Pollination biology of cantharophilous and melittophilous Annonaceae and Cyclanthaceae in French Guiana

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Summary

Pollination biology of cantharophilous and melittophilous Annonaceae and Cyclanthaceae in French Guiana

Thesis topic
Due to human impact tropical forests become fragmented and their preservation and sustainable management receive more attention. Functioning plant-animal interaction are crucial for long-term preservation of biodiversity in every rainforest. Therefore, understanding the plant-animal interactions is the key to sustainable management of this ecosystem. In undisturbed ecosystems, these interactions exist in their most preserved state and provide the true account on the ecology of the species concerned. The relationship between plants and their insect pollinators is one of the most important interactions in the evolutionary history of angiosperms. Most tropical plants are pollinated by insects, but only a fraction has been investigated and information is completely missing for many evolutionary important taxonomical groups.

The aim of the present study was to estimate pollination mechanisms of selected species belonging to the families Annonaceae and Cyclanthaceae in their original and undisturbed habitat. Annonaceae is an evolutionary old family, and their members form an important component of tropical rainforests because of their species richness and high abundance.

The Cyclanthaceae occur only in the Neotropics. Their live form include mostly terrestrial herbs or (hemi)-epiphytes.

The following questions were addressed:
1) How do the plant-pollinator relationships function in the selected Annonaceae and Cyclanthaceae?
What is the pollinator spectrum during anthesis?
How is cross-pollination ensured?
What is the timing of individual floral events during the pollination process?
2) What are the morphological and ecological traits that the plants have evolved which improve their relationship with their pollinators?
How do the plants attract the pollinators?
Is flowering phenology adjusted to fit the behavior of the pollinators?
What is the reward for pollinators?

3) How can the interactions investigated be placed in the context of the evolutionary history of pollination modes?
How advanced are these plant-pollinator relationships as compared to other pollination modes?
How do the pollination syndromes fit into the evolutionary history of the two families?

**Introduction**

*The family Annonaceae*

Annonaceae is one of the most diverse and largest family of the “basal” angiosperms. Annonaceae are included in the Magnoliidae. For a long time Magnoliidae have been considered to be the most primitive angiosperm group and sister group to all other angiosperms (Cronquist 1981). Within the Magnoliidae the Annonaceae is the most successful family, because of the large number of genera and species it has (van Heusden 1992). In tropical forests, Annonaceae contribute significantly to plant diversity, both in terms of species richness and abundance (Ter Steege et al. 2000).

The family comprises 135 genera and around 2500 species. Except for two North American genera, *Asimina* and *Deeringothamnus*, all members of the family occur in the Neo- and Paleotropics. The family has a high level of continental generic endemism. Some genera are only present in tropical America, others are restricted to Africa or Asia. Only a few genera (*Xylopia*) have a Pantropical distribution and occur in more than one continent. *Annona* is a Neotropical genus but a few species also occur in Africa. The combined Afro-Asian distribution is common in several genera. *Anaxagorea* is the only genus that has a disjunct distribution in tropical America and Asia and lacks representatives in Africa (Doyle and Le Thomas 1997). Recently, combined morphological and molecular analyses have provided evidence that the family originated either in South America or in
western Gondwana. From there the members have spread to Asia and Australia (Doyle et al. 2004, Richardson et al. 2004).

Among the Annonaceae, medium-sized trees are the most common growth form, but shrubs, lianas and trees of all sizes occur as well. In general, Annonaceae have semi-spiral flowers which exhibit protogynous anthesis. The flowers are mostly borne on leafy branches, on leafless parts of the branches, on the trunk, or on shoots departing from the trunk. The perigon is more or less clearly differentiated into sepals and petals. The petals and sepals are arranged in whorls. Most genera have six petals in two whorls and three sepals in one whorl. The stamens and carpels are usually numerous and spirally arranged. In a few genera either the inner or the outer stamens are modified into staminodes (van Heusden 1992).

There is information on pollination systems from Annonaceae of the Neotropics, Southeast Asia and Australia, but there are nearly no data from African species. The most reports on pollination biology come from the Neotropics (Gottsberger 1970, 1989a, 1989b, 1999, Webber and Gottsberger 1985, Andrade et al. 1996, Webber 1996, Armstrong and Marsh 1997, Carvalho and Webber 2000, Silberbauer-Gottsberger et al. 2003) and only a few are available for Asia/Australia (Nagamitsu and Inoue 1997, Silberbauer-Gottsberger et al. 2003, Ratnayake et al. 2006a, Ratnayake et al. 2006b, Ratnayake et al. 2007). Perhaps more than ninety percent of the species studied are well adapted to beetle pollination and possess features characteristic for this pollination syndrome, i.e., strong floral odor, often thermogenesis, fleshy petals and a pollination chamber formed over the reproductive organs. In consequence, cantharophily has received most attention. More recent works have pointed out that other pollination modes also occur in the family. In particularly, pollination by flies, thrips, cockroaches and bees has been documented. Moreover, several documentations exist on specialized pollination patterns in non-beetle pollinated species (see references above). These results show that the family has gone through a strong evolutionary radiation and some advanced pollination features have evolved during the 82 millions of years of its evolutionary history.
The family Cyclanthaceae

The distribution of the Cyclanthaceae is purely Neotropical. They range from southern Mexico through the rest of Central America including several Islands of the Lesser Antilles, to northern South America. A few species occur along the Brazilian coast. The center of diversity is in Colombia. All species require wet and more or less shady habitats and are concentrated in rainforests. The family comprises 12 genera and about 230 species. It includes terrestrial, as well as hemi- or epiphytic life forms. The family is divided into two subfamilies based on the inflorescence morphology. Most genera belong to Carludovicoideae, while the Cyclanthoideae are monotypic. The structure of the Carludovicoideae inflorescence is unique among angiosperms (Fig. 1). It consists of an unbranched spadix bearing free to connate flowers. The flowers are arranged in a regular chessboard mosaic, where each pistillate flower is surrounded by four staminate flowers. In pistillate flowers, staminodes are fused with the petals and can reach up to 25 cm in length (Harling 1958).

The pollination biology of Cyclanthaceae species has ever been studied only by Gottsberger (1991), Eriksson (1994) and Franz (2007). They have documented that the exclusive pollination mode within the family is cantharophily. While the one Cyclanthoideae species is pollinated by scarab beetles, all Carludovicoideae are pollinated by small Curculionidae beetles. It seems to be the only monocotyledonous family which is pollinated exclusively by beetles.

Fig. 1. Inflorescence of *E. funifer* with their long staminodes (illustrated by V. Kutschera).
Beetle pollination

Beetle pollination has for a long time been regarded as the oldest pollination mode among angiosperms, because it often occurred in the basal taxonomical groups, e.g., Magnoliaceae, Eupomatiaceae, Calycanthaceae, Monimiaceae, Winteraceae and Nymphaeaceae. Today we know that most insect groups that participate in pollination had already existed before the origin of the flowering plants. It seems that the early angiosperms were more generalistic in their choice of pollinators, and specialized plant-animal interactions have evolved much later. Beetle pollination is also known from more recent plant families. Cantharophily sometimes is a derived pollination syndrome, because it is associated with specialized floral features, e.g., fleshy petals, strong floral odor and thermogenesis (Gottsberger 1977, Thien 1980, Bernhardt 2000). The species investigated in this work possess most of these characteristics.

Summary chapter 1

Observations showed that *Unonopsis stipitata* (Annonaceae) is pollinated by males of two scent-collecting euglossine bees, *Euleama bombiformis* and *Euglossa imperialis*. This is the first detailed account of this pollination mode in a member of a basal angiosperm. The flowers showed a two day-lasting protogynous anthesis with an overlapping of female and male stages on the second day. The petals remain open during the whole anthesis.

The odor of *U. stipitata* has a spearmint-like smell. Odor analysis using GC-MS showed that the bouquet mainly consists of monoterpenes which are known to be attractive to male euglossine bees. One of the main compounds is trans-carvone oxide and this is a scent compound considered to be evolved convergently in euglossine-pollinated plants, because it is also found in species of Orchidaceae, Euphorbiaceae and Araceae. One of the main genera that gets attracted by trans-carvone oxide is *Eulaema*. *Eulaema bombiformis* was the most abundant species on flowers of *U. stipitata*. Both bee species showed different arrival times at the flowers. Temporal pattern of visits showed that the smaller *E. imperialis* arrived about one hour earlier on individual flowers than the larger *E. bombiformis*, but both were more abundant on the second day of anthesis. Another significant difference
was evident in the mean time of fragrance collecting. *Eulaema bombiformis* remained longer while fragrance collecting than *E. imperialis*. This can be either explained by competition of both bee species, or a change in the odor bouquet during anthesis. EAD revealed a clear reaction of the bees antennae to the main odor compounds of *U. stipitata*.

Morphological and histochemical studies showed that the adaxial surface of the inner petal is responsible for scent release; this coincides with the bees’ behavior of fragrance collection. A corrugated surface with a strong accumulation of trichomes exists exclusively at the petal of the inner whorl.

**Summary chapter 2**

*Anaxagorea prinoides* (Annonaceae) has a diurnal two day-lasting protogynous anthesis. The flowers seem to be the smallest of the genus, but as the other species also form a pollination chamber over their reproductive organs. There, the pollinators are sheltered. *Anaxagorea prinoides* is pollinated by small nitidulid beetle, belonging to the genus *Colopterus*, and a few staphylinid beetles.

Observations on the phenology within a population of 21 individuals of *A. prinoides* showed that the population consist of two different morphs with different chronologies of flowering. This flowering rhythm affords the beetles to change within the population, because when on morph releases the beetles in the male stage, the other morph is in the female stage. Flowers of one morph emit scent always half an hour before the others release their beetles. When two reciprocal mating types occur in the population with one part of the individuals being in the male stage and the other in the female stage then this system is called heterodichogamy. This system allows holding the pollinators within the population. This is supported by the finding that always more beetles visited the morph with less tree individuals and less beetles visited the morph with a higher number of tree individuals. The beetles get accumulated in the morph with few individuals, whereas in the morph with higher number of individuals the pollinators are distributed among a larger amount of flowers.

During the observation time single individuals of *A. prinoides* produced up to 104 flowers per flowering time. The mean number of flowers per day was 25 ± 8.4
The flowering intensity increased in the beginning of October with a peak in mid October. During flowering of *A. prinoides* the trees simultaneously carried fruits. These fruits are probably the result of developments that began the year before. This would indicate that fruit development and ripening lasts about one year. The seed release needs only up to three weeks. Observations on dispersal showed that no animals are attracted to the seeds of *A. prinoides* and that the dispersal mode apparently is ballistic.

The scent bouquet of *A. prinoides* mostly contains volatiles belonging to fatty acid derivates and herein to the ester group. The two main compounds are ethyl 3-methylbutanoate and isobutyl isovalerate. They are characteristic mostly by a fruity smell. *Anaxagorea prinoides* as well as *A. dolichocapa*, a further investigated species in the Nouragues natural reserve, showed a similar composition in their bouquet, and similarity also with other investigated *Anaxagorea* species in Central Amazonia (Jürgens et al. 2000). All those species are pollinated by the same genus of nitidulid beetles, namely *Colopterus*. Species of this genus are known to live and eat on rotten fruits and barks. The scent compounds of the flowers also occur in volatiles of fruits. The knowledge that the pollinator is eating on fruits, and that the main volatiles of *A. prinoides* are fruity esters, allows the presumption that the scent of *A. prinoides* evolved as a fruit mimic. The performed olfactory Y-tube test should give information of the attractiveness of the main odor compounds to the pollinators. Results of the test showed no exclusive preference of the pollinator to anyone of the offered compounds. Therefore, it seems that the beetles have an opportunistic behavior and are attracted by a wide range of scent compounds.

**Summary chapter 3**

*Duguetia cadaverica* is pollinated by small mycetophagous nitidulid beetles. The pollinator is an undescribed species of the genus *Pycnocnemus* and belongs to the tribe Cyllodini, which is known to feed on mushrooms. Investigation on the scent bouquet with GC-MS showed that the scent of *D. cadaverica* contains eight compounds which were mainly alcohols and are also found in mushroom scents, namely \((Z)-1\text{-octen}-5\text{-ol}\) and \((E)-2\text{-octen}-1\text{-ol}\). Further detected compounds were 4-methylpentanoic acid (isocapron acid), dimethyltrisulfide and dimethyltetrasulfide.
These compounds are also found in plants that mimic carrion or dung and have an unpleasant smell. A further adaptation of this pollination mode is the presentation of flowers on the ground, like in other dung-, carrion- or mushroom-mimicking flowers.

*Duguetia cadaverica* presents flowers on long flagelliform twigs. These twigs mostly originate at the base of the stem and creep in various directions away from the stem. These flagella are up to 2 meters long and can bear several flowers which are originating successively along the flagella. The outer petals of the flowers are uniformly red, contrary to the inner petals which are red on the tips and include a pronounced white fleshy swelling at the base. The color as well as the scent of *D. cadaverica* fits with its pollination mode.

It is known that flowers with such mushrooms scent can attract fungus gnats (Mycetophilidae, Diptera). To the best of our knowledge the pollination mode of *D. cadaverica* seems to be the first description of a beetle-pollinate flower mimicking fungi.

During the observation time in 2004, 35 trees within a population had 201 flagella with a mean length of 57.36 ± 38.9 cm. The total number of buds was 364, from which only 99 developed to a flower, and these 99 flowers resulted in 20 fruits. During flowering time, 55 buds were shed, mostly on flagella which developed a flower and this is a hint of re-allocation of limited resources, a common phenomenon in tropical rainforests. The mean number of flowers produced by 35 individuals per day was 2.5. During the flowering time never more then six flowers were open within the population and also days with no open flowers occurred. The pollinating beetles were very rare. In 2004 only a few beetle individuals were observed at the flowers. In the following year no beetle could be observed visiting *D. cadaverica*.

The flowers of *D. cadaverica* have a diurnal protogynous anthesis, which lasts only one day. After ending of anthesis in the male stage, the petals were retained and the pollination chamber existed until the next day when petals dropped.
Summary chapter 4

Observation on *Evodianthus funifer* and *Ludovia lancifolia* showed that both species are pollinated by small derelomine beetles (Curculionidae). The inflorescences of Cyclanthaceae species are rather similar. Each pistillate flower which is inserted along the spadix is surrounded by four staminate flowers arranged in a chessboard mosaic. From each pistillate flower four long staminodes are originating, which can be up to 9 cm long like in *E. funifer*. The funnel shaped staminate flowers together form a small pollination chamber above each pistillate flower. The entrances to the pistillate flowers are restricted for small beetles only. The two day-lasting protogynous anthesis started early in the morning when the inflorescence was in the female stage and dozens of small weevils were attracted which all arrived during a small time span. Observations showed that not all weevils acted as pollinators and only four of the six attracted species were entering the pollination chamber and had contact with the receptive stigmas. The weevils which were present only on the outer side of the inflorescence, gnawed on the staminodes. The pollinators showed the same behavior, but gnawed staminodes inside the inflorescence. Both, pollinators as well as non-pollinators used the inflorescence for feeding and as an oviposition site. The non-pollinators cut the staminodes and took them away and later laid their eggs onto them. Conversely, the pollinators laid most of their eggs on staminate flowers, which were aborted soon after anthesis.

Analysis of nutritional composition should give clarity about resources that can be used by the weevils, either for themselves when feeding on them or for the first larval stages when they developed on this brood substrate. Former observations questioned this behavior, because less eggs were laid in pistillate flowers, which seems to be the better substrate because of fruit development. The staminodes are the parts of the plant with the highest energy content. They have also high contents of nitrogen and a small C/N ratio, which indicates a good utilization for the beetles. The staminate flowers are also rich in energy and nitrogen content and biomass is higher then in staminodes. The results showed that the moderately digestible brood substrate seems to be a “first aid package” of food resource for larvae before eating on poorly utilizable detritus.

The staminodes have the function of osmophores, which is proved by smelling and staining with neutral red. Cross sections showed that staminodes
contain ducts mostly along the whole length. Stainings of these sections with alcian blue showed that the staminodes transport a kind of mucilage. The nature of this mucilage is not known but it is presumed that it is a substance involved in scent release.

Scent was collected from three species of the Cyclanthaceae. Each bouquet is dominated by mainly two compounds. The odor bouquet of *E. funifer* is dominated by a new natural scents belonging to the sesquiterpenes. *Ludovia lancifolia* is dominated by jasmone and *Stelystylis surinamensis* by trans-cinnamyl acetate. Although most investigated species of Cyclanthaceae are pollinated by the same spectrum of derelomine beetles, all of them have different compositions of odor.

**Discussion**

The studies on Annonacea and Cyclanthaceae species showed that they are all pollinated by beetles; the exception is *Unonopsis stipiata*, which is pollinated by male euglossine bees. The pollination of a member of Annonacea by male euglossine bees, has been observed before (Carvalho and Webber 2000, Silberbauer-Gottsberger et al. 2003), but details on this plant-pollinator relationship provided in this study are the first of its kind. *Anaxagorea prinoides* and *Duguetia cadaverica* (Annonacea) are pollinated by small Nitidulidae beetles, which are also known to be pollinators of other species in this genera (Webber 1996, Silberbauer-Gottsberger et al. 2003). The two Cyclanthaceae species are pollinated by small curculionid beetles, which are the exclusive pollinators.

The abundance of pollinators in flowers during anthesis is variable. In anthetic flowers of *A. prinoides* there are up to 35 beetles per flower, while in *D. cadaverica* only a few pollinators could be noticed. In both Cyclanthaceae species there were up to 100 beetles observed on one inflorescence.

All observed beetle-pollinated species have a protogynous anthesis with female and male stages well separated. The beetles arrived at flowers in female stage carrying pollen grains and deposited pollen on the receptive stigmas. All beetles arrived at approximately the same time and remained in the flowers for the rest of anthesis. Later, the flower changed to the male stage, pollen was shed and beetles got dusted with pollen; they departed to search another flower in a female stage.
Anthesis of a flower finished by the end of the male stage, when petals or whole flowers dropped. On a population level, the system prevents self-fertilization in individual flowers and promotes outcrossing. A single individual at a given time carried flowers that were all in the same stage of anthesis. Beetles departing from a flower in male stage will find flowers in female stage only at a different individuals.

The abundance of euglossine pollinators at the flowers of *U. stipitata* does not have such a regular pattern. On the first day of anthesis, the bees visited the flowers only sporadically. But their abundance increased considerably on the second day of anthesis. Because of an overlapping of female and male phase on the second day of anthesis, outcrossing is possible. The bees can carry pollen either to first-day flowers which are in the female stage or to second-day flowers which are in both phases.

Scent plays an important role in beetle- as well as in euglossine-pollinated plant species (Vogel 1966, Dressler 1982, Gottsberger 1990). In the species under study, the flowers or inflorescences started to emit a strong scent at the beginning of anthesis which was perceptible over a long distance. The scent bouquet of the beetle-pollinated species contains a small number of compounds and is dominated by only few of them. In contrast to scent bouquets of other *Anaxagorea* and *Duguetia* species analyzed by Jürgens (2000), the bouquets of the species under study show only a few similarities in their main scent compounds. The floral scent chemistry of beetle-pollinated Annonaceae seems to be very variable not only on the family but on generic level. A similarly high variation in floral scent chemistry of beetle-pollinated taxa has been documented also by Thien et al. (1975) in Magnoliaceae species and also in palms (Ervik et al. 1999).

The scent of *U. stipitata* contains several compounds which are unique in the Annonaceae species investigated so far. The scent is dominated by monoterpenes which are known to be attractive to male euglossine bees. They are known to occur in scents of Orchidaceae and few other families in which male euglossine bees are the pollinators. (Vogel 1966, Whitten et al. 1986, Armbruster et al. 1989, Hentrich et al. 2007).

The species showed almost all the general features characteristic in beetle-pollinated flowers. They all form a pollination chamber over their reproductive organs, either by their petals (the Annonaceae species), or with the aid of staminate
flowers (the Cyclanthaceae species). In this chambers the beetles stay during the whole anthesis; there they also find mating partners. During their permanence in these chambers the beetles are also save for being predated. Nectar never is present in flowers but beetles can feed on pollen. The Cyclanthaceae species also offer food in form of easily-digestible tissue of staminodes. *Evodianthus funifer* heats up its inflorescence during the attractive phase as well as at the end of anthesis. This recurrent heating during the staminate phase stimulates the weevils’ activity rate just before they leave to fly to receptive inflorescences.

A liquid perfume, such as the one produced by flowers of *U. stipitata* is another highly specialized resource for pollinating insects, which is only known to be produced in plants pollinated by male euglossine bees. Male euglossine bees collect a variety of scents and the choice is often species specific (Eltz et al. 1999). These semiochemicals are used for territorial display or for attracting sexual partners (Eltz et al. 1999, Bembé 2004, Zimmermann et al. 2006).

**Conclusion:**

In my work I present some not yet known pollination modes of Annonaceae. Although the Annonaceae seem to be a very old family (at least 82 Million years), they still retained their plasticity and showed a high radiation towards specialized cantharophilous flower characteristics and other pollination modes. To talk about primitiveness in the case of Annonaceae is not totally correct, because this basal family has also specialized pollination syndromes, including beetle pollination. Primitive flowers are more generalists and have open accessible flowers, with a mixed pollinator spectrum. Also Cyclanthaceae are highly specialized. The different flower parts have nutritional value for beetles and are consumed by pollinators as well as non-pollinators.
Literature


Summary


Zusammenfassung

Bestäubungsbiologie von cantharophilen und melittophilen Annonaceen und Cyclanthaceen in Französisch Guayana


In dieser Arbeit wurden die Bestäubungsmechanismen einiger ausgewählter Arten der Pflanzenfamilien Annonaceae (Schuppenapfelgewächse) und Cyclanthaceae (Scheibenblumengewächse) an ihren natürlichen und ungestörten Standorten untersucht. Annonaceen gehören zu einer evolutionär alten Familie und ihre Vertreter (Bäume, Sträucher, Lianen) machen einen wichtigen Teil tropischer Regenwälder weltweit aus, da sie zum einen sehr viele Vertreter haben, und zum anderen sehr häufig sind. Die Cyclanthaceen mit ihren 230 Arten sind eine rein neotropische Gruppe die meist als terrestrische Kräuter oder (Hemi)-Epiphyten vorkommen.

Die folgenden Fragen sollten erarbeitet werden:
Wie funktionieren diese Pflanze-Tier Interaktionen in den ausgesuchten Arten der Annonaceen und Cyclanthaceen?
Wie ist das Besucherspektrum während der Anthese?
Wodurch ist Fremdbestäubung gewährleistet?
Zusammenfassung

Wie ist der zeitliche Ablauf der Einzelereignisse während des Bestäubungsvorganges?

Welche sind die morphologischen und ökologischen Anpassungen, die die Arten entwickelt haben, um die Interaktionen mit den Bestäubern zu gewährleisten?

Wie locken die Pflanzen Ihre Bestäuber an?

Ist der Blühverlauf an das Verhalten der Bestäuber angepasst?

Welche Ressourcen werden den Bestäubern angeboten?

Welche Bestäubungssyndrome haben sich bei Annonaceen und Cyclanthaceen entwickelt?


Die Besucherhäufigkeit der Prachtbienen Männchen zeigten nicht dieses Muster. Am ersten Tag der Anthese wurden Besuche an Blüten nur sporadisch festgestellt, wohingegen sich die Besucherhäufigkeit am zweiten Tag deutlich intensivierte.


Morphologische Anpassungen an Käferbestäubung sind an den cantharophilen Arten festzustellen. Die Blüten oder Infloreszenzen formen mit ihren


Perfume-collecting male euglossine bees as pollinators of a basal angiosperm: the case of *Unonopsis stipitata* (Annonaceae)
Abstract: Pollination of *Unonopsis stipitata* (Annonaceae) by males of two perfume-collecting bees, *Euglossa imperialis* and *Eulaema bombiformis* (Euglossini), is described. This is the first detailed account of this pollination mode in a member of a basal angiosperm family. Pollinator behavior, identification of the odor bouquet and electrophysiological reaction of one of the two pollinators to the odor bouquet was determined. The collected odor is produced by “osmophores” located adaxially on petals. Starch and polysaccharides accumulated in petals were metabolised during odor emission. Mainly monoterpenes were detected in the scent samples, among them trans-carvone oxide. This molecule is thought by several authors to be the key attractant for male *Eulaema* bees and may be pivotal for convergent evolution of the perfume-collecting syndrome among dicotyledonous and monocotyledonous plants. It is speculated that *Unonopsis*, which on the basis of molecular age dating is considered a relatively recent genus of the Annonaceae, has diversified in relation to male euglossine bee pollinators.

Introduction

Annonaceae are a Pantropical family of trees, shrubs and lianas. Approximately 135 genera and more than 2500 species are known, of which about 900 species occur in the Neotropics (Chatrou et al. 2004). Annonaceae are mesothermic plants and grow predominantly in lowland tropical regions. In tropical forests, Annonaceae contribute significantly to plant diversity, both in terms of species richness and abundance (Ter Steege et al. 2000).

The floral biology of Neotropical Annonaceae has been increasingly studied in the last decades (e.g., Gottsberger 1970, 1989a, 1989b, 1999, Webber and Gottsberger 1995, Webber 1996, Silberbauer-Gottsberger et al. 2003). Among the studied Neotropical genera (e.g. *Guatteria*, *Duguetia*, *Annona*, *Bocageopsis*, *Xylopia*, *Anaxagorea*, *Cymbopetalum*), flowers are frequently protogynous and usually hermaphroditic. During anthesis, fruity and spicy or unpleasant floral odors attract specific pollinators. In several species thermogenesis enhances floral odor emission. Flowers provide nutritious floral tissue and pollen but usually no nectar to visitors. During anthesis a “pollination chamber” is often formed by the petals, enclosing the reproductive organs. Most Annonaceae are pollinated by medium-
sized to large (approx. 1.5 cm) beetles, which enter the interior of the pollination chamber and stay there until the petals drop. Inside the pollination chamber the flower visitors are sheltered from predators and find a nutritious and odoriferous place for mating. In a few Neo- and Paleotropical Annonaceae species, thrips, flies, cockroaches, or occasionally bees have been found to be pollinators as well (Webber 1996, Nagamitsu and Inoue 1997, Gottsberger 1999, Silberbauer-Gottsberger et al. 2003).

An extraordinary pollination mode involving male euglossine bees was recently reported for *Unonopsis guatterioides* (A. DC.) R.E. Fries (Carvalho and Webber 2000, Silberbauer-Gottsberger et al. 2003), these authors observed the pollination process of this plant in Central Amazonia and described the behavior of the exclusive pollinators, male *Eulaema bombiformis* Packard bees, when visiting flowers.

*Unonopsis* is a small genus (28 species) of Neotropical annonaceous trees, which grow from 3 to 20 meters. Their center of distribution is the Amazon lowland. Only two species have been studied with respect to pollination mode, *U. guatterioides* (Carvalho and Webber 2000, Silberbauer-Gottsberger et al. 2003) and herein, *U. stipitata*. The fruits are believed to be dispersed by birds. Flower color is uniformly cream-colored, white or yellow and flowers are uniform in their appearance and morphology (van Heusden 1992).

“Orchid” or “perfume” bees (tribe Euglossini, Apidae) are restricted to the Neotropics, where they live mostly in tropical forest; one-third of all species are found in Costa Rica and Panama. Five genera and approximately 200 species are known (Roubik and Hanson 2004). Male euglossines are renown for collecting perfume (liquid scent) from orchid flowers (Vogel 1963a). Since this early discovery, it has become clear that besides orchids, male euglossines also collect perfume from flowers of other monocotyledons, as for instance certain species of Araceae (Vogel 1963a, 1963b, 1966a, 1966b, Dressler 1968, Williams and Dressler 1982, Schwerdtfeger et al. 2002, Roubik and Hanson 2004). Also some members of dicotyledonous families (e.g., Euphorbiaceae, Solanaceae, Bignoniaceae, Gesneriaceae) exhibit this phenomenon (Vogel 1966a, Dressler 1982, Armbruster et al. 1989, Sazima et al. 1993, Roubik and Hanson 2004, Gottsberger and Silberbauer-Gottsberger 2006). In addition, scent is collected by these male bees from other, non
floral sources such as leaves, fruits, bird droppings, plant sap, rotting wood and stored perfume from the cadavers of other males (Williams and Whitten 1983, Roubik 1998, Pemberton and Wheeler 2006). The male bees take up the liquid volatiles usually while standing on the flower or otherwise perched upon it and brushing the fragrance-producing tissue with special brushes on the tarsus of the front leg. Thereafter, they hover in front of the flower and transfer the liquid odor from the first pair of legs to the middle pair and, finally, to the tibial storage organ on their hind legs. It is now known that bees use the “perfume” to attract species-specific male euglossine bees, demark their territories, and presumably also attract females (Zimmermann et al. 2006). Orchid bees are known as fast, long distance flyers and trapliners, and it has been estimated that during foraging they can “range” up to 40 km (Roubik and Hanson 2004), and Janzen (1971) reported 23 km for one continuous flight. The bees range from solitary to communal but some of the species are weakly eusocial (Roubik and Hanson 2004).

In an effort to better understand this unusual pollination mode, in French Guiana we undertook detailed observations of flowers of Unonopsis stipitata Diels and the behavior of visiting bees, then collected and chemically analyzed floral scent and tested bee antennae for perception of scent using gas chromatography coupled with electroantennography.

**Material and Methods**

**Observations and plant characteristics**

Observations were carried out in the Nouragues Natural Reserve (4°5’ N, 52°41’ W) in French Guiana. The reserve is located about 100 km southwest of Cayenne. Most of its area is covered by tropical lowland rainforest largely undisturbed by human activity. The climate is permanently humid and annual precipitation may reach 3000 mm (Grimaldi and Riera 2001).

Floral phenology and behavior of the flower visitors of Unonopsis stipitata were recorded during September and October 2005 at four tree individuals. The flowering time of U. stipitata is from June to October (Mori et al. 2002). Total observation time of 18 flowers was 20 hours for the first day flowers and 40 hours for the second day flowers. The observation time on the first day extended from the
morning when flowers opened at 10 AM and ended with the fading of scent production at 3 PM. On the second day observation started with beginning of scent production at 9 AM and ended with the dropping of the petals at 2 PM. The frequency and behavior of the euglossine bees at flowers, and also the scent-collecting time for each bee was recorded.

For further laboratory studies, flowers were collected and stored in 50% formol. Several fresh flowers were immersed into 0.01% solution of neutral red to visualize the scent-producing parts (Vogel 1963b). Neutral red is a weak catatonic dye that penetrates membranes by non-ionic diffusion and accumulates intracellularly. This method provides a quick and selective staining of intact tissue that contains osmophores. For investigations of the morphology of scent-producing organs, SEM photos were made after dehydrating the flower material in 70 and 100% isopropanol, critical point drying and sputter-coating with gold (Balzers Union, Liechtenstein) with a ZEISS DSM 249.

Further investigations on osmophores were done with histological cuts examined under the light microscope. The stored flowers were dehydrated through an ethanol series. Tert-butanol was used as an intermedium-solution. After dehydration, the samples were embedded in paraffin wax and cut (7 µm) with a Leitz microtome. The sections were stained with periodic acid-Schiff’s (PAS) reagent (Feder and O’Brien 1968) to visualize starch and complex polysaccharides within the osmophile layers.

Odor sampling and identification
Scent was always collected from single, individual flowers when the scent production started, by a standard dynamic headspace method (Knudsen and Tollsten 1993, Raguso and Pellmyr 1998, Knudsen et al. 2006). The flowers were enclosed in ovenbags to accumulate emitted scent, and the scented air was drawn with a battery-operated pump for 3 hours (150 ml/min), through a glass tube, filled with the absorbents Tenax TA 60/80 (25 mg) and Carbopack B 60/80 (40 mg). The absorbed scent was subsequently eluted with 0.3 ml of high grade acetone (Merck, Germany). The compounds were identified by GC-MS at the University of Bayreuth. Before analysing the samples in a Varian Saturn 2000 mass spectrometer and a Varian 3800
gas chromatograph fitted with a 1079 injector (Varian Inc., Palo Alto, USA), 100 µg of nonadecane was added as internal standard. One µl of the samples was injected using a Varian 8200 autosampler. The injector heated initially with 150°C, and the split rate was 1:10. During the injection the temperature increased with a rate of 200°C min⁻¹ to 250°C and was held for 2 min. For the analyses a ZB-5 (60 m x 0.25 mm i.d., film thickness 0.25 µm, Phenomenex) column was used. Helium was used as a carrier gas with a constant flow rate of 1.8 ml min⁻¹. The temperature of the GC oven was held at 40°C for 2 min, and thereafter the temperature increased with 5°C min⁻¹ to 240°C and held for 3 min. The MS interface had a temperature of 260°C and the ion trap 175°C. The mass spectra were taken at 70 eV with a scanning speed of 1 scan/s⁻¹ from m/z 40 to 350. To process the GC/MS data a Saturn Software package 5.2.1 was used. Component identification was carried out using the NIST 02 mass spectral data base (NIST algorithm), or MassFinder 3.0, and confirmed by comparison of retention times with published data (Adams 1995). Some of the components were identified by comparison of mass spectra and GC retention data with those of authentic standards.

Electrophysiological recordings

Gas chromatography with electroantennographic detection (GC-EAD) was performed with male Euglossa imperialis (Hymenoptera: Apidae: Euglossini), one of the two flower-visiting species collected in Nouragues, April 2006, to identify the electrophysiologically active compounds of the flower fragrances. For EAD an excised antenna was mounted between two glass electrodes filled with insect Ringer solution. The electrode holding the base of the antenna was connected to a grounded Ag-AgCl wire. The electrode holding the tip of the antenna was connected via an amplifier to a signal interface board (Syntech, Hilversum, Netherlands) for signal transfer to a PC. One µl of the odor sample was injected splitless into a gas chromatograph HP6890 (Hewlett-Packard, Palo Alto, USA) at 50°C. After one min the split valve was opened and the temperature increased by 10°C min⁻¹ up to 310°C. The GC was equipped with a DB-5MS capillary column (30 m x 0.25 mm i.d.; J&W Scientific, Folsom, USA) and a flame ionisation detection (FID); hydrogen was used as the carrier gas (2ml/min). A variable GC effluent splitter
(SGE, Darmstadt, Germany), with a split ratio FID:EAD = 1:3 was used and the outlet for the EAD was placed in a purified and humidified air stream. This air stream was directed over the male *Euglossa* bee’s antenna. EAD and FID signals were recorded simultaneously on a PC running a GC-EAD program (Syntech, Hilversum, Netherlands). To verify the reproducibility of the detection four electrophysiological recordings were done, whereof three were reproducible.

**Breeding system**

The P/O-Rate was determined according to Cruden (1977) to get an idea on the breeding system. Cruden (1977) showed that the pollen-ovula ratio of species decreases with the degree of self fertilization. Seven flowers were used of which all pollen grains from three anthers of each flower were prepared and counted with a cell-counter Casy®1 (Schärfe System, Germany) and this number then multiplied with the number of anthers. The number of ovules was counted by opening all carpels.

**Statistics**

To evaluate the difference in the presence of the two bee species at flowers we used the circular distribution (Zar 1999) and calculated the mean angle for the date of each bee species. The Watson Williams test for two samples was used to compare the two species.

To test the significance of differences in fragrance collecting time the Mann-Whitney U-Test was applied. Statistical tests were performed using the Statistica program, Rel. 6.0 (StatSoft Inc., Tulsa, Oklahoma, USA).

**Results**

**Plant material**

*Unonopsis stipitata* Diels is a small tree with reproductively mature individuals having a height of 3 to 12 meters. Bisexual flowers formed on the main trunk and on leafy branches. Flowers have cream-colored petals; they are free and arranged in two
valvate whorls. The flowers do not form a closed floral chamber, but remain open and accessible with petals curved towards the center. The free carpels (mean number 86 ± 7 SD) are densely aggregated on the floral axis. The stamens (mean number 221 ± 16 SD) bear a shield-like apical expansion of the connective. The flowers have diurnal proterogynous anthesis which lasts for two days. On the first day of anthesis, between 10 AM and 3 PM the flowers are in the pistillate stage and emit a spearmint-like odor. At 9 PM of the second day the spearmint-like odor production is resumed. From 11 AM on, with an overlapping of around one to two hours of both floral phases, the flowers change from the pistillate to the staminate stage, when the anthers open and pollen is shed. Anthesis ends with the dropping of petals at 2 PM on this second day.

Observations on flower visitors
The main flower visitors of *U. stipitata* were male bees of *Euglossa imperialis* Cockerell, and *Eulaema bombiformis* Packard. Only once the presence of a male *Eulaema cingulata* Fabricius was recorded. On the first day of anthesis the bees visited the flowers at very irregular intervals and always only for a few seconds. At the second day of anthesis *Euglossa imperialis* visited flowers from about 9:30 AM to 12:30 PM; *Eulaema bombiformis* was not so frequent in the early morning but became more abundant around 11:30 AM and visited the flowers until the end of anthesis (Fig. 1).

![Frequency of bees on flowers in half-hour intervals during both days of anthesis](image-url)

**Figure 1**: Frequency of bees on flowers in half-hour intervals during both days of anthesis: *Euglossa imperialis*, n=2, *Eulaema bombiformis*, n=5 (1st day), *Euglossa imperialis*, n=40, *Eulaema bombiformis*, n=88 (2nd day).
The two bee species showed a significant difference in frequency during the second day of anthesis when female and male stages begin to overlap (Watson-Williams test for two samples; $F=64.53$, $df=127$, $p<0.001$). Temporal pattern of visits at individual flowers showed that the smaller *E. imperialis* arrived about one hour earlier ($10:43$ AM ± 4.8 min, mean vector ± circular SD) than the larger *E. bombiformis* ($11:53$ AM ± 8.1 min, mean vector ± circular SD). The bees flew directly to the flowers, hovering for a short time in front of them before landing. The bees then were hanging upside down on the petals of the inner whorl. With their hind tarsi the bees held themselves and with their foretarsal organs brushed the osmophores at the inner petal surface. During these movements the bees had intensive contact with the stigmas and anthers. While visiting transitional female-male-stage flowers the bees got dusted with pollen on their head and mouthparts. After a while, they flew up and hovered in front of the flower and transferred the collected fragrances from their front tarsi over the midlegs to the hind tibia. The bees repeated this behavior several times and changed between the different petals. In this way they had contact with most anthers which are arranged spirally below the carpels (Fig. 2).
Another significant difference (Mann-Whitney U-Test, p<0.05) on the second day of anthesis was evident in the mean time of fragrance collection per flower. *Eulaema bombiformis* remained 1 up to 140 min on individual flowers, whereas *E. imperialis* collected only 1 and maximum to 38 min (Fig. 3).
Morphological and histochemical studies of the scent-producing area

Detached flowers, stained with neutral red, were used to visualize the areas where osmophores apparently are present. The inner whorl of petals after staining showed the typical pattern described by this method (Vogel 1963b). The reddish coloring was most intensive at the inner surface of the inner petals and the petals margin. Such coloring was absent in the outer petals.

Observations by light-microscopy and scanning electron-microscopy revealed details about the scent-emanating adaxial surface of the petals of the inner whorl, which is characterized by a corrugated surface. Over the whole surface trichomes are frequently embedded in depressions. They are one-celled, and elongated. Also the lateral part of the petals showed a strong accumulation of such trichomes.

Investigations with the scanning electron microscope did not show any glandular features of these trichomes. Furthermore, over the surface of the inner and outer whorl stomatas are frequently occurring. In the petals of the inner whorl and outer whorl a hypodermis cell layer is inserted below the epidermis. The cell walls of these two layers are up to twice as thick as the cell walls of the parenchyma cells. Parenchyma and hypodermis layers of the inner and outer petals differ in layer height. These two layers in the inner petals are 10.6 µm (26.7%) higher than in the
outer petals. Also the epidermis and hypodermis cells are larger (epidermis 7.3 µm; 35%; hypodermis 15.7 µm; 17.4%) in the inner petals (Fig 4).

Figure 4: Anatomical characteristic of tissues of an inner petal. Adaxial tissue (a) with characteristic morphology of the osmophore. The outer cell layers show a much larger cell volumes than the abaxial tissue (b). Scanning-electron pictures.

Histochemical differences were revealed by staining with PAS-reagent, comparing petals from buds with such from flowers in anthesis. In this way it was possible to visualise the consumption of energy-rich starch and complex polysaccharides during scent production. Differences were found in the intensity of staining in the upper cell layers. Epidermis cell layers of buds showed a considerable stronger staining than the identical cell layers from anthetic and post-anthetic flowers. Also the trichomes and the cuticle showed stronger accumulation of the PAS-reagent in buds as compared to open flowers. Their cells contain numerous accumulated starch-rich plastids, apparent as red regions in the trichomes and upper cell layers.

Scent

The total amount of floral scent was 249.8 µg/flower. We identified 25 components in the odor samples of flowers of *U. stipitata* (Table 1), mainly monoterpenoids. Most of the compounds were only found in low percentage amounts, and only six compounds reached a percentage amount of at least 5%, i.e. α-pinene (12%),
limonene (27%), trans-limonene oxide (9%), 1,8-cineole (7%), carvone (9%) and trans-carvone-oxide (19%).

Tab. 1: Mean percentage amount of floral fragrance from Unonopsis stipitata. The compounds are listed according to class, relative retention time (RRt). (* active compounds in GC-EAD)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>RRt</th>
<th>Relative amounts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty acid derivatives</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isobutenyl methyl ketone</td>
<td>671</td>
<td>0.35</td>
</tr>
<tr>
<td>Aromatics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-Cymene</td>
<td>1133</td>
<td>0.20</td>
</tr>
<tr>
<td>Monoterpenes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α -Thujene</td>
<td>935</td>
<td>0.07</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>954</td>
<td>12.10</td>
</tr>
<tr>
<td>unknown</td>
<td>980</td>
<td>0.05</td>
</tr>
<tr>
<td>Camphene</td>
<td>986</td>
<td>0.14</td>
</tr>
<tr>
<td>Sabinene</td>
<td>1032</td>
<td>3.69</td>
</tr>
<tr>
<td>β -Pinene</td>
<td>1043</td>
<td>2.45</td>
</tr>
<tr>
<td>Myrcene</td>
<td>1058</td>
<td>3.45</td>
</tr>
<tr>
<td>unknown</td>
<td>1094</td>
<td>0.38</td>
</tr>
<tr>
<td>α -Terpinene</td>
<td>1118</td>
<td>0.09</td>
</tr>
<tr>
<td>Limonene *</td>
<td>1143</td>
<td>27.45</td>
</tr>
<tr>
<td>β-Phellandrene</td>
<td>1147</td>
<td>0.24</td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>1150</td>
<td>6.72</td>
</tr>
<tr>
<td>Trans-β-Ocimene</td>
<td>1172</td>
<td>0.03</td>
</tr>
<tr>
<td>γ-Terpinene</td>
<td>1200</td>
<td>0.24</td>
</tr>
<tr>
<td>Terpinolene</td>
<td>1259</td>
<td>3.59</td>
</tr>
<tr>
<td>Cis-Limonene oxide</td>
<td>1346</td>
<td>1.02</td>
</tr>
<tr>
<td>Trans-Limonene oxide*</td>
<td>1354</td>
<td>9.31</td>
</tr>
<tr>
<td>α-Terpineol</td>
<td>1452</td>
<td>0.02</td>
</tr>
<tr>
<td>Cis-dihydro Carvone</td>
<td>1465</td>
<td>0.05</td>
</tr>
<tr>
<td>Trans-dihydro Carvone</td>
<td>1480</td>
<td>0.15</td>
</tr>
<tr>
<td>Carvone*</td>
<td>1547</td>
<td>8.75</td>
</tr>
<tr>
<td>Trans-Carvone oxide*</td>
<td>1582</td>
<td>18.91</td>
</tr>
<tr>
<td>Unknown Carvone oxide</td>
<td>1605</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Perception of U. stipitata volatiles by male bees

Electroantennographic detections gave a first indication which compounds can be sensed by Euglossa imperialis males. Four of the five major compounds of the odor bouquet from U. stipitata triggered receptor potentials in bee’s antennae, namely limonene, trans-limonene oxide, carvone and trans-carvone oxide (Fig. 5).
Breeding system

The outcrossing index of *U. stipitata* was determined to be four, which is equal to a xenogamic breeding system. The mean number of pollen grains was 152.982 ± 17.125 and 86 ± 7 ovula per flower. This has resulted a mean P/O ratio of 1778 ± 273 what is indicative of a facultative xenogamic breeding system.

Discussion

Prior to the discovery of pollination by male euglossine bees in *Unonopsis* (Carvalho and Webber 2000, Silberbauer-Gottsberger et al. 2003), only cantharophily and pollination by thrips, flies or cockroaches was known in the Annonaceae. Bees, although occasionally noticed in Annonaceae, only collected pollen during the staminate stage at the end of anthesis, but never during the pistillate stage (Webber 1996). There is only one further species, *Uvaria concava* from North Queensland, for which pollination by small pollen-collecting meliponine bees is described (Silberbauer-Gottsberger et al. 2003). The scent bouquets of the
few investigated beetle-pollinated species differ considerably in their composition from that of *U. stipitata*; their main compounds are esters, aliphatic acids, benzenoids, naphthalene, and a few monoterpenes (Jürgens et al. 2000). The compounds of *U. stipitata* on the other hand consist mainly of monoterpenes, which obviously are attractive for a completely different range of flower visitors. One of the main scent compounds of *U. stipitata* is trans-carvone oxide. Whitten et al. (1986) presumed that carvone oxide is a scent convergently evolved in euglossine-pollinated plants. They suggested trans-carvone oxide to be the key attractant of many euglossine-pollinated species, which before was found already in *Catasetum* spp., *Notylia latilaba*, *Aspasia variegata* (Orchidaceae), *Dalechampia spathula* (Euphorbiaceae) (Armbruster and Webster 1979) and species of *Anthurium* (Araceae) (Schwerdtfeger et al. 2002). Most species containing carvone oxide in their bouquet attract various species of *Eulaema*. Trapping experiments with baits of synthetic trans-carvone oxide attracted several *Eulaema* species in Panama (Whitten et al. 1986). Studies on hind leg extracts from two different male *Eulaema* species by Zimmermann et al. (2006) showed that in 43 of 48 investigated individuals, carvone oxide was detected. Our results showed that the main pollinator of *U. stipitata*, which is a further carvone oxide-producing plant, is an *Eulaema* species as well. The electrophysiological investigations revealed a clear reaction of the bees to the odor bouquet of *U. stipitata*. Among the 25 compounds there were four compounds that elicited reaction to the bee’s antenna, viz. both carvone compounds, limonene and trans-limonene oxide. These four major compounds of the floral scent bouquet are strongly related in their biosynthetic pathway and represent more than 50% of the total floral scent amount. The monocyclic monoterpenic carvone is biosynthesized by a three-step pathway from limonene and is described in the leaves of spearmint (*Mentha spicata*) as well as in the fruits of caraway (*Carum carvi*) (Gershenzon et al. 1989, Bouwmeester et al. 1998). The result of a further step by the alkaline epoxidation of carvone, is trans-carvone oxide (Lindquist et al. 1985).

The staining with neutral red showed that the adaxial surface of the inner petal whorl is the main area where osmophores are present. This coincides with the bees’ behavior of fragrances collecting. SEM-microscopy showed that the stained areas have osmophores, and that polysaccharides and starch are consumed in the
petals during scent production. The trichomes themselves probably enlarge the area of scent emission.

Differences in arrival time of the two bee species might indicate temporal displacement between them or, eventually, could be the result of a change in the odor bouquet. The number of *Euglossa* individuals diminished as the larger *Eulaema* individuals became more abundant. Our observations showed that the larger *Eulaema* was displacing the smaller *Euglossa* when it visited flowers. The prolonged visits of *E. bombiformis* at flowers apparently also largely excluded *Euglossa imperialis* from visiting flowers. *Eulaema bombiformis* appears to be the better pollen vector of the two bees because of its longer presence at flowers and its more intense contact with the flowers’ reproductive organs.

The possibility exists that *U. stipitata* is self-compatible, but this could not be proved unequivocally by bagging flowers. The P/O ratio after Cruden (1977) suggests that the breeding system might be facultatively xenogamous. Of 18 observed flowers only one developed a fruit. This could be a consequence of low visitation rates during the pure pistillate stage on the first day of anthesis. We do not know if during other unobserved flowering periods, *U. stipitata* would have a better fruit set. The observed relatively good fruit-set of trees when we started our observations, is a hint that fruit-set might not always be as low as during our observation period.

Pemberton and Wheeler (2006) questioned the notion of co-evolution between euglossine bees and perfume orchids. These authors demonstrated that the bees can survive without their associated orchids, because they can find complementary perfume resources in other plant species. Our observations on *Unonopsis stipitata* and further observations by several other authors on non-orchid groups (Vogel 1966a, Dressler 1982, Armbruster et al. 1989, Sazima et al. 1993, Schwerdtfeger et al. 2002, Roubik and Hanson 2004, Gottsberger and Silberbauer-Gottsberger 2006) clearly indicate that male euglossine bees are not at all exclusive “orchid bees”, but can find “perfume-flowers” in several angiosperm families. That a basal group such as Annonaceae also have members that evolved such a sophisticated pollination syndrome, however, is most surprising.

*Unonopsis* is a well defined genus of 28 species with a New World distribution in Central and South America and the Antilles, with the largest number
of species occurring in lowland Amazonia (Fries 1959, van Heusden 1992). There are data on pollination of two species of *Unonopsis* (Carvalho and Webber 2000, Silberbauer-Gottsberger et al. 2003, and this paper), which are both pollinated by males of euglossine bees. Future work will reveal whether *Unonopsis* is uniformly bee-pollinated or not. Tentative molecular age dating of Annonaceae genera gives a likely origin of *Unonopsis* between the middle of the Oligocene and the middle of Miocene, or an age between 30 to 15 million years; in any case, according to Richardson et al. (2004), *Unonopsis* belongs to the more recent groups of the Annonaceae.

The fossil record shows that long-tongued euglossine bees already existed 20 millions years ago, but unfortunately, this does not give indications about the absolute age of this group of bees. The combined fossil and biogeographic evidence suggests that the orchid bees perhaps originated sometime between 100 and 20 million years ago (Roubik and Hanson 2004). Unfortunately, this is a quite large-ranging space of time. On the other hand, it is tempting to speculate that the genus *Unonopsis* diversified exclusively in relation to male euglossine bee pollinators. It will be necessary to study the pollination in the remaining species of this genus to verify this. Maybe in the future we will be able also to obtain a more accurate dating of the group; together with more information on pollination and seed dispersal this could refine the existing molecular-based data on phylogeny of the Annonaceae.

Although the Annonaceae appear to be fairly basal in angiosperms, this does not mean that all the component genera are ancient. It is a family that looks as if it is still diversifying vigorously. It seems that *Unonopsis* is just such a recent genus, even if it belongs to an old angiosperm lineage.
Literature


Heterodichogamy within a population of *Anaxagorea prinoides* (Annonaceae) pollinated by nitidulid beetles
Abstract: Heterodichogamy in a natural population of the Annonaceae family is described for the first time from rainforests in French Guiana. *Anaxagorea prinoides* has bisexual flowers and both morphs within the studied population are protogynous. Observations showed that when one of the morph ends anthesis in the male stage, the complementary morph starts its anthesis with flowers being in the female stage. About one hour before a morph finishes anthesis, flowers of the reciprocal morph start to emit scent. The temporal separation of the female and the male stages of the two different morphs is for a few hours only. Identification of the banana-like scent indicated that six of the seven detected compounds are esters and one is isoamylalcohol. The examined main compounds have previously also been found as scent compounds of fruits. The attractiveness of the three main volatiles was tested with Y-tube experiments and showed no exclusive preference of the pollinators for anyone of the compounds. Nitidulidae beetles are the pollinators of *A. prinoides* and during flowering are maintained within an *Anaxagorea* population. This is not only due to the fact that the beetles are kept and sheltered in the flowers’ respective “pollination chamber”, but also because upon release at the end of flower anthesis from individuals of male-stage flowers they are attracted by the odoriferous female-stage flowers of other individuals of the same population.

Introduction

The genus *Anaxagorea* comprises 26 species mainly distributed in Central and South America, except for four species that occur in Asia (Maas and Westra 1984, 1985, Maas et al. 1986, Berry et al. 1999).

Studies on the floral biology of Neotropical Annonaceae showed that their predominant pollination mode is cantharophily, with a few species being pollinated by thrips and flies (Gottsberger 1970, 1989a, 1989b, 1999, Webber and Gottsberger 1985, Webber 1996, Silberbauer-Gottsberger et al. 2003). In two further species of the genus Unonopsis, pollination by perfume-collecting male euglossine bees was verified (Carvalho and Webber 2000, Silberbauer-Gottsberger et al. 2003, Teichert et al., submitted). Among the studied Neotropical genera, flowers are usually hermaphroditic and bear protogynous dichogamy.

Dichogamy, the temporal separation of both genders is often interpreted as an outcrossing mechanism that prevents self-fertilization. Two modes of dichogamy
occur in hermaphroditic plants and depend on the different time of pollen presentation and stigma receptivity during anthesis. Shedding of pollen before receptivity of stigmas is known as protandry while stigma receptivity before pollen shedding is called protogyny. Dichogamy can reach several levels, such as the degree of separation of pollen and stigmas or the degree of synchronization of flowers within one plant (Lloyd and Webb 1986). An even more specialized type of dichogamy is heterodichogamy in which two reciprocal mating types occur in one population with one part of the individuals being in the male and the other in the female stage.

Another important character in flowers of Annonaceae species is the emitting of strong floral fragrances that attract the pollinators, which seems to play an important role in early reproductive systems (Gottsberger 1970, 1974, 1989b, 1990, Thien et al. 2000). Fragrance is one of the most important attractants in many beetle-pollinated plants. In Annonaceae which are pollinated by beetles, mostly fruity, spicy and unpleasant floral fragrances occur (Armstrong and Marsh 1997, Gottsberger 1999, Jürgens et al. 2000). These fragrances consist mostly of essential oils and are known as mono- and sesquiterpenes or aromatic compounds (Santos et al. 1998).

Most beetle-pollinated magnoliids are known to form chamber blossoms (Gottsberger 1970, 1989a, 1989b, 1990, 1999, Endress 1990). In beetle-pollinated Annonaceae the petals form a “pollination chamber” enclosing the reproductive organs of flowers. There, the beetles are sheltered from predators and can find mating partners and feeding tissue (Gottsberger 1974, 1989a, 1990, 1999, Webber 1996, Armstrong and Marsh 1997). These floral chambers receive or exclude beetles according to their size. Small floral chambers can be visited by small beetles only, such as Nitidulidae, Chrysomelidae, Staphylinidae or Curculionidae, whereas larger flower chambers are visited usually by large beetles only such as species of the Scarabaeidae, subfamily Dynastinae (Gottsberger 1990, 1999).

In this paper the pollination and reproductive mode of A. prinoides, a heterodichogamous species within the basal angiosperms is presented and discussed. Pollinator attractants, especially floral fragrances, were analyzed and tested for their olfactory attractiveness to the beetle pollinators. For the first time heterodichogamy was verified for a species of the Magnoliales at a natural site.
Material and Methods

Plant characteristics and observation of flower visitors

All observations took place in the Nouragues Natural Reserve (4°5’ N, 52°41’ W) in French Guiana. The area is situated in a mostly pristine tropical lowland forest with a mean annual rainfall of 2990 mm and a mean temperature of 26°C. Between September and November a dry period occurs with a monthly rainfall of as low as 88 mm, while the average rainfall ranges around 246 mm per month during the rest of the year (Grimaldi and Riera 2001).

From September to October in 2004 and 2005, the floral phenology of *Anaxagorea prinoides* (Dunal) A. DC. was studied within a population of 21 individuals. In 2004, during a one month period, open flowers were counted every day. In 2005, open flowers as well as fruits were counted once a week. Because of an asynchronous rhythm of flowering within the population observed in 2004, the beginning of the flowering period and the timing of floral anthesis of each individual was examined in 2005.

Thirty-nine buds of a size between 2 to 4 mm in size were marked and measured weekly until their anthesis to study their development and to calculate the percentage of abortion. The structure of the population was described by measuring the height of the individuals as well as their diameter at breast height (dbh), and the distance between them. During numerous days the behavior and the number of flower visitors at several flowers was recorded. For basic morphological descriptions, flowers were collected and preserved in 50% formol. Voucher specimens are deposited in the herbarium ULM.

Odor sampling and identification

Scent was always collected by a standard dynamic headspace method from single, individual flowers when scent production started. The flowers were enclosed in ovenbags to accumulate emitted scent, and the scented air was drawn with a battery-operated pump for 3 hours (150 ml/min), through a glass tube, filled with the absorbents Tenax TA 60/80 (25 mg) and Carbopack B 60/80 (40 mg). The absorbed scent was subsequently eluted with 0.3 ml of high grade acetone. The compounds
were identified by GC-MS. Before analyzing the samples in a Varian Saturn 2000 mass spectrometer and a Varian 3800 gas chromatograph fitted with a 1079 injector (Varian Inc., Palo Alto, USA), 100 µg of nonadecane was added as internal standard. One µl of the samples was injected using a Varian 8200 autosampler. The injector heated initially with 150°C, and the split rate was 1:10. During the injection the temperature increased with a rate of 200°C min⁻¹ to 250°C and was held for 2 min. For the analyses a ZB-5 (60 m x 0.25 mm i.d., film thickness 0.25 µm, Phenomenex) column was used. Helium was used as a carrier gas with a constant flow rate of 1.8 ml min⁻¹. The temperature of the GC oven was held at 40°C for 2 min, and thereafter the temperature increased with 5°C min⁻¹ to 240°C and held for 3 min. The MS interface had a temperature of 260°C and the ion trap 175°C. The mass spectra were taken at 70 eV with a scanning speed of 1 scan/s⁻¹ from m/z 40 to 350. To process the GC/MS data a Saturn Software package 5.2.1 was used. Component identification was carried out using the NIST 02 mass spectral data base (NIST algorithm), or MassFinder 3.0, and confirmed by comparison of retention times with published data (Adams 1995). Some of the components were identified by comparison of mass spectra and GC retention data with those of authentic standards.

**Breeding system**

The P/O ratio was determined according to Cruden (1977). From five flowers, four anthers were opened each, and all pollen grains were counted using a cell-counter Casy® 1 (Schärfe System, Germany). The number of pollen grains was estimated for a whole flower. All ovules were counted from dissected ovaries.

**Y-tube experiments**

Y-tube experiments were performed to test the attractiveness of the main odor compounds of *A. prinoides* to the pollinators. The three compounds which represent 95 % of the bouquet were tested against each other and against the solvent. In each of the short arms of the Y-tube, 10 µl of the synthetic scent and a mixture of all main compounds which composes the scent (ethyl 3-methylbutanoate, isobutyl isovalerate and ethyl tiglate) were placed on filter paper, all diluted to flower equivalents. The air stream (150 ml/min) directed on both arms of the Y-tube was produced by a
battery operated pump (inverted), and pre-cleaned by active charcoal filter. The beetles which were used for the test were captured just before the end of flower anthesis, because this is the time when the beetles normally change from one flower to another. The beetles were released in the long arm of the Y-tube. The first choice of direction the beetles used was noted. Each test series included 15 beetle individuals. To avoid side specific preferences of the beetles, the location of the two different test solutions was changed after every test. Each test was performed with a new clean Y-tube, new filter papers and new beetles.

Voucher specimens of beetles were collected and compared with the reference collection in the herbarium ULM. They are deposited in the private collection of the first author.

**Results**

**Plant material and floral biology**

*Anaxagorea prinoides* (Dunal) A. DC. generally forms small trees and grows mainly in small populations. The population under study extended over 0.03 hectare and contained 21 reproductively mature individuals. Only one other smaller population was found in the region about 800 m distant from the population under study. The structure of the population is shown in Fig 1. The trees were 1.7-4.5 m tall. The dbh of the individuals within the population ranged from 0.5-3.5 cm. The distances from bole to bole extended from 0.6 up to 17.6 m whereas the mean distance to the nearest conspecific neighbor was $1.62 \pm 1.34$ m (mean $\pm$ SD).
The flowers of *A. prinoides* originate on leafy branches or on the main trunk. The pendant flowers consist of 3 yellow sepals (7 x 5 mm), and 6 yellow, fleshy petals arranged in two whorls. Unlike the outer petals (11 x 5 mm) the three petals of the inner whorl (11 x 4.5 mm) are keeled adaxially and thickened on their apical tips. The carpels (12 ± 2, mean ± SD) are densely aggregated at the floral apex. Each carpel contains two ovules. The carpels are surrounded by a whorl of 11 ± 2 (mean ± SD) pointed staminodes. The outermost row of the reproductive part of the flower consists of 14 ± 2 (mean ± SD) pointed stamens. During anthesis, the petals of the inner whorl form a dome shaped pollination chamber, which is covered by the three outer spoon-shaped petals. Principally, the thickened tips of the inner petals are forming a pollination chamber.

The examined flowers had 14,736 ± 4,094 pollen grains and 25 ± 2 (mean ± SD) ovula per flower. In consequences, the P/O ratio was 680 ± 144 (mean ± SD) indicating a facultative xenogamic breeding system (Cruden 1977).
The light-green apocarpous fruits consist of several club-shaped monocarpous fruitlets with a stipe-like peduncle. Each fruitlet contains two hard, black, shinny seeds, which are tightly pressed together. There are no arils in *Anaxagorea* species or they are rudimentary. At ripening the dehiscent monocarps dry out, open on the lateral side and catapult the seeds a short distance (Fig 2).

**Figure 2:** Flower of *A. prinoides* during end of anthesis being in the male stage. Inside the flower there are several *Colopterus* individuals dusted with pollen (A). Fruit of *A. prinoides* with fruitlets opening laterally, showing the two seeds (B).

**Population phenology**

During the observation period in 2004 the 21 monitored trees produced in total 538 flowers. Six trees produced flowers almost every day, whereas the remaining trees flowered only sporadically. Three trees produced no flowers at all. The highest number of produced flowers during the flowering period per individual was 104. Total numbers of flowers during the observed period and per day are shown in Fig. 3. Trees of *A. prinoides* produced a large number of flowers over a time span of several weeks. On average, all trees produced $25 \pm 8.4$ (mean $\pm$ SD) flowers per day. Flowering intensity increased during the first two weeks of October and peaked in mid October. At this time more than 40 flowers per day were counted. With an average of seventy percent, subpopulation A produced almost three-quarters of all produced flowers. 

From the 38 marked buds, 26 (86%) developed to open flowers, the remaining were aborted. The development from bud to flower required about 56 days.

During the flowering period in September and October 2005 the trees simultaneously carried fruits. During September only a few fruitlets produced ripe
seeds. The remaining 75% seeds were released more rapidly in the following three weeks (Fig 4). When fruits are ripe, the globose fruitlets desiccate and open on the lateral side. Then by pressure they eject the seeds for a certain distance. Seeds at the ground were never removed indicating their lack of attractiveness or any animal. After seed release the fruitlets completely dried and finally dropped.

**Floral anthesis and floral visitors**

Within the population two different morphs with different chronologies of flowering were found. Twelve of the 21 observed individuals belong to one phenotypical morph (called morph A), and seven to the other (B). Individuals of each morph flowered synchronously but reciprocal to the other morph. While one morph is in the female stage, the other starts to be in the male stage. On the first day of anthesis, morph A extended its petals around 9 AM and remained so without producing any noticeable scent until noon. Around 1:30 PM the production of a strong banana-like scent started which could be noticed over a distance of several meters. The flowers remained in this odoriferous stage throughout the night. At 10 AM of the second day these flowers changed from the pistillate to the staminate stage, i.e. anthers opened and pollen was shed. During the staminate stage the staminodes inclined and curved towards the pistils, this way preventing pollen being deposited on them. Around 11 AM anthesis ended, the flowers opened completely and the petals dropped. The individuals of the complementary morph B extended their petals around 5 PM, and remained so during the night. On the next morning around 9:30 AM, precisely one and a half hour before the end of anthesis of morph A, the flowers of morph B started to emit scent. Their flowers changed around 1 PM from the pistillate to the staminate stage and petals dropped at about 2 PM, half an hour before new flowers of morph A started to emit odor (Fig. 5).
**A. prinoides**

Figure 3: Number of open flowers of the studied population during the year 2004.

Figure 4: Number of fruits and fruitlets in the population remaining on the trees in September - November 2005.
Figure 5: Timing of anthesis of both morphs of *A. prinoides* (Annonaceae). 1 = flowers spreading their petals, 2 = initiation of scent production, 3 = arrival of pollinators.

The flowers were visited and pollinated by nitidulid beetles belonging to the genus *Colopterus*. The beetles were attracted only during the odoriferous period of anthesis. The beetles entered the flowers by passing through the small openings between the petals. They remained inside the pollination chamber until anthesis ended by dropping of the petals. During the change from the pistillate to the staminate stage, the beetles became dusted with pollen. When the petals detached, the beetles partly were released from the flower, fell to the ground and could also be seen to flying to flowers of individuals of the complementary morph which were in the pistillate stage. Beetles only were seen arriving at flowers one hour after the complementary morph had ended anthesis. Several of the arriving beetles were visibly dusted with pollen. Flowers of the morph A in total lasted 24 hours and flowers of morph B only 21 hours. Although both morphs flowered complementary, the time in which the beetles were attracted by the scent, and the time they remained inside the flowers differed considerable. In morph A the beetles were attracted at 13:30 PM and stayed for 21 hours inside the flowers. Conversely, morph B attracted the beetles at around 9:30 AM, and they stayed inside the flowers for only 4 hours.
The number of beetles which were counted inside individual flowers of the two morphs differed significantly, too (Mann-Whitney U-Test, p<0.001). Morph A harbored 2.5 (median) beetles per flower, while morph B sheltered 15 beetles per flower (Fig. 6). The maximum number of counted beetles inside individual flowers in morph A was 10 and in morph B 25. No marks of gnawing neither on the fleshy petals nor on the staminodes could be observed on any post-anthetic flower parts. The pollination chamber is probably a meeting and mating place, since a few of the caught male beetles showed an extended aedeagus.

![Figure 6: Total number of beetles inside the flowers per morphs (A and B). Median, 10, 25, 75 and 90 percentiles, and extreme values are plotted.](image)

**Scent**

The bouquet of *A. prinoides* mainly consists of aliphatic esters. Seven compounds were identified and half of them occurred only in low percentage amounts (Tab.1). The bouquet is dominated by ethyl 3-methylbutanoate which represents 80% of the total volatiles, followed by isobutyl isovalerate (13%) and ethyl tiglate (2%).
Tab. 1: Mean percentage amount of floral fragrances of *Anaxagorea prinoides*. The compounds are listed according to class and relative retention time (RRt).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>RRt</th>
<th>Relative amounts [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alcohols</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoamyl alcohol</td>
<td>546</td>
<td>1.21</td>
</tr>
<tr>
<td><strong>Esters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl 3-methylbutanoate</td>
<td>760</td>
<td>81.72</td>
</tr>
<tr>
<td>Ethyl tiglate</td>
<td>902</td>
<td>2.31</td>
</tr>
<tr>
<td>Propyl isovalerate</td>
<td>952</td>
<td>0.08</td>
</tr>
<tr>
<td>Isobutyl isovalerate</td>
<td>1062</td>
<td>13.68</td>
</tr>
<tr>
<td>Isoamyl isovalerate</td>
<td>1256</td>
<td>0.35</td>
</tr>
<tr>
<td>Amyl isovalerate</td>
<td>1262</td>
<td>0.48</td>
</tr>
</tbody>
</table>

**Y-tube experiments**

The performed Y-tube experiments showed no clear results of attraction to any compound of the odor. As is shown in Fig. 7, there are only three significant results (Binominal test, p<0.05) to the offered compounds. The ethyl tiglate is significantly more attractive than the solvent. However, the attractiveness of ethyl tiglate decreases when it is not offered as a single compound. In comparison with the mixture, ethyl tiglate is significantly less attractive than the mixture. The solvent was significantly more attractive when it was tested against isobutyl isovalerate.
**Discussion**

Protogynous dichogamy is well known in plants that keep their pollinators in their blossoms for several hours. Flowers in which pollinators remain inside the whole anthesis function well when being protogynous, because foreign pollen can be deposited on the receptive stigmas on entry and new pollen taken away during the staminate stage on exit (Lloyd and Webb 1986).

In many families with dichogamous mechanisms the species tend to be either wholly protandrous or wholly protogynous. The distribution of dichogamy is characteristic also in taxa above the family level. A widespread distribution of protogyny is present in many species of the Magnoliales (Bertin and Newman 1993). The frequent occurrence of protogyny in basal angiosperms is probably an ancestral condition and often found in beetle- or wind-pollinated species (Bernhardt and Thien 1987, Lloyd and Webb 1986, Thien et al. 2000). In Annonaceae there are several reports of protogynous dichogamy in which all flowers of an individual are flowering synchronously (Gottsberger 1974, 1989a, Silberbauer-Gottsberger et al.}
A. prinoides

1977, Murray and Johnson 1987, Andrade et al. 1996, Armstrong and Marsh 1997, Nagamitsu and Inoue 1997, Carvalho and Webber 2000). This so-called synchronous dichogamy occurs when flowers on a single individual open at the same time (Lloyd and Webb 1986). A variant of synchronous dichogamy is heterodichogamy which seems to be an evolutionary development of synchronous dichogamy (Gleeson 1982, Renner 2001). In heterodichogamous populations two reciprocal mating types occur with one part of the individuals being in the male and the other in the female stage. The flowers of heterodichogamous species can be either unisexual or bisexual. Most of the plants with unisexual flowers are monoecious and populations consist of two different morphs in which one morph is protogynous and the other protandrous. In bisexual heterodichogamous flowers protogyny dominates and only the time of flowering is shifted for several hours (Endress and Lorence 2004, Kubitzki and Kurz 1984, Li et al. 2001, Stout 1927). Until now, heterodichogamy was observed in 12 plant families, including two orders of basal angiosperms, namely Laurales and Magnoliidae (Renner 2001). The only description of heterodichogamy in Magnoliidae was in the family Annonaceae in a cultivated Annona squamosa (Wester 1910). In all heterodichogamous systems that have been already observed, only members of the Magnoliidae and Laurales group show absolute protogyny in both morphs (Endress and Lorence 2004, Renner 2001). Conversely, in families which belong to higher orders, the populations consist of one morph which is protogynous and the other protandrous. Differences in the separation of stigma receptivity and pollen shedding in the two different morphs are not in a daily rhythm. In those species, the receptivity of the stigmas of pistillate flowers could be up to several weeks before staminate flowers start anthesis, or the opposite way. As is shown in the review of Renner (2001), only in plants of the two basal orders the completion of the two phases is in a daily rhythm, whereas in all other orders the separation lasts from several days up to several weeks.

Contrary to other heterodichogamous plant populations, in Annonaceae the pollinators are kept in the flowers and are concentrated there during the whole anthesis. Starting of flower scent production shortly before the complementary morph releases the beetles provides a direct attraction of the pollinators to the new flowers in the female stage. This synchronization supports that the beetles completely change within the population. In heterodichogamous populations which
have open accessible flowers, the flowers get visited more or less sporadically. This “open systems” do not guarantee to hold the pollinators within the system, whereas in the “closed systems” of trap flowers the pollinators are in a steady number during the whole flowering because beetles change between individuals of the different morphs. The fact of differences in the mean number of beetle individuals in the flowers of the two morphs shows that the pollinators were kept more or less totally within the population. In the morph with only a few individuals (B), the beetles got accumulated in the few flowers, whereas in the morph with a higher number of individuals (A) the beetles are distributed among a larger amount of flowers. Beetles were never observed by us to arrive on new flowers before the complementary morph released its beetles, indicating that they indeed came from and changed within the population.

Observations on another population of *A. prinoides* in the Nouragues reserve showed that one population might have more than two morphs; this other population contained individuals with four different antheses types. The four different flower antheses were acting complementary to each other. This surprising observation differs from all other known ones, in which only two different reciprocal morphs occur.

The scent bouquet of *A. prinoides* consists of only seven compounds. These compounds are also dominating the odor bouquet of *Anaxagorea dolichocarpa*, a widespread distributed South American species. Odor samples of *A. dolichocarpa* from natural populations (Teichert, unpublished data) and others from greenhouse plants (Jürgens et al. 2000) show that their odor bouquet is similar to that of *A. prinoides*. In both species, ethyl 3-methylbutanoate is the compound that dominates the scent by more than 50%. Isobutyl isovalerate, the second main compound in *A. prinoides*, also was found in relative high amounts in *A. dolichocarpa*. Both plant species have a characteristically fruity odor, which can be described in *A. prinoides* as banana like, whereas the odor of *A. dolichocarpa* tends towards a more pineapple scent. These differences in smell can be the result of the dissimilar amounts of the specific compounds in the two species. Observations showed that both species are pollinated by beetles belonging to the same genus, viz. *Colopterus* (Nitidulidae).

The three main floral compounds identified in *A. prinoides* are also produced by other flowers that attract beetles, such as Arecaceae, Cycadaceae and
Magnoliaceae. Ethyl 3-methylbutanoate is additionally found in Asteraceae and ethyl tiglate in Rubiaceae, whereas isobutyl isovalerate was only found in flowers of another Annonaceae (Knudsen et al. 2006).

Studies on the pollination of several *Anaxagorea*, *Duguetia* and *Guatteria* species showed that the main pollinators belong to the genus *Colopterus* (Webber 1996). Jürgens et al. (2000) wondered whether the flower visitors have opportunistic behavior, since it appeared that the beetles got attracted by a wide range of compounds, or whether these interactions are more specific. The performed Y-tube experiments with the main compounds of the floral scent did not give a clear indication about preferences of the beetles to one of the offered compounds. Moreover, it seems that the beetles are attracted by several different volatile compounds which are causing the fruity smell of quite a number of Annonaceae species. It was suggested that several Annonaceae attract Nitidulidae beetles which normally live and eat on rotten bark and fruits (Gottsberger 1974, Thien et al. 1975). Thus, scent compounds of the flowers might also occur in fruits. Indeed, volatile flavor compounds from cashew apple juice (*Anacardium occidentale*) has 3-methyl butanoate (Garruti et al. 2003), the main compound in the flower bouquet of *A. prinoides*. Ethyl tiglate (ethyl (E)-2-methyl-2-butanoate), the third most prominent amount in the scent bouquet of *A. prinoides*, is an important compound in the fragrances of analyzed apple fruits also. Hauck et al. (2000) showed that ethyl tiglate which was injected in apple fruits caused the formation of small amounts of ethyl 2-methylbutanoate and indicates a relationship of both compounds in their biosynthesis pathway. Ethyl 2-methylbutanoate was also detected in high amounts in the bouquet of *Anaxagorea brevipes* (Jürgens et al. 2000), another *Colopterus* (Nitidulidae)-pollinated *Anaxagorea* species.

The relatively high fruit set that was observed during the flowering period in 2005 probably was the result of developments that began the year before. The presumption that the fruits are the result from the former flowering period would indicate that fruit development and ripening lasts about one year. It is known that fruit ripening of Annonaceae can be a long-lasting process. For example, studies in *Cymbopetalum baillonii* (Coates-Estrada and Estrada 1988) showed that the fruits needed up to nine months for ripening. After ripening of the fruits of *A. prinoides*, the seeds were released over a time span of one month. Preliminary observations
during several days showed that neither animals nor wind were involved in seed dispersal. The seeds of *A. prinoides* as well as that other *Anaxagorea* species are dispersed exclusively ballistically (Maas and Westra 1984, Armstrong and Marsh 1997), which could explain the small and relatively close distribution pattern of the population and the few seedlings just around reproductively mature trees. The ballistic dispersal mode of *Anaxagorea* contrasts with the dispersal of most other Annonaceae. There is some information on seed dispersal in the family. The most prominent dispersal mode in Annonaceae is endozoochory. Most of the fleshy fruits are eaten by monkeys, squirrels and scatter-hoarding rodents; fruit consumption by fish was also observed in some *Annona* and *Duguetia* species (Gottsberger 1978, Roosmalen 2003 and references therein). Gottsberger (1970, 2006) showed that fruits can be also dispersed by birds and lizards. *Annona glabra* fruits are eaten by iguanas and alligators (“Alligator apple”). Some other genera with fleshy closed monocarps are dispersed by bats and the fruitlets of *Asimina pygmaea* are eaten by turtles (Kral 1960, Coates-Estrada and Estrada 1988, Kessler 1993).

*Anaxagorea* is a well defined genus with a disjunct distribution in tropical America and Southeast Asia. The distribution of *Anaxagorea prinoides* is restricted to the Guianas and the state of Pará in northern Brazil. The genus *Anaxagorea* seems to be a very early lineage in the Annonaceae and is sister group to the rest of the family. Several morphological features of *Anaxagorea* are in common with other Magnoliales (Doyle and Le Thomas 1994, 1996, Doyle et al. 2000, Sauquet et al. 2003). The presence of inner staminodes and laminar stamens is such a character which is shared with other Magnoliales but apparently was lost in the rest of the Annonaceae. Morphological and molecular studies of Scharaschkin and Doyle (2005, 2006) indicate that *A. prinoides* is the sister group of the rest of the genus, and therefore is also the most basal recent species of the whole Annonaceae. One differential character of *A. prinoides* are the pointed staminodes and stamens, that are more rounded or truncate in the other species of the genus.

Until now, pollination in *Anaxagorea* was studied in four species only; three from South America and one from Central America. They all emit fruity and spicy odors and attract mostly nitidulid beetles and a few Staphylinidae. The three species from Central Amazonia (*A. brevipes*, *A. manausensis*, *A. phaeocarpa*) are mainly pollinated by several different *Nitidulidae* species of the genus *Colopterus* (Webber
For *A. crassipetala* from Costa Rica there is no information on pollinator genera (Armstrong and Marsh 1997). The *Anaxagorea* species investigated with respect to their pollination system have flowers sizes that vary from 1.4 up to 2.5 cm in length. *Anaxagorea prinoides* seems to be the smallest one with a flower length of only 1.1 cm. Warming up of flowers (thermogenesis) is reported from several genera in the family (Gottsberger 1970, 1989a, 1989b, 1999, Webber and Gottsberger 1993 Küchmeister et al. 1998, Silberbauer-Gottsberger et al. 2003) and might be also a reward for floral visitors (Seymour and Schultze-Motel 1996). The three *Anaxagorea* species from Central Amazonia also showed slight thermogenesis (Webber 1996, Küchmeister et al. 1998), but this was never observed in *A. prinoides*. 
Literature


A. prinoides


A novel pollination mode: mushroom-like floral characteristics in *Duguetia cadaverica* (Annonaceae) attract mycetophagous nitidulid beetles
Abstract: A novel pollination mode in *Duguetia cadaverica* (Annonaceae) is shown and analyzed; small mycetophagous nitidulid beetles are attracted by the stench-emitting, red- and white-colored flowers. *Duguetia cadaverica* presents its upright flowers on up to 2 m long flagelliform twigs growing along the ground. The flowering phenology of a population of 35 individuals as well as bud and flower abortion rates were studied. The analysis of the scent bouquet by GC-MS revealed the presence of alcohols mostly found in mushroom scent, unpleasant-smelling isocapronic acid and sulphur-containing compounds. The pollinator of *D. cadaverica* was found to be *Pycnocnemus* sp., an as yet undescribed beetle belonging to the tribe Cyllodini (Nitidulidae), a group known to be mycetophagous. Pollinator abundance was low and the beetles were only observed during one of the three observation periods. The typical mushroom-like scent emissions and the attraction of mycetophagous beetles are characteristics of mushroom mimicking flowers.

Introduction
Pollination with developments towards flower specialization are common evolutionary trends in angiosperms. One example of remarkable specialization is floral mimicry, in which flowers may mimic flowers of the other sex, or flowers of other plant species or even several unusual or non-flower substrates. The so-called reproductive mimicry includes several phenomena, such as pseudocopulation of orchids, brood substrate selection or the imitation of the pollinator’s food resource (Dafni 1984). This kind of imitation includes flower shape as well as color or olfactory attraction. Some Araceae, Apocynaceae and Rafflesiaeaceae can attract certain beetles and flies by odor and flower shape that imitate the smell of dung or carrion. Others, such as many Orchidaceae and Aristolochiaceae species attract fungus gnats (Mycetophylidae) by emitting fungal scent (van der Pijl 1961, Kaiser 2006). These flower mimicries usually deceive pollinators by simulating resources that do not exist in the respective flower.

The present study about pollination of *Duguetia cadaverica* (Annonaceae) shows that mycetophagous nitidulid beetles are attracted by the flowers. *Duguetia* is the third-largest genus of the Annonaceae, a family with Pantropical distribution. It
D. cadaverica comprises 93 species, of which 89 occur in the Neotropics and four in Africa (Maas et al. 2003). The presenting of flowers on long flagelliform twigs such as in the plant under study, is also known from two other species in this genus, namely D. flagellaris and D. sessilis. Apart from Duguetia species, flagelliflory in Neotropical Annonaceae is also found in species of Anaxagorea (Maas and Westra 1984, 1985), Hornschuchia (Johnson and Murray 1995) and Stenanona (Maas et al. 2003). In Neotropical Annonaceae, beetle pollination (cantharophily) is the predominant pollination mode with characteristic floral features. The flowers are protogynous, they emit a strong floral scent and form a pollination chamber in which the beetles are sheltered during floral anthesis (Gottsberger 1970, 1989, 1990, 1999).

The aim of this paper is to explore the flowering phenology of Duguetia cadaverica both on the level of individuals and populations. The floral odor composition is analyzed and the relationship with the mycetophagous pollinators is shown.

Material and methods

Study site and analyses

The study was undertaken during the rainy season from February to April 2004 in the Nouragues Natural Reserve in French Guiana (4°5’N, 52°41’W). The area is located in a mostly undisturbed tropical rainforest with an annual rainfall of 2990 mm and a mean annual temperature of 26.3°C (Grimaldi and Riera 2001).

From the end of February until the beginning of April 2004, the floral phenological development of Duguetia cadaverica Huber was followed in a population of 35 individuals growing in an area of 1,500 m². The tree individuals were measured (height and diameter at breast height, dbh). In addition, the flagella length of each individual was measured as well as its origination along the stem. Initially, all buds occurring on the flagella were counted and measured. Afterwards, development of all buds was followed over the whole observation period. Buds were classified as small (apparent - 8 mm), medium (8 - 15 mm) and large (>15 mm). On every day, the open flowers were counted. Flower visitors were observed, caught, and stored in alcohol for later identification. Voucher specimens of the pollinator are
deposited in the collection of A. Kirejtschuk (St. Petersburg, Russia) and plant material in the collection of the herbarium ULM.

Scent

Scent was always collected from single, individual flowers when the scent production started, by a standard dynamic headspace method (Knudsen et al. 1993, Raguso and Pichersky 1995, Knudsen et al. 2006). The flowers were enclosed in ovenbags to accumulate emitted scent, and the scented air was drawn with a battery-operated pump for 3 hours (150 ml/min), through a glass tube, filled with the absorbents Tenax TA 60/80 (25 mg) and Carbopack B 60/80 (40 mg). The absorbed scent was subsequently eluted with 0.3 ml of high grade acetone (Merck, Germany). The compounds were identified by GC-MS at the University of Bayreuth. Before analyzing the samples in a Varian Saturn 2000 mass spectrometer and a Varian 3800 gas chromatograph fitted with a 1079 injector (Varian Inc., Palo Alto, USA), 100 µg of nonadecane was added as internal standard. One µl of the samples was injected using a Varian 8200 autosampler. The injector heated initially with 150°C, and the split rate was 1:10. During the injection the temperature increased with a rate of 200°C min-1 to 250°C and was held for 2 min. For the analyses a ZB-5 (60 m x 0.25 mm i.d., film thickness 0.25 µm, Phenomenex) column was used. Helium was used as a carrier gas with a constant flow rate of 1.8 ml min-1. The temperature of the GC oven was held at 40°C for 2 min, and thereafter the temperature increased with 5°C min-1 to 240°C and held for 3 min. The MS interface had a temperature of 260°C and the ion trap 175°C. The mass spectra were taken at 70 eV with a scanning speed of 1 scan/s-1 from m/z 40 to 350. To process the GC/MS data a Saturn Software package 5.2.1 was used. Component identification was carried out using the NIST 02 mass spectral data base (NIST algorithm), or MassFinder 3.0, and confirmed by comparison of retention times with published data (Adams 1995). Some of the components were identified by comparison of mass spectra and GC retention data with those of authentic standards.
Results

Plant analysis

The average height of individuals of *Duguetia cadaverica* was $3.21 \pm 0.81$ m (mean ± SD, n=35). The dbh was $8.65 \pm 2.7$ cm (mean ± SD). *Duguetia cadaverica* presents its flowers on long flagelliform twigs. These twigs originate mostly on the base of the stem, but some originate also higher up. These twigs creep in various directions along the ground and sometimes branch from one to several orders. The flowers originate successively along the flagella. The flowering period is in January, September, October and November (Mori et al. 2002), but flowering individuals were also found until May. The three sepals of the flowers are sordid brownish to green. The reproductive part of the flower is surrounded by six petals arranged in two whorls. The outer petals are red, ovoid and somewhat pointed at the apex. The inner petal whorl has the same red color including a pronounced white fleshy swelling at the base. The petals close over the reproductive parts but leave a small entrance (Fig. 1). The reproductive organs of a flower consist of $18 \pm 4.5$ carpels (mean ± SD), which are densely aggregated on the floral apex. The carpels are surrounded by $91 \pm 21$ (mean ± SD) stamens with red filaments and two white anthers.

The examined flowers had $18,396 \pm 1,053$ pollen grains and $23 \pm 4$ (mean ± SD) ovula per flower. The P/O ratio was $1,778 \pm 273$ (mean ± SD) indicating a facultative xenogamic breeding system (Cruden 1977). The fruits of *D. cadaverica* are brown and subglobose.
Figure 1: a) Flowers of *Duguetia cadaverica* on long flagelliform twigs. b) Several flagelliform twigs at the base of the stem c) Flower showing the small entrance into the pollination chamber.

Figure 2. Correlation between tree height and flagella length, showing that higher trees have longer flagella (Pearson correlation, p<0.05).
The 35 observed trees in total had 201 flagella with a mean length of 57.36 ± 38.9 cm. The longest flagellum had a length of 191 cm, whereas the shortest was only 3 cm long. Fig. 2. shows that flagella length correlated with the tree height (Pearson correlation, p<0.05). The number of flagella ranged from 2 to 14 per tree with an average of 5.7. The flagella originate 30.7 ± 44.9 cm (mean ± SD) above the ground.

**Phenology of individuals and the population**

The flowers had an accentuated protogynous anthesis. In the afternoon, the flowers opened; at that time no scent releases was perceived. On the following morning, between 5 and 6 AM the flowers started to emit a very unpleasant scent. At this time the stigmas were receptive and shiny exudates were visible on the stigmas. The flowers remained in this female stage until 4 PM. Between 4 and 5 PM the anthers opened and pollen was shed. The petals are retained until the next morning when they dropped and the pollination chamber opened.

At the end of February and the beginning of April 2004, 364 buds on 201 flagella were counted. Of the counted 20 fruits, only eight could be followed until fruit ripening. Observations showed that some flagella that developed several buds also aborted quite a number of them; on other flagella buds developed well. During observations 83 small, 22 middle-sized and 13 large buds were aborted from the flagella. At the end of the observation time in April 2004, 147 buds were still present at the flagella. Between the end of February and the beginning of April 2004, 99 out of 217 buds developed to flowers; during this time 118 buds were shed. The percentage proportion of buds which developed into flowers and buds which were shed was 45 to 55%. About 8% of flowers developed to fruits and only 3.8 % of buds developed to fruits.

<table>
<thead>
<tr>
<th>flagella length [cm]</th>
<th>flagella [n]</th>
<th>buds [n]</th>
<th>buds/flagella</th>
<th>flowers [n]</th>
<th>flowers/flagella</th>
<th>fruits [n]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-50</td>
<td>103</td>
<td>184</td>
<td>1.8</td>
<td>51</td>
<td>0.5</td>
<td>9</td>
</tr>
<tr>
<td>50-100</td>
<td>68</td>
<td>128</td>
<td>1.9</td>
<td>33</td>
<td>0.5</td>
<td>6</td>
</tr>
<tr>
<td>100-150</td>
<td>25</td>
<td>45</td>
<td>1.8</td>
<td>15</td>
<td>0.6</td>
<td>5</td>
</tr>
<tr>
<td>150-200</td>
<td>5</td>
<td>7</td>
<td>1.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>total number</td>
<td>201</td>
<td>364</td>
<td>1.7</td>
<td>99</td>
<td>0.4</td>
<td>20</td>
</tr>
</tbody>
</table>
The number of flowers that were produced by the 35 tree individuals was relatively low during the observations time (Fig. 3). The highest number of flowers produced per day was six, but there were always several days in between in which no open flower was present. The mean number of flowers per day of all 35 individuals was 2.5. No pattern of flowering within the population was observed. Mostly only one or two flowers per day were produced on one tree, but it was very rare to find flowers on the same individual on subsequent days.

Figure 3: Number of open flowers in the population during 2004.

**Flower scent**

The scent of *D. cadaverica* flowers was very unpleasant and can be described as mouldy to cheesy. The main two volatiles were the alcohols (*E*)-2-octen-1-ol and (*Z*)-1-octen-5-ol, followed by 4-methylpentanoic acid (isocapronic acid) and two sulphur-containing compounds.
Tab. 2: Relative amount of floral fragrance from *Duguetia cadaverica*. The compounds are listed according to class, relative retention time (RRt).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>RRt</th>
<th>Relative amounts [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-methylpentanoic acid</td>
<td>961</td>
<td>16.83</td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E) 1-octen-5-ol</td>
<td>988</td>
<td>39.6</td>
</tr>
<tr>
<td>(Z)-2-octen-1-ol</td>
<td>1056</td>
<td>0.29</td>
</tr>
<tr>
<td>(Z) 1-octen-5-ol</td>
<td>1068</td>
<td>0.57</td>
</tr>
<tr>
<td>(E)-2-octen-1-ol</td>
<td>1075</td>
<td>37.98</td>
</tr>
<tr>
<td>Sulfides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dimethyltrisulfide</td>
<td>988</td>
<td>16.84</td>
</tr>
<tr>
<td>dimethyltetrasulfide</td>
<td>1240</td>
<td>0.16</td>
</tr>
</tbody>
</table>

**Pollinators**

Only individuals of *Pycnocnemus* sp. (Nitidulidae) were present on the female- and male-stage flowers of *D. cadaverica*. *Pycnocnemus* sp. is an undescribed species of the family Nitidulidae, tribe Cyllodini (Fig. 4). This tribe is mycetophagous, with exception of species of the genus *Camptodes* which feed on rotten flowers of Cactaceae species (Kirejtshuk, pers. comm.). The beetles apparently were attracted by the strong scent. The beetles arrived in the morning at pistillate-stage flowers, entered and stayed until pollen was shed in the afternoon. They seemed staying in the pollination chamber even after the beginning of the staminate stage. Most of them did not leave the flowers until the next day when the petals were dropped. In 2004 only a few beetle individuals were observed at the flowers. In the following year no beetle could be observed visiting *D. cadaverica* flowers.

Figure 4. Dorsal and lateral view of *Pycnocnemus* sp. impregnated with pollen of *D. cadaverica* on their elytra.
**Discussion**

The presentation of flowers on long flagella is known from many chiropterophilous as well as ornithophilous plant species. Flowers are well exposed and accessible for their pollinators (van der Pijl 1961, Hopkins 1984). Representatives of Asclepiadaceae, Araceae and Rafflesiaaceae with carrion flowers are exposed on the ground, a level where faeces and carrion may occur (Beaman et al. 1988, Meve and Liede 1994, Beath 1996). The unpleasant smelling *D. cadaverica* also uses this kind of flower presentation. For trees it is much easier to present flowers on the ground if they are growing on flagella, like in *Aristolochia arborea*. Controversely, herbaceous plants already grow at the ground and produce their flowers there.

Another advantage to produce flagella is the presentation of flowers on a large area. Flagella originate and creep in different directions away from the stem. Webber (1996) showed for *D. flagellaris*, that the flagella also have the capacity to aid in asexual reproduction. These flagella sometimes grow below the ground and develop roots, and can separate themselves from the main tree forming clones. This phenomenon was observed to occur also in *D. cadaverica*.

Three of the detected scent compounds in *D. cadaverica*, especially 4-methyl pentanoic acid as well as the sulphur-containing compounds were also found in plants which specially mimic carrion or dung (Borg-Karlson et al. 1994, Kite and Hetterschied 1997, Stránský and Valterová 1999). These plants are mostly visited and pollinated by species of the families Silphidae and Scarabaeidae. Furthermore, scent bouquets with high amounts of sulphur-containing compounds are often associated with plants pollinated by bats (Knudsen and Tollsten 1995, von Helversen et al. 2000). The most interesting scent compounds of *D. cadaverica* are the mushroom-like volatiles. These volatiles indeed were described from bouquets of mushrooms. It is known that flowers with such mushroom scents can attract fungus gnats (Mycetophilidae, Diptera). Knudsen and Tollsten (1993) consider the presence of mushroom volatiles in flower bouquets that are not pollinated by fungus gnats as an adaptation to dry environments, in which the flowers imitate a humid milieu. Both isomers of 2-octen-1-ol are found in headspace samples of mushrooms, the trans-isomer in cultivated white mushrooms (Buchbauer et al. 1993), and the cis-isomer is responsible for the moldy smell of several mushrooms and also occurs in *Aspergillus* species (Kaminski et al. 1972).
Each individual of *D. cadaverica* produces only a few flowers per day. Most other Annonaceae species produce considerable more flowers during their flowering time (pers observ.). The reason why *D. cadaverica* produces fewer flowers than other Annonaceae species might be its dependency on its pollinator species. In Annonaceae that produce larger numbers of flowers, such as *Anaxagorea* or other *Duguetia* species in the same locality in French Guiana, we found up to 25 pollinator individuals within one flower, whereas the event to meet a pollinator of *D. cadaverica* was very rare. The high proportion of aborted buds in early developmental stages is a phenomenon which is well known in commercial fruit cultivation (Stephenson 1981). Re-allocation of limited resources, a common phenomenon in tropical forests, is generally suspected to be the proximate reason for these abortions, as resources are usually translocated out of a plant part before abscission and the remaining fruit benefit from this surplus resource (Stephenson 1981).

The pollinator *Pycnocnemus* sp. ( Nitidulidae) belongs to a tribe of beetles that feed on mushrooms. The identified scent compounds with mostly mushroom volatiles are a clear hint that the *Duguetia cadaverica/Pycnocnemus* system is a mushroom mimicry system. To the best of our knowledge the present observation is the first description of a pollination system in which beetles pollinate a flower mimicking fungi.
**Literature**


Ecological relationships of

*Evodianthus funifer* and other

Cylanthaceae with their associated
derelomine (Curculionidae) beetles
Abstract: The pollination of *Evodianthus funifer* by small derelomine beetles in French Guiana is described. The weevils are attracted by the scent producing staminodes. The analysis of the scent bouquet of three Cyclanthaceae species (*Evodianthus funifer, Ludovia lancifolia, Stelystylis surinamensis*) using GC-MS demonstrated that they are all dominated by only two compounds, which were not found in the other species. Observations showed that *E. funifer* and *L. lancifolia* are visited by pollinator as well as non-pollinators, which are gnawing on plant parts and ovipositing on staminodes or on staminate flowers. An investigation of nutritional chemistry and energy content of all inflorescence organs proved that the weevils placed their eggs on the energy and carbon-rich parts. The highest energy content was found in the staminodes. Histological investigations showed that ducts are present along them, and some liquid is transported. It is assumed that this liquid is involved in scent emission.

Introduction

Pollination studies in members of the family Cyclanthaceae are scarce. Only a few species of *Asplundia, Sphaeradenia, Carludovica, Chorigyne, Cyclanthus, Dicranopygium, Evodianthus* and *Ludovia* were investigated in this respect (Beach 1982, Schremmer 1982, Gottsberger 1991, Eriksson 1994, Franz 2007). As can be seen from these references, most investigations were published 15 years ago or are even older. These investigations show that members of the subfamily Carludovicoideae are pollinated by small weevils, while *Cyclanthus bipartitus* (subfamily Cyclanthoideae) is pollinated by dynastid scarab beetles. As Gottsberger (1991) emphasized, the Cyclanthaceae appear to be the only exclusive cantharophilous family among monocotyledons, a fact confirmed by other authors.

The inflorescences of Cyclanthaceae have characteristic adaptations for beetle pollination. Similar syndromes are also present in other beetle-pollinated families, such as in Annonaceae and Araceae, in which the flowers or inflorescences are protogynous, form pollination chambers, have synchronized dichogamy with protogyny, strong floral fragrances, pale floral organs, thermogenesis and no apparent liquid resources for the pollinators (Gottsberger 1991, Eriksson 1994, ...
Franz 2007). The family Cyclanthaceae has an exclusive Neotropical distribution. The 230 species are divided into two subfamilies, based on inflorescence morphology. The subfamily Cyclanthoideae comprises only Cyclanthus bipartitus, the remaining species belong to the Carludovicoidae. The life forms of this family vary from permanently terrestrial plants to root climbers, primary and secondary hemiepiphytes, and holoepiphytes (Harling 1985).

**Material and Methods**

**Species investigated, their characteristics and phenology**

All observations were conducted in the Nouragues Natural Reserve (4°5’ N, 52°41’ W) in French Guiana. The area lies in a mostly undisturbed tropical lowland forest with an average rainfall of 2990 mm per year, and a 3 months dry period between September and October (Grimaldi and Riera 2001).

*Evodianthus funifer* (Poiteau) Lindman and the other studied species belong to the subfamily Carludovicoidae. The distribution of *E. funifer* extends from Central America through tropical South America. *Evodianthus funifer* is a root-climbing liana with a mostly unbranched stem. The leaves are spirally arranged and the leaf blade is bifid and rather large. The inflorescence of the Carludovicoidae species is a monoecious spadix. The three spathes are inserted directly below the spadix. The staminate and the pistillate flowers are arranged on the spadix in a regular chessboard mosaic. Each pistillate flower has four funnel-shaped staminodes. The pistillate flowers have well developed tepals opposite the long staminodes. The fruits are dispersed endozoochorously by bats (Harling 1958).

Additional analyses were done in *Ludovia lancifolia* Brongniart. The morphology of the inflorescence is similar to that of *E. funifer*. In contrast to *E. funifer*, in which the perianth of the staminate flowers hides the stamens, the stamens of *L. lancifolia* are exposed already in early-stage flowers.

Floral phenology and behavior of the flower visitors of *E. funifer* were recorded during September and October 2005. The flowering time of *E. funifer* is during September (Mori et al. 2002). Observations on the floral anthesis started at 4
AM in the first morning of flowering of a spadix and ended at 9 AM on the second day when anthesis finished.

For general morphological investigations in the laboratory, fresh flowers of Evodianthus funifer, Ludovia lancifolia and Asplundia heteranthera Harling were collected and stored in 70% alcohol. Several fresh flowers were immersed into 0.01% solution of neutral red to localize the scent-producing tissue (Vogel 1963). For further morphological investigations scanning electron microscope (SEM) photos were made. The samples were dehydrated in 70% isopropanol for one day and in 100% isopropanol for a second day. After dehydration the samples were critical point dried and sputter-coated with gold (Balzer Union, Liechtenstein). The observations were made using a scanning electron microscope (Zeiss DSM 240, Germany). Histological investigations on cross sections of the staminodes were executed to investigate whether staminodes have a secretion tissue and later were examined under the light microscope. The stored flowers were dehydrated through an ethanol series. Tert-butanol was used as an intermediate solution. After dehydration, the samples were embedded in paraffin wax and cut (7 µm) with a Leitz microtome. The cuts were stained with alcian blue to visualize mucosubstances.

Thermogenesis during anthesis of the flower was measured with a multi-channel thermometer (Bioblock Scientific, Logging Thermometer, 16200). The temperature of the inflorescences as well as the temperature of the surrounding air was measured in parallel.

**Odor sampling and identification**

Scent was collected from E. funifer and L. lancifolia at their natural site; additionally scent was collected from Stelestylis surinamensis which is cultivated in the greenhouse of the Botanical Garden of Ulm University.

Scent was always collected from the whole inflorescence when the scent production started, by a standard dynamic headspace method. The flowers were enclosed in ovenbags to accumulate emitted scent, and the scented air was drawn with a battery-operated pump for 3 hours (150 ml/min), through a glass tube, filled with the absorbents Tenax TA 60/80 (25 mg) and Carbopack B 60/80 (40 mg). The
absorbed scent was subsequently eluted with 0.3 ml of high grade acetone. The compounds were identified by GC-MS using a Thermo Finningen Voyager Mass Spectrometer combined with a Trace GC 2000 Series and the Xcalibur software. For the analyses a DB-WAX column (30m x 0.32 mm i.d., film thickness 0.25µm, J&W Scientific) was used. Component identification was carried out by comparison of their mass spectra and retention times with those of authentic references samples available from the collection of references compounds.

**Nutritional chemistry of inflorescences and sterile plant parts**

Our own observations and that of other authors showed that the visiting weevils use the inflorescence for feeding and as an oviposition site. To examine the resources, the nutritional composition and energy content of the flower parts and additionally of some leaves was measured. The key nutritional substance nitrogen and water, as well as carbon and the energy content were studied. Water content (%) of the reproductive organs, such as pistillate and staminate flowers, staminodes, as well as spathe and leaves was examined. Plants were collected and separated into their respective flower parts and fresh weight was measured. Thereafter, the samples were dried (60°C) in an oven and re-weighted to calculate the water loss.

Nitrogen content was determined with the Kjeldahl method. Of each part 50 mg dry material was used for analysis. The carbon content of the plant parts was measured from 50 mg dry material using the carbon measure CS 125/225 (Leco). To measure the energy budget of each flower part 50 mg dry material was completely burned in a calorimeter (C 7000). For calibration a defined burning was executed.

**Results**

**Plant material and floral biology**

*Evodianthus funifer* (Poit.) Lindman is a root-climbing liana. The plants occurred at a height from 2 up to several meters on their phorophyte. The inflorescence is about 3 to 4 cm long and has 1.9 cm in diameter. Along the spadix are 41 ± 0.2 (mean ± SD) pistillate flowers inserted, surrounded by 187 ± 16.6 (mean ± SD) staminate
flowers. Observations on inflorescences of *E. funifer* showed that the anthesis is protogynous and lasts for two days. In the evening before anthesis started, the spathes opened somewhat and the staminodes were visible but still folded. In the following night the light orange staminodes began to stretch and the spathes opened. Between 4 and 5 AM, the inflorescences started to emit a strong fruity and spicy scent. The staining with neutral red indicated that only the staminodes are involved in scent releasing. During this time dozens of weevils were attracted to an inflorescence. The weevils crawled on the staminodes before entering between the funnel-shaped staminate flowers which form a “pollination chamber” over the pistillate flowers. Beetles either entered the inflorescence and contacted pistillate and staminate flowers or visited the surface of the inflorescence and the staminodes only. The differential observation and collections of visiting beetles showed that not all visitors are pollinators. Four of the six determined morpho-species (Curculionidae: Derelomini) were entering the interior of the spadix and contacted the receptive stigmas with its shiny stigmatic coat. The weevils which were not entering these chambers were present on the staminodes only where they were running up and down and also flew to other staminodes. The inflorescences were crowded with dozens of weevils, which were moving on and between the staminodes during this time. Often these weevils were observed mating, but they generally did not change their rapid movements. The beetles also fed on the staminodes, they gnawed on them and often staminodes fell to the ground. Serial photographs of staminodes lying on the ground showed that they were cut up into small pieces and taken away by the weevils. These beetles never came in contact with any receptive floral organ. The weevils which entered the “pollination chambers” occupied this space and defended it aggressively against other weevils that tried to enter. In the late morning the staminodes were all bitten off, either by the weevils outside the spadix or by others inside the pollination chambers; the last ones were gnawing on them directly at their basal portion. Fresh staminodes showed drops of secreted liquids on their surface. On scares from cut staminodes also liquid extruded. After the staminodes had dropped, the activity of the pollinators concentrated towards parts in the interior of the inflorescence. During the day the activity of the beetles was restricted to the “pollination chamber”.

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Cyclanthaceae
Observations showed that the pollinators used the inflorescence as an oviposition site. Eggs were found deposited on the perianth of the staminate flowers. During the following night, around 3 to 4 AM the inflorescence changed to the staminate phase. The perianth opened and the anthers released pollen. The open perianth of the staminate flowers was touching each other, and only a few small holes offered space for the pollinators to crawl out. During this process, they became completely dusted with pollen. Afterwards, the weevils changed to inflorescences in the pistillate stage on other individuals. During the days following anthesis the staminate flowers dried and dropped from the spadix (Fig. 1).

There was evidence of floral temperature elevation in *E. funifer*, which initiated just before the female stage. The temperature within the floral chamber reached 24.1°C which was only around 1°C higher than the ambient temperature. During the day, the inflorescence temperature was lower than ambient temperature.
but rose again to 24.2°C during the staminate stage (1.2 °C above ambient temperature) (Fig. 2).

Anthesis of *Ludovia lancifolia* started at 6 AM. In this species too, weevils (Curculionidae: Derelomini) acted as pollinators or were present only at the staminodes. Between 5 and 6 AM on the next morning, the flowers changed to the male stage and the beetles got dusted with pollen.

**Figure 2:** Thermogenesis in *Evodianthus funifer*: course of air and inflorescence temperature over the whole anthesis. Arrows mark time of heating of inflorescence.

### Scent analyses

The scent bouquet of the three analyzed Cyclanthaceae species differs considerably in its composition. All of them were dominated by one or two main compounds which were not found in the other species. Only three compounds, which occurred in *L. lancifolia* as well as in *E. funifer*, could be identified. The scent of *E. funifer* mainly consisted of four compounds of sesquiterpenes. The main compound was the hitherto undescribed natural scent compound (E,E)-alpha-farnesene 2(3), 9(10)-diepoxid, with a percentage of 59%. The two other main compounds could not be identified and represented 15 and 7% of the floral fragrance, followed by (E,E)-alpha-farnesene with 3% and (E)-alpha-farnesene epoxid with 1.1%. The bouquet of
L. lancifolia was dominated by two compounds, namely 3-methylene-2-(2(z)-pentenyl)cyclopentanol (46.3%) and jasmone (52%). Trans-cinnamyl acetate was found to be the major compound in Stelystylis surinamensis with (89.25%) followed by benzyl acetate with 8.5% (Tab.1).

### Tab. 1: Relative amount of floral fragrances of three Cyclanthaceae species: *Evodianthus funifer*, *Ludovia lancifolia*, *Stelystilis surinamensis*. The compounds are listed according to their class.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Relative amount [%]</th>
<th>E. funifer</th>
<th>L. lancifolia</th>
<th>S. surinamensis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alcohols</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E)-Nerolidol</td>
<td>-</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(E)-Cinnamyl alcohol</td>
<td>-</td>
<td>-</td>
<td>0.25</td>
<td>-</td>
</tr>
<tr>
<td>(Z)-2-Pentenol</td>
<td>-</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-Methylene-2-(2(Z)-pentenyl)-cyclopentanol</td>
<td>-</td>
<td>46.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Ketones</strong></td>
<td></td>
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<tr>
<td>Jasmone</td>
<td>-</td>
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<td>-</td>
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<td><strong>Aldehydes</strong></td>
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</tr>
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<td>0.02</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Octanal</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(E)-Cinnamaldehyde</td>
<td>-</td>
<td>-</td>
<td>0.01</td>
<td>-</td>
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<td><strong>Acids</strong></td>
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<td>Acetic acid</td>
<td>0.01</td>
<td>0.02</td>
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<td>-</td>
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<td><strong>Esters</strong></td>
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<td>Ethyl-3-methyl butanoate</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>(E)-Methyl cinnamate</td>
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<td>-</td>
<td>0.06</td>
<td>-</td>
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<tr>
<td>cis-Cinnamyl acetate</td>
<td>-</td>
<td>-</td>
<td>0.06</td>
<td>-</td>
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<tr>
<td>trans-cinnamyl acetate</td>
<td>-</td>
<td>-</td>
<td>89.25</td>
<td>-</td>
</tr>
<tr>
<td>Methyl benzoate</td>
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<td>-</td>
<td>1.6</td>
<td>-</td>
</tr>
<tr>
<td>Benzyl acetate</td>
<td>-</td>
<td>-</td>
<td>8.57</td>
<td>-</td>
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<td><strong>Alkanes</strong></td>
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<tr>
<td>(E)-7-methyl-1,6-dioxaspiro(4,5)Decane</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Benzenoids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Styrene</td>
<td>0.01</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenylpropanoides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzenepropyl acetate</td>
<td>-</td>
<td>-</td>
<td>0.18</td>
<td>-</td>
</tr>
<tr>
<td><strong>Monoterpenes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Linalool</td>
<td>-</td>
<td>0.07</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

89
Table 1. continued

<table>
<thead>
<tr>
<th>Compounds</th>
<th>E. funifer</th>
<th>L. lancifolia</th>
<th>S. surinamensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,8-Cineol</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carvone</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(E)-Ocimene</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Sesquiterpene</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E)-β-Farnesene</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(Z,E)-α-Farnesene</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(E,E)-α-Farnesene</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(Z)-α-Farnesene epoxide</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(E)-α-Farnesene epoxide</td>
<td>1.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(E,E)-α-Farnesene-2(3),9(10)-diepoxid</td>
<td>59</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Nitrogen compounds</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl anthranilate</td>
<td>-</td>
<td>0.01</td>
<td>-</td>
</tr>
<tr>
<td>Indole</td>
<td>-</td>
<td>0.01</td>
<td>-</td>
</tr>
<tr>
<td><em>Irregular terpenes</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E)-4,8-Dimethyl-1,3,7-nonatriene</td>
<td>0.01</td>
<td>0.02</td>
<td>-</td>
</tr>
<tr>
<td>Unknown compounds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Unknown</td>
<td>15</td>
<td>-</td>
<td>-</td>
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</table>

*Morphological and histochemical studies of the staminodes*

After staining with neutral red, the staminodes showed the typical pattern described by Vogel (1963) indicating scent emitting areas. These red spots were visible on the whole surface of the staminodes.

The light orange staminodes of *E. funifer* which are responsible for scent production were up to 9 cm long. Sometimes, rudimentary anthers were found on the end of the staminodes. Cross sections showed that the staminodes of *E. funifer* and *A. heteranthera* had a biconcave, plane shape. In each of the two outer swalloes a continuous duct was present. The ducts were laterally as well as dorsally restricted by only a few layers of parenchyma cells. The center of the staminodes was occupied by a vascular bundle. Cross sections as well as longitudinal sections of female flowers showed, that the staminodes adnate close to the petals of the pistillate
flowers. The sections also proved that the origin of these ducts was not at the base of the staminodes but initiates somewhat above. The whole surface of the staminodes was uniformly corrugated with regular distributed stomata over the whole staminode.

Histochemical analyses by colored cross sections with alcian blue showed in all observed species a strong staining of the ducts. The whole content of this ducts stained blue as well as the intercellular spaces between the parenchyma cells. The epidermis cells also showed such a strong accumulation of alcian blue. Scanning electron microscope pictures of cross sections sometimes showed a solidified substance in these channels (Fig 3).

![Cross section pictures of Asplundia heteranthera showing ducts within the staminodes. SEM picture show the solidified liquid substance within the ducts. (Bar represents 90 µm) (a). Staining with alcian blue show that mucosubstance is present within the ducts (50x magnificated) (b).](image)

**Nutritional chemistry**

In both analyzed plant species, the staminodes had always high levels of nitrogen and carbon and were energy-rich. The staminate flowers were also energy-rich and contained a relatively high content of the analyzed nutrients. In *E. funifer* the pistillate flowers had significantly more nitrogen then other plant tissues. Pistillate flowers of *L. lancifolia* instead had lower content of nitrogen than staminate flowers and staminodes. The spathes of both plant species were rich in water content but poor in all other nutrients. Of the two evaluated plant species the leaves had mostly less content of nutrients than the staminate and pistillate flowers, as well as the staminodes. In both plant species the lowest C/N ratio was calculated in the
staminodes and the pistillate flowers. Energy content in *E. funifer* as well as in *L. lancifolia* was always high in staminodes and staminate flowers.
Tab. 2: Nitrogen, water, carbon and energy content of different plant species and inflorescence organs (n=3). Different letters denote significant differences between plant parts within species (Anova, Tukey’s test p<0.05) (Due to loss of material, leaves are n=1)

<table>
<thead>
<tr>
<th>Plant part</th>
<th>E. funifer Nitrogen [mg g(^{-1})dm]</th>
<th>L. lancifolia Nitrogen [mg g(^{-1})dm]</th>
<th>E. funifer Carbon [mg g(^{-1})dm]</th>
<th>L. lancifolia Carbon [mg g(^{-1})dm]</th>
<th>E. funifer C/N</th>
<th>L. lancifolia C/N</th>
<th>E. funifer Water content [%]</th>
<th>L. lancifolia Water content [%]</th>
<th>E. funifer Energy content [J g(^{-1})dm]</th>
<th>L. lancifolia Energy content [J g(^{-1})dm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>leaves</td>
<td>17.8(^{\text{c}})</td>
<td>9.9(^{\text{ab}})</td>
<td>433(^{\text{b}})</td>
<td>418.7(^{\text{a}})</td>
<td>24</td>
<td>42</td>
<td>85.3(^{\text{b}})</td>
<td>82.5(^{\text{bc}})</td>
<td>14370(^{\text{bc}})</td>
<td>15656(^{\text{b}})</td>
</tr>
<tr>
<td>spathe</td>
<td>12.8±1.7(^{\text{d}})</td>
<td>5.7±1.4(^{\text{a}})</td>
<td>400.1±6.9(^{\text{d}})</td>
<td>416.0±7.4(^{\text{a}})</td>
<td>31</td>
<td>73</td>
<td>91.6±0.4(^{\text{b}})</td>
<td>89.8±0.6(^{\text{c}})</td>
<td>13604±831(^{\text{c}})</td>
<td>13129±921(^{\text{b}})</td>
</tr>
<tr>
<td>staminodes</td>
<td>23.3±0.3(^{\text{b}})</td>
<td>19.2±4.8(^{\text{c}})</td>
<td>479.9±8.4(^{\text{a}})</td>
<td>508.6±3.1(^{\text{c}})</td>
<td>21</td>
<td>26</td>
<td>87.2±1.9(^{\text{b}})</td>
<td>80.3±2.1(^{\text{bc}})</td>
<td>18172±1333(^{\text{a}})</td>
<td>18020±863(^{\text{c}})</td>
</tr>
<tr>
<td>staminate flowers</td>
<td>18.4±0.3(^{\text{c}})</td>
<td>16.6±1.2(^{\text{bc}})</td>
<td>458.0±3.0(^{\text{c}})</td>
<td>507.2±1.8(^{\text{c}})</td>
<td>25</td>
<td>31</td>
<td>81.4±0.7(^{\text{c}})</td>
<td>62.3±0.8(^{\text{a}})</td>
<td>16286±809(^{\text{ab}})</td>
<td>18569±138(^{\text{d}})</td>
</tr>
<tr>
<td>pistillate flowers</td>
<td>27.2±0.9(^{\text{a}})</td>
<td>15.9±2.5(^{\text{bc}})</td>
<td>403.1±5.2(^{\text{a}})</td>
<td>456.6±2.3(^{\text{b}})</td>
<td>15</td>
<td>28</td>
<td>85.9±1.5(^{\text{b}})</td>
<td>73.9±1.2(^{\text{b}})</td>
<td>13200±952(^{\text{c}})</td>
<td>14898±54(^{\text{b}})</td>
</tr>
</tbody>
</table>
Discussion

As other investigated Cyclanthaceae species, also *Evodianthus funifer* and *Ludovia lancifolia* are mainly pollinated by small weevils belonging to the tribe Derelomini (Coleoptera: Curculionidae) (Gottsberger 1991, Eriksson 1994, Franz 2007). The morphology of inflorescences of members of the Carludovicioideae allows only small beetles to be pollinators. This results from the small entrances to the reproductive pistillate flowers between the staminate flowers.

Six beetle morpho-species are associated with the inflorescence of *E. funifer*, but not all of them are acting as pollinators. Only four are real pollinators and were found in the pistillate- and male-stage inflorescences. This concurs with former observations on other Cyclanthaceae species in Costa Rican forests. There are up to nine different species of derelomine flower weevils associated with one host plant, in which only four act as pollinators (Franz 2007).

Curculionidae beetles are often found to be florivorous, they are eating on buds, fresh or decayed flowers or on fallen flower organs (Armstrong and Marsh 1997, Franz 2003, Frame 2003, Held and Potter 2004, Neto and Teixeira 2006, Feinstein et al. 2007). Therefore it is not surprising to find them on their host plants eating on flower- and inflorescence-parts.

The plant itself does not suffer by this behavior. Although the weevils eat on floral organs, they do not cause drastic damages of organs which are involved in fruit maturation. Florivory on Cyclanthaceae species is not only restricted to feeding on flowers, but extends to oviposition on staminate flowers. Most eggs were laid on the perianth of staminate flowers, which were shed soon after anthesis. Staminodes are further food resources; they act as the most attractive organs to the pollinators and non-pollinators. When they are damaged this has no influence on the reproductive success of the plant. The staminodes as well as the staminate flowers are used for oviposition, both by weevil species which have no influence on pollination and others which act as pollinators.

The behavior of the non-pollinators, which are feeding and ovipositing on staminodes was recently described by Franz (2003). The beetles detach the staminodes, fall together with them to the ground, and oviposite later onto them. On the other hand, weevils which act as pollinators use staminate flowers for
ovipositing. This is surprising since these flowers are shed soon after anthesis. Eriksson (1994) explained this as a behavior to minimize a loss of investigated energy and increases the success of fruit development when the eggs are laid on staminate flowers that are not nourished after anthesis. Franz (2007) discovered that eggs laid on pistillate flowers were not surviving when the infructescences matured successfully. The lower risk for the larvae to survive therefore is to lay eggs on staminate flowers.

The nutritional composition of flower organs is quite variable. The staminate flowers of the two studied species mostly had a higher energy content than the other plant parts. They are also rich in carbon, but have less nitrogen and a higher C/N ratio than pistillate flowers. However the results offered not a clear indication for egg-laying on staminate flowers because of nutrient content. As Eriksson (1994) mentioned, the morphology of the pollination chamber above the pistillate flowers promotes egg-laying on the staminate flowers. Another reason for egg-laying onto staminate flowers could be the exposition of the infructescences since they can easily be reached by predators. If the development is between the leaf litter then the larvae are more secured from potential predators. But anyway, when eggs are laid on staminate flowers, the larvae are developing fast within less then two weeks (Franz 2005) and find energy and carbon rich moderately digestible brood substrate, on which they can feed later on. This seems to be a “first aid package” of food resources before feeding on poorly utilizable detritus on the ground.

If the eggs are laid on staminodes, the resources of the brood substrate are much higher than on staminate or pistillate flowers. In both plant species under study, the highest energy content was found in the staminodes. In the same way the nitrogen and carbon content was significantly higher as compared with other plant parts. The choice to use staminodes for ovipositing seems to be an adaptation to utilize the rich content of the staminodes. Nitrogen plays an important role in metabolic processes. Plant parts which are growing fast and have high turnover rates like flowers usually contain high nitrogen concentrations (Mattson 1980). Maynard and Loosli (1969) observed that in plant tissues which are growing fast, non-protein nitrogenous compounds are more common than proteins. And such plant tissues are preferred by herbivores (Feeny 1970).
The plant themselves do not profit from this relationship because these visitors never come in contact with reproductive organs, but they themselves have a much better brood substrate for their offspring. Although the staminodes are important for the attraction of beetles, their detaching does not have a deleterious effect since most pollinators have already arrived. Both pollinators and non-pollinators use these high energy rich resources of staminodes for feeding (Gottsberger 1991, Eriksson 1993, Franz 2007). The attractiveness of the inflorescences seems to be altered by the beetles’ destructive behavior. The pollinators as well as the non-pollinators usually arrive in a high number of individuals and at about the same time at the inflorescence. Conversely, in long-lived flowers attraction has to be for several days, and any damage could decrease the success of reproduction (Karban and Strauss 1993, Krupnick and Weis 1999).

The staminodes have several functions and play an important role in pollination. First of all the staminodes are the organs that emit the strong scent. Furthermore, staminodes probably increase the visual attraction to the inflorescence (Gottsberger 1991, Franz 2007). The morphological investigations showed that the staminodes have features which allow the transport of a kind of liquid. Although we do not know the nature of this liquid, coloring with alcian blue indicated that this channels transport some mucilage. Staining of cross sections of staminodes of *Evodianthus funifer* and *Asplundia heteranthera* indicated that this mucilage is transported between the cells and is accumulated in the epidermis cells. It is not clear how the liquid is secreted. The surface of the staminodes is very roughly corrugated, and seems to be an enlargement of the liquid-secreting area. Especially in scent-emitting surfaces such features are often found (Vogel 1963, Effmert et al. 2005). Another possibility is a liquid-release via the stomata; this is indicated by drops forming on the staminodes’ surface. The chemical investigations showed that the staminodes are the most energy-rich parts of the inflorescence and are also rich in carbon and nitrogen. The relatively high energy content could be explained by oily substances within the staminodes (Kazda, pers. comm.). The liquid secretion that is transported and secreted from the staminodes might therefore reveal to be liquid perfume oil. Observations by Gottsberger (1991) showed that male euglossine bees were also observed on staminodes, apparently collecting liquid perfume.
The scent bouquets of the three investigated Cyclanthaceae have different major constituents. All of them are dominated by two compounds. Similar observations are known from palm trees (Evrik et al. 1999) and from the scent analysis of another representative of the Cyclanthaceae (Schultz et al. 1999). Both major compounds in *E. funifer* and *L. lancifolia* were unknown in natural scents. Just as in Cyclanthaceae, derelomine beetles play an important role also in pollination of Arecaceae. Also in palms the scent is dominated by a few major compounds. Although the taxonomy of these beetles is not yet cleared-up, it could be possible that they are attracted by a wide range of diverse volatile compounds.
Cyclanthaceae

Literature


Curriculum vitae

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Place of birth  Aalen

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2008             Teaching assistant of an undergraduate student ecological field course in Banyang Mbo, Cameroon, University of Rostock
Presentation at scientific symposia


Publications


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Erklärung

Hiermit erkläre ich, die vorliegende Dissertationsarbeit selbständig angefertigt und keine anderen als die in der Arbeit aufgeführten Hilfsmittel verwandt zu haben. Wörtlich oder inhaltlich übernommene Stellen wurden als solche gekennzeichnet.

Ulm, den 12. Februar 2008