner petals produced the strongest scent emissions, followed by the outer petals that apparently produced lower amounts of scent.

The total amount of scent trapped using dynamic headspace varied among flowering stages and also among replicates within specific stages. 1–2 μg per 2 min and flowers were trapped in the pre-pistillate, 3–83 μg in the pistillate, 1 μg in the pre-staminate, 16–70 μg in the staminate, and 8 μg in the late-staminate stage.

In total 54 different compounds were detected in the samples, but only nine contributed more than 5% to the total scent of any sample, and only these compounds are listed in Table 1 (see Appendix A for a list of all components). Among them are two C5-branched chain compounds, one aliphatic, one benzenoid, and five monoterpenoids. All samples collected during the pistillate and staminate stages were strongly dominated (61–100% of total scent)

Table 1
Total and percentage amount of scent compounds found in scent samples collected from different flowering stages of eight *Magnolia ovata* flowers (F1–F8). The daytime time of the scent collection is also given. Only compounds that contribute at least in one sample more than 5% to the total amount are given. A detailed table comprising all the compounds detected in the samples can be found as Appendix A. tr.: the percentage amount was less than 0.5%. Values above 5 are printed in bold.

Time of scent collection (start)	Pre-pistillate			Pistillate			Pre-staminate	Staminate		Late-staminate
	F1[b,c] 18:00	F2[d,e] 22:00	F3[d,e] 23:00	F4[d] 20:20	F3[d] 20:30	F5[b] 21:00	F6[b] 18:05	F7[d] 20:10	F8[b] 18:45	F3[d] 21:00
Total amount of scent trapped (μg per 2 min)	1	1	2	3	83	13	1	70	16	8
C5-branched chain compounds										
Methyl 2-methyl butyrate[a]	–	**37**	**28**	**61**	**99**	**84**	**28**	**100**	**89**	**40**
2-Methylbutanoic acid	–	–	–	–	1	**8**	tr	–	**7**	**15**
Aliphatics										
Methyl hexanoate	–	1	4	**29**	tr	2	**9**	tr	1	1
Benzenoids										
Methyl benzoate[a]	–	–	–	tr	tr	tr	–	tr	1	**7**
Monoterpenoids										
α-Pinene[a]	**100**	–	–	3	–	–	–	–	–	**11**
α-Terpinene	–	**7**	4	1	tr	1	tr	tr	–	tr
p-Cymene[a]	–	**35**	**26**	–	–	3	–	–	–	–
γ-Terpinene[a]	–	**17**	**14**	2	tr	1	–	tr	–	1
Linalool[a]	–	–	–	tr	tr	tr	**39**	tr	tr	**19**
∑	**100**	**98**	**76**	**96**	**100**	**99**	**76**	**100**	**98**	**95**

[a] Identification of compounds is based on authentic standards.
[b] *In situ* collection.
[c] Scent was collected 2 days before flower opening.
[d] *Ex situ* collection.
[e] Scent was collected 1 day before flower opening.

by methyl 2-methyl butyrate, a C5-branched chain compound. In addition to this, other stages emitted other compounds high in relative amounts, including α-pinene, *p*-cymene, and γ-terpinene in the pre-pistillate stage, methyl hexanoate in the pistillate stage, linalool in the pre-staminate stage, and 2-methylbutanoic acid, α-pinene as well as linalool in the late-staminate stage. One sample collected from a pre-pistillate stage, 2 days before flower opening did not yet contain methyl 2-methyl butyrate.

Behavior of pollinating beetles

The only beetle species attracted and visiting the flowers of *Magnolia ovata* during all years was *Cyclocephala literata* Burm. (Dynastinae, Scarabaeidae; Figs. 1D, F and 2A, B). Approximately 1 h after sunset (air temperature between 20 °C and 21 °C) the beetles begin to fly around the flowering trees and visit the flowers. The period of most movement activity of *C. literata* (flying and visiting *M. ovata* flowers) lasted approximately 1¼ h (between 19:45 and 21 h), a period of time which correlated with maximum scent emissions. The beetles were most actively flying from flower to flower, in some nights "like bees", i.e., they did not stay long in a flower, but frequently switched to another flower. They indiscriminately visited pistillate and staminate stage flowers, changing from one stage to the other or moving between flowers of the same sexual stage. During this period of most movement activity the beetles more and more assembled in the flowers (Fig. 1F), especially in the pistillate stage (up to 6 or 8 individuals), and were observed to mate, to lick on stigmas and petal exudates, and to gnaw on the tissue of the inner petals. The feeding activity, especially when several beetle individuals were involved, caused notable damage to petal tissue (Fig. 2B and C). In the staminate stage flowers, before leaving or upon visiting them anew, the beetles ate pollen (Fig. 2A). After 21 h, flight- and flower changing-activity of the beetles diminished, until late in the night (observed until 23 h). Beetles inside the first-night pistillate flowers become trapped for the next period of time, when the petals close tightly. Inside these closed flowers the beetles continue licking exudates (as long as they are available), feed on petal tissue, and mate (Fig. 1F). Sometimes flowers in this stage have a hole at the tip of the closed petals, possibly allowing some beetles to escape from the chamber. In the next evening, upon flower opening (staminate stage), the beetles are finally released. When entering other flowers they eat pollen (in staminate stage flowers), and feed on nutritious tissue in newly opened pistillate stage flowers, and continue mating with new partners. Their smooth bodies carry the sticky pollen grains. The pollen has small droplets of pollenkitt; beetles additionally became sticky from exudates produced by stigmas and petals.

Flowers visited by beetles sometimes had their inner and outer petals severely damaged and largely eaten, and with the interior full of stinking detritus and the beetles' excrement (Fig. 2B and C). However, the gynoecium of *M. ovata* was never gnawed by the beetles. Occasionally, a gynoecium was partly eaten and destroyed by other insects (Fig. 1B), that obviously found their way into the flowers; buds were frequently pierced by insects and with unidentified larvae feeding on the gynoecium and stamens.

Attraction of beetles to synthetic methyl 2-methyl butyrate

On 5 days in October and November 2009 attempts were made to attract the pollinating beetles with synthetic methyl 2-methyl butyrate, the main compound of the scent of *Magnolia ovata*. On October 30 at 20:15 h one individual of *Cyclocephala literata* approached one of the two scented model flowers and settled on it. In this night there were no open flowers on any *M. ovata* trees in the population. Further tests were made during November, also between 19:45 and ca. 21:00 h, the "hot phase" of beetle activity, with trees having several anthetic flowers. On November 21 and 22 successful trials with synthetic methyl-2-methyl butyrate (always only on scented model flowers) were done when four and five individuals, respectively, became attracted and settled on the scented model flowers. Because scent had to be added frequently during the experiments, the models and the dummy were positioned close to the ground at about one meter height, although the beetles showed a preference to visit flowers in the higher part of the canopy, usually at 6–10 m. Nevertheless, 10 beetles were lured in five short evening periods. Not any other visitors were attracted.

Seed-dispersal by birds

Upon ripening, the thick woody external layer of the fruit splits and the red seeds are exposed. The seeds with their red fleshy sarcotesta are very attractive to birds, their apparent seed dispersing agents, which eat them entirely. At two sites, one in Botucatu and the other in Pardinho, several species of birds (e.g., *Schistochlamys ruficapillus*, Cinnamon Tanager, *Thraupis sayaca*, Sayaca Tanager, both Thraupidae; cf. *Cnemotriccus fuscatus*, Fuscous Flycatcher, Tyrannidae) could be observed, eating the seeds. They waited until seeds were fully exposed and partly dangling on their vessel thickenings after splitting of the woody layer, and swallowed them.

Discussion

Flowering, anthesis, thermogenesis, and beetle behavior

Onset of flowering of *Magnolia ovata* in the study region occurs in mid of October and usually ends in early January; main flowering period, however, is November (for comparable sites in São Paulo and Minas Gerais States see Gibbs et al., 1977). Despite year-depending shifts in the flowering period, its duration usually does not exceed 6 weeks. It is interesting that this flowering period corresponds with the beginning of the warm rainy season, similar as in other species of the region pollinated by dynastid scarab beetles, e.g., with *Philodendron selloum*, other *Philodendron* species, *Caladium striatipes* (Araceae) (Gottsberger and Amaral, 1984; Gottsberger, 1992) and *Annona* (Annonaceae) species (e.g., *A. crassiflora*, *A. coriacea* and *A. dioica*; Gottsberger, 1989). Many dynastid beetles (tribe Cyclocephalini) develop in the soil and their larvae feed on litter and roots (García et al., 2009; Villegas et al., 2008). Obviously, the beetles finish their development at the onset of the rainy season and visit as adults specific plants, functioning as pollinators of nocturnal flowers. They have been observed emerging from the soil, e.g., below *Philodendron selloum* plants, and then fly directly to inflorescences (Gottsberger and Amaral, 1984). Therefore, there is a correlation between the appearance of these beetles and flowering of the species they visit (occasional flowering can occur in the dry season, e.g., of *Philodendron selloum*, but then no beetles visit the inflorescences: Gottsberger and Amaral, 1984).

A protogynous, two-night pattern of anthesis with thermogenesis occurs in practically all dynastid beetle-pollinated plants; as these beetles have crepuscular and nocturnal activities the plant species visited by them have adapted to this insect behavior (Beach, 1982; Gibernau et al., 1999, 2000; Gottsberger and Amaral, 1984; Gottsberger, 1989; Prance and Arias, 1975; Schatz, 1990; Seymour and Matthews, 2006; Silberbauer-Gottsberger et al., 2001, 2003; Webber, 1996). Usually, flowers visited by dynastid beetles produce heat during the first evening (pistillate stage) and attract beetles in a short interval of time, in some cases in a few minutes. Temperature is then maintained at a lower level throughout the night. It rises again above ambient temperature in the staminate stage. However, duration of (for short time more strongly elevated) high

temperature and intensity of scent emission are much lower during the second night. Long-range attraction of dynastid beetles occurs in the first night by scent, intensively volatilized by the heating at the beginning of the pistillate stage.

In *M. ovata* likewise as in other beetle-pollinated plants of the region olfactory stimuli are replaced at close-range by visual cues, and the beetles orient themselves toward the light-colored flowers (or inflorescences – Gottsberger and Silberbauer-Gottsberger, 1991). The temperature increase in the pistillate and staminate stages of a flower or inflorescence is always associated with enhancement of insect activity. In the warm flower or spathe vessel (as in Araceae), the beetles are better able to carry out their activities such as eating, digesting and competing for mates (heat is a resource). The warm floral environment during the first night permits the beetles to expend less energy to keep them warm and to promote their activities, thus saving them energy and reducing their food requirements (Seymour et al., 2003, 2009). Thermogenesis is less in the second evening and the insects are encouraged to depart with pollen loads.

There are a few documented cases in Annonaceae, in which flowers in both, the pistillate and the staminate stages produce high temperatures, e.g., reaching 15 °C in *Annona coriacea* and 10 °C in *A. cornifolia* above air in both nights (Gottsberger, 1989). On the other hand, *Magnolia ovata* is the first species known in which flower heat production is higher – and even longer in time – in the staminate than the pistillate stage (Seymour et al., 2010).

Magnolia ovata emits the same floral scent on both evenings, intensified by thermogenesis, and since the flowers of a tree and of the individuals of a population exhibit a staggered opening and closing pattern over an extended period, the same beetle individuals can repeatedly visit several flowers of both sexual stages on the same evening. This is distinct in comparison with the majority of dynastid scarab beetle-pollinated species that produce heat strongly and emitting the beetle-attracting scent compounds only in the pistillate stage, and thus receive only one load of beetles. By contrast, *M. ovata* flowers can receive several loads of beetles and pollen during the pistillate stage. The special floral rhythm of *M. ovata* and the unusual behavior of *C. literata* in flying and visiting several flowers may distinctly enhance the reproductive success of the self-compatible species.

Magnolia ovata flowers are morphologically and physiologically highly specialized, so that beetle pollination is efficiently improved. This is enabled by provision to the beetles of a place for nourishment, hiding and mating. Indeed the genetic heterozygosity of the beetles is probably increased as a result of switching mates in visits to different flowers. In particular, the petals fulfil several functions. They are thick enough to restrain for about 1 day several voracious beetles inside the floral chamber, and prevent access of beetle predators. Petals need to contain sufficient nutritious tissues to nourish the beetles during their stay, and they produce scent. Finally, they are important as the main source of energy for thermogenesis and as insulation to limit the loss of heat from the flower and keeping the beetles warm (Seymour, 2010). Such large and strongly constructed flowers also occur in other dynastid-pollinated flowers, e.g., the Annonaceae (Gottsberger, 1989).

The pollination of *Magnolia schiedeana*, a rare tree species in the cool cloud forests in Veracruz, eastern Mexico, was described by Dieringer and Espinosa (1994) and Dieringer and Delgado (1994) as having diurnal, protogynous anthesis flowers that are functional for only 1 day (opening in the morning and becoming staminate the same afternoon). The authors mentioned to have found many staphilinid beetles (*Stenagria* sp.) and a few individuals of *Cyclocephala jalapensis* inside the flowers from 9:00 to 19:00 h. On the other hand, they indicated that the beetles entered the flowers the night before and the flowers become staminate in the late afternoon, continuing to produce a strong scent and receiving pollinators throughout the second night. No measurements were made to determine if these flowers produced heat. It is possible that the authors did not document the full extent of anthesis. Their observations that beetles approached the flowers in the night hours and also that scent was strong during this period is a hint that flowers in reality might be nocturnal with a two-night rhythm; also thermogenesis is a likely but possibly overlooked character of this dynastid scarab beetle-attracting species. The staphylinid beetles appeared to be an effective pollinator in addition to *C. jalapensis*.

Magnolia tamaulipana, from the cloud forests in Tamaulipas and eastern Mexico, has distinct, protogynous, nocturnal, two-night flowers, and besides *Cyclocephala caelestis* also attracts staphylinid beetles. Heat production was very high in the pistillate stage, reaching 1.0–9.3 °C above ambient air, while staminate stage flowers reached an excess temperature ranging from 0.2 to 5.0 °C (Dieringer et al., 1999). Thus, this Mexican species and the Brazilian *M. ovata* are the only two Neotropical *Magnolia* species unequivocally documented to have protogynous, nocturnal, thermogenic, two-night flowers with dynastid scarab beetles being prominent or even the sole pollinators.

Flower scent and attraction of Cyclocephala literata *by methyl 2-methyl butyrate*

More than 50 different scent compounds were detected in *M. ovata*, but only a few of them contributed more than 5% to the total scent. The most abundant compound produced was a C5-branched chain compound, methyl 2-methyl butyrate, which is derived from amino acids (Bergström et al., 1991). This chemical was found to be behaviorally attractive to *C. literata*. Synthetic methyl 2-methyl butyrate smells to the human nose like the scent of pistillate-stage flowers, the scent of staminate stage flowers smells similarly, but has in addition the odor of lemon grass. The total amount of scent substances trapped per flower was very high (up to 80 μg per 2 min of scent collection) though flowers were not bagged for scent collection and therefore only a small amount of the scent produced and emitted was trapped. The flowers therefore emit a very high amount of scent, which seems to be higher than in other strong-scented *Magnolia* species. On a per hour basis, a maximum of 1021 μg was collected from bagged flowers of *M. dealbata*, 350 μg and 270 μg were collected from *M. mexicana* and *M. hypoleuca*, respectively (Azuma et al., 1997, 2004). The butyrate of *M. ovata* is emitted in both flowering stages, which might explain why *C. literata* shows no preference for visiting either pistillate or staminate stage flowers. Olfactorially, the two stages have the same chemical appeal to the beetles. The tests to lure *C. literata* with synthetic methyl 2-methyl butyrate were successful and demonstrate that this compound alone can attract the beetles. It remains to be tested whether also other compounds, occurring only as minor or trace constituents, play a role in attracting *C. literata* or whether this is olfactory "noise" or eventually may have some importance as repellents against predatory flower-destroying insects (Junker and Blüthgen, 2010). Apparently the flowers of *M. ovata* do not possess the "push–pull" manipulation of insect attraction and repulsion as it occurs in some cycads (Terry et al., 2007).

On the ca. 25 investigated species of *Magnolia*, analyzed for floral odors, more than 100 volatile compounds have been identified (e.g., Azuma et al., 1997, 2004; Thien et al., 1975; Yasukawa et al., 1992). Many of these compounds were also found in *Magnolia ovata* and most of the compounds identified in *M. ovata* were also found in other *Magnolia* species including methyl 2-methyl butyrate. However, in most other magnolias, methyl 2-methyl butyrate only occurs in small relative amounts (Azuma et al., 1997, 2004). Instead, they are characterized by other main compounds, such as methyl benzoate, veratrole, α-farnesene or 2-phenylethyl alcohol (Azuma et al., 1999a, 2004). Also the other two *Magnolia* species

attracting *Cyclocephala* beetles, i.e., *M. tamaulipana* (*C. caelestis*) and *M. schiedeana* (*C. jalaupensis*) both of which occur in Mexico (Dieringer et al., 1999; Dieringer and Espinosa, 1994), have a different scent composition compared to *M. ovata*. Both are dominated by the monoterpene geranyl methyl ether (85–88%; Azuma et al., 1999b), a compound not occurring in *M. ovata*, and *M. tamaulipana* as well as *M. schiedeana* do not emit methyl 2-methyl butyrate (they only emit terpenoids; Azuma et al., 1997, 2004). Only in one other *Magnolia* species, *M. coco* (distributed in Asia), methyl 2-methyl butyrate also was the most abundant compound (≥92%, Azuma et al., 2004). Though pollinators of this species are unknown, it was suggested to be nocturnally pollinated and methyl 2-methyl butyrate may also be important for attracting pollinators to flowers of this species.

Azuma et al. (1999b) mentioned the lineage of *Magnolia grandiflora*, a wide ranging species in eastern North America, and the two endemic Mexican species *M. tamaulipana* and *M. schiedeana*, all three belonging to the section *Theorhodon*, as examples for the possibility that pollinators affect the composition of floral scents. Molecular phylogenetic data (Azuma et al., 1999b) indicate a close relationship among the three species, with *M. grandiflora* having plesiomorphic characters. The floral scent of *M. grandiflora* has 25 compounds (mainly terpenoids and aliphatic esters), in which geraniol comprises 20% as the main compound (Azuma et al., 1997), and the flowers attract at least 11 species of small-sized beetles (Thien, 1974). The floral scent of the two Mexican species with the predominant single compound geranyl methyl ether, a compound not occurring in *M. grandiflora*, is considered to be an adaptation in specialized dynastid beetle pollinators (Azuma et al., 1999b). Still, evidence is missing that this compound is indeed attractive for the *Cyclocephala* pollinators. Thus, methyl 2-methyl butyrate is the first compound known to be attractive for a Cyclocephalini (dynastid) pollinator of *Magnolia*, but apparently other *Magnolia* species produce other attractive compounds.

Methyl 2-methyl butyrate not only occurs in *Magnolia* species but has been detected as a floral scent compound in several species in other plant families, e.g., Araceae, Nymphaeaceae, Brassicaceae and Orchidaceae (Knudsen et al., 2006). In *Victoria amazonica* × *cruziana* (Nymphaeaceae), a hybrid between the two Amazonian *Victoria* species, both being pollinated by *Cyclocephala* beetles, and in the Australian *Eupomatia laurina* (Eupomatiaceae), another magnolialian representative, pollinated by *Elleschodes* (Curculionidae), methyl 2-methyl butyrate [(2S)-enantiomer in *E. laurina*] was also found as the main floral scent compound (Bergström et al., 1991; Kite et al., 1991). This compound therefore may be also important in attracting *Cyclocephala* and other beetles to plants other than Magnoliaceae.

Systematic position of Magnolia species in the tropical American section Talauma and remarks on the diversification of the Magnolia flower

Studies of Cretaceous and Tertiary fossils and molecular studies have shaped and revolutionized our understanding of the diversity and phylogeny of early angiosperms, including Magnoliaceae. The fossil *Archaeanthus*, from near the Albian-Cenomanian boundary in Kansas, was the first mid-Cretaceous flower to be described in detail. Dilcher and Crane (1984) demonstrated that *Archaeanthus* was a bisexual flower with close correspondence to Magnoliaceae. Doyle and Endress (2010) showed that this plant differs from living Magnoliaceae only in having a sessile stigma (i.e., no style), which supports its position on the stem lineage of the family. It appears to be either the sister group of Magnoliaceae as a whole, or it is sister to either of the two subfamilies of the family, Magnolioideae or Liriodendroideae. This fossil also provides a minimum age of latest Albian (ca. 100 MY) for the node connecting Magnoliaceae with *Degeneria* (Degeneriaceae), *Galbulimima* (Himantandraceae), *Eupomatia* (Eupomatiaceae) and Annonaceae. Unfortunately, there is no way to know the pollination of such an old fossil, but since the living Magnoliaceae are basically beetle pollinated, as are *Degeneria*, *Eupomatia* and the Annonaceae (for *Galbulimima* cantharophily is suspected; Endress, 2010), one probably is not wrong to assume that the archaic magnoliaceous *Archaeanthus* was also a beetle-pollinated species. *Degeneria* is pollinated by small beetles (Thien, 1980), *Eupomatia* is pollinated by beetles of the genus *Elleschodes*/Curculionidae (Hamilton, 1898), and species of the early-divergent annonaceous genus *Anaxagorea* have *Colopterus* species (Nitidulidae) pollinating them (Teichert et al., 2011; Webber, 1996), thus all relatives of Magnoliaceae have relatively small-sized beetles as pollinators, which is true also for the basal members of the otherwise considerably diversified and species-rich Annonaceae (see, e.g., Gottsberger et al., 2011; Silberbauer-Gottsberger et al., 2003). On the basis of these comparative data, one tentatively can assume that the early representatives of Magnoliaceae, such as *Archaeanthus*, also had small beetles associated with their flowers, which is the more probable because large flower-visiting beetles, like Dynastinae, apparently did not exist in mid-Cretaceous times (see below).

Despite the long history of Magnoliaceae, diversification of extant members of the family apparently occurred recently, during the Tertiary. In the middle Eocene (ca. 42 MY), the tropical American section *Talauma* of *Magnolia* (31 recent spp. according to Nooteboom, 2000), to which *M. ovata* belongs, branched off first, and then both the tropical Asian group and the West Indies group diverged (Azuma et al., 2001). Azuma et al. (2001) also affirm that their data show that tropical disjunctions occurred prior to the disjunctions of temperate taxa. Nie et al. (2008) confirmed and extended the data of Azuma et al. (2001), but infer an earlier diversification of the extant magnolias at the early Eocene, at about 54.8 MY. This earlier diversification and quite complicated evolutionary history of *Magnolia* (Nie et al., 2008), correlate with the "Paleocene-Eocene thermal maximum" event, which at the start of the Eocene marks the most rapid and extreme global warming recorded in geological history (Lourens et al., 2005).

The diversification of *Magnolia* at the early Tertiary, initially by members of the tropical American section *Talauma*, allows speculations about diversification and/or some functional trends of flowers within the genus *Magnolia* as a whole. *Magnolia ovata*, the subject of the present paper and the only species of the section *Talauma* studied up to now, is pollinated by dynastid scarab beetles, and the relationship with these beetles is imprinted on its flower characteristics. We certainly can expect more species of this basal American section (or subsection according to Nie et al., 2008) of *Talauma* to be dynastid beetle-pollinated and showing similar characteristics as *M. ovata.* The above-mentioned two Mexican taxa (*M. tamaulipana* and *M. schiedeana*) although belonging to a different lineage, in a convergent way also show the characteristics of dynastid scarab beetle-pollinated magnolias.

The flower-visiting members of the tribe Cyclocephalini (subfamily Dynastinae, family Scarabaeidae) (to which the genus *Cyclocephala* with more than 300 species belongs) is nearly exclusively American, with only one species occurring in Africa (Endrödi, 1985). The relatively modern subfamilies of Scarabaeidae, the more night-active Dynastinae and the more day-active Cetoniinae, both with many known flower-visiting species, do not seem to have evolved before the Tertiary (Crowson, 1981, and pers. comm. in 1987). Therefore, this is the earliest they could have assumed their role as pollinators of Magnoliaceae and other groups. Based on the data available, it can be hypothesized that *Magnolia* evolution

started in the early Tertiary in tropical America and in association and under the influence of flower-visiting Cyclocephalini, most probably of the genus *Cyclocephala.*

It has always been a riddle to explain the large and robust *Magnolia* flowers of temperate and tropical Southeast Asian members of this genus on the basis of their relatively small pollinating beetles (mainly Nitidulidae, Staphylinidae, Chrysomelidae, Curculionidae, Oedemeridae, Scraptiidae, 2–5 mm in length; e.g., Ishida, 1996; Thien, 1974), flies and bees, which both eat or collect pollen grains, but do not further harm the flower. The large and robust magnolia flower probably is best explained as being an archaic structure which stems from the initial association of tropical American species of the section *Talauma* with dynastid scarab beetles and which was maintained during diversification of the genus. Other characteristics, such as nocturnal anthesis and thermogenesis had to be abandoned by other lineages when branching off and establishing in Asia or the temperate zones, which are regions outside the range of the tropical American flower-visiting dynastid beetles.

Prominent reproductive characteristics of the early-divergent members of the genus *Magnolia* appear to be the large, robust flowers with ornithochorous seeds bearing a fleshy sarcotesta. Both characteristics apparently were maintained during the evolutionary history of the genus (for fruits and seeds and their dispersal see Nooteboom, 1993; Oppel and Mack, 2010). On the other hand, the types of beetle pollination apparently modified and adapted when *Magnolia* radiated from the Neotropics (large dynastid beetles) to the Old World tropics and temperate zones (small beetles and other insects). A re-adaptation to dynastid beetles was possible when members of the genus radiated back to the warmer American regions and the Neotropics. In this context it would be interesting and rewarding to study the floral ecology of other tropical American representatives of *Magnolia*.

Acknowledgements

This work was supported by the German Research Council (DFG), the German Federal Ministry for Education and Research (BMBF), the Australian Research Council and the Alexander von Humboldt Foundation. The first two authors additionally thank the authorities of their former university in Botucatu for support during the years of their employment (1968–1981). We greatly appreciate the cooperation of Silvia R. Machado of the Departamento de Botânica, Instituto de Biologia, Universidade Estadual Paulista, Campus de Botucatu, Brazil. We thank Robin Seymour, Cristina Mattos, Edy de Lello Montenegro, Carmen Regina Marcati, Luzia Gonçalves, José Angelo (Zico) Gonçalves, Oswaldo Rodrigues for assistance in Botucatu and Pardinho, and Marcus Braun, Ulm, for the identification of seed-eating birds from color slides. Richard Saunders, Hong Kong and anonymous reviewers have kindly commented on the manuscript. The late Sebo Endrödi, Budapest, and Brett C. Ratcliffe, Lincoln, kindly identified and confirmed the pollinating beetle species.

Appendix A.

Percentage amount of all scent compounds found in scent samples collected from different flowering stages of eight *Magnolia ovata* flowers (F1–F8). tr.: the percentage amount was less than 0.5%. ST: unknown sesquiterpenoid.

	Pre-pistillate			Pistillate			Pre-staminate	Staminate		Late- staminate
	F1	F2	F3	F4	F3	F5	F6	F7	F8	F3
C5-branched chain compounds										
Methyl 2-methyl butyrate	–	37	28	61	99	84	28	100	89	40
Methyl 2-hydroxy-2-methyl butyrate	–	–	–	1	–	–	–	tr	–	–
Methyl tiglate	–	–	–	tr	–	–	–	tr	–	–
2-Methylbutanoic acid	–	–	–	–	1	8	tr	–	7	15
Methyl 3-hydroxy-2-methyl butyrate	–	–	–	1	tr	tr	tr	tr	tr	–
Aliphatics										
Methyl 2-methyl pentanoate	–	–	–	tr	–	–	–	tr	–	–
Methyl hexanoate	–	1	4	29	tr	2	9	tr	1	1
Methyl (3Z)-3-hexenoate	–	–	–	1	tr	tr	tr	tr	tr	–
Methyl heptanoate	–	–	–	1	tr	–	tr	–	tr	–
Methyl (2E)-2-heptenoate	–	–	–	–	–	–	–	–	tr	–
a Methyl octenoate	–	–	–	tr	–	tr	tr	–	tr	–
Methyl ocatanoate	–	–	tr	1	tr	tr	tr	tr	tr	–
Benzenoids										
Methyl benzoate	–	–	–	tr	tr	tr	–	tr	1	7
Veratrole	–	–	–	–	–	–	tr	–	tr	–
Phenylpropanoids										
Eugenol	–	–	–	tr	–	–	–	–	–	3
N-bearing compounds										
2-Aminobenzaldehyde	–	tr	tr	–	–	tr	tr	–	tr	–
2-Aminobenzyl alcohol	–	–	–	–	–	–	tr	–	tr	–
Homoterpenes										
(E)-4,8-Dimethyl-1,3,7-nonatriene	–	–	–	–	–	–	tr	tr	tr	tr
Monoterpenes										
α-Pinene	100	–	–	3	–	–	–	–	–	11
β-Myrcene	–	1	1	tr	tr	tr	1	tr	tr	tr
α-Terpinene	–	7	4	1	tr	1	tr	tr	–	tr
p-Cymene	–	35	26	–	–	3	–	–	–	–
Limonene	–	–	–	–	–	tr	2	–	tr	–
(E)-β-Ocimene	–	–	–	tr	tr	–	2	tr	tr	1
γ-Terpinene	–	17	14	2	tr	1	–	tr	–	1
(Z)-Linalool oxide furanoid	–	–	–	–	–	tr	tr	tr	tr	–
(E)-Linalool oxide furanoid	–	–	–	tr	tr	tr	2	tr	tr	tr
Linalool	–	–	–	tr	tr	tr	39	tr	tr	19

	Pre-pistillate			Pistillate			Pre-staminate	Staminate		Late- staminate
	F1	F2	F3	F4	F3	F5	F6	F7	F8	F3
Sesquiterpenes										
δ-Elemene	–	–	tr	–	–	–	–	tr	tr	–
α-Cubebene	–	tr	tr	–	–	tr	tr	tr	tr	–
α-Ylangene	–	–	–	–	–	–	1	–	tr	–
α-Copaene	–	tr	4	tr	tr	tr	2	tr	tr	tr
ST, *m/z*: 105, 77, 161, 81, 79, 91	–	tr	1	–	–	tr	–	–	tr	–
(Z)-α-Bergamotene	–	tr	tr	–	–	tr	1	tr	tr	–
α-Santalene	–	tr	tr	tr	–	tr	1	tr	tr	tr
β-Caryophyllene	–	tr	1	tr	tr	tr	1	tr	tr	tr
(E)-α-Bergamotene	–	tr	1	–	tr	tr	1	tr	tr	tr
α-Guaiene	–	tr	2	tr	tr	tr	1	tr	tr	tr
α-Caryophyllene	–	tr	1	–	tr	tr	tr	tr	tr	–
9-epi-(E)-Caryophyllene	–	–	–	–	–	tr	tr	–	tr	–
ar-Curcumene	–	–	–	–	–	–	1	–	tr	–
γ-Muurolene	–	tr	tr	–	–	tr	1	–	tr	–
ST, *m/z*: 41, 39, 69, 91, 79, 133	–	–	1	–	tr	–	tr	tr	tr	tr
Germacrene D	–	tr	1	–	tr	tr	–	tr	tr	tr
β-Bisabolene	–	tr	4	–	tr	tr	2	tr	tr	tr
ST, *m/z*: 93, 121, 91, 79, 105, 107	–	tr	4	–	tr	–	–	tr	tr	tr
α-Bulnesene	–	tr	1	–	tr	–	tr	tr	tr	tr
ST, *m/z*: 161, 91, 105, 119, 77, 133	–	–	tr	–	–	–	tr	–	tr	–
δ-Cadinene	–	tr	1	–	tr	tr	1	tr	tr	tr
(E)-γ-Bisabolene	–	tr	1	–	tr	–	tr	tr	tr	tr
α-Calacorene	–	–	tr	–	–	–	1	–	tr	–
β-Calacorene	–	–	tr	–	–	–	1	–	tr	–
ar-Bisabolol	–	–	–	–	–	tr	tr	–	tr	–
ST, *m/z*: 161, 105, 204, 81, 162, 119	–	–	–	–	–	–	tr	–	tr	–

References

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Allured Publishing Corporation, Carol Stream, IL.

Amirav, A., Dagan, S., 1997. A direct sample introduction device for mass spectrometry studies and gas chromatography mass spectrometry analyses. Eur. Mass Spectrom. 3, 105–111.

Azuma, H., et al., 1997. Chemical divergence in floral scents of *Magnolia* and allied genera (Magnoliaceae). Plant Species Biol. 12, 69–83.

Azuma, H., Thien, L.B., Kawano, S., 1999a. Floral scents, leaf volatiles and thermogenic flowers in Magnoliaceae. Plant Species Biol. 14, 121–127.

Azuma, H., Thien, L.B., Kawano, S., 1999b. Molecular phylogeny of *Magnolia* (Magnoliaceae) inferred from cpDNA sequences and evolutionary divergence of the floral scents. J. Plant Res. 112, 291–306.

Azuma, H., García-Franco, J.G., Rico-Gray, V., Thien, L.B., 2001. Molecular phylogeny of the Magnoliaceae: the biogeography of tropical and temperate disjunctions. Am. J. Bot. 88, 2275–2285.

Azuma, H., Rico-Gray, V., García-Franco, J.G., Toyota, M., Asakawa, Y., Thien, L.B., 2004. Close relationship between Mexican and Chinese *Magnolia* (subtropical disjunct of Magnoliaceae) inferred from molecular and floral scent analyses. Acta Phytotax. Geobot. 55, 167–180.

Beach, J.H., 1982. Beetle pollination of *Cyclanthus bipartitus* (Cyclanthaceae). Am. J. Bot. 69, 1074–1081.

Bergström, G., Groth, I., Pellmyr, O., Endress, P.K., Thien, L.B., Hübener, A., Francke, W., 1991. Chemical basis of a highly specific mutualism: chiral esters attract pollinating beetles in Eupomatiaceae. Phytochemistry 30, 3221–3225.

Crowson, R.A., 1981. The Biology of the Coleoptera. Academic Press, London.

Dieringer, G., Delgado, L., 1994. Notes on the biology of *Cyclocephala jalapensis* (Coleoptera, Scarabaeidae): an endemic of eastern Mexico. Southwest. Entomol. 19, 309–311.

Dieringer, G., Espinosa, S.J.E., 1994. Reproductive ecology of *Magnolia schiedeana* (Magnoliaceae), a threatened cloud forest tree species in Veracruz, Mexico. Bull. Torrey Bot. Club 121, 154–159.

Dieringer, G., Cabrera, L., Lara, M., Loya, L., Reyes-Castillo, P., 1999. Beetle pollination and floral thermogenicity in *Magnolia tamaulipana* (Magnoliaceae). Int. J. Plant Sci. 160, 64–71.

Dilcher, D.L., Crane, P.R., 1984. *Archaeanthus*: an early angiosperm from the Cenomanian of the western interior of North America. Ann. MO Bot. Gard. 71, 351–383.

Dötterl, S., Wolfe, L.M., Jürgens, A., 2005. Qualitative and quantitative analyses of flower scent in *Silene latifolia*. Phytochemistry 66, 203–213.

Doyle, J.A., Endress, P.K., 2010. Integrating early cretaceous fossils into the phylogeny of living angiosperms: Magnoliidae and eudicots. J. Syst. Evol. 48, 1–35.

Endress, P.K., 2010. The evolution of floral biology in basal angiosperms. Phil. Trans. R. Soc. B 365, 411–421.

Endrödi, S., 1985. The Dynastinae of the World. Akadémiai Kiadó, Budapest.

Figlar, R.B., 2006. Taxonomy topics – a new classification for *Magnolia*. In: Royal Horticultural Society (Ed.), Rhododendrons with Camellias and Magnolias. MPG Books Ltd., Bodmin, Cornwall, pp. 69–82.

Figlar, R.B., Nooteboom, H.P., 2004. Notes on Magnoliaceae IV. *Blumea*. J. Plant Taxon. Plant Geogr. 49, 87–100.

Frodin, D.G., Govaerts, R., 1996. World Checklist and Bibliography of Magnoliaceae. Royal Botanic Gardens, Kew.

García, G.L., Ortega-Arenas, L., Hernández, H.G., García, A.A., Nápoles, J.R., Cortés, R.R., 2009. Description of Melolonthidae (Coleoptera) third instar larvae associated to *Agave tequilana* var. Azul and their population fluctuation in Jalisco Mexico. Neotrop. Entomol. 38, 769–780.

Gibbs, P.E., Semir, J., da Cruz, N.D., 1977. Floral biology of *Talauma ovata* St. Hil. (Magnoliaceae). Ciênc. Cult. 29, 1436–1441.

Gibernau, M., Barabé, D., Cerdan, P., Dejean, A., 1999. Beetle pollination of *Philodendron solimoesense* (Araceae) in French Guiana. Int. J. Plant Sci. 160, 1135–1143.

Gibernau, M., Barabé, D., Labat, D., 2000. Flowering and pollination of *Philodendron melinonii* (Araceae) in French Guiana. Plant Biol. 2, 330–333.

Gottsberger, G., 1989. Beetle pollination and flowering rhythm of *Annona* spp. (Annonaceae) in Brazil. Plant Syst. Evol. 167, 165–187.

Gottsberger, G., 1992. Diversität der Bestäubung und Reproduktionsbiologie von ursprünglichen Angiospermen. Stapfia 28, 11–27.

Gottsberger, G., Amaral Jr., A., 1984. Pollination strategies in Brazilian *Philodendron* species. Ber. Deut. Bot. Ges. 97, 391–410.

Gottsberger, G., Silberbauer-Gottsberger, I., 1991. Olfactory and visual attraction of *Erioscelis emarginata* (Cyclocephalini Dynastinae) to the inflorescences of *Philodendron selloum* (Araceae). Biotropica 23, 23–28.

Gottsberger, G., Silberbauer-Gottsberger, I., 2006. Life in the Cerrado. Vol. I. Origin, Structure, Dynamics and Plant Use. Reta, Ulm.

Gottsberger, G., Meinke, S., Porembski, S., 2011. First records of flower biology and pollination in African Annonaceae: *Isolona*, *Piptostigma*, *Uvariodendron*, Monodora and Uvariopsis. Flora 206, 498–510.

Hamilton, A.G., 1898. On the fertilisation of *Eupomatia laurina* R. Br. Proc. Linn. Soc. N. S. W. 22, 48–55.

Heiser, C.B., 1962. Some observations on pollination and compatibility in *Magnolia*. Proc. Indiana Acad. Sci. 72, 259–266.

Heywood, V.H., Brummitt, R.K., Culham, A., Seberg, O., 2007. Flowering Plant Families of the World. Royal Botanic Gardens, Kew.

Hirota, M.M., 2003. Monitoring the Brazilian Atlantic forest cover. In: Galindo-Leal, C., Camara, I.G. (Eds.), The Atlantic Forest of South America: Biodiversity Status, Threats, and Outlook. Island Press, Washington/Covelo/London, pp. 60–65.

Ishida, K., 1996. Beetle pollination of *Magnolia praecocissima* var. *borealis*. Plant Species Biol. 11, 199–206.

Junker, R.R., Blüthgen, N., 2010. Floral scent repel facultative flower visitors but attract obligate ones. Ann. Bot. 105, 777–782.

Kite, G., Reynolds, T., Prance, G.T., 1991. Potential pollinator-attracting chemicals from *Victoria* (Nymphaeaceae). Biochem. Syst. Ecol. 19, 535–539.

Knudsen, J.T., Eriksson, R., Gershenzon, J., Ståhl, B., 2006. Diversity and distribution of floral scent. Bot. Rev. 72, 1–120.

Kornerup, A., Wanscher, J.H., 1961. Taschenlexikon der Farben. Musterschmidt, Zürich-Göttingen.

Lourens, L.J., et al., 2005. Astronomical pacing of late Palaeocene to early Eocene global warming events. Nature 435, 1083–1087.

Nie, Z.-L., et al., 2008. Phylogenetic and biogeographic complexity of Magnoliaceae in the Northern Hemisphere inferred from three nuclear data sets. Mol. Phylogenet. Evol. 48, 1027–1040.

Nooteboom, H.P., 1993. Magnoliaceae. In: Kubitzki, K., Rohwer, J.G., Bittrich, V. (Eds.), The Families and Genera of Vascular Plants, vol. II. Springer, Berlin/Heidelberg/New York, pp. 391–401.
Nooteboom, H.P., 2000. Different looks at the classification of the Magnoliaceae. In: Liu, Y.-H., Fan, H.-M., Zhen, Q.-W. (Eds.), Proc. Intern. Symposium on the Family Magnoliaceae. Science Press, Beijing, pp. 26–37.
Oppel, S., Mack, A.L., 2010. Bird assemblage and visitation pattern at fruiting *Elmerrillia tsiampaca* (Magnoliaceae) trees in Papua New Guinea. Biotropica 42, 229–235.
Prance, G.T., Arias, J.R., 1975. A study of the floral biology of *Victoria amazonica* (Poepp.) Sowerby (Nymphaeaceae). Acta Amazon. 5, 109–139.
Schatz, G.E., 1990. Some aspects of pollination biology in Central American forests. In: Bawa, K.S., Hadley, M. (Eds.), Reproductive Ecology of Tropical Forest Plants. UNESCO, Paris and The Parthenon Publishing Group, Carnforth, pp. 69–84.
Seymour, R.S., 2010. Scaling of heat production by thermogenic flowers: limits to floral size and maximum rate of respiration. Plant Cell Environ. 33, 1474–1485.
Seymour, R.S., Matthews, P.G.D., 2006. The role of thermogenesis in the pollination biology of the Amazon waterlily *Victoria amazonica*. Ann. Bot. 98, 1129–1135.
Seymour, R.S., White, C.R., Gibernau, M., 2003. Heat reward for insect pollinators. Nature 426, 243–244.
Seymour, R.S., White, C.R., Gibernau, M., 2009. Endothermy of dynastine scarab beetles (*Cyclocephala colasi*) associated with pollination biology of thermogenic arum lily *Philodendron solimoesense*. J. Exp. Biol. 212, 2960–2968.
Seymour, R.S., Silberbauer-Gottsberger, I., Gottsberger, G., 2010. Respiration and temperature patterns in thermogenic flowers of *Magnolia ovata* under natural conditions in Brazil. Funct. Plant Biol. 37, 870–878.
Silberbauer-Gottsberger, I., Webber, A.C., Küchmeister, 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. Dissertat. Bot. 346, Borntraeger, Berlin-Stuttgart, pp. 165–183.
Silberbauer-Gottsberger, I., Gottsberger, G., Webber, A.C., 2003. Morphological and functional flower characteristics of New and Old World Annonaceae with respect to their mode of pollination. Taxon 52, 701–718.
Teichert, H., Dötterl, S., Gottsberger, G., 2011. Heterodichogamy and nitidulid beetle pollination in *Anaxagorea prinoides*, an early divergent Annonaceae. Plant Syst. Evol. 291, 25–33.
Terry, I., Walter, G.H., Moore, C., Roemer, R., Hull, C., 2007. Odor-mediated push–pull pollination in cycads. Science 318, 70.
Thien, L.B., 1974. Floral biology of *Magnolia*. Amer. J. Bot. 61, 1037–1045.
Thien, L.B., 1980. Patterns of pollination in the primitive angiosperms. Biotropica 12, 1–13.
Thien, L.B., Heimermann, W.H., Holman, R.T., 1975. Floral odors and quantitative taxonomy of *Magnolia* and *Liriodendron*. Taxon 24, 557–568.
Villegas, N.P., Gaigl, A., Vallejo, L.F., 2008. The white grub complex (Coleoptera: Melolonthidae) associated with onion and pasture in Risaralda, Colombia. Rev. Colomb. Entomol. 34, 83–89.
Vogel, S., 1962. Duftdrüsen im Dienste der Bestäubung. Über Bau und Funktion der Osmophoren. Akad. Wiss. Lit. Mainz. Abh. Math.-Nat. Kl. 10, 598–763.
Webber, A.C., 1996. Biologia Floral, Polinização e Aspectos Fenológicos de Algumas Annonaceae na Amazônia Central. Doctoral thesis. Instituto Nacional de Pesquisas da Amazônia, Fundação Universidade do Amazonas, Manaus.
Yasukawa, S., Kato, H., Yamaoka, R., Tanaka, H., Arai, H., Kawano, S., 1992. Reproductive and pollination biology of *Magnolia* and its allied genera (Magnoliaceae). I. Floral volatiles of several Magnolia and Michelia species and their roles in attracting insects. Plant Species Biol. 7, 121–140.
Zhao, X., Sun, W., 2009. Abnormalities in sexual development and pollinator limitation in *Michelia coriacea* (Magnoliaceae), a critically endangered endemic to Southeast Yunnan, China. Flora 204, 463–470.