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**THE REPRODUCTIVE BIOLOGY OF  
EUGLOSSINE-POLLINATED PLANTS IN THE  
NATURAL RESERVE NOURAGUES, FRENCH GUIANA**

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**DISSERTATION**

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vorgelegt von

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Tag der Promotion:	

*Legend to pictures of the title page:*

A	B	A: <i>Euglossa</i> cf. <i>chalybeata</i> approaching flower of <i>Voyria clavata</i> (Gentianaceae)
C	D	B: <i>Euglossa</i> cf. <i>chalybeata</i> approaching flower of <i>Rapatea paludosa</i> (Rapateaceae)
		C: <i>Exaerete frontalis</i> sucking nectar at a flower of <i>Bonafousia disticha</i> (Apocynaceae)
		D: <i>Eulaema bombiformis</i> collecting floral perfume at an inflorescence of <i>Spathiphyllum humboldtii</i> (Araceae)

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## SUMMARY

### **The reproductive biology of euglossine-pollinated plants in the natural reserve Nouragues, French Guiana**

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#### GENERAL INTRODUCTION

Tropical *lowland* rainforests are characterized by a high plant diversity and low population densities (Kress & Beach 1994, Myers *et al.* 2000). The wide spacing between conspecifics (Hubbel & Foster 1983, Ashton 1984, Bawa *et al.* 1985a) leads to more intense selection for long-distance pollen flow than in other communities (Janzen 1970, Bawa 1990). Hereby, plant species of different forest strata have evolved different ways to attain pollen flow, which is generally attributed to environmental factors (particularly light intensity) and the stratification of pollen vectors (Bawa 1983, Bawa 1990, Kress & Beach 1994, Kato 2005). In the forest canopy mass-flowering species dominate, which attract a broad species spectrum of foraging insects, and are either visited by large numbers of small diverse insects or medium- to large-sized bees (Frankie *et al.* 1983, Bawa *et al.* 1985b, Bawa 1990, Kress & Beach 1994, Momose *et al.* 1998). Interplant movement of pollinators is attained by the exact timing of flowering and nectar production of the conspecifics (Frankie *et al.* 1976, Frankie *et al.* 1983, Bawa *et al.* 1985b). Usually, blooming of an individual plant takes only a few days and different plant species are supposed to minimize competition for shared pollinators and interspecific gene-flow by short, non-overlapping flowering periods (Bawa 1983, Frankie *et al.* 1983). Since the risk of inbreeding depression caused by geitonogamy is high, most species have evolved self-incompatibility or dioecy (Heinrich & Raven 1972, Bawa 1990, Kress & Beach 1994). In the forest understory flower visitors are not attracted by large floral displays. Instead, plants are highly specialized in their reproductive biology to specific pollinators that primarily belong to the guilds of long-billed nectarivorous birds, long-tongued bees, and beetles (Bawa 1990, Kress & Beach 1994). Beetle-pollinated plants provide their visitors with food tissue, and achieve cross-pollination by the exact timing of the female and male blooming periods of different protogynous flowers or protogynous and monoecious inflorescences (Gottsberger 1970, Gottsberger & Amaral Jr. 1984, Bawa *et al.* 1985a, Young 1988, Gottsberger 1989, Gottsberger & Silberbauer-Gottsberger 1991, Kress & Beach 1994, Gottsberger & Silberbauer-Gottsberger 2006). Bird- and bee-pollinated plants produce few hermaphroditic flowers daily, which supply their visitors with large amounts of nectar. In order to prevent exploitation of the flowers by indiscriminate foragers, floral resources are

hidden in long corolla tubes that are only accessible to the valid pollinators (Heinrich & Raven 1972, Stiles 1975, Feinsinger 1983, Momose *et al.* 1998). However, nectar amounts secreted by each plant are too low to saturate the daily energy requirements of the pollinators (Heinrich & Raven 1972, Stiles 1975, Bawa *et al.* 1985a). These have to visit various populations consecutively and cover several kilometers during foraging, which contributes to cross-pollination and large-distance gene flow (Stiles 1975, 1978). Thereby, the pollinators follow established flight routes that connect their host plants ('trap-lining'; Janzen 1971, Stiles 1978, Sakai *et al.* 1999). The hosts represent reliable food resources as they produce multi-flowered inflorescences that bloom over extended time periods ('steady state flowering'; Gentry 1974, Bawa 1983). Self-compatibility in this plants is very common but outcrossing seems to prevail since the offspring of selfed plants usually shows a reduced fecundity (Bawa *et al.* 1985a, Kress & Beach 1994).

Specialization to pollinators is suggested to be an important driving force in the speciation of plant species (Stebbins 1974, Grant 1981, Feinsinger 1983). It permits the co-occurrence and the simultaneous flowering of various, even closely related plant species without interfering in each others reproductive biology (Stiles 1975, Sakai *et al.* 1999, Kay & Schemske 2003). Further, it may promote adaptive divergence in floral morphology (Sakai *et al.* 1999, Kay & Schemske 2003). However, tropical understory communities have been poorly studied since extremely low population densities of plant species and high logistical efforts make the exploration of tropical rainforests very difficult. Therefore, most theory about speciation mechanisms and the reproductive ecology of these plants has not been verified by field research yet. The only in-depth community investigations on the reproductive ecology of understory species were made on hummingbird-pollinated plants (Stiles 1975, Feinsinger 1978). These studies showed that pollination guilds are probably much more fine-grained as it has been supposed. Reproductive isolation was not only achieved by differences in spatial and temporal blooming patterns but also by floral morphology and ethological mechanisms (Stiles 1975, 1978).

In my thesis, I investigated the reproductive biology of a community of plants pollinated by euglossine bees. The tribe Euglossini (Hymenoptera: Apidae) represents one of the major groups of most bee communities in Central- and South American tropical forests, and in some localities it is even the major group (Roubik & Hanson 2004). It consists of 184 exclusively Neotropical distributed species and is divided into five genera (*Aglae*, *Eufriesea*, *Euglossa*, *Eulaema*, and *Exaerete*; Kimsey 1987, Ramírez *et al.* 2002). The animals are solitary or communal and have a life-span of two (*Euglossa* spp.) to five months (*Eulaema*



spp., *Exaerete frontalis*; Dodson 1966, Ackerman & Montalvo 1985, Zimmerman & Madriñan 1988), which is rather long for bees. A very conspicuous characteristic of all species in the tribe is their long proboscis, which sometimes even exceeds the bee's body and reaches up to a maximum length of 43 mm (Roubik & Hanson 2004). No other bee group in the Neotropics possesses comparable tongue sizes. Therefore, many euglossines are enabled to exploit nectar sources that are inaccessible for most other insects. Similar to some hummingbird species, euglossines are supposed to show a 'trap-line' foraging behavior (Janzen 1971, Ackerman *et al.* 1982). It has been demonstrated that the bees are able to cover large distances of more than 20 km on one foraging trip (Janzen 1971, Williams & Dodson 1972, Roubik & Hanson 2004). Another unusual peculiarity of the tribe is the scent-collecting behavior of the males, which is unique in the animal kingdom. The males have a broad spectrum of sources to gather scent, including rotten wood, injured trees, fungi, faeces, and also scents of flowers. Many Neotropical plants, which are also known as 'perfume flowers', have adapted to the behavior of the males and are exclusively pollinated by them (e.g., Vogel 1966, Dodson *et al.* 1969, Roubik & Hanson 2004). The final purpose of scent-collection is still uncertain. Though, latest studies indicate that it is related with the territorial and probably also with the mating behavior of the bees (Roubik & Hanson 2004, Zimmermann *et al.* 2006, Eltz *et al.* 2007).

Perfume flowers as well as flowers of euglossine food hosts show strong adaptations in their floral ecology towards the bees. Perfume flowers usually produce neither nectar nor fodder pollen and often have complex pollination mechanisms (e.g., Vogel 1966, Dressler 1968). Flowers of their nectar hosts generally possess long narrow corolla tubes or spurs and often show a flowering pattern of 'steady-state' (Ackerman 1985). Informations about the ecology of their pollen hosts are very scarce so far, since most pollen plants were discovered in palynological analyses of the bees' brood cells (Arriaga & Hernández 1998) or during visitor observations of pollen-collecting euglossine bees (Frankie & Coville 1979, Buchmann 1980, Roubik *et al.* 1982, Frankie *et al.* 1983).

Owing to the strong specialization towards their flower visitors, euglossine bees are generally supposed to be the only pollinators of the perfume- and nectar-flowered plants (Roubik & Hanson 2004). Therefore these plants highly depend on their pollination service. Considering the total amount of euglossine-pollinated species known so far, one can imagine the importance this bee group plays for Neotropical rainforests. About 800 species of perfume-flowered plants are known from the orchid family alone (Gerlach 1995). However, their importance as pollinators of nectar- and pollen-flowered plants is supposed to be even

much greater. Roubik & Hanson (2004) list more than 170 genera from 68 families, in which at least one euglossine-pollinated species occurs. Many of these species are sympatric and show convergent adaptations in their floral biology towards the bees (Roubik & Hanson 2004). It has been suggested that the tribe plays an important role in the diversification and reproductive isolation of these plants (Dodson *et al.* 1969, Williams & Dodson 1972, Dressler 1982, Kay & Schemske 2003, Roubik & Hanson 2004). However, most present studies focus on single plant taxa or genera. Therefore, our knowledge about the plant-euglossine interactions of entire communities is scarce. The only survey across taxonomic families was made by Ackerman (1985), who compared the seasonality of the euglossine community with the flowering phenology of their nectar hosts at BCI, Panama. He showed that plants adapted to euglossine-pollination are primarily found in the understory and principally flower in the mid wet season when large long-tongued euglossines predominate, while unspecialized mass-flowering plants in the canopy had a blooming peak in the dry season when smaller euglossines with short tongues dominated.

In the present work, I investigated the reproductive biology of several euglossine-pollinated species in the natural reserve Nouragues, French Guiana. Among the studied plants were perfume-flowers as well as nectar and pollen hosts of the bees.

The following questions were addressed:

- Does floral scent serve as reproductive isolation mechanism in sympatric perfume-flowered Araceae species?
- Are plants of the myco-heterotrophic genus *Voyria* insect-pollinated or obligately autogamous?
- What are the reproductive characteristics of an euglossine-pollinated plant community?
- Do sympatrically occurring euglossine-pollinated plants differ in their visitors spectrum and why is that so?

## SUMMARY OF CHAPTERS

In the FIRST CHAPTER I studied the pollination biology of three perfume-flowered Araceae species, which occur sympatrically in Nouragues and show an overlap in their flowering period.

Floral scent generally plays a major role in the pollination biology of Araceae. In the genus *Arum*, for example, small flies are attracted to trap-flowers by carrion-like scents (e.g., Kite 1995), while the inflorescences of the genus *Philodendron* allure large *Dynastinae* beetles by the emission of fruity and spicy floral perfumes (e.g., Young 1988, Gottsberger & Silberbauer-Gottsberger 1991). In the genera *Anthurium*, *Spathiphyllum*, and *Xanthosoma*, perfume-flowered species have evolved, which are pollinated by male euglossine bees (Williams & Dressler 1976). Frequently, several perfume-flowered aroid species occur sympatrically and the question arises how these plants manage to separate from each other in their reproductive biology. Studies on perfume-flowered orchids have shown that the attraction of the flower visiting species by floral scent is so specific that several plant species are able to grow in the same location and to flower at the same time without overlapping in their visitor spectrum. In contrast to orchids, the pollination biology of the perfume-flowered Araceae has been poorly studied (Williams & Dressler 1976, Croat 1980, Schwerdtfeger *et al.* 2002). It is supposed that similar isolation mechanisms exist (Williams & Dressler 1976). However, this has not been proved yet, since only few visitor observations exist and since the only published floral scent analyses were made on plant individuals that are cultivated in a botanical garden (Schwerdtfeger *et al.* 2002).

In order to test whether isolation mechanisms by floral scent exist, the pollination biology of the perfume-flowered species *Anthurium rubrinervium*, *A. thrinax*, and *Spathiphyllum humboldtii* in the reserve Nouragues was studied. All three species occur in the same habitat and show an overlap in their flowering time. Flower visitors were observed and sampled over several days and floral scent was collected in the field and subsequently analyzed by gas chromatography and mass spectrometry (GC/MS). The results showed that every plant species had a specific visitor spectrum of three to seven euglossine species. In each case, two bee species quantitatively dominated the flower visits. These were not observed at the inflorescences of the other plants or occurred there only sporadically. *Spathiphyllum humboldtii* possessed the broadest visitor spectrum of all three species, which might be related to its relatively short flowering period of only three months. A high specialization towards few visitor species would threaten the reproduction of the plants if the

bees were not present during the flowering period. Since *A. rubrinervium* and *A. thrinax* flower the entire year, the risk of pollination failure is much lower and the species were able to develop a higher specialization. Significant differences between the plant species also occurred with regard to the visiting time of the bees. *Spathiphyllum humboldtii* was almost exclusively visited in the morning and showed a visitation peak at 9:00 h, while the two *Anthurium* species were visited throughout the day. In *A. rubrinervium* the first bees arrived at about 9:30 h, then the visitor number constantly raised until 11:45 h and decreased until zero at about 15:00 h. In contrast, the first visitors of *A. thrinax* were already present in the early morning. Here, the visitor rates showed two peaks, one at 9:00 h and another at 11:15 h. After 11:15 h bee numbers declined and ceased in the afternoon.

The differences in visitor spectrum and visiting times are probably attributed to the floral scent of the plants. While floral scent could be olfactorily perceived at the inflorescences of *Anthurium thrinax* throughout the day, in *Spathiphyllum humboldtii* this was only the case in the morning and in *A. rubrinervium* from 9:30 h onwards. The analysis of the scent samples showed that the composition and the main compounds of the floral bouquet was specific for each plant species. Multidimensional scaling of the composition of the samples demonstrated clearly separated clusters for each plant species with a stress-factor of 0.04. Additionally, Sørensen indices of the interspecific similarity of the scent bouquets exhibited low values around 50%. A total of 66 substances out of four different chemical classes was detected in the scent samples (terpenoids, benzenoids, lipid-derived compounds, and nitrogen-containing compounds). These substances are largely known from the floral scent of other perfume-flowered species (Williams & Whitten 1983, Gerlach & Schill 1991, Knudsen & Tollsten 1993, Knudsen *et al.* 2006) and their attractiveness to male euglossines has partly been tested in baiting experiments (Ramírez *et al.* 2002, Roubik & Hanson 2004). The specific attraction of the bees is explained by the species-specific scent spectrum each species collects (Eltz *et al.* 1999, Eltz *et al.* 2006). One could expect that it was possible to make clear predictions of each plant's visitor spectrum by analyzing its floral scent and studying the bees' preferred substances. Unfortunately, the story is not as easy as it seems. The perception of scent involves complex processes in the bee's higher nervous system. Thereby, one scent compound can influence the perception of another (Eltz & Lunau 2005). Since floral scents never consist of a single substance, but always represent a mixture of several compounds, a prediction of the visitor spectrum is impossible so far. The floral scent bouquet of each of the studied plant species has, in this meaning, consequently to be regarded as a unit. Perhaps, the complexity of the floral scent influences the species number of attracted bees. The scent

bouquet of the rather generalist species *S. humboldtii* was exclusively composed of terpenoids, while the scent of the two *Anthurium* species contained substances of various chemical classes.

While scent samples of *Spathiphyllum humboldtii* and *Anthurium thrinax* were quite uniform within each species, the floral scent of *A. rubrinervium* showed some qualitative variability between different individuals. Interspecific variability of floral scent in perfume flowers has only been studied in *Geonoma macrostachys* so far (Knudsen 2002). Larger changes in fragrance composition may lead to the attraction of different pollinator species and could therefore be of major importance for the speciation of the plants. However, the variability in *A. rubrinervium* did not lead to different pollinator spectra. It has been reported that the continuous collection of the same fragrance substances results in a lower attractiveness of these substances to the bees (Eltz *et al.* 1999). Since *A. rubrinervium* is flowering year round and the bees have a life span of several months, slightly altered scent bouquets might be a means to remain attractive for the bees.

Finally, this study showed that the investigated Araceae possess similar isolation mechanisms as perfume-flowered orchids. The species-specific scent composition conducts to a clear separation of each plant species' pollinators and permits a directed pollen flow. Moreover, the study let to suppose that additionally phenological isolation mechanisms have developed.

The SECOND CHAPTER deals with the first observation of an *Aglae caerulea* male collecting floral scent at the flowers of *Anthurium rubrinervium* (Araceae) and the analysis of the plant's floral scent.

The tribe Euglossini is divided into five genera, namely, *Aglae*, *Eufriesea*, *Euglossa*, *Eulaema* and *Exaerete* (Kimsey 1987). *Aglae* is a monotypic genus whose only species, *Aglae caerulea*, seems to be extremely rare even in its natural habitat (Michener 2000, Cameron 2004). Therefore, only few observations of the bee exist, and while males of the other genera have been seen during fragrance-collection at perfume-flowers, *Aglae* was the only exception. Since male *Aglae* are provided with the same morphological features as their relatives in the other genera (Sakagami 1965, Vogel 1966, Kimsey 1987, Michener 2000) and since *A. caerulea* males could already be attracted to artificial scent baits (Williams & Dodson 1972, Morato 2001, Ramírez *et al.* 2002, Anjos-Silva *et al.* 2006), it has been presumed that they also collect scent at natural resources.

In the course of the pollination ecology study of the sympatric perfume-flowered Araceae, an *Aglae caerulea* male was observed at the inflorescence of an *Anthurium rubrinervium* plant. The bee stayed for ca. one minute at the inflorescence and showed the typical perfume-collecting behavior of the Euglossini (Evoy & Jones 1971). However, its importance in the pollination of the plant species is probably rather low, since the bee species was only observed once in the whole studying period, and since other flower visitors were present in much higher numbers.

The floral scent of several *Anthurium rubrinervium* individuals was collected in the field and analyzed in the laboratory by means of gas chromatography and mass spectrometry (GC/MS). A total of 36 compounds was found that belong to the chemical classes of terpenoids, benzenoids and lipid-derived substances. Main compounds of all analyzed samples were (Z)-8-heptadecene, methyl salicylate, 1,8-cineole, benzyl benzoate, and linalool. Besides these, single samples additionally contained large amounts of (E)-ocimene and methyl (E)-cinnamate. The majority of the detected scent compounds of *A. rubrinervium* is known from the floral scent of other perfume-flowered species (Williams & Whitten 1983, Gerlach & Schill 1991, Knudsen & Tollsten 1993, Knudsen *et al.* 2006) and has partly been demonstrated in baiting experiments to be attractive for male euglossine bees (Ramírez *et al.* 2002, Roubik & Hanson 2004). However, (Z)-8-heptadecene has not been reported from other perfume-flowered species yet. This compound is known to be a semiochemical of mites that is used in apiculture to reduce the offspring of *Varroa destructor* (Nazzi *et al.* 2002, Milani *et al.* 2004). Although male euglossine bees do not participate in brood care, the presence of this substance in floral scents could be a hint that scent-collecting is related with the prevention of diseases and parasites of the bees. Nevertheless, (Z)-8-heptadecene has never been used in baiting experiments. Therefore, its attractiveness to male euglossines has still to be tested.

In the THIRD CHAPTER the question is tackled whether plants of the myco-heterotrophic genus *Voyria* (Gentianaceae) are obligately autogamous.

While most plants acquire organic compounds entirely by photo-autotrophy, few hundred species of various plant families gain their energy from an associated fungus partner (Leake 1994). These species are called myco-heterotrophic (formerly ‘saprophytic’) plants and are characterized by the loss of chlorophyll and by strong adaptations in morphology and anatomy to their way of life (Maas 1986, Leake 1994). Such adaptations are also found in the reproductive biology of the plants. Many species are able to reproduce vegetatively. Nevertheless, sexual reproduction is still very common. In contrast to their autotrophic

relatives, their inflorescences, flowers and floral organs are usually reduced in size and complexity (Maas 1979, Maas 1986, Leake 1994). In most studies about the pollination biology of myco-heterotrophic plants, flower visitors were rare and only irregularly observed. Moreover, it has been demonstrated that many species are able to self-pollinate (e.g., Takahashi *et al.* 1993, Zhang & Saunders 2000, Lehnebach *et al.* 2005). Therefore, it appeared that these plants were obligately autogamous and that obligate autogamy is a typical adaptation to the myco-heterotrophic way of life. However, this has never been proved since the reproductive biology of only few myco-heterotrophic plants has been studied yet and since the pollination biology of entire myco-heterotrophic families and genera is still unknown (Maas & Ruyters 1986, Maas *et al.* 1986, Maas & Rübsamen 1986).

The myco-heterotrophic genus *Voyria* Aubl. (Gentianaceae) comprises 19 species, of which 18 are distributed in tropical and subtropical America (Maas & Ruyters 1986, Albert & Struwe 1997). The pollination biology of the genus has been poorly studied. Oehler (1927) examined the anatomy and morphology of *Leiphaimos* sp. (= *V. rosea*) on alcohol-preserved material and discovered that pollen had already germinated within the anthers. Moreover, the pollen tubes had penetrated the stigma and conducted to the self-fertilization of the ovules. Although not having done any visitor observations, Oehler (1927) assumed that the species was strongly autogamous. The only record of flower visitors in the genus was made by Imhof *et al.* (1994). They observed *Brachycera* (Diptera) at the flowers of *V. tenella* and considered outcrossing possible in this species since the tongue length of the insects corresponded to the flowers' corolla tube length. Finally, the question became apparent, how important flower visitors are for outcrossing in this genus in direct comparison to self-pollination?

In order to find out if pollinators exist and if they play a role for the reproduction of the plants, I studied the reproductive biology of three *Voyria* species, namely, *V. caerulea*, *V. clavata* and *V. rosea* in the reserve Nouragues, French Guiana. *Voyria rosea* is quite common in the reserve and often occurs in larger populations, while the other two species are less frequent and plant individuals are usually separated from each other by several hundreds of meters. Interestingly, most plants only grew in areas of the reserve whose soil was of 'Carribean' type granite origin and represented very sandy organic-rich Cambisols (Grimaldi & Riéra 2001). The large and conspicuously-colored flowers of the plants developed directly on the ground. They had an average flowering time of 6-10 days, produced nectar and emitted floral scent. *Voyria caerulea* and *V. rosea* had long and narrow trumpet flowers that were arranged in 2-4-flowered cymes. The flowers of *V. clavata*, on the other hand, were solitary and had a long broad corolla tube whose diameter strongly decreased in the lower, nectar-

bearing section, and whose corolla lobes did not form a landing platform for visiting insects. The nectar of all species was sucrose-dominant and had average sugar concentrations between 16.4% and 23.9%. *Voyria caerulea*, *V. clavata* and *V. rosea* emitted a pleasant, rosy-citrus-like floral perfume that was dominated by terpenoids. Major compounds of *V. clavata* were (E)-8-oxo- $\beta$ -farnesene, (E,E)-farnesyl acetate, and (E)- $\beta$ -farnesene, while the scent of *V. rosea* contained high amounts of (E)-nerolidol, geraniol, and linalool. Unfortunately, the concentration of the scent samples of *V. caerulea* was too low for the GC/MS-analysis. However, the floral scent of this species was previously analyzed by Kaiser (2006), who detected large amounts of (E)-nerolidol, geraniol, and nerol. Thereby it shows a high similarity to the floral scent of *V. rosea*.

All studied floral characteristics indicated that the plants were adapted to long-tongued flower visitors and indeed they could be observed. *Voyria rosea* was visited by Lepidoptera and Euglossini, while the visitors of *V. caerulea* were only Lepidoptera, and those of *V. clavata* Euglossini. Nevertheless, flower visits to the first two species were very rare and irregular (e.g., visitors of *V. rosea* were only observed on two out of 15 days), while visits to *V. clavata* were more frequent and regular (one visit within 1-2 days).

The majority of the plants in the study area developed fruits and showed average seed sets of 36-49%. However, seed set varied strongly between one and 100% within each species. Strong autogamous plants usually have a very high seed set (Ornduff 1969), but some studies indicate that the energetic dependency of myco-heterotrophic plants to their fungus partner might also affect reproductive success (Oehler 1927, Ushimaru & Imamura 2002). Therefore it could be possible that the observed seed number does not directly correspond to the number of fertilized ovules. Seed dispersal in all three species has previously been suggested to be performed by water (Maas & Ruyters 1986, Leake 1994). This is probably true for *Voyria rosea* since their fruits are hard and the capsule wall gets thinner in the course of seed maturation. In *V. caerulea* and *V. clavata*, however, the capsules are fleshy and the seeds are immersed in the swollen placenta tissue. Additionally, the fruits often showed bite marks, indicating that the seed are dispersed endozoochorously, probably by rodents.

In order to estimate the importance of self-pollination in the three plants, the morphology of the reproductive organs was studied, the pollen/ovule ratio (P/O ratio) was determined, and several flowers of *V. rosea* were enclosed by nylon nettings over the entire study period to exclude pollinators. All species had a very low P/O ratio (mean: 33-78), which indicates that pollen transfer in these plants is highly efficient (Cruden 1977). P/O ratios of this dimension are predominantly found in facultative autogamous species. However, several



obligate xenogamous plants with low P/O ratios have been reported as well. These either disperse their pollen in small packages or have a high area ratio between stigma surface and pollen-bearing area of the flower visitor (Cruden 1977, Cruden & Jensen 1979, Cruden & Miller-Ward 1981). Both characteristics were present in the species studied. First, they possess disc- to cone-shaped stigmas that were much larger than the pollen-bearing part of the insect (proboscis in *V. caerulea* and *V. rosea*; dorsal mesosoma and metasoma in *V. clavata*). Second, it is very likely that the pollen is distributed in packages. In all species, germinated pollen could be found in the anthers of mature flower buds. The pollen tubes were interwoven with each other and formed large bundles that also included non-germinated pollen. In *V. clavata* the anthers were further covered by a jelly-like liquid that also contributed to the clumping of the pollen. The transfer of these pollen-bundles from the visitors' body to the stigma was probably facilitated by the papillous texture of all species' stigma surfaces.

Despite the large stigma area and the distribution of pollen in packages, the floral morphology of *Voyria caerulea* and *V. rosea* let to assume that facultative self-pollination by germinating self-pollen is very likely. In these two species, the anthers closely surround the stigma and touch its rim with their apices. This is further supported by the fruit set of the enclosed flowers of *V. rosea*. Conversely, the probability of self-pollination by germinating self-pollen can be excluded in *V. clavata* as its anthers are located several millimeters below the stigma and lean against the style.

The study showed that all three *Voyria* species were visited by insects that contributed to the outcrossing of the plants. Hereby, the grade of specialization towards certain pollinators and their reliability were probably decisive for the plant to be autogamous or xenogamous. *Voyria clavata* had a specialized floral morphology that only permitted access to the nectar for few euglossine species. Since these bees have more or less determined flight routes and cover large distances during foraging (Janzen 1971, Ackerman *et al.* 1982), they represent highly reliable pollinators, which ensure the reproduction of the plants. Thus, straight xenogamous plants were able to evolve. The floral morphology of the other two species, however, was less restrictive in terms of flower visitors. Any long-tongued insect was able to reach the nectar. A lower specialization probably led to a less narrow plant-pollinator relationship, which again resulted in irregular and less frequent flower visits. Since pollen transfer was not guaranteed by the visitors, competing self-pollination ensured the seed set, which means that pollen germinated in the anthers and led to self-fertilization when no outcrossing had taken place during flowering. It is suggested that other myco-heterotrophic plants might also have developed self-pollination as a kind of emergency pollination.

In the FOURTH CHAPTER I compared the reproductive biology of several sympatric euglossine-pollinated nectar- and pollen-flowered species.

The pollination systems of plants in tropical understory communities are usually characterized by a high specialization towards reliable long-distance pollinators (Bawa 1983, Bawa *et al.* 1985b, Bawa 1990, Kress & Beach 1994, Kato 2005). The two dominating pollinator groups of these plants are nectarivorous birds and long-tongued bees. Both are strong fliers that follow established foraging routes ('trap-lining'; Janzen 1971, Stiles 1978, Sakai *et al.* 1999). The specialization to different pollinator groups is supposed to assure reproductive isolation of sympatric conspecifics and to increase the directed pollen flow of heterospecifics of a plant community (Stiles 1975, Sakai *et al.* 1999, Kay & Schemske 2003). However, in-depth studies on the reproductive biology of these plant communities remain scarce and most hypotheses still need to be verified by field research.

In the Neotropics, the guild of long-tongued bees is represented by the Euglossini (Apidae: Hymenoptera). These bees particularly became famous for the pollination of flowers that offer floral scent as their sole resource ('perfume flowers'; Vogel 1966, Dodson *et al.* 1969). Research on the reproductive biology of plants pollinated by euglossines had a strong emphasis on these perfume flowers, almost ignoring that the bees also visit a large number of nectar and pollen hosts (Roubik & Hanson 2004). Although extended lists of euglossine-pollinated plants exist (Dodson 1966, Ackerman 1985, Ramírez *et al.* 2002, Roubik & Hanson 2004), in-depth studies are scarce and generally focus on single plant taxa or genera (Kennedy 1978, Schemske 1981, Ackerman *et al.* 1982, Schemske & Horvitz 1984, Kay & Schemske 2003, Borrell 2005, Almeida Barreto & Freitas 2007, Leite & Machado 2007). Likewise, isolation mechanisms and the importance of pollinator specificity for speciation were largely discussed in perfume-flowered species (a bibliography is given by Ramírez *et al.* 2002, Roubik & Hanson 2004), but are poorly understood in euglossine food-hosts.

In the present study, I tried to characterize the reproductive biology of an understory community of euglossine-pollinated plants. Seven plant species were studied, which belong to the most frequent euglossine-pollinated understory taxa at the study site. These were *Bonaifousia disticha* (Apocynaceae, understory treelet), *Mandevilla rugellosa* (Apocynaceae, liana), *Costus congestiflorus* (Costaceae, erect shrub), *Calathea erecta*, *Ischnosiphon martianus* (Marantaceae, erect shrubs), *Episcia sphalera* (Gesneriaceae, herb), and *Rapatea paludosa* (Rapateaceae, erect shrub). While the first five species develop exclusively nectar flowers, *E. sphalera* is visited for both nectar and pollen, and *R. paludosa* offers pollen only. The studied plants represent ca. 10-20% of all euglossine-pollinated understory species at the

study site, which flower during the rainy season. Additionally, pollinator data of 29 further taxa were collected, summing up to a total of ca. 70-80% of all euglossine-pollinated understory species at the study site, which flower during the rainy season.

The inflorescences of all species consisted of various, sometimes dozens of flowers. Only one or few new flowers opened a day in each inflorescence, conducting to a prolonged blooming period of several days or weeks per inflorescence (dependent on the plant species and the flower number). With the exception of *Episcia sphalera* whose anthesis took two days, individual flowers of all species were open for one day only. This kind of flowering phenology ('steady state flowering'; Gentry 1974, Bawa 1983), is supposed to be an adaptation to the trap-line foraging behavior of the euglossines (Frankie *et al.* 1983). The blooming period of *Bonafousia disticha*, *Costus congestiflorus*, and *E. sphalera* was restricted to few weeks in the rainy season, while the other species flowered over several months, or perhaps even over the entire year. Several studies have shown that the number of long-tongued euglossines reaches its annual peak at the beginning of the rainy season, which is probably also true for Nouragues (Ackerman 1985, Roubik & Ackerman 1987). Therefore, the flowering time of the former plants could be an adaptation to the increasing nectar demand in the rainy season, while the latter represent keystone species, maintaining the bee population over the year.

The visitor spectrum of the species studied was clearly dominated by euglossine bees. They were responsible for more than 80% of all flower visits (only exception: *Calathea erecta* with about 50%) and can be regarded as pollinators of the plants as they had contact to anthers and stigmas and approached several flowers of the same species successively. Besides this, Bombyliidae, Lepidoptera, and Meliponinae occurred. These usually played a minor role as pollinators or were no pollinators at all since they occurred in smaller quantities and often had no contact to anthers or stigmas or only came for pollen-robbing. Further, their foraging behavior was less directed than that of the Euglossini, so that the number of flowers visited by each individual was clearly smaller.

The prevalence of euglossines at the flowers can for the most part be attributed to the floral morphology (long corolla tubes or spurs) that permitted nectar access only to long-tongued insects, sometimes even specifically to euglossines. Additionally, nectar characteristics might have played a role. The nectar was always sucrose-dominant and showed average sugar concentrations of 25.5-29.7%. Flowers visited by long-tongued bees were demonstrated to produce a sucrose-dominant nectar (Percival 1961, Baker & Baker 1983), with sugar concentrations of 22-48% (Roubik *et al.* 1995). Surprisingly, also the free

accessible disc-shaped pollen flowers of *Rapatea paludosa* were almost exclusively visited by euglossine bees. This might be attributed to the fact that pollen was located in poricidally opening anthers and could only be harvested by vibration ('buzz-pollination'; Michener *et al.* 1978, Buchmann 1983). Nevertheless, many other bee groups at the study area would have been able to exploit the flowers (Michener *et al.* 1978, Buchmann 1983). Maybe they have specialized to collect pollen from other plant species since individuals of this species grew in low densities and offered only small amounts of pollen per inflorescence.

With regard to the euglossine visitor spectrum, specificity of the plants towards pollinators was similar to perfume flowers. One bee species or a bee subgenus dominated the visits of one plant species. In almost all plants the major visitors were individuals of the *Euglossa* subgenus *Glossura* (mainly the species *E. piliventris* and *E. chalybeata*). Several long-term baiting experiments have shown that bees of this subgenus are the most abundant euglossines in Neotropical rainforests (Ackerman 1983a, Pearson & Dressler 1985, Powell & Powell 1987, Roubik & Ackerman 1987, Roubik 1989, Roubik & Hanson 2004). Due to their large and stable populations, they probably represent good and reliable pollinators. Therefore many plant species might have evolved and specialized under their influence. In *Mandevilla rugellose* and *Bonafousia disticha* large bees of the species *Exaerete frontalis* and the *Eulaema* subgenus *Eulaema* s.s dominated the flower visits, while *Episcia sphalera* was pollinated by females of one so far undetermined small *Euglossa* species only. The differences in the plant species' visitor spectrum were probably related to the nectar amounts offered by the flowers, which showed significant differences between species. The values ranged between averages of 3.7  $\mu$ l in *E. sphalera* and 51.1  $\mu$ l in *M. rugellose* and were positively correlated with the average body size of the visitors. This hypothesis is further supported by the occurrence of significantly different visitor spectra at different individuals of *B. disticha*. Plants of this species that developed few flowers and consequently supplied low nectar totals were almost exclusively visited by bees of the *Euglossa* subgenus *Glossura*, while large bees (*Exaerete frontalis*, *Eulaema* subgenus *Eulaema* s.s ) predominated at many-flowered treelets. Since the foragers cross large distances between plant populations, probably a minimum caloric reward exists the flowers have to offer in order to be included in the bees' foraging route (Heinrich & Raven 1972). Larger euglossine species have a higher energy consumption than smaller ones (Casey *et al.* 1985). Therefore, the minimal threshold of nectar amounts should also be higher for them. Pyke (1978) already demonstrated on bumblebees that there is a positive correlation between the size of a bee and the average amount of nectar it obtained per flower. My study let to suppose that this is also true for the Euglossini.

Another flower characteristic that was decisive besides nectar amounts, is the floral morphology. Length of the corolla tube was positively correlated with the proboscis length of the visitors. Moreover, narrow entrances in gullet flowers (flowers, where the bee has to crawl into the corolla tube) or small landing platforms often restricted the exploitation of the resources to small euglossine species.

While the plants showed a high specificity in pollinator choice, each bee species usually visited a diverse spectrum of flowers. This phenomenon was already observed in other studies and is explained by the fact that the lifetime of a bee often exceeds the flowering period of a plant (Feinsinger 1983) and that a single plant species is not able to attract and to feed a pollinator group (Schemske 1981, Sakai *et al.* 1999). Feinsinger (1983) suggests that rare plants might converge on one another in signal, morphology, and reward in order to be included in the diet of the same long-distance foragers. When several species are pollinated by the same visitor, selection might favor divergence in the placement of pollen on the flower visitor (Feinsinger 1983). This was observed in several hummingbird-pollinated *Heliconia* species (Stiles 1975) and also in euglossine-pollinated perfume orchids (Ackerman 1983b). In the studied species, pollen was placed on the proboscis (Apocynaceae, Marantaceae), the dorsal mesosoma (*Costus congestiflorus*, *Episcia sphalera*) and the ventral mesosoma (*Rapatea paludosa*). Since the same bees visited the flowers of four species that deposited pollen on the proboscis, heterospecific pollen was inevitably mixed. This overlap might particularly be critical in closely related species. Here, selection for strong barriers to hybridization may occur (Schemske 1981). The absence of different pollen placement could be explained by the size of the visitors. In contrast to hummingbirds, euglossine bees are quite small and the exact placement of pollen at different positions on the bee might be impossible. Perfume orchids, on the other hand, possess pollen-packages that are attached to the pollinator's body by a small adhesive disc, which enables orchids to place the pollen at determined spots, also on small bees (Ackerman 1983b).

Considering the sexes of the flower visitors, all nectar plants but *Episcia sphalera* were visited by female and male bees. However in *E. sphalera* and *Rapatea paludosa* only females were observed. Since *R. paludosa* is only offering pollen as a resource and since males usually do not collect pollen, this result was not very surprising. *Episcia sphalera* on the contrary provided both pollen and nectar. Maybe the nectar amounts were too small to attract males, whereas in combination with pollen-collection, visits were profitable for the females. Another possible explanation was that the number of females in the population of this species was temporarily higher than that of males.

The bees' main activity at the nectar flowers was between 9:00 h and 16:00 h. Usually, more visitors were observed in the morning than in the afternoon. This phenomenon was already observed in other studies, but the authors did not find any correlation to climatic parameters that could explain it (Armbruster & McCormick 1990, Armbruster & Berg 1994). Visitor numbers only corresponded to time of day or an undetected variable (Armbruster & Berg 1994). The results of the studied plants let to suppose that the available nectar amount could be responsible for the decline in the afternoon. Highest nectar secretion was performed in the morning or before bud opening and ceased until 14:00 h. These results are in accordance with measurements in other species of the studied plant families and seem to be a widespread phenomenon (Madrigal & Girón 1982, Torres & Galetto 1998, Leite & Machado 2007, Almeida Barreto & Freitas 2007). Besides nectar secretion, the higher probability of rainfall in the afternoon could have had an influence on the bees' diel activity since strong downpours destroyed many open flowers and hindered the bees from foraging.

No significant differences in the visitation time were found neither between bee species nor between plant species. This result was rather surprising as clear differences between small and large bees, caused by diverging cooling capacities, were expected (May & Casey 1983). Moreover, the plants grew in different habitats with contrasting microclimates. Some plant species were distributed at open sites, exposed to sunlight, whereas others preferred the dense shady understory of the forest. Probably microclimate plays a minor role in the diel activity of the bees, at least during the rainy season.

In contrast to the nectar-offering species, *Rapatea paludosa* was exclusively visited in the early morning hours. Here, the first bees already arrived at sunrise (ca. 6:15 h). Flower visits peaked at 8:00 h and ceased until midday. The differing visiting peak is probably related to the limited pollen number offered by each flower, which leads to a strong competition among the bees. Therefore, the first visitors already arrive at flower-opening and collect pollen as long as it is available. In *E. sphalera* this phenomenon was not observed since the pollen could not be actively collected from the anthers and since nectar was offered as an additional resource.

With regard to pollen number and size, strong differences between nectar- and pollen-flowered plants were found. While the former produced few hundred large pollen grains per flower, several ten thousand tiny pollen grains were counted in the latter. This is a typical characteristic of pollen flowers, which might be attributed to the small openings of the anthers, and the fact that pollen is offered as a resource (Cruden 2000). The observed negative

correlation between pollen grain size and number is generally considered to be a trade-off between number and size (Charnov 1982, Vonhof & Harder 1995).

In their foraging bouts, the euglossines showed a variety of different behaviors that were not related to the bee species or the visited plants. Some bees principally visited all flowers of a plant or a population and made a control flight before leaving, obviously in order to assure that no flower was missed. These individuals even alighted on wilted flowers or flower buds. However, other bees only visited single flowers and then flew away quickly. These differences might be related to learning processes of the bees. Experienced foragers know the most profitable flowers of a population and their handling, while younger animals still have to establish their flight routes and learn to discriminate between ‘good’ and ‘bad’ inflorescences. In flower choice, some bees were less directed but very selective. They inspected several flowers without landing on them and then chose one to exploit. The same behavior is reported from other bees of closely related groups, namely *Apis mellifera*, *Bombus* spp. or *Xylocopa virginica texana* (Frankie & Vinson 1977, Stout & Goulson 2001). It has been shown that these bees leave odor marks on the flowers they visit, which are used by conspecifics and heterospecifics to assess potential foraging profits at the flowers without landing (Frankie & Vinson 1977, Stout *et al.* 1998, Goulson *et al.* 1998, Stout & Goulson 2001). In the Euglossini, the use of odor marks has not been studied yet, but their close phylogenetic relationship to the other bee groups (Michener 2000, Cameron 2004) and my observations could be a hint that they also do so.

All species but *Rapatea paludosa* showed a very low P/O ratio. According to Cruden (1977), these values indicate that the plants possess a facultative autogamous reproductive system. For *Episcia sphalera* this is certainly true since several individuals cultivated in the greenhouse, where no natural pollinators are present, developed mature fruits. The other species however are probably not facultative autogamous. Low P/O ratios were also discovered in several other species of the Apocynaceae, Costaceae, and Marantaceae (Ramirez & Seres 1994, Torres & Galetto 1999, Lopes & Machado 1999, Löhne *et al.* 2004, Leite & Machado 2007). Nevertheless, it was demonstrated that autonomous self-pollination occurred in none or only very few plant individuals (Schemske 1981, Ramirez & Seres 1994, Torres & Galetto 1999, Lopes & Machado 1999, Löhne *et al.* 2004, Leite & Machado 2007, Almeida Barreto & Freitas 2007). These families were therefore considered to be an exception to the rule. Here, low P/O ratio are supposed to be the consequence of highly efficient pollination mechanisms, large pollen grains, and large stigma areas. This again

implies that the entire fruit set of all studied species but *E. sphalera* was achieved by the successful pollen transfer of the euglossine bees.

Fruit set in the studied species ranged broadly. While about half of the flowers of an inflorescence developed into fruits in *Episcia sphalera* and *Costus congestiflorus*, fruit set was only 25% in *Rapatea paludosa* and *Ischnosiphon martianus*, 10% in *Mandevilla rugellosea*, and ca. 1% in *Bonafousia disticha* and *Calathea erecta*. Low fruit sets were often coupled with florivory and frugivory. Additionally, Apocynaceae were reported to drop fruits when resource limitations occur (Lopes & Machado 1999, Torres & Galetto 1999). Therefore, low fruit set in these plants was not necessarily linked with a low pollination success. In *Calathea erecta*, low fruit sets can be explained by the high number of visits by nectar thieves. These probably released the pollination mechanism without coming into contact with the stigma. Thereby, the flowers were not able to be pollinated any more. Schemske & Horvitz (1984) already showed in *Calathea ovadensis* that butterfly nectar thieves exerted a strong negative effect on fruit production.

## CONCLUSION

Tropical understory plants have the fate to accomplish reproduction in a dark, energetically poor environment, in which conspecifics are separated for several hundreds of meters from each other. My study has shown that euglossine-pollinated plants have evolved reproductive traits, which enable them to achieve a highly efficient pollen flow even in this unfavorable habitat. Two pollination systems can be distinguished: perfume flowers on the one hand, and nectar or pollen flowers on the other. Both groups were characterized by a highly species-specific pollinator spectrum and by low daily energy costs for the provision of floral resources. In perfume flowers, the selective attraction of visitors was achieved by the emission of species-specific floral scent. Therefore, no large-scaled floral displays were necessary and it was sufficient to provide floral scent as major attractant for the foragers. In nectar hosts, the offered nectar amounts and the floral morphology were decisive for the restriction of visitors. Since the nectar could only be exploited by a limited number of pollinators it was sufficient to produce one or few flowers daily. This is also true for the studied pollen hosts. However it could not be revealed how these plants manage to attain a limitation in the pollinating species. However, the results of my study let to suppose that some euglossine bees might be oligolectic, at least temporally or/and spatially.



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## **ZUSAMMENFASSUNG**

### **Die Reproduktionsbiologie euglossinenbestäubter Pflanzen im Reservat Nouragues, Französisch Guayana**

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#### ALLGEMEINE EINLEITUNG

Tropische Tieflandregenwälder sind durch eine hohe pflanzliche Artenvielfalt bei gleichzeitig niedriger Individuendichte charakterisiert (Hubbel & Foster 1983, Ashton 1984, Bawa *et al.* 1985a, Myers *et al.* 2000). Große Distanzen zwischen den Individuen einer Art führen dazu, dass die Selektion für Pollenübertragung über große Entfernungen weitaus stärker ist, als in anderen Pflanzengesellschaften (Janzen 1970, Bawa 1990). Zwischen den Pflanzenarten verschiedener Waldschichten haben sich hierbei unterschiedliche Strategien zur Realisierung der Pollenübertragung entwickelt, welche überwiegend auf abiotische Faktoren (vor allem auf die Strahlungsintensität) und die Stratifikation der Pollenvektoren zurückgeführt werden (Bawa 1983, Bawa 1990, Kress & Beach 1994, Kato 2005). In den Baumkronen dominieren Pflanzen, die durch Massenblüte ein breites Artenspektrum an Blütenbesuchern anlocken. Bei diesen handelt es sich entweder um verschiedene kleine Insekten oder um große bis mittelgroße Bienen (Frankie *et al.* 1983, Bawa *et al.* 1985b, Bawa 1990, Kress & Beach 1994, Momose *et al.* 1998). Der Wechsel der Blütenbesucher zwischen den Pflanzen einer Art wird dabei durch das exakte Timing ihrer Blütezeit und ihrer Nektarsekretion erreicht (Frankie *et al.* 1976, Frankie *et al.* 1983, Bawa *et al.* 1985b). Meistens dauert die Blüte eines Pflanzenindividuums nur wenige Tage und man geht davon aus, dass Pflanzen verschiedener Arten den interspezifischen Genaustausch und die Konkurrenz um die gleichen Bestäuber durch kurze, sich nicht überschneidende Blühperioden reduzieren (Bawa 1983, Frankie *et al.* 1983). Da das Risiko der Inzuchtsdepression durch Geitonogamie stark erhöht ist, hat sich in den meisten Pflanzenarten Selbstinkompatibilität oder Dichogamie entwickelt (Heinrich & Raven 1972, Bawa 1990, Kress & Beach 1994). Im Unterwuchs werden Blütenbesucher nicht durch große Blütenmassen angelockt. Stattdessen sind die Pflanzen in ihrer Reproduktionsbiologie stark auf spezifische Bestäuber spezialisiert, die überwiegend zur Gilde der langgeschnäbelten nektarivoren Vögel, der langzüngigen Bienen oder der Käfer gehören (Bawa 1990, Kress & Beach 1994). Käferbestäubte Pflanzen bieten ihren Besuchern Futtergewebe an und erreichen eine Fremdbestäubung durch das genaue Timing kurzer weiblicher und männlicher Blühphasen verschiedener protogynen Blüten oder protogynen und monözischer Blütenstände (Gottsberger 1970, Gottsberger & Amaral Jr. 1984, Bawa *et al.*

1985a, Young 1988, Gottsberger 1989, Gottsberger & Silberbauer-Gottsberger 1991, Kress & Beach 1994, Gottsberger & Silberbauer-Gottsberger 2006). Vogel- und bienenbestäubte Arten produzieren täglich wenige hermaphrodite Blüten, die ihren Besuchern große Mengen an Nektar zur Verfügung stellen. Um eine Ausbeutung der Blüten durch beliebige Blütenbesucher zu verhindern, liegen ihre Ressourcen in langgestreckten Kronröhren verborgen, die nur den Bestäubern zugänglich sind (Heinrich & Raven 1972, Stiles 1975, Feinsinger 1983, Momose *et al.* 1998). Nichtsdestotrotz ist die angebotene Nektarmenge eines einzelnen Pflanzenindividuums zu niedrig, um den täglichen Energiebedarf seiner Bestäuber zu decken (Heinrich & Raven 1972, Stiles 1975, Bawa *et al.* 1985a). Diese sind daher darauf angewiesen mehrere, weit voneinander entfernte Pflanzenpopulationen hintereinander zu besuchen, was wiederum zur Fremdbestäubung und einem erhöhten Genaustausch über große Distanzen führt (Stiles 1975, 1978). Hierbei folgen die Tiere festgelegten Flugrouten, die ihre Wirtspflanzen miteinander verbinden ("trap-lining"; Janzen 1971, Stiles 1978, Sakai *et al.* 1999). Die Wirtspflanzen wiederum besitzen vielblütige Infloreszenzen, die über lange Zeiträume blühen und daher verlässliche Nahrungsquellen darstellen ("steady state flowering"; Gentry 1974, Bawa 1983). Obwohl die meisten Pflanzen selbstkompatibel sind, wird angenommen, dass Fremdbestäubung überwiegt, da die Nachkommen selbstbestäubter Pflanzen, in der Regel eine deutlich reduzierte Fruchtbarkeit aufweisen (Bawa *et al.* 1985a, Kress & Beach 1994).

Die starke Spezialisierung auf Bestäuber wird als wichtige Triebfeder der Speziation angesehen (Stebbins 1974, Grant 1981, Feinsinger 1983). Sie ermöglicht es selbst nah verwandten Pflanzenarten im gleichen Gebiet vorzukommen und zur selben Zeit zu blühen, ohne dass es zur gegenseitigen Beeinflussung ihrer Fortpflanzungsbiologie kommt (Stiles 1975, Sakai *et al.* 1999, Kay & Schemske 2003). Außerdem begünstigt sie adaptive Konvergenzen der Blütenmorphologie (Sakai *et al.* 1999, Kay & Schemske 2003). Da die niedrige Populationsdichte der meisten Arten und ein oft hoher logistischer Aufwand die Erforschung tropischer Regenwäldern erschweren, sind Unterwuchsgesellschaften bisher nur ansatzweise untersucht. Ein Großteil der genannten Hypothesen konnte daher noch nicht durch Feldforschung verifiziert werden. Die einzigen ausführlichen Studien wurden über die Reproduktionsbiologie kolibribestäubter Pflanzengesellschaften angefertigt (Stiles 1975, Feinsinger 1978). Diese Arbeiten zeigen, dass die Bestäubungsgilden viel komplexer und feinstrukturierter sind als man bisher annahm. Reproduktive Isolation wurde in diesem Fall nicht nur durch räumliche und zeitliche Unterschiede der Blühpatterne erreicht, sondern auch

durch die Blütenmorphologie und durch verhaltensbiologische Mechanismen (Stiles 1975, 1978).

Meine Dissertation beschäftigt sich mit der Reproduktionsbiologie einer Unterwuchsgesellschaft von Pflanzen, die von Euglossinen bestäubt werden. Die Tribus Euglossini (Hymenoptera: Apidae) ist eine der größten Bienengruppen Mittel- und Südamerikas (Roubik & Hanson 2004). Sie besteht aus 184 Arten, die ausschließlich in den Neotropen vorkommen und sich zu fünf Gattungen gliedern (*Aglae*, *Eufriesea*, *Euglossa*, *Eulaema*, und *Exaerete*; Kimsey 1987, Ramírez *et al.* 2002). Die Tiere sind solitär oder kommunal und haben eine für Bienen relativ lange Lebensspanne von zwei (*Euglossa* spp.) bis fünf Monaten (*Eulaema* spp., *Exaerete frontalis*; Dodson 1966, Ackerman & Montalvo 1985, Zimmerman & Madriñan 1988). Das auffälligste Merkmal der Euglossinen ist ihre lange Proboscis, die in manchen Fällen sogar den Körper der Tiere überragt und eine maximale Länge von 43 mm erreichen kann (Roubik & Hanson 2004). Keine weitere Bienengruppe der Neotropen besitzt vergleichbar lange Zungen. Daher sind Euglossinen in der Lage Nektarquellen auszubeuten, die anderen Insekten verschlossen bleiben. Man geht davon aus, dass Euglossinen, ähnlich wie manche Kolibriarten, ein „trap-line“ Verhalten bei ihrer Futtersuche aufweisen (Janzen 1971, Ackerman *et al.* 1982). Außerdem wurde gezeigt, dass sie auf einem Nahrungsflug Entfernungen von über 20 km zurücklegen (Janzen 1971, Williams & Dodson 1972, Roubik & Hanson 2004). Eine weitere Eigenheit der Tribus, die im Tierreich nur bei diesen Bienen vorkommt, ist das Duftsammelverhalten der Männchen. Diese besitzen in der Regel ein breites Spektrum an Duftquellen. Neben faulem Holz, verwundeten Bäumen, Pilzen oder Fäkalien, sammeln sie auch Düfte von Blüten. Viele neotropische Pflanzenarten, die auch als Parfümblumen bezeichnet werden, haben sich an das Verhalten der Männchen angepasst und werden ausschließlich von diesen bestäubt (z.B. Vogel 1966, Dodson *et al.* 1969, Roubik & Hanson 2004). Die Bedeutung des Duftsammelns ist noch nicht vollständig geklärt. Es gibt jedoch Hinweise darauf, dass es mit dem Territorialverhalten und vermutlich auch mit dem Paarungsverhalten der Bienen in Zusammenhang steht (Roubik & Hanson 2004, Zimmermann *et al.* 2006, Eltz *et al.* 2007).

Sowohl Parfümblumen als auch die Nahrungspflanzen der Euglossinen zeigen starke blütenökologische Anpassungen an die Bienen. Parfümblumen produzieren in der Regel weder Nektar noch Futterpollen und weisen häufig komplexe Bestäubungsmechanismen auf (z.B. Vogel 1966, Dressler 1968). Die Nektarpflanzen haben meistens Blüten mit langen, engen Kronröhren oder Spornen und besitzen vielfach eine „steady state“ Blühphänologie (Ackerman 1985). Über die Ökologie ihrer Pollenpflanzen existieren nur wenige

Informationen. Meistens wurden nur die Brutzellen palynologisch untersucht (Arriaga & Hernández 1998) oder die Besuche pollensammelnder Euglossinen registriert (Frankie & Coville 1979, Buchmann 1980, Roubik *et al.* 1982, Frankie *et al.* 1983).

Durch die starken Anpassungen an ihre Blütenbesucher sind Euglossinen in der Regel die einzigsten Bestäuber der Parfüm- und Nektarpflanzen. Daher hängen diese Pflanzen in ihrer sexuellen Reproduktion stark von ihnen ab (Roubik & Hanson 2004). Betrachtet man die Anzahl aller bisher bekannter euglossinenbestäubter Pflanzenarten, so wird die Bedeutung dieser Bienen für die neotropischen Regenwälder deutlich. Alleine in der Familie der Orchidaceae kennt man ca. 800 Arten, die zu den Parfümblumen zählen (Gerlach 1995). Von weitaus größerer Bedeutung dürften Euglossinen jedoch für die Bestäubung von Nektar- und Pollenpflanzen sein. Roubik & Hanson (2004) zählen mehr als 170 Pflanzengattungen aus 68 Familien auf, in denen eine oder mehrere euglossinenbestäubte Arten vorkommen. Viele dieser Pflanzenarten kommen sympatrisch vor und weisen konvergente Anpassungen in ihrer Blütenbiologie an die Bienen auf (Roubik & Hanson 2004). Man nimmt an, dass Euglossinen eine wichtige Rolle für die Pflanzenvielfalt und die reproduktive Isolation verwandter Arten in tropischen Unterwuchsgesellschaften spielen (Dodson *et al.* 1969, Williams & Dodson 1972, Dressler 1982, Kay & Schemske 2003, Roubik & Hanson 2004). Bisherige Studien beschäftigten sich jedoch überwiegend mit einzelnen Taxa oder Gattungen. Daher existiert nahezu keinerlei Information über die Interaktionen zwischen Pflanzen und Euglossinen innerhalb ganzer Pflanzengesellschaften. Die einzige Untersuchung, die sich über mehrere Pflanzenfamilien erstreckt, stammt von Ackerman (1985), der die Saisonalität der Euglossinenpopulation mit der Blühphänologie ihrer Nektarpflanzen auf BCI in Panama verglich. Er konnte zeigen, dass Pflanzen, die an die Bestäubung von Euglossinen angepasst waren, überwiegend im Unterwuchs vorkamen und ihre Hauptblüte in der mittleren Regenzeit hatten, zu einer Zeit, in der langzüngige Bienen die Bienenpopulation dominierten. Unspezialisierte Pflanzenarten des Kronendaches hingegen, hatten ihre Massenblüte in der Trockenzeit, wenn kurzzüngige Bienen überwogen.

In der vorliegenden Arbeit untersuchte ich die Reproduktionsbiologie verschiedener euglossinenbestäubter Pflanzenarten im Reservat Nouragues, Französisch Guayana. Zu den ausgewählten Pflanzen zählten sowohl Parfümblumen, als auch Nektar- und Pollenpflanzen.

Folgende Fragestellungen sollten beantwortet werden:

- Dient Blütenduft als reproduktionsbiologischer Isolationsmechanismus bei sympatrischen Parfümblumenarten aus der Familie der Araceae?
- Werden Pflanzen der myco-heterotrophen Gattung *Voyria* von Insekten bestäubt oder sind sie obligat autogam?
- Welche reproduktionsbiologischen Eigenschaften besitzen die Arten einer euglossinenbestäubten Pflanzengemeinschaft?
- Gibt es Unterschiede im Besucherspektrum verschiedener euglossinenbestäubter Pflanzenarten und worauf sind diese Unterschiede zurückzuführen?

Im ERSTEN KAPITEL befasste ich mich mit der Bestäubungsbiologie dreier sympatrisch vorkommender Parfümblumenarten aus der Familie der Araceae.

Blütendüfte spielen in der Bestäubungsbiologie der Araceae allgemein eine wichtige Rolle. Man denke nur an die Fallenblumen der Gattung *Arum*, die mit ihrem aasähnlichen Duft kleine Fliegen anlocken (z.B. Kite 1995) oder die Thermogenese-betreibenden Infloreszenzen der Gattung *Philodendron*, deren fruchtig bis würzig riechender Blütenduft große *Dynastinae* Käfer anzieht (z.B. Young 1988, Gottsberger & Silberbauer-Gottsberger 1991). In den Gattungen *Anthurium*, *Spathiphyllum* und *Xanthosoma* haben sich wiederum Spezies entwickelt, die Parfümblumen ausbilden und von Euglossinen bestäubt werden (Williams & Dressler 1976). Häufig kommen mehrere dieser Parfümblumenarten sympatrisch vor und es stellt sich die Frage, wie sich die Pflanzen reproduktionsbiologisch voneinander abgrenzen. Untersuchungen an Parfümorchideen haben gezeigt, dass die Anlockung der Bestäuber durch den Blütenduft in der Regel so spezifisch ist, dass mehrere Pflanzenarten im gleichen Gebiet vorkommen und gleichzeitig blühen können, ohne dass es Überschneidungen im Besucherspektrum gibt. Im Gegensatz zu den Orchideen ist die Bestäubungsbiologie der Parfümblumen in der Familie der Araceae noch kaum untersucht (Williams & Dressler 1976, Croat 1980, Schwerdtfeger *et al.* 2002). Man nimmt an, dass bei dieser Familie ähnliche Isolationsmechanismen wie bei den Parfümorchideen vorkommen. Dies konnte bisher jedoch noch nicht nachgewiesen werden, da es nur wenige Besucherbeobachtungen gibt und die

einzig existierenden Duftanalysen von Pflanzenindividuen aus botanischen Gärten stammen (Schwerdtfeger *et al.* 2002).

Um festzustellen, ob Blütendüfte auch bei Araceen der reproduktiven Isolation dienen, wurde die Bestäubungsbiologie der Parfümblumenarten *Anthurium rubrinervium*, *A. thrinax* und *Spathiphyllum humboldtii* in Nouragues untersucht. Alle drei Arten kommen im gleichen Habitat vor und überschneiden sich in ihrer Blütezeit. Es wurden Besucherbeobachtungen durchgeführt und an den Infloreszenzen der selben Pflanzen Duftproben genommen, die mittels Gaschromatographie und Massenspektrometrie (GC/MS) analysiert wurden. Die Untersuchung zeigte, dass jede der drei Pflanzenarten ein spezifisches Besucherspektrum von drei bis sieben Euglossinenarten besaß. Jeweils zwei Bienenarten dominierten die Blütenbesuche jeder Pflanzenart und kamen an den Infloreszenzen der anderen Pflanzenarten nicht oder nur sehr selten vor. *Spathiphyllum humboldtii* besaß das umfangreichste Besucherspektrum aller drei Spezies, was eventuell auf die relativ kurze Blühperiode dieser Art von ca. drei Monaten zurückzuführen ist. Eine Spezialisierung auf wenige Bienenarten könnte, im Falle dass die Bienen während der Blütezeit nicht vorkommen, die Reproduktion der Pflanzen gefährden. *Anthurium rubrinervium* und *A. thrinax* hingegen blühen das ganze Jahr über. Dadurch ist das Risiko eines Bestäubungsausfalles geringer, was den Pflanzen ermöglichte, sich stärker zu spezialisieren. Signifikante Unterschiede zwischen den Pflanzenarten traten auch in den Besuchszeiten der Euglossinen auf. *Spathiphyllum humboldtii* wurde fast ausschließlich in den frühen Morgenstunden, mit einem Besucherpeak um 9:00 Uhr, besucht, während die Bienen an den beiden *Anthurium* Arten über den ganzen Tag Duft sammelten. Bei *A. rubrinervium* trafen die ersten Euglossinen um 9:30 Uhr ein. Die Zahl der Besucher stieg daraufhin bis 11:45 Uhr an und fiel danach kontinuierlich ab, bis gegen 15:00 Uhr keine weiteren Besucher mehr kamen. Bei *Anthurium thrinax* hingegen, waren die ersten Bienen schon am frühen Morgen präsent. Hier gab es zwei Besucherpeaks: einen um 9:00 Uhr und den anderen um 11:15 Uhr. Nach 11:15 Uhr gingen auch hier die Besucherzahlen bis zum Nachmittag konstant zurück.

Die Ursache für die Unterschiede im Besucherspektrum und den Besuchszeiten sind mit hoher Wahrscheinlichkeit im Blütenduft der Pflanzen zu finden. Während an den Infloreszenzen von *Anthurium thrinax* Blütenduft olfaktorisch den ganzen Tag festgestellt werden konnte, war dies bei *Spathiphyllum humboldtii* nur morgens und bei *A. rubrinervium* erst ab 9:30 Uhr der Fall. Die Analyse der Duftproben ergab, dass die Zusammensetzung und die Hauptkomponenten der Duftbouquets für jede Pflanzenart spezifisch waren. Eine multidimensionale Skalierung der Komposition der Duftproben zeigte deutlich voneinander

getrennte Cluster jeder Art mit einem Stress-Faktor von 0,04. Zusätzlich lag der Sørensen Index der interspezifischen Ähnlichkeit der Duftbouquets bei nur etwa 50%. Insgesamt wurden 66 Substanzen aus vier verschiedenen chemischen Klassen in den Duftproben nachgewiesen (Terpenoide, Benzenoide, Lipid-verwandte und Stickstoff-enthaltende Verbindungen). Diese sind großteils aus den Duftbouquets anderer Parfümblumenarten bekannt (Williams & Whitten 1983, Gerlach & Schill 1991, Knudsen *et al.* 1993, Knudsen *et al.* 2006) und ihre Attraktivität auf Euglossinen-Männchen wurde teilweise in Köderexperimenten nachgewiesen (Ramírez *et al.* 2002, Roubik & Hanson 2004). Die spezifische Anlockung der Bienen wird damit erklärt, dass jede Euglossinenart ein artspezifisches Spektrum an Duftsubstanzen sammelt (Eltz *et al.* 1999, Eltz *et al.* 2006). Demnach könnte man annehmen, dass die Analyse des Blütenduftes und die Kenntnis über die bevorzugten Substanzen der Bienen Klarheit darüber verschaffen, welche Bienenarten angelockt werden. Dies ist jedoch leider nicht der Fall. Die Perzeption der Düfte ist mit komplexen Prozessen im höheren Nervensystem der Biene verbunden. Ein Duftstoff kann dabei die Wahrnehmung anderer Duftstoffe beeinflussen (Eltz & Lunau 2005). Da Blütendüfte nie aus einer Reinsubstanz bestehen, sondern immer eine Mischung verschiedener Komponenten darstellen, ist eine Vorhersage des Besucherspektrums daher leider unmöglich. Der Blütenduft jeder der untersuchten Parfümblumenarten muss in diesem Sinne wohl eher als Einheit verstanden werden. Eventuell beeinflusst jedoch die Komplexität des Blütenduftes die Anzahl der angelockten Bienenarten. Das Duftbouquet der eher generalistischen Spezies *S. humboldtii* bestand ausschließlich aus Terpenoiden, während sich der Duft der beiden *Anthurium*-Arten aus Substanzen mehrerer Stoffklassen zusammensetzte.

Während die Duftproben von *Spathiphyllum humboldtii* und *Anthurium thrinax* jeweils relativ einheitlich waren, zeigten sich leichte Unterschiede in der Zusammensetzung des Blütenduftes einzelner Individuen von *A. rubrinervium*. Die innerartliche Variabilität des Blütenduftes bei Parfümblumen wurde bisher nur bei *Geonoma macrostachys* untersucht (Knudsen 2002). Sie könnte eine wichtige Bedeutung für die Speziation der Pflanzen haben, da größere Veränderungen des Duftbouquets zur Anlockung anderer Bestäuberarten führen. Im Falle der untersuchten *A. rubrinervium* Individuen wurden jedoch keine Unterschiede im Besucherspektrum festgestellt. Man weiß, dass das Sammeln der gleichen Duftstoffe über einen längeren Zeitraum bei den Bienen zu einer Sättigung führt und dann andere Substanzen bevorzugt werden (Eltz *et al.* 1999). Da *A. rubrinervium* das ganze Jahr über blüht und die Bienen eine Lebensdauer von mehreren Monaten besitzen, könnte eine geringe Variabilität des Blütenduftes zu einer länger anhaltenden Attraktivität der Infloreszenzen beitragen.

Zusammenfassend hat diese Studie gezeigt, dass sich in den untersuchten Araceenarten ähnliche Isolationsmechanismen entwickelt haben wie in Parfümorchideen. Spezies-spezifischer Blütenduft führte zur deutlichen Abgrenzung der bestäubenden Euglossinen jeder Pflanzenart und ermöglichte dadurch einen zielgerichteten Pollentransfer. Darüber hinaus gibt es Hinweise, dass sich auch phänologische Barrieren herausgebildet haben.

Das ZWEITE KAPITEL handelt von der Erstbeobachtung eines *Aglae caerulea*-Männchens beim Sammeln von Blütenduft an *Anthurium rubrinervium* (Araceae) und der Analyse dieses Blütenduftes.

Die Tribus Euglossini besteht aus fünf Gattungen, *Aglae*, *Eufriesea*, *Euglossa*, *Eulaema* und *Exaerete* (Kimsey 1987). *Aglae* ist eine monotypische Gattung, deren einzige Spezies, *Aglae caerulea*, wahrscheinlich ausschließlich in den Tieflandregenwäldern Südamerikas vorkommt und dort relativ selten ist (Michener 2000, Cameron 2004). Daher existieren auch nur wenige Beobachtungen über diese Art, und im Gegensatz zu den Bienen aller anderen Gattungen der Tribus, wurde *Aglae caerulea* noch nie beim Duftsammeln an Parfümblumen gesehen. Da Männchen dieser Art jedoch mit den gleichen morphologischen Merkmalen ausgestattet sind wie ihre Verwandten anderer Euglossinen-Gattungen (Sakagami 1965, Vogel 1966, Kimsey 1987, Michener 2000), und da *Aglae caerulea* bereits mit künstlichen Duftködern angelockt werden konnte (Williams & Dodson 1972, Morato 2001, Ramírez *et al.* 2002, Anjos-Silva *et al.* 2006), ging man davon aus, dass auch sie an natürlichen Duftquellen sammeln.

Im Rahmen der Studie über die Bestäubungsbiologie sympatrischer Parfümblumen aus der Familie der Araceae, wurde ein *Aglae caerulea*-Männchen an einer Infloreszenz von *Anthurium rubrinervium* beobachtet. Dieses hielt sich dort für etwa eine Minute auf und zeigte das für Euglossinen typische Duftsammelverhalten (Evoy & Jones 1971). Da *Aglae caerulea* im Zeitraum der Studie nur einmal an einer Infloreszenz beobachtet wurde und im Vergleich zu den anderen Blütenbesuchern sehr selten ist, spielt sie für die Bestäubung der Pflanzenart eher eine untergeordnete Rolle.

Der Blütenduft mehrerer Pflanzenindividuen von *Anthurium rubrinervium* wurde im Feld gesammelt und mittels Gaschromatographie und Massenspektrometrie (GC/MS) analysiert. Insgesamt konnten 36 Substanzen detektiert werden, die zu den chemischen Klassen der Terpenoide, Benzenoide und der lipidverwandten Substanzen gehören. Hauptkomponenten aller untersuchter Blütendüfte waren (Z)-8-Heptadecen, Methylsalicylat, 1,8-Cineol, Benzylbenzoat und Linalool. Daneben gab es Duftproben, die zusätzlich noch große Mengen an (E)-Ocimen und Methyl (E)-cinnamat enthielten. Die Mehrzahl der



Duftkomponenten von *A. rubrinervium* sind bekannte Bestandteile des Blütenduftes anderer Parfümblumenarten (Williams & Whitten 1983, Gerlach & Schill 1991, Knudsen *et al.* 1993, Knudsen *et al.* 2006) und werden teilweise als Reinsubstanzen zur Anlockung von Euglossinen verwendet (Ramírez *et al.* 2002, Roubik & Hanson 2004). (Z)-8-Heptadecen wurde jedoch bisher für keine weitere Parfümblumenart beschrieben. Diese Substanz ist als innerartlicher Botenstoff („Semiochemical“) von Milben bekannt und wird zur Bekämpfung von *Varroa destructor*-befallenen Nestern in der Bienenzucht eingesetzt (Nazzi *et al.* 2002, Milani *et al.* 2004). Obwohl Euglossinen-Männchen nicht an der Brutfürsorge beteiligt sind, könnte das Vorkommen dieser Substanz ein Hinweis darauf sein, dass das Duftsammeln in Zusammenhang mit der Bekämpfung von Krankheiten und Parasiten steht. Allerdings wurde (Z)-8-Heptadecen noch nicht als Ködersubstanz getestet, so dass nicht feststeht, ob diese Substanz überhaupt attraktiv für die Bienen ist.

Im DRITTEN KAPITEL setzte ich mich mit der Frage auseinander, ob Pflanzen der myko-heterotrophen Gattung *Voyria* (Gentianaceae) obligat autogam sind.

Während die Mehrzahl der Samenpflanzen ihre organischen Verbindungen photo-autotroph gewinnt, gibt es wenige hundert Arten aus verschiedenen Familien, die einen anderen Weg eingeschlagen haben und ihren Energiehaushalt durch die Vergesellschaftung mit einem Pilz sicherstellen. Diese Pflanzen werden als myko-heterotroph (auch „saprophytisch“) bezeichnet und sind dadurch charakterisiert, dass sie keinerlei Chlorophyll besitzen und sowohl morphologisch als auch anatomisch stark an ihre Lebensweise angepasst sind (Maas 1986, Leake 1994). Derartige Anpassungen findet man auch in der Reproduktionsbiologie der Pflanzen wieder. Viele Arten sind zwar in der Lage, sich vegetativ zu vermehren; nichtsdestotrotz ist sexuelle Fortpflanzung weit verbreitet. Im Vergleich zu ihren autotrophen Verwandten sind die Blütenstände und Blüten, als auch die Blütenorgane dieser Arten stark reduziert und weitaus einfacher aufgebaut (Maas 1979, Maas 1986, Leake 1994). In den meisten Studien über die Bestäubungsbiologie myko-heterotropher Pflanzen kamen Blütenbesucher nur sehr selten und unregelmäßig vor. Zudem konnte bei vielen Arten nachgewiesen werden, dass sie zu Selbstbestäubung befähigt sind (z.B. Takahashi *et al.* 1993, Zhang & Saunders 2000, Lehnebach *et al.* 2005). Dadurch entstand der Eindruck, dass diese Pflanzen obligat autogam sind und dass obligate Autogamie eine charakteristische Anpassung an die myko-heterotrophe Lebensform darstellt. Dies wurde allerdings nie bewiesen, da nur wenige myko-heterotrophe Pflanzen bisher in ihrer Reproduktionsbiologie untersucht wurden

und die Bestäubungsbiologie ganzer Familien und Gattungen noch unbekannt ist (Maas & Ruyters 1986, Maas *et al.* 1986, Maas & Rübsamen 1986).

Die Gattung *Voyria* Aubl. (Gentianaceae) setzt sich aus 19 rein myko-heterotrophen Arten zusammen, von denen 18 im tropischen und subtropischen Amerika vorkommen (Maas & Ruyters 1986, Albert & Struwe 1997). Die Bestäubungsbiologie der Pflanzen wurde bisher nur ansatzweise studiert. Oehler (1927) untersuchte die Anatomie und Morphologie von *Leiphaimos* sp. (= *V. rosea*) an alkoholkonserviertem Material und stellte fest, dass der Pollen der Blüten noch in den Antheren ausgekeimt war. Die Pollenschläuche wuchsen von dort aus in die Narbe und führten zur Befruchtung der Samenanlagen. Obwohl er keinerlei Besucherbeobachtungen durchgeführt hatte, folgerte Oehler (1927) daraus, dass die Art stark autogam sei. Der einzige Nachweis über Blütenbesucher der Gattung geht auf Imhof *et al.* (1994) zurück, welche Brachyceren (Diptera) an den Blüten von *V. tenella* beobachteten. Da die Rüsselllänge dieser Insekten der Kronröhrenlänge der besuchten Blüten entsprach, hielten die Autoren eine Fremdbestäubung für durchaus möglich. Allerdings machten sie keinerlei Angaben über die Häufigkeit der Blütenbesuche und über die Fähigkeit der Pflanze sich selbst zu bestäuben. Letztendlich stellt sich die Frage, welche Bedeutung Blütenbesucher im Vergleich zur Selbstbestäubung in dieser Pflanzengattung überhaupt haben.

Um dieser Frage nachzugehen, wurde die Reproduktionsbiologie dreier *Voyria*-Arten, *V. caerulea*, *V. clavata* und *V. rosea*, im Reservat Nouragues in Französisch Guayana untersucht. *Voyria rosea* ist in Nouragues weit verbreitet und kommt häufig in größeren Populationen vor, während *V. caerulea* und *V. clavata* eher selten sind und die Pflanzenindividuen meist einzeln, mehrere 100 m voneinander entfernt, stehen. Interessanterweise wuchsen nahezu alle Pflanzen ausschließlich in den Gebieten des Reservats, deren Böden aus karibischem Granit entstanden sind und daher sehr viel Sand und organische Stoffe enthalten („Cambisol“, Grimaldi & Riéra 2001). Die Blüten der untersuchten Arten entwickelten sich direkt am Waldboden und waren groß und auffällig gefärbt. Sie hatten eine durchschnittliche Anthesezeit von 6-10 Tagen, produzierten Nektar und emittierten Blütenduft. *Voyria caerulea* und *V. rosea* besaßen lange, engröhrige Stieltellerblüten, die in kleinen Trugdolden von durchschnittlich 2-4 Blüten zusammenstanden. Die Blüten von *V. clavata* hingegen waren einzelnstehende, langgestreckte Röhrenblüten, deren Durchmesser in der oberen Hälfte deutlich größer war als im unteren nektartragenden Teil und deren Kronblätter keine Landeplattform für besuchende Insekten bildeten. Der Nektar aller Arten war saccharosedominant und hatte eine durchschnittliche Konzentration von 16,4-23,9%. *Voyria caerulea*, *V. clavata* und *V. rosea* verströmten einen

angenehm rosig-zitrusartigen, von Terpenen dominierten Blütenduft. Hauptkomponenten des Duftbouquets von *V. clavata* waren (E)-8-oxo- $\beta$ -Farnesen, (E,E)-Farnesylacetat und (E)- $\beta$ -Farnesen, während hohe Anteile an (E)-Nerolidol, Geraniol und Linalool das Bouquet von *V. rosea* prägten. Die Duftproben von *V. caerulea* waren leider zu niedrig konzentriert für eine Analyse. Allerdings wurde der Blütenduft dieser Art schon zuvor von Kaiser (2006) untersucht, der darin große Mengen an (E)-Nerolidol, Geraniol und Nerol fand. Damit zeigt der Blütenduft eine starke Ähnlichkeit zum Bouquet von *V. rosea*.

Sämtliche untersuchten Blütenmerkmale wiesen darauf hin, dass die Pflanzen an die Besuche langzüngiger Insekten angepasst sind. Tatsächlich konnten diese auch beobachtet werden. *Voyria rosea* wurde von Lepidopteren und Euglossinen besucht, *V. caerulea* ausschließlich von Lepidopteren und *V. clavata* ausschließlich von Euglossinen. Blütenbesuche an den ersten zwei Pflanzenarten waren jedoch sehr selten und unregelmäßig (Besucher von *V. rosea* wurden beispielsweise nur an zwei von 15 Tagen beobachtet). Bei *V. clavata* traten Blütenbesucher häufiger und regelmäßiger auf (ein Besuch innerhalb von 1-2 Tagen).

Die Mehrzahl der Pflanzen im Untersuchungsgebiet produzierte Früchte und zeigte einen durchschnittlichen Samenansatz von 36-49%. Dieser variierte jedoch bei jeder Art zwischen einem und nahezu 100%. Bei streng autogamen Pflanzen hätte man einen sehr hohen Samenansatz erwartet (Ornduff 1969). Verschiedene Studien haben jedoch gezeigt, dass die starke energetische Abhängigkeit myko-heterotropher Pflanzen von ihrem Pilzpartner auch zur Beeinträchtigung ihres Fortpflanzungserfolges führen kann, so dass die beobachtete Samenanzahl nicht unbedingt der Anzahl der befruchteten Eizellen entspricht (Oehler 1927, Ushimaru & Imamura 2002). Man nimmt an, dass die Samen der drei untersuchten Arten durch Wasser ausgebreitet werden (Maas & Ruyters 1986, Leake 1994). Dies trifft bei *Voyria rosea*, deren Früchte sehr hart sind und deren Kapselwand im Laufe der Samenreife immer dünner wird, auch sicherlich zu. Die Früchte von *V. caerulea* und *V. clavata* bestehen jedoch aus weichem, fleischigen Plazentengewebe, in dem die Samen tief eingebettet liegen. Dies und die häufige Beobachtung von Fraßspuren an den Kapseln lassen vermuten, dass die Samen endozoochor, vermutlich durch Nagetiere, ausgebreitet werden.

Um die Bedeutung von Selbstbestäubung bei den drei Pflanzenarten abschätzen zu können, wurde die Morphologie der reproduktiven Organe untersucht, das Pollen/Ovula-Verhältnis (P/O-Verhältnis) bestimmt und mehrere Blüten von *V. rosea* über den Verlauf der Blütezeit in engmaschigen Gazebeuteln eingeschlossen. Alle drei Arten wiesen ein sehr niedriges P/O-Verhältnis auf ( $\bar{O}$ : 33-78), was nach Cruden (1977) ein Hinweis auf sehr

effiziente Pollenübertragung ist. P/O-Verhältnisse dieser Größenordnung treten überwiegend bei fakultativ autogamen Pflanzen auf. Allerdings sind auch obligat xenogame Arten mit niedrigen P/O-Werten bekannt. Diese breiten ihren Pollen entweder in Paketen aus oder besitzen Narben, welche im Verhältnis zur pollentragenden Stelle des Insektes relativ groß sind (Cruden 1977, Cruden & Jensen 1979, Cruden & Miller-Ward 1981). Beides war bei den untersuchten Arten gegeben. Zum einen besitzen sie konisch oder tellerförmig geformte Narben, deren Oberfläche sehr viel größer ist als die pollentragende Stelle der Besucher (Proboscis bei *V. caerulea* und *V. rosea*; dorsales Mesosoma und Metasoma bei *V. clavata*). Zum anderen wird der Pollen mit hoher Wahrscheinlichkeit in kleinen Paketen übertragen. In allen drei Arten wurde auskeimender Pollen in den Antheren reifer Knospen gefunden. Die Pollenschläuche waren stark miteinander verwoben und schlossen auch ungekeimten Pollen mit ein. In *V. clavata* waren die Antheren zusätzlich mit einer viskosen Substanz überzogen, die zu einer weiteren Verklumpung des Pollens führte. Die Übertragung dieser Pollenpakete, vom Körper der Blütenbesucher auf die Narbe, wird vermutlich durch die bei allen drei Arten papillös entwickelte Narbenoberfläche erleichtert.

Trotz großer Narbenoberfläche und der Pollenausbreitung in Paketen legt die Blütenmorphologie von *Voyria caerulea* und *V. rosea* nahe, dass fakultative Selbstbestäubung durch auskeimende Pollenkörner mit hoher Wahrscheinlichkeit möglich ist. In diesen beiden Arten liegen die Antheren dicht an der Narbe und berühren deren Rand mit ihren Spitzen. Außerdem zeigten die isolierten Blüten von *V. rosea* Fruchtsatz. Bei *V. clavata* hingegen schließe ich die Möglichkeit einer Selbstbestäubung aus, da die Antheren hier mehrere Millimeter unterhalb der Narbe am Griffel ansetzen.

Letztendlich konnte durch diese Studie gezeigt werden, dass alle drei *Voyria* Arten von Insekten besucht werden, die zur Fremdbestäubung der Pflanzen beitragen. Die Besucherart und ihre Zuverlässigkeit als Bestäuber spielen jedoch eine entscheidende Rolle dabei, ob die Pflanze rein xenogam oder fakultativ autogam ist. *Voyria clavata* besitzt eine Blütenmorphologie, die nur wenigen Euglossinenarten Zugang zum Nektar gewährt. Da diese Bienen auf ihrer Nahrungssuche täglich feste Routen abfliegen und dabei weite Strecken zurücklegen (Janzen 1971, Ackerman *et al.* 1982), stellen sie höchst zuverlässige Bestäuber dar, die die Reproduktion der Pflanzen absichern. Daher konnte sich bei *V. clavata* reine Xenogamie entwickeln. Die Blütenmorphologie der anderen beiden Arten ist weniger restriktiv in Bezug auf ihre Blütenbesucher. Sämtliche langzüngigen Insekten haben die Möglichkeit an den Nektar zu gelangen. Eine geringere Spezialisierung könnte dazu geführt haben, dass diese Insekten die Blüten nur unregelmäßig aufsuchen und daher nicht immer

eine Pollenübertragung garantieren. Um die Samenproduktion trotzdem zu gewährleisten, sichern sich diese Pflanzen durch konkurrierende Selbstbestäubung ab. Dabei keimt der Pollen zur Blütenöffnung in den Antheren aus, und es kommt zur Selbstbestäubung, falls keine Pollenübertragung mittels Besucher erfolgte. Da die Lebensumstände der meisten myko-heterotrophen Pflanzen sehr ähnliche sind, ist es durchaus denkbar, dass sich auch in anderen Arten Selbstbestäubung als Notfallmechanismus für eine ausgebliebene Fremdbestäubung entwickelt hat.

Im VIERTEN KAPITEL verglich ich die Reproduktionsbiologie mehrerer euglossinenbestäubter Nektar- und Pollenpflanzen in Nouragues.

Viele Pflanzenarten des Unterwuchses tropischer Regenwälder haben sich in ihrer Bestäubungsbiologie auf zuverlässige Bestäuber spezialisiert, die in der Lage sind, Pollen auf sichere Weise über große Entfernungen zu transportieren (Bawa 1983, Bawa *et al.* 1985b, Bawa 1990, Kress & Beach 1994, Kato 2005). Nektarivore Vögel und langzüngige Bienen stellen hierbei die wichtigsten Bestäubergruppen dieser Pflanzen dar. Beide sind gute Flieger, die auf ihrer Futtersuche einer festen Route folgen („trap-lining“; Janzen 1971, Stiles 1978, Sakai *et al.* 1999). Man nimmt an, dass sich nahverwandte Pflanzenarten durch eine Spezialisierung auf verschiedene Bestäubergruppen reproduktionsbiologisch voneinander abgrenzen können und es generell innerhalb einer Pflanzengesellschaft zu einer effizienteren Pollenübertragung kommt (Stiles 1975, Sakai *et al.* 1999, Kay & Schemske 2003). Leider sind tropische Pflanzengesellschaften nur wenig untersucht, so dass viele dieser Theorien rein hypothetisch sind und erst noch durch Feldstudien belegt werden müssen.

Die Gilde der langzüngigen Bienen wird in den Neotropen von der Tribus der Euglossini (Apidae: Hymenoptera) repräsentiert. Diese Bienen sind vor allem durch die Bestäubung sogenannter „Parfümblumen“ bekannt (Vogel 1966, Dodson *et al.* 1969). Hierbei handelt es sich um Pflanzenarten, deren Blüten ausschließlich Duft als Ressource anbieten. Die Erforschung der Reproduktionsbiologie euglossinenbestäubter Pflanzen konzentrierte sich vorwiegend auf diese Parfümblumen (Roubik & Hanson 2004). Die große Anzahl an Nektar- und Pollenpflanzen, die ebenfalls von den Bienen besucht werden und deren Bestäubung von mindestens gleicher, wenn nicht sogar größerer Bedeutung ist, wurde hierbei größtenteils ignoriert (Roubik & Hanson 2004). Zwar sind etliche dieser Pflanzenarten bekannt (Dodson 1966, Ackerman 1985, Ramírez *et al.* 2002, Roubik & Hanson 2004), aber nur wenige wurden bisher näher untersucht und vorhandene Studien beschränken sich in der Regel auf einzelne Taxa oder Gattungen (Kennedy 1978, Schemske 1981, Ackerman *et al.* 1982,

Schemske & Horvitz 1984, Kay & Schemske 2003, Borrell 2005, Almeida Barreto & Freitas 2007, Leite & Machado 2007). Isolationsmechanismen und deren Bedeutung für die Speziation der Pflanzen wurden bislang ebenso fast ausschließlich an Parfümblumen erforscht (Literaturübersicht: Ramírez *et al.* 2002, Roubik & Hanson 2004), während in den Futterpflanzen der Euglossinen darüber nur wenig bekannt ist.

In der vorliegenden Studie versuchte ich die Reproduktionsbiologie einer euglossinenbestäubten Pflanzengesellschaft des Unterwuchses zu charakterisieren. Hierzu wurden sieben Pflanzenarten ausgewählt, die zu den am häufigsten vorkommenden euglossinenbestäubten Spezies des Unterwuchses im Untersuchungsgebiet gehören. Bei diesen handelt es sich um *Bonafousia disticha* (Apocynaceae, Unterwuchsbaum), *Mandevilla rugellosa* (Apocynaceae, Liane), *Costus congestiflorus* (Costaceae, Staude), *Calathea erecta*, *Ischnosiphon martianus* (Marantaceae, Stauden), *Episcia sphalera* (Gesneriaceae, Kraut) und *Rapatea paludosa* (Rapateaceae, Staude). Während die ersten fünf Arten reine Nektarpflanzen sind, wird *E. sphalera* sowohl wegen ihres Nektars als auch wegen ihres Pollens besucht und *R. paludosa* ausschließlich wegen ihres Pollens. Die ausgewählten Pflanzen stellen etwa 10-20% aller in der Regenzeit blühender, euglossinenbestäubter Unterwuchsarten des Untersuchungsgebietes dar. Zusätzlich wurden Besucherbeobachtungen an weiteren 29 Spezies durchgeführt, so dass insgesamt Daten von ca. 70-80% sämtlicher zur Regenzeit blühender euglossinenbestäubter Unterwuchsarten des Untersuchungsgebietes vorliegen.

Die Infloreszenzen aller Pflanzenarten setzten sich aus mehreren, häufig sogar Dutzenden Blüten zusammen. Von diesen öffneten sich jedoch täglich nur eine oder wenige, was zur Folge hatte, dass sich die Blühperiode einer Pflanze über etliche Tage bis Wochen hinzog. Bis auf *Episcia sphalera*, deren Anthese zwei Tage dauerte, betrug die Anthesezeit der Einzelblüten in sämtlichen Pflanzenarten nur einen Tag. Man vermutet, dass diese Blühphänologie, die auch als „steady state flowering“ bezeichnet wird (Gentry 1974, Bawa 1983), eine Anpassung an das Futtersuchverhalten der Euglossinen ist (Frankie *et al.* 1983). Die Blütezeit von *B. disticha*, *C. congestiflorus* und *E. sphalera* beschränkte sich auf wenige Wochen in der Regenzeit, während die anderen Pflanzenarten über mehrere Monate, möglicherweise sogar über das ganze Jahr hinweg, blühten. Verschiedene Studien zeigten, dass die Anzahl langzüngiger Euglossinen zu Beginn der Regenzeit im Jahresmittel am höchsten ist, was vermutlich auch in Nouragues der Fall war (Frankie *et al.* 1983, Ackerman 1985, Roubik & Ackerman 1987). Die Blütezeit der ersteren Arten könnte daher eine Anpassung an den gestiegenen Nahrungsbedarf der Bienen sein, während die letzteren

Schlüsselarten darstellen, die die Grundversorgung der Bienenpopulation über das Jahr hinweg absichern.

Das Besucherspektrum der untersuchten Pflanzen wurde deutlich von Euglossinen dominiert. Diese waren in der Regel für mehr als 80% aller Blütenbesuche verantwortlich (einzigste Ausnahme: *Calathea erecta* mit ca. 50%) und können als Bestäuber der Pflanzen angesehen werden, da sie sowohl Kontakt mit Antheren als auch Narben hatten und mehrere Blüten einer Art anflogen. Weitere Besucher waren Bombyliiden, Lepidopteren und Meliponinen. In den meisten Fällen spielten diese jedoch als Bestäuber keine oder nur eine untergeordnete Rolle, da sie in kleiner Anzahl auftraten, häufig keinen Kontakt mit Antheren oder Narben hatten, oder nur den Pollen raubten. Überdies war ihr Nahrungssuchverhalten weniger gerichtet als das der Euglossinen, so dass deutlich weniger Blüten von jedem Individuum angeflogen wurden.

Die Besucherdominanz der Euglossinen ist vermutlich überwiegend auf die Morphologie der Blüten zurückzuführen (langgezogene enge Kronröhren oder Sporne), die dazu führte, dass der Zugang zum Nektar auf langzüngige Insekten und in einigen Fällen sogar spezifisch auf Euglossinen beschränkt war. Ferner könnten auch die Eigenschaften des Nektars eine Rolle gespielt haben. Dieser war ausnahmslos Saccharose-dominant und zeigte durchschnittliche Zuckerkonzentrationen von 25,5-29,7%. Es ist bekannt, dass Blüten, die von langzüngigen Bienen besucht werden, meistens saccharosedominanten Nektar produzieren (Percival 1961, Baker & Baker 1983), der eine Konzentration von 22-48% besitzt (Roubik *et al.* 1995). Überraschenderweise wurden die offenen Scheibenblumen von *R. paludosa* ebenfalls fast ausschließlich von Euglossinen besucht. Dies lag eventuell daran, dass die Blüten der Art lediglich Pollen anbieten, der jedoch nicht frei zugänglich ist, sondern nur durch Vibrationen an den sich porizid öffnenden Antheren gesammelt werden kann („buzz-pollination“; Michener *et al.* 1978, Buchmann 1983). Allerdings kommen im Untersuchungsgebiet verschiedene Bienengruppen vor, die in der Lage gewesen wären, die Blüten auszubeuten. Möglicherweise sind diese jedoch auf andere Pollenpflanzen spezialisiert, da *R. paludosa* nur eine geringe Populationsdichte aufweist und jede Infloreszenz nur wenig Pollen anbietet.

Das Euglossinen-Besucherspektrum jeder Pflanzenart zeigte eine ähnlich hohe Spezifität wie bei Parfümblumen. Eine Bienenart oder eine Bienenuntergattung dominierte die Besuche jeweils einer Pflanzenart. In nahezu allen Fällen waren dies Bienen der *Euglossa*-Untergattung *Glossura* (vor allem der Spezies *E. piliventris* und *E. chalybeata*), welche zu den individuenreichsten Euglossinengruppen neotropischer Regenwälder zählen (Ackerman

1983, Pearson & Dressler 1985, Powell & Powell 1987, Roubik & Ackerman 1987, Roubik 1989, Roubik & Hanson 2004). Aufgrund ihrer großen und stabilen Populationen stellen diese Bienen vermutlich gute und zuverlässige Bestäuber dar, so dass sich viele Pflanzenarten unter ihrem Einfluss entwickelt und spezialisiert haben könnten. Bei *Mandevilla rugellosa* und *Bonaifousia disticha* dominierten hingegen große Bienen der Spezies *Exaerete frontalis* und der *Eulaema*-Untergattung *Eulaema* s.s. die Blütenbesuche, während *Episcia sphalera* ausschließlich von Weibchen einer noch unbestimmten kleinen *Euglossa*-Art bestäubt wurde. Die divergierenden Besucherspektren der Pflanzenarten sind mit hoher Wahrscheinlichkeit auf die angebotenen Nektarmengen der Blüten zurückzuführen, die zwischen den Arten signifikant verschieden waren. Durchschnittliche Nektarmengen variierten zwischen 3,7 µl bei *E. sphalera* und 51,1 µl bei *M. rugellosa* und korrelierten positiv mit der mittleren Körpergröße der Blütenbesucher. Ein weiterer Hinweis, der diese Hypothese unterstützt, sind signifikante Unterschiede in den Besucherspektren einzelner *B. disticha* Individuen. Pflanzen dieser Art, die wenige Blüten und damit ein geringes Nektarangebot besaßen, wurden fast ausschließlich von Bienen der *Euglossa*-Untergattung *Glossura* besucht, während an vielblütigen Pflanzen große Bienenarten (*Exaerete frontalis*, *Eulaema*-Untergattung *Eulaema* s.s.) dominierten. Aufgrund der langen Flugstrecke, die eine Biene zwischen den besuchten Pflanzenpopulationen zurücklegen muss, gibt es vermutlich eine minimale Nektarmenge, ab der es sich für sie lohnt, Blüten in ihre tägliche Flugroute zu integrieren (Heinrich & Raven 1972, Pyke 1984). Da große Euglossinen einen höheren Energieverbrauch haben als kleine (Casey *et al.* 1985), dürfte der Schwellenwert bei ihnen dementsprechend höher liegen. Pyke (1978) konnte schon bei Hummeln eine Korrelation zwischen Körpergröße und mittlerem Nektarangebot einer Blüte nachweisen, und meine Studie zeigt, dass dies vermutlich auch für Euglossinen zutrifft. Neben der Nektarmenge hat auch die Blütenmorphologie zur Eingrenzung des Besucherspektrums beigetragen. So war die Kronröhrenlänge positiv mit der durchschnittlichen Proboscislänge der Blütenbesucher korreliert, was ein Hinweis darauf ist, dass Bienen überwiegend diejenigen Blüten besuchten, deren Kronröhrenlänge ihrer Zungenlänge entsprach. Darüber hinaus war der Eingang von Rachenblüten häufig so eng oder die Landemöglichkeit auf der Blüte so klein, dass nur kleine Bienen die Ressourcen ausbeuten konnten.

Während die Pflanzen in ihrem Bestäuberspektrum sehr selektiv waren, besaß jede Bienenart eine große Bandbreite verschiedener Futterpflanzen. Dies könnte damit verbunden sein, dass die Lebensspanne einer Biene häufig länger ist als die Blühperiode einer Pflanzenart (Feinsinger 1983) und dass eine einzelne Pflanzenart durch die geringe



Populationsdichte und die niedrige Anzahl an Blüten wahrscheinlich nicht in der Lage wäre, die gesamte Population ihrer Bestäuber zu ernähren (Schemske 1981, Sakai *et al.* 1999). Feinsinger (1983) vermutet, dass es bei Pflanzenarten, die eine geringe Populationsdichte aufweisen, zur konvergenten Entwicklung der Reizmittel, der Morphologie und der Ressourcen der Blüten kommt, damit sie in die Flugrouten von Blütenbesuchern übernommen werden, die große Distanzen zurücklegen. Bei Pflanzen, die vom gleichen Bestäuber besucht werden, könnten Arten von der Selektion begünstigt werden, die ihren Pollen artspezifisch an bestimmten Stellen des Bestäubers auftragen. Dies wurde schon für verschiedene kolibribestäubte *Heliconia* Arten (Stiles 1975) und euglossinenbestäubte Parfümorchideen nachgewiesen (Ackerman 1983). Blüten von Pflanzenarten meiner Studie trugen ihren Pollen auf der Proboscis (Apocynaceae, Marantaceae), dem dorsalen Mesosoma (*Costus congestiflorus*, *Episcia sphalera*) und dem ventralen Mesosoma (*Rapatea paludosa*) ihrer Bestäuber auf. Da sämtliche Arten, die ihren Pollen auf der Proboscis auftragen, von den gleichen Bestäubern besucht wurden, kam es unweigerlich zu einer Vermischung heterospezifischen Pollens. Diese Überschneidung ist vor allem bei nah verwandten Arten bedenklich, da sie zur Hybridbildung führen kann. Aus einer Studie zweier sympatrischer *Costus*-Arten, die von derselben Biene bestäubt werden ist bekannt, dass sich starke Hybridisierungsbarrieren entwickelt haben (Schemske 1981). Dies müsste bei den Pflanzenarten meiner Studie noch untersucht werden. Das Ausbleiben der Entwicklung verschiedener Aufbringungsorte des Pollens könnte mit der geringen Körpergröße der Bestäuber verbunden sein. Im Vergleich zu Kolibris sind Euglossinen relativ klein, so dass eine exakte Positionierung des Pollens eventuell gar nicht möglich ist. Parfümorchideen hingegen breiten ihren Pollen in kleinen Paketen aus, die mit einer Klebescheibe an dem Bestäuber angebracht werden (Ackerman 1983). Dies ermöglicht ihnen, den Pollen exakt an beliebigen Stellen selbst kleiner Euglossinen anzubringen.

Hinsichtlich der Geschlechterverteilung der Blütenbesucher wurden bei allen Nektarpflanzen bis auf *Episcia sphalera* sowohl männliche als auch weibliche Bienen beobachtet. Bei *E. sphalera* und *Rapatea paludosa* hingegen, waren es ausschließlich Weibchen. Da *R. paludosa* nur Pollen als Ressource anbietet und männliche Bienen in der Regel keinen Pollen sammeln, ist diese Beobachtung nicht weiter verwunderlich. *Episcia sphalera* hingegen besitzt neben Pollen auch Nektar. Möglicherweise waren die Nektarmengen aber so gering, dass sich ein Blütenbesuch nur in Kombination mit dem Pollensammeln lohnte. Denkbar wäre auch, dass zum Zeitpunkt der Untersuchung nur weibliche Bienen vorkamen.

Die Hauptaktivitätszeit der Bienen an den Nektarblüten erstreckte sich von ca. 9:00 Uhr bis 16:00 Uhr. Generell wurden am Morgen jedoch mehr Besucher beobachtet als am Nachmittag. Dieses Phänomen wurde schon in anderen Studien festgestellt, in denen man versuchte, eine Korrelation zwischen der Häufigkeit der Besucher und klimatischen Faktoren zu finden, was jedoch nicht gelang (Armbruster & McCormick 1990, Armbruster & Berg 1994). Die Besuchsfrequenzen am Nachmittag richteten sich lediglich nach der Tageszeit oder einer unbekannten Variablen (Armbruster & Berg 1994). Meine Nektaruntersuchungen lassen vermuten, dass die sinkenden Besucherzahlen mit dem Verlauf der täglichen Nektarproduktion zusammenhängen. In den untersuchten Pflanzenarten waren die Nektarmengen morgens oder kurz vor Blütenöffnung stets am höchsten und fielen bis um 14:00 Uhr auf nahezu null ab. Ähnliche Tagesverläufe wurden zuvor schon bei anderen Pflanzenarten der untersuchten Familien beobachtet und scheinen bei diesen sehr weit verbreitet zu sein (Madrigal & Girón 1982, Torres & Galetto 1998, Leite & Machado 2007, Almeida Barreto & Freitas 2007). Neben der Nektarproduktion könnte zusätzlich auch die zunehmende Regenwahrscheinlichkeit am Nachmittag von Bedeutung sein, da starker Regen viele Blüten zerstörte und die Bienen am Blütenbesuch hinderte.

In den Besuchszeiten an den Nektarpflanzen gab es weder signifikante Unterschiede zwischen Bienenarten, noch zwischen Pflanzenarten. Dieses Ergebnis ist durchaus überraschend, da Unterschiede zwischen großen und kleinen Bienenarten aufgrund verschiedener Kühlungskapazitäten zu erwarten gewesen wären (May & Casey 1983). Außerdem standen die Pflanzen an unterschiedlichen Standorten mit gegensätzlichem Mikroklima (offene, stark sonnenexponierte Flächen oder dichter, schattiger Wald). Offenbar scheinen mikroklimatische Faktoren, zumindest in der Regenzeit, nur eine untergeordnete Rolle für die Aktivitätszeiten der Bienen zu spielen.

Im Gegensatz zu den Nektarpflanzen wurde *Rapatea paludosa* ausschließlich am frühen Morgen besucht. Die ersten Bienen trafen schon kurz nach Sonnenaufgang ein (ca. 6:15 Uhr). Um ca. 8:00 Uhr erreichten die Besucherzahlen ihren Höhepunkt und gingen daraufhin bis zum Mittag auf null zurück. Durch die geringe Menge des angebotenen Pollens entsteht vermutlich ein starker Konkurrenzdruck unter den Bienen. Diese treffen daher sofort zum Zeitpunkt der Blütenöffnung ein und besuchen die Pflanzen so lange, bis sämtliche Blüten restlos ausgebeutet sind. Bei *E. sphalera* trat dieses Phänomen nicht auf, da der Pollen nicht aktiv von den Antheren abgesammelt werden konnte, sondern beim Hineinkriechen in die Blüte auf dem dorsalen Mesosoma der Biene aufgetragen und erst hinterher in den

Hörschen verstaubt wurde. Außerdem boten diese Pflanze neben dem Pollen auch zusätzlich Nektar an.

In Bezug auf die Anzahl und Größe der Pollenkörner der Blüten gab es deutliche Unterschiede zwischen Nektar- und Pollenpflanzen. Während die Ersteren nur wenige hundert, meist sehr großer Pollen produzierten, waren es bei den Letzteren einige zehntausend kleiner Pollen. Dies ist ein typisches Charakteristikum von Pollenpflanzen, welches vermutlich auf die kleinen poriziden Öffnungen der Antheren und das Anbieten des Pollens als Ressource zurückzuführen ist (Cruden 2000).

Während der Blütenbesuche konnten unterschiedliche Herangehensweisen einzelner Bienenindividuen zur Ausbeutung der Blüten beobachtet werden, die unabhängig von Bienenart oder besuchter Pflanze waren. Einige Bienen besuchten sämtliche Blüten einer Pflanze oder einer Population und absolvierten vor dem Verlassen dieser nochmals einen Kontrollflug, offensichtlich um sicher zu gehen, keine Blüte ausgelassen zu haben. Manche Tiere landeten sogar auf verschlossenen Knospen oder verwelkten Blüten und stellten erst dann fest, dass keine Ressourcen vorhanden waren. Andere Bienen wiederum suchten gezielt nur einzelne Blüten auf, um im Anschluss sofort weiterzufliegen. Die beobachteten Unterschiede sind vermutlich auf Lernprozesse der Bienen zurückzuführen. Erfahrene Tiere kennen durch das tägliche Abfliegen der gleichen Strecke die ertragreichsten Blüten einer Population und sind mit deren Handhabung vertraut, während junge Tiere erst noch ihre Flugroute etablieren und lernen müssen, „gute“ von „schlechten“ Blütenständen zu unterscheiden. In ihrer Blütenauswahl gingen manche Bienen zwar weniger zielgerichtet, dafür aber sehr selektiv vor. Sie flogen mehrere Blüten hintereinander an, um für kurze Zeit vor ihnen zu schweben, landeten jedoch nur auf einigen wenigen. Dieses Verhalten wurde schon bei *Apis mellifera*, *Bombus* spp. oder *Xylocopa virginica texana* beobachtet (Frankie & Vinson 1977, Stout & Goulson 2001) und wurde darauf zurückgeführt, dass die Tiere bei ihrem Blütenbesuch Duftmarken hinterlassen, die von art eigenen und teilweise auch artfremden Individuen erkannt werden, welche die Blüte daraufhin meiden (Frankie & Vinson 1977, Stout *et al.* 1998, Goulson *et al.* 1998, Stout & Goulson 2001). Ob dies für Euglossinen ebenfalls zutrifft, wurde noch nicht untersucht. Meine Beobachtungen und die nahe Verwandtschaft zu Bienengruppen die Duftmarken einsetzen (Michener 2000, Cameron 2004) sind jedoch ein Hinweis darauf, dass dies bei Euglossinen ebenfalls der Fall ist.

Der Fruchtansatz der untersuchten Pflanzenarten variierte stark. Während sich bei *Episcia sphalera* und *Costus congestiflorus* etwa die Hälfte der Blüten einer Infloreszenz zu Früchten entwickelten, waren es bei *Rapatea paludosa* und *Ischnosiphon martianus* nur noch

ein Viertel, bei *Mandevilla rugelosa* 10% und bei *Bonaifousia disticha* und *Calathea erecta* ca. 1%. Niedrige Fruchtansätze waren häufig durch starke Florivorie und Frugivorie bedingt. Apocynaceen scheinen außerdem dazu zu neigen bei limitierter Nährstoffversorgung einen Teil ihrer bestäubten Blüten vorzeitig abzuwerfen (Lopes & Machado 1999, Torres & Galetto 1999). Daher ist der niedrige Fruchtansatz einiger Pflanzenarten nicht unbedingt mit einem niedrigen Bestäubungserfolg gleichzusetzen. *Calathea erecta* wurde häufig von Nektardieben aufgesucht, die vermutlich den nur einmalig funktionierenden Bestäubungsmechanismus auslösten, ohne jedoch die Blüten zu bestäuben. Schemske & Horvitz (1984) zeigten schon an *Calathea ovadensis*, dass dies zu einer deutlichen Herabsetzung des Fruchtansatzes führt.

Bis auf *Rapatea paludosa* wiesen sämtliche Arten sehr niedrige P/O-Verhältnisse auf, die nach Cruden (1977) auf die fakultativ autogame Fortpflanzung der Pflanzen schließen lassen. Für *Episcia sphalera* trifft dies auch sicherlich zu. Mehrere im Gewächshaus kultivierte Pflanzenindividuen dieser Art fruchteten, ohne zuvor Kontakt mit einem Bestäuber gehabt zu haben. Bei den anderen Pflanzen ist dies jedoch mit hoher Wahrscheinlichkeit nicht der Fall. Niedrige P/O-Verhältnisse wurden schon bei verschiedenen Arten der Apocynaceae, Costaceae und Marantaceae festgestellt (Ramirez & Seres 1994, Torres & Galetto 1999, Lopes & Machado 1999, Löhne *et al.* 2004, Leite & Machado 2007). In diesen und weiteren Studien konnte jedoch nachgewiesen werden, dass es bei keinen oder nur sehr wenigen Pflanzenindividuen zu autonomer Selbstbestäubung kam (Schemske 1981, Ramirez & Seres 1994, Torres & Galetto 1999, Lopes & Machado 1999, Löhne *et al.* 2004, Leite & Machado 2007, Almeida Barreto & Freitas 2007). Vielmehr handelte es sich bei Pflanzen dieser Familien um Ausnahmen, deren niedriges P/O-Verhältnis aus sehr effektiven Bestäubungsmechanismen, mit großen Pollenkörnern und großen Narben resultiert. Dies würde wiederum bedeuten, dass der Fruchtansatz aller untersuchter Arten auf den Pollentransfer durch Euglossinen zurückzuführen ist und diese demnach als sehr effektive Bestäuber einzustufen sind.

## SCHLUSSFOLGERUNG

Tropische Unterwuchspflanzen teilen sich das Schicksal, dass sie ihre Fortpflanzung in einer dunklen, energiearmen Umwelt bewerkstelligen müssen, in welcher arteigene Individuen meist Hunderte von Metern voneinander entfernt wachsen. Meine Studie hat gezeigt, dass euglossinenbestäubte Pflanzen reproduktionsbiologische Eigenschaften besitzen, die es ihnen erlauben trotz der widrigen Umstände einen sehr effizienten Pollenaustausch zu erreichen. Hierbei können zwei verschiedene Bestäubungssysteme unterschieden werden: zum einen

Parfümblumen und zum anderen Nektar- und Pollenpflanzen. Beide Gruppen zeichneten sich durch ein hochspezifisches Bestäuberspektrum jeder Pflanzenart und einen relativ geringen täglichen Energieaufwand zur Produktion der Blütenressourcen aus. Die selektive Anlockung der Bestäuber wurde bei den Parfümblumen durch artspezifische Blütendüfte erzielt. Deshalb waren keine aufwendigen Blütenschauapparate nötig und der Blütenduft genügte als Hauptattraktanz. Bei den Nektarpflanzen führte eine restriktive Blütenmorphologie und Unterschiede in der angebotenen Nektarmengen zur Einschränkung des Besucherspektrums. Dadurch, dass der Nektar nur von einer begrenzten Anzahl von Bestäubern ausgebeutet werden konnte, reichte es aus täglich nur eine oder wenige Blüten zu produzieren. Dies trifft auch für die untersuchten Pollenpflanzen zu. Bei diesen ist jedoch noch unklar, wie es zu einer Beschränkung der Besucherarten kam. Die Ergebnisse meiner Studie deuten darauf hin, dass manche Euglossinenarten möglicherweise oligolektisch sind, zumindest für einen gewissen Zeitraum oder Ort.

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Several male *Eufriesea surinamensis* gnawing at the roots of *Esenbeckia* sp. (Rutaceae) with their mandibles probably to collect leaking fragrance substances.



Several male *Eulaema* cf. *bombyiformis* robbing scent from the hindtibiae of a dying conspecific.

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## REPRODUCTIVE ISOLATION BY FLORAL SCENT IN THREE SYMPATRIC EUGLOSSINE-POLLINATED *ANTHURIUM* AND *SPATHIPHYLLUM* (ARACEAE) SPECIES IN FRENCH GUIANA

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### ABSTRACT

In French Guiana, we studied the reproductive biology of three sympatric Araceae species, i.e., *Anthurium rubrinervium*, *A. thrinax*, and *Spathiphyllum humboldtii*, pollinated by male euglossine bees. In total, every plant species was visited by 3-7 euglossine species; in each case, two euglossine species accounted for at least 80 percent of all flower visits, and were plant species specific. Floral scent bouquets of the plant species differed mainly in the presence of different principal compounds and in the distribution of the fragrance compounds among different chemical classes. Each bouquet consisted of 6-10 main compounds, which made up 76-94% of the total amount of volatiles. There was both intraspecific and interspecific variation in floral scent composition. *S. humboldtii* and *A. thrinax* scent variation differed solely by component amounts, whereas among individuals of *A. rubrinervium* there were additionally differences in scent sample components. A limited degree of intraspecific variability in the qualitative composition of floral scent in *A. rubrinervium* may be a strategy of a year-round flowering euglossine-pollinated plant to remain attractive for the long-lived bees, because the collection of the same fragrances over a long period of time leads to a decreasing attractiveness of these compounds. Emission of different floral scent bouquets between the three sympatric plant species ensures a clear separation of the main pollinating euglossine species and thus a directed pollen-flow leading to the reproductive isolation of the plants. Additionally, differences in bees' daily visiting times to inflorescences might have further contributed to reproductive separation.

Key words: *Anthurium rubrinervium*; *Anthurium thrinax*; *Spathiphyllum humboldtii*; Araceae; euglossine bee pollination; Euglossini; French Guiana; intraspecific variation in floral fragrances.

## INTRODUCTION

Floral scent plays a major role for the attraction of pollinators in the reproductive biology of Araceae. The floral fragrance spectrum in this family broadly ranges from unpleasant foul or decaying odors to agreeable sweet or fruity scents, dependent on the flower visitors of the respective species (Mayo *et al.* 1997). Aroids are known for their fly-pollinated flowers, which attract visitors by odors resembling those of feces, carrion or mushrooms (Vogel 1978, Kite 1995, Mayo *et al.* 1997), and for their beetle-pollinated flowers, which produce a pleasant fruity or spicy scent (Gottsberger & Silberbauer-Gottsberger 1991). In both pollination types, inflorescence heating (thermogenesis) is used to volatilize odor compounds (Meeuse & Raskin 1988, Mayo *et al.* 1997) and the inflorescence morphology is quite complex, with female and male flowers spatially separated on a spike ('spadix'). Additionally, a large bract ('spathe') subtends the spike which forms a trap or a pollination chamber at the spike base. The minute timing of flower maturation and scent emission in these flowers is often decisive for a successful pollination.

In the rather basal groups of Araceae (*Pothoideae*, *Monsteroideae*, *Lasioideae*), the inflorescence structure is more simple, with bisexual flowers uniformly arranged on a spadix, and a spathe that does not fulfill such complex functions. In some species the spathe serves as a visual attractant to the pollinators or as a rain-protecting umbrella, in others it probably just represents a green leaf with little contribution to pollination (Mayo *et al.* 1997, Schwerdtfeger *et al.* 2002). While the 'trap'-flowered species have attracted much attention to scientist, the pollination biology of most basal Araceae has been studied to a much smaller extent. Only a handful of reports exist on the pollination of the families' largest genus, *Anthurium* (*Pothoideae*), which comprises about 1000 species (Croat 1998). The few existing studies show that *Anthurium* species obviously have adapted to a rather large spectrum of pollinator groups, including Drosophilidae, Cecidiomyiidae, Curculionidae, Euglossini and hummingbirds (Williams & Dressler 1976, Croat 1980, Kraemer & Schmitt 1999, Schwerdtfeger *et al.* 2002, Franz 2007). Similar to 'trap'-flowered aroids, floral odor composition is also supposed to be decisive for the attraction of distinct pollinators (Croat 1980, Schwerdtfeger *et al.* 2002). The flowers of species visited by Drosophilidae emit yeasty scents resembling that of rotten fruits, while euglossine- and weevil-pollinated plants produce a sweet perfume-like odor (Williams & Dressler 1976, Croat 1980, Schwerdtfeger *et al.* 2002, Franz 2007). However Cecidiomyiidae- and hummingbird-pollinated flowers are completely scentless to the human nose (Kraemer & Schmitt 1999, Schwerdtfeger *et al.* 2002). In many regions, various *Anthurium* species occur sympatrically and specialization towards different



pollinators seems to contribute to their reproductive isolation (Croat 1992, Schwerdtfeger *et al.* 2002). This hypothesis is further supported by the fact that although most *Anthurium* species can readily hybridize with species in a given section, natural hybrids are very seldom in the genus and only occur in the section *Porphyrochitonium* (T. Croat, pers. comm.).

Reproductive isolation of sympatric *Anthurium* species is especially interesting in euglossine-pollinated plants. Euglossine pollination is characterized by the attraction of male euglossine bees through floral odors, which serve as both resource and attractant to the visitors. No additional nectar, fodder pollen or other food tissue is offered. For these reasons, the bees are in most cases the only flower visitors and the respective plants are highly dependent on their pollination services. The floral odor composition determines the bee species that are attracted to the flowers (Vogel 1963, Dressler 1968, Dodson *et al.* 1969). By emitting different scent bouquets, several euglossine-pollinated species may grow in the same area without interfering in each others pollination biology (Hills *et al.* 1972, Whitten & Williams 1992). In fact, many euglossine-pollinated *Anthurium* species overlap in their distribution areas and previous studies have shown that some of these species emit different floral scent bouquets and consequently are visited by different pollinators (Williams & Dressler 1976, Schwerdtfeger *et al.* 2002). Unfortunately, the present knowledge on scent emission and pollinator activity of these plants is mostly based upon scent samples collected from single plant individuals cultivated in greenhouses (sometimes of unknown origin), and upon pollinator data which are often based on anecdotal information or on observations of single visiting events. So far, no detailed *in situ* studies on the pollination biology of sympatric species and on the intraspecific variation in floral scent composition are available.

In the almost pristine rainforest site Nouragues, French Guiana, several *Anthurium* species and one *Spathiphyllum* species occur sympatrically. *Spathiphyllum* is a rather small genus (41 species) of the basal aroid subfamily *Monsteroideae* (Bunting 1960, Mayo *et al.* 1997). Flowers of the genus are known to emit a pleasant, sweet odor, and pollination by euglossine bees and stingless bees is reported (Williams & Dressler 1976, Montalvo & Ackerman 1986). Similar to *Anthurium*, some *Spathiphyllum* species show an overlap in their distribution areas and reproductive isolation by the emission of species-specific floral scent bouquets is suggested (Williams & Dressler 1976).

We studied the pollination biology of the euglossine-pollinated aroids *Anthurium rubrinervium*, *A. thrinax* and *Spathiphyllum humboldtii* at Nouragues. All plants are terrestrial herbs and grow in small populations of 1-8 plants in swampy areas along creeks at the Inselberg site of Nouragues. While *Anthurium rubrinervium* and *S. humboldtii* occur

throughout the Amazon basin, from the wet lowland forests of southern Venezuela to the northern part of Peru (Bunting 1960, Croat 1998), *A. thrinax* has a more limited distribution and is restricted to the Guianas (Tropicos 2007). At the Inselberg site, all three species are common elements of the local flora.

Our working hypothesis was that each species possesses a distinctive floral scent bouquet that assures the attraction of different pollinator species. To test our hypothesis, we recorded and sampled the flower visitors of each plant species over several days and collected and analyzed floral scent composition both within (intraspecific) and between (interspecific) the three species.

## MATERIAL & METHODS

### *Study site*

The study was conducted during the early- to midrainy season (January-June) of 2005 and 2006 in the environs of the Inselberg station of the Nouragues Natural Reserve, French Guiana (04°05' N, 52°41' W, 120 m asl.). Nouragues is located in primary lowland tropical rainforest with an annual rainfall of 2990 mm and a mean annual temperature of 26.3°C (Grimaldi & Riéra 2001).

### *Floral morphology and flowering phenology*

The floral morphology and flowering phenology of individual inflorescences of *Anthurium rubrinervium*, *A. thrinax* and *Spathiphyllum humboldtii* was studied in the field. *Anthurium rubrinervium* and *A. thrinax* are supposed to flower the year round in French Guiana (Croat 1997), while *Spathiphyllum humboldtii* has a flowering period that is restricted to the late dry and early rainy season (November-April; Croat 1997). Voucher specimens of the investigated species were collected and are deposited in the herbaria CAY and ULM (Hentrich, FGIC 165, 209, 211).

### *Visitor observations*

We observed flower visitors to several individuals of each of the study species. Initially, two observations were made between 0800-1500 h for each plant species to gather information on the main insect visiting hours. Subsequently, flower visitors of *Anthurium rubrinervium*, were recorded from 1000-1500 h on eight days between 09 and 29 March 2006; *A. thrinax* was observed from 0900-1400 h on seven days between 08 May and 01 June 2005 and *Spathiphyllum humboldtii* from 0830-1200 h on twelve days between 31 January and 04 March 2006. Minimum observation-distance to the flowers was 2 m. Voucher specimens of

the visiting bees were collected from each plant species on separate days to avoid disturbance to pollinator activities. The bees were identified using the reference collection of G. Gerlach (Botanical Garden Nymphenburg, Munich) and are deposited in the collection of the first author and the insect collection of the herbarium ULM (*Hentrich* FGIC 4, 12, 13, 16-18, 20-23, 25, 26, 28, 31, 32, 47).

### *Scent sampling*

Onset and termination of daily fragrance emission of the flowers was tested by the observer sniffing at the spadices. With the exception of *Anthurium thrinax*, five scent samples for each plant species were collected in the field by the headspace method (Williams & Whitten 1983, Hills & Schutzman 1990, Raguso & Pellmyr 1998). For *A. thrinax* two of the five samples were collected in the greenhouse from a direct clone of an individual that has been studied previously in the field. The samples of *A. rubrinervium* are from four different plants, the samples of *A. thrinax* from three (including the clones), and those of *S. humboldtii* from five different plants. Inflorescences were covered with an inert oven-bag during collection. The scented air was drawn through a sampling cartridge by a battery-operated membrane pump for four hours at a flow rate of 150 ml/min. The sampling cartridge was prepared with 25 mg of Tenax TA (mesh 80-100, Macherey Nagel, Düren, Germany) and 40 mg of Carboxen 100 (mesh 20-40, Supelco, Bellefonte, Pennsylvania, USA). The adsorbents were conditioned by washing with methanol (purity 99.8%, Merck, Darmstadt, Germany) and acetone (purity 99.9%, Merck, Darmstadt, Germany) and heated out for 30 min at 220°C before sampling. The adsorbed scent was recovered by elution with 200 µl acetone into glass vials. The samples were analyzed by GC/MS using a Thermo Finnigan Voyager Mass Spectrometer combined with a Trace GC 2000 Series and the Xcalibur software. The analyses were made on a DB-WAX column (J&W Scientific) 30 m x 0.32 mm i.d., film thickness 0.25 µm; splitless injection, temperature programme was 50°C-2'iso-2.5°C/min-230°C-40'iso. Compounds were identified by comparison of their mass spectra and retention times with those of authentic reference samples available from the Givaudan reference compound collection. Substances that had a mean value of > 1% of all volatiles in a scent sample of the respective species were classified as major compounds.

### *Statistical analysis*

A  $\chi^2$ -test was used to calculate if there were significant differences in visitor spectrum and visiting time between the three plant species. For the comparison of the visitor spectrum, the

Bonferroni correction was applied on  $\alpha$  to reduce overall experimentwise error rate, which occurs through the repeated use of a variable (Zar 1998).

Intraspecific and interspecific qualitative similarity of the scent samples were calculated using Sørensen's index of similarity (Magurran 1988), considering all compounds detected. For the calculation of interspecific differences, mean scent sample values of the respective plant species were used. Additionally, similarity of the floral scent bouquets was visualized by non-metric Multidimensional Scaling (MDS) using the PRIMER v5 Software (Clarke & Gorley 2001). The MDS plot was based on a similarity matrix calculated with the Bray-Curtis similarity index (Clarke & Gorley 2001). In ideal MDS plots, rank order of distances between samples correspond exactly to the ranked similarities in the similarity matrix. Deviations from an exact match are expressed in terms of 'stress', with values  $< 0.15$  indicating a good fit concerning the overall structure of the plot.

## RESULTS

### *Floral morphology and flowering phenology*

The studied species have bisexual protogynous flowers, of which several hundreds are closely arranged in spirals on a cylindrical spadix. Female and male phases of all flowers within an inflorescence are temporally separated, with the anthers not emerging until the stigmas of all flowers of the spadix are wilted. With the exception of *A. thrinax*, which has a scattered, irregular flowering pattern throughout the spadix, maturation of the flowers normally starts at the base of the inflorescence and proceeds regularly towards the distal apex.

Although the anthers of most *Anthurium* species cover the wilted stigma in the male phase, self-pollination rarely occurs (Croat 1980, Mayo *et al.* 1997) and was not observed in *A. rubrinervium* and *A. thrinax*. In *Spathiphyllum*, anthers do not reach the elongated pistil; it is reported that *S. friedrichsthali* is self-compatible but does not self-pollinate (Montalvo & Ackerman 1986). This is probably true for most species of the genus, based on the observation that individuals grown in greenhouses, without natural pollinators, do not develop fruits.

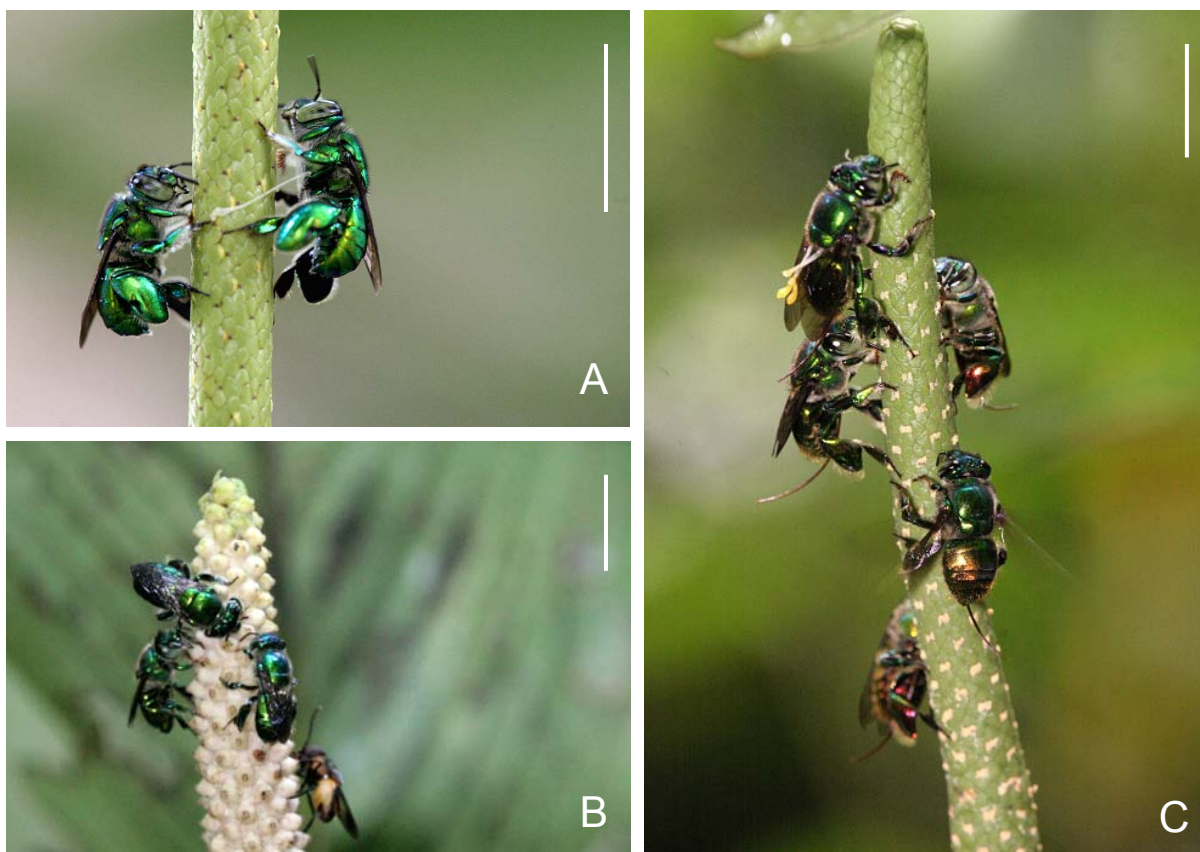


FIG. 1. Main flower visitors to the investigated Araceae: A: *Euglossa prasina*/*Euglossa variabilis* at *Anthurium thrinax*; B: *Euglossa stilbonota* and *Lepidomyia* sp. at *Spathiphyllum humboldtii*; C: *Euglossa piliventris* at *Anthurium rubrinervium*. Scale bar = 1 cm.

#### Visitor observations

Male euglossine bees were the principal flower visitors to the investigated plant species (Fig. 1). Apart from these, small numbers (< 7/d) of Meliponinae (*Trigona williana*, *Tetragona perangolata*, *Plebeia* sp. 1, *Plebeia* sp. 2), Syrphidae (*Lepidomyia* sp., *Salpingogaster* sp.), and Curculionidae were also observed at the spadices. Euglossine bees landed on female-phase as well as on male-phase inflorescences. The other visitors were only observed at male-phase inflorescences and are considered here as non-pollinating florivores. The Curculionidae and the Syrphidae stayed for several h on a spadix to feed on pollen or on other floral tissue. Meliponinae also collected pollen, but their visits to each inflorescence were usually shorter (< 1 h).

Euglossine bees, the main visitors, showed typical fragrance-collecting behavior, i.e., brushing the spadix-surface with their forelegs and hovering in the air to transfer the scent to the inflated hind tibiae (Vogel 1966, Evoy & Jones 1971, Whitten et al. 1989). The bees usually started collecting fragrances at the lower part of the inflorescence and moved upward. When they reached the top of the spadix, they flew downwards again and continued their

behavior. While brushing at the spadix-surface, the bees' body came in contact with the anthers or the stigma. Pollen grains accumulated especially at the long hairs of the foretarsi, which were used to wipe off the scent. Visitor behavior was the same at both female and male inflorescences and visits to individual inflorescences took between several min and one h or more. In *Anthurium rubrinervium* the visitor activity at individual inflorescences was highly variable (mean number of bees per inflorescence/d:  $15.2 \pm 6.8$ ;  $n = 10$ ).

The euglossine visitor spectrum to the study species varied; three, six and seven euglossine species visited *Anthurium rubrinervium*, *A. thrinax* and *Spathiphyllum humboldtii*, respectively (Fig. 2). For each plant species, visits were dominated by two euglossine species, which accounted for more than 80 percent of all euglossine visitors. The other sampled euglossine bees made rare visits during the studying period or came in small numbers to the inflorescence (i.e., one or two visits of a bee species a day).

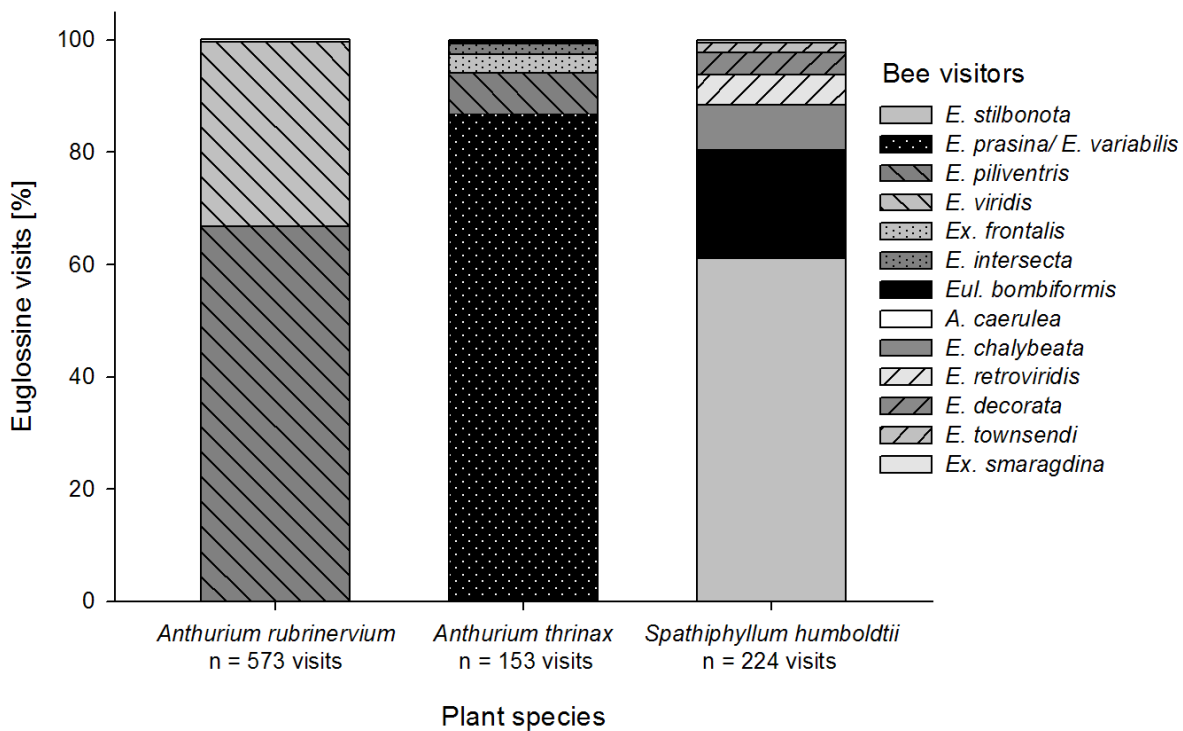


FIG. 2. Visitor spectra of the investigated Araceae species. The total number of observed bees is given as n in each figure. Values are percent of all flower visits. A. = *Aglae*, E. = *Euglossa*, Eul. = *Eulaema*, Ex. = *Exaerete*.

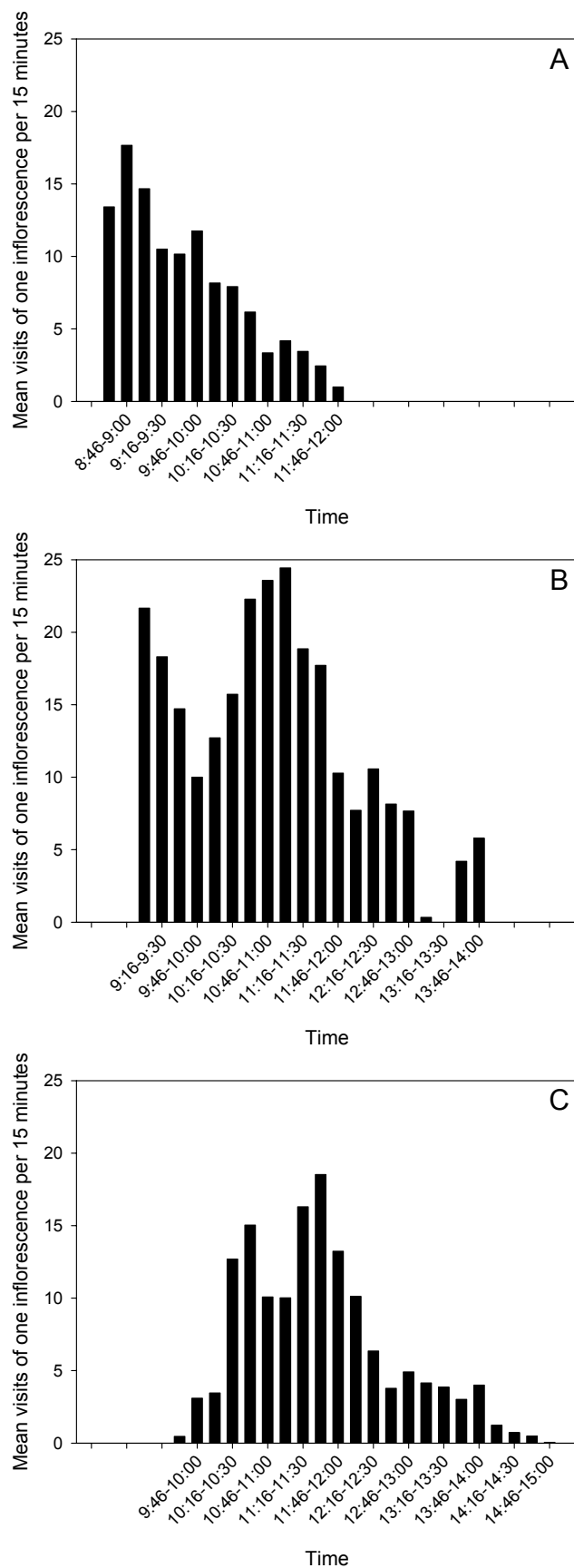


FIG. 3. Visitor activity of euglossine bees (all species) at the inflorescences of *Spathiphyllum humboldtii* (A; n = 12 observation days), *Anthurium thrinax* (B; n = 7 observation days), and *Anthurium rubrinervium* (C; n = 8 observation days). The results are presented as mean visits of one inflorescence in groups of 15 minutes.

There were significant differences in the euglossine visitor spectra of the three plant species (*A. rubrinervium* vs. *A. thrinax*:  $\chi^2 = 662$ ; df = 6;  $P < 0.001$ ; *A. rubrinervium* vs. *S. humboldtii*:  $\chi^2 = 797$ ; df = 9;  $P < 0.001$ ; *A. thrinax* vs. *S. humboldtii*:  $\chi^2 = 373$ ; df = 10;  $P < 0.001$ ; Bonferroni corrected). Principal visitors were specific to each Araceae species. For *A. rubrinervium* the main visitors were *Euglossa viridis* and *E. piliventris* while flower visits at *A. thrinax* were dominated by *E. prasina* and *E. variabilis* and those at *S. humboldtii* by *E. stilbonota* and *Eulaema bombiformis* (Fig. 2). An overlap in plant use only occurred in the case of *E. piliventris* and *Eulaema bombiformis*. These two species were sporadically also observed at *Anthurium thrinax* and made up 7.2 percent of its euglossine visitor spectrum in the case of *E. piliventris* and 0.7 percent in the case of *Eulaema bombiformis*.

Significant differences were also found in the visitation time of the euglossine bees to the spadices of the three plant species (*A. rubrinervium* vs. *S. humboldtii*:  $\chi^2 = 82$ ; df = 11;  $P < 0.001$ ; *A. thrinax* vs. *S. humboldtii*:  $\chi^2 = 27$ ; df = 11;  $P < 0.01$  *A. rubrinervium* vs. *A. thrinax*:  $\chi^2 = 61$ ; df = 19;  $P < 0.001$ ; Fig. 3). Flower visits at *S. humboldtii* began shortly after sunrise, peaked between 0830 h and 1000 h and ceased at noon. In *A. thrinax* euglossine bees were also observed in the early morning but main visitation hours were between 1030 h and 1130 h and visitation ceased in the early afternoon. In *A. rubrinervium* flower visitation did not start before 0930 h, showed peaks at 1030 h and 1130 h and ceased between 1400 and 1500 h (Fig. 3).

### Scent composition

All species produced a strong pleasant floral scent during anthesis, in both female and male phase. *Anthurium thrinax* was fragrant between sunrise and sunset, while *S. humboldtii* emitted fragrances only between sunrise and late noon and *A. rubrinervium* only between 0930 h and dusk. Floral scent presentation in form of crystals or liquids as it is reported from a number of flowers in ‘perfume orchids’ (orchids characterized by the euglossine syndrome; Williams et al. 1985) was not observed in the studied plants, but spadix surfaces of the *Anthurium* species were very waxy and brilliant.

A total of 66 compounds was detected in the 15 scent samples (Table 1). The basic pattern of scent composition was similar for the three species. Each bouquet consisted of 6-10 major compounds, summing up to 76-94 percent of all volatiles in the bouquet. Besides this, a large number of minor compounds were present, that often were shared between species. The main compounds (bold letters in Tab. 1) were specific for each plant species and, with the exception of 1,8-cineol, limonene and (E)-ocimene, could only be found in small amounts in the other sympatric species.



Striking differences existed in the qualitative composition, especially when regarding the distribution of compounds in the chemical classes. While all plants possessed many terpenoids, which were often shared between species, the occurrence of compounds from other chemical classes was mostly limited to one species. In *A. rubrinervium*, many benzenoids and lipid-derived compounds occurred, which quantitatively dominated the blend. *A. thrinax* only produced small amounts of benzenoids and lipid-derived compounds. Instead, nitrogen-containing compounds appeared in this species. *S. humboldtii*, however, exclusively emitted terpenoids.

All three species showed considerable qualitative and quantitative intraspecific variation in the composition of the scent samples. Intraspecific qualitative similarity varied between 74-98 percent in *A. rubrinervium*, 62-91 percent in *A. thrinax* and 88-98 percent in *S. humboldtii*. In *A. rubrinervium* large amounts of (E)-ocimene, (E, Z)-2,6-dimethyl-2,5,7-octatrien-1-ol and methyl (E)-cinnamate were detected in single fragrance samples and did not appear in the others. A similar tendency was found in *A. thrinax* for (Z)-8-heptadecene. In contrast, scent samples of *S. humboldtii* were quite uniform. Fragrance samples of an *A. rubrinervium* inflorescences in male and female phase differed only quantitatively; qualitative composition was the same. In scent samples of the *A. thrinax* greenhouse clone, several substances in small amounts occurred (especially nitrogen-containing compounds) which were not detected in the sample of the *in situ* plant. Further, the sample of the study site plant did not contain the major compound limonene.

The Sørensen indices showed low values in the interspecific similarity of the scent bouquets (*A. rubrinervium* vs. *A. thrinax*: 52%, *A. rubrinervium* vs. *S. humboldtii*: 53%, *A. thrinax* vs. *S. humboldtii*: 55%), and MDS produced clearly separated species-specific clusters for the fragrance samples with a stress of 0.04 (Fig. 4).

TABLE 1. Floral fragrance compounds of the investigated Araceae species. Substances are divided into chemical classes and within each class in the order of their GC retention time. Sample numbers refer to individual scent samples. With the exception of AR3 and AR4, all samples were taken from different inflorescences. AR3 and AR4 were taken from one inflorescence in the female (AR3) and male phase (AR4). AT6 and AT7 are samples taken in the greenhouse from a direct clone of a plant at study site of which sample AT5 was collected. Values are percent of total fragrance and major compound values are printed in bold face.

Plant species	<i>Anthurium rubrinervium</i>					<i>Anthurium thrinax</i>					<i>Spathiphyllum humboldtii</i>				
Compound/Sample No.	AR2	AR3	AR4	AR5	AR6	AT2	AT4	AT5	AT6	AT7	SH2	SH3	SH4	SH5	SH6
<b>Terpenoids</b>															
$\alpha$ -Pinene	2.09	0.17	0.04	1.20	0.30	0.20	1.14	1.76	0.90	0.20	0.04	0.03	0.01	0.75	0.81
$\beta$ -Pinene	0.74	0.17	0.05	0.60	0.20	<b>2.00</b>	<b>1.43</b>	<b>3.52</b>	<b>2.03</b>	<b>2.70</b>	0.01	0.04	0.01	0.43	0.51
Sabinene	0.61	0.14	0.04	1.30	1.70	<b>1.50</b>	<b>0.86</b>	<b>2.93</b>	<b>1.81</b>	<b>1.80</b>	0.01	0.05	0.02	0.32	0.51
Myrcene	0.61	0.69	0.41	0.60	0.20	0.60	0.57	1.76	1.13	0.90	<b>27.18</b>	<b>20.70</b>	<b>28.00</b>	<b>14.98</b>	<b>23.99</b>
Dehydroeucalyptol	-	-	-	-	-	0.01	-	-	0.03	0.01	-	0.02	0.02	-	-
Limonene	<b>3.81</b>	<b>1.72</b>	<b>0.55</b>	<b>1.30</b>	<b>1.10</b>	<b>2.00</b>	<b>4.28</b>	-	<b>7.23</b>	<b>6.30</b>	0.05	0.20	0.10	0.07	0.10
$\beta$ -Phellandrene	0.37	0.14	0.04	-	-	-	-	-	-	-	-	-	-	-	-
1,8-Cineole	<b>33.32</b>	<b>19.45</b>	<b>4.66</b>	<b>18.50</b>	<b>15.00</b>	<b>4.00</b>	<b>11.28</b>	<b>24.85</b>	<b>25.53</b>	<b>26.00</b>	<b>1.82</b>	<b>2.70</b>	<b>1.40</b>	<b>2.03</b>	<b>2.73</b>
(Z)-Ocimene	-	-	-	-	1.10	0.10	0.57	0.20	0.34	0.10	0.40	0.60	0.30	0.21	0.51
$\gamma$ -Terpinene	-	-	-	-	-	-	-	-	0.02	-	-	0.01	0.02	-	-
(E)-Ocimene	-	-	-	-	<b>9.50</b>	<b>2.50</b>	<b>3.28</b>	<b>3.91</b>	<b>3.50</b>	<b>1.50</b>	0.08	0.10	0.05	0.05	0.10
2-Methyl-6-methylen-1,3,7-octatriene	-	-	-	-	-	-	-	-	-	-	<b>1.41</b>	<b>1.70</b>	<b>1.50</b>	<b>0.53</b>	<b>1.52</b>
Terpinolene	1.48	0.34	0.14	0.60	0.50	0.20	0.43	1.17	1.02	0.90	-	0.02	0.50	-	-
(E)-4,8-Dimethyl-1,3,7-nonatriene	-	-	-	-	-	-	0.29	-	-	-	0.01	0.01	0.01	0.01	0.01
(E,E)-2,6-Dimethyl-1,3,5,7-octatetraene	-	-	-	-	-	-	-	-	-	-	0.40	0.70	0.40	0.11	0.61
6,7-Epoxy myrcene (2,2-Dimethyl-3-(3-methylene-4-penten-1-yl)-oxirane	-	-	-	-	-	-	-	-	-	-	<b>7.98</b>	<b>9.50</b>	<b>4.20</b>	<b>4.60</b>	<b>10.43</b>
3,10-Epoxy myrcene (2-Ethenyl-2-(4-methyl-3-pentenyl)-oxirane	-	-	-	-	-	-	-	-	-	-	0.20	0.20	0.20	0.09	0.20
6-Methyl-5-hepten-2-one	0.05	0.10	0.05	0.01	0.01	-	0.14	-	0.09	0.07	-	-	-	-	-
Ipsenone (2-Methyl-6-methylene-7-octen-4-one)	-	-	-	-	-	-	-	-	-	-	0.02	0.09	0.02	0.03	0.10

trans-Sabinene hydrate	-	-	-	0.05	0.06	-	-	-	-	0.05	0.10	0.30	0.10	0.02	0.10
1,2-Epoxy myrcene (5-Methyl-1-methylene-4-hexenyl)-oxirane)	-	-	-	-	-	-	-	-	-	-	0.01	0.02	0.02	0.01	0.02
cis-Sabinene hydrate	-	-	-	-	-	-	-	-	0.08	-	0.01	0.07	0.05	0.05	-
2-Methyl-6-methylene-7-octen-4-ol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04
2-Methyl-6-methylene-1,7-octadien-3-one	-	-	-	-	-	-	-	-	-	-	0.02	0.04	0.03	0.04	0.02
(E)-Ocimene epoxide (2,2-Dimethyl-3-((E)-3-methyl-2,4-pentadien-1-yl)-oxirane	-	-	-	-	0.10	-	-	-	-	-	-	-	-	-	-
Linalool	<b>4.55</b>	<b>8.44</b>	<b>5.34</b>	<b>0.05</b>	<b>0.04</b>	-	0.04	-	0.23	0.03	0.10	0.10	0.05	0.05	0.02
Ipsdienone (2-Methyl-6-methylene-2,7-octadien-4-one)	-	-	-	-	-	-	-	-	-	-	<b>1.01</b>	<b>2.00</b>	<b>0.90</b>	<b>1.18</b>	<b>1.62</b>
Ipsdienyl acetate	-	-	-	-	-	-	-	-	-	-	0.20	0.20	0.10	0.21	0.61
Terpinen-4-ol	0.12	-	-	0.05	0.05	-	-	-	-	-	-	0.02	0.10	-	-
δ-Terpineol	0.25	0.01	-	0.01	0.04	-	0.04	-	-	0.20	-	0.03	0.02	-	-
2-Methyl-6-methylen-3,7-octadien-2-ol	-	-	-	-	-	-	-	-	-	-	-	0.30	0.10	-	-
Ipsdienol (2-Methyl-6-methylene-2,7-octadien-4-ol)	-	-	-	-	-	-	-	-	-	-	<b>57.28</b>	<b>57.70</b>	<b>59.95</b>	<b>71.68</b>	<b>54.06</b>
Tetrahydrogeranylacetone	-	-	-	-	-	-	-	-	0.79	0.30	-	-	-	-	-
α-Terpineol	3.44	0.14	0.02	0.20	0.70	-	<b>1.00</b>	<b>1.76</b>	<b>2.82</b>	<b>2.80</b>	0.30	0.70	0.30	0.40	0.20
(E,Z)-2,6-Dimethyl-2,5,7-octatrien-1-ol	-	-	-	-	<b>5.80</b>	-	-	-	-	-	-	-	-	-	-
trans-Pinocarveol	-	-	-	-	-	-	-	-	-	-	0.10	0.20	0.10	0.10	0.10
6,10,14-Trimethylpentadecan-2-one	-	-	-	-	-	<b>77.00</b>	<b>32.84</b>	<b>49.89</b>	<b>46.32</b>	<b>45.00</b>	-	-	-	-	-
6,10,14-Trimethylpentadecan-2-ol	-	-	-	-	-	-	0.03	-	0.02	0.03	-	-	-	-	-
<b>Benzenoids</b>															
Styrene	0.06	0.03	0.07	0.01	0.02	-	-	-	-	-	-	-	-	-	-
Benzaldehyde	0.07	0.10	0.41	0.02	0.20	-	-	-	-	-	-	-	-	-	-
Methyl salicylate	<b>4.67</b>	<b>44.93</b>	<b>42.87</b>	<b>23.90</b>	<b>13.30</b>	-	0.07	-	0.23	0.30	-	-	-	-	-
Methyl benzoate	0.49	3.44	3.15	0.10	0.50	-	-	-	-	-	-	-	-	-	-
Benzyl alcolhol	0.06	0.17	0.96	0.05	0.10	-	-	-	-	-	-	-	-	-	-
p-Cymen-8-ol	-	-	-	-	-	-	-	-	0.03	0.04	-	-	-	-	-

Phenylethyl alcohol	-	-	-	-	-	-	-	-	0.02	-	-	-	-	-	-
Amyl benzoate	-	-	-	0.02	0.03	-	-	-	-	-	-	-	-	-	-
Methyl (Z)-cinnamate	0.49	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methyl 2-methoxybenzoate	0.04	0.52	0.55	0.02	0.08	-	-	-	-	-	-	-	-	-	-
Methyl (E)-cinnamate	<b>5.29</b>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
p-Cresol	-	-	-	0.01	0.01	-	-	-	-	-	-	-	-	-	-
Eugenol	-	-	-	0.03	0.03	-	-	-	-	-	-	-	-	-	-
(Z)-Isoeugenol	-	-	-	0.02	0.02	-	-	-	-	-	-	-	-	-	-
(E)-Isoeugenol	0.12	-	-	1.20	0.80	-	-	-	-	-	-	-	-	-	-
Benzyl benzoate	<b>1.90</b>	<b>8.95</b>	<b>19.86</b>	<b>8.40</b>	<b>4.00</b>	-	-	-	-	-	-	-	-	-	-
Benzyl salicylate	0.25	0.69	0.96	0.10	0.20	-	-	-	-	-	-	-	-	-	-
<b>Lipid-derived compounds</b>															
Nonanal	0.01	0.17	0.07	0.01	0.01	-	0.11	-	-	-	-	-	-	-	-
Acetic acid	0.37	0.52	0.55	-	-	-	0.71	-	-	-	-	-	-	-	-
Decanal	-	-	-	-	-	-	0.14	-	-	-	-	-	-	-	-
(Z)-8-Heptadecene	<b>31.48</b>	<b>1.55</b>	<b>14.79</b>	<b>35.00</b>	<b>43.00</b>	-	34.84	1.96	-	-	-	-	-	-	-
<b>Nitrogen-containing compounds</b>															
Isovaleronitril	-	-	-	-	-	<b>1.20</b>	<b>0.14</b>	<b>0.20</b>	<b>2.71</b>	<b>2.20</b>	-	-	-	-	-
1-Nitro-3-methylbutane	-	-	-	-	-	0.02	-	-	0.06	0.04	-	-	-	-	-
Isobutyraldoxime (E/Z)	-	-	-	-	-	-	-	-	0.11	0.10	-	-	-	-	-
2-Methylbutyraldoxime (E/Z)	-	-	-	-	-	-	0.03	-	0.45	0.40	-	-	-	-	-
Isovaleraldoxime (E/Z)	-	-	-	-	-	-	0.06	-	0.79	0.60	-	-	-	-	-
Phenylacetoneitrile	-	-	-	-	-	-	-	-	0.02	0.01	-	-	-	-	-
Indole	-	-	-	-	-	0.10	0.43	0.20	0.06	0.20	-	-	-	-	-
<b>Total</b>	<b>96.74</b>	<b>92.58</b>	<b>95.58</b>	<b>93.36</b>	<b>98.70</b>	<b>90.11</b>	<b>94.32</b>	<b>93.91</b>	<b>98.31</b>	<b>92.58</b>	<b>98.74</b>	<b>98.35</b>	<b>98.58</b>	<b>97.95</b>	<b>98.92</b>



FIG. 4. Two-dimensional non-metric Multidimensional Scaling plot of the 15 floral scent samples of the investigated Araceae species based on the Bray-Curtis similarity index. Floral scent composition of the three species differed distinctly in producing separated clusters.

## DISCUSSION

### *Scent composition*

Our results show that three euglossine-pollinated sympatric Araceae species in the Nouragues reserve of French Guiana are pollinated by different euglossine species. To understand the reasons for the differences in the visitor spectra, we certainly have to take a closer look at the floral fragrance composition of the plants. Our similarity analysis of the scent samples showed that each plant species emitted a specific floral bouquet that clearly differs from the bouquets of the other sympatric species. Most of the main odor compounds are common fragrances in perfume flowers and many of them have been proved to be attractive to male euglossine bees in baiting experiments (Williams & Whitten 1983, Gerlach & Schill 1991, Ramirez *et al.* 2002). 1,8-Cineol, which was a main compound of the two *Anthurium* species, for example, is one of the most wide-spread fragrance compounds in perfume flowers and the best general attractant for male euglossine bees (Dodson *et al.* 1969, Roubik & Hanson 2004). Large amounts of myrcene which were detected in the floral fragrance of *Spathiphyllum humboldtii* can also be found in the floral fragrance of the euglossine-pollinated *Geonoma macrostachys*, *Coryanthes trifoliata* and *Anthurium ochranthum* (Whitten *et al.* 1988, Gerlach & Schill 1991, Knudsen *et al.* 1999). The floral bouquet of *A. rubrinervium* with its high quantities of methyl salicylate and benzyl benzoate is similar to that of *Stanhopea panamensis* (Whitten & Williams 1992).

In the two *Anthurium* species, long chained molecules, i.e., 6,10,14-trimethylpentadecan-2-one and (Z)-8-heptadecene, dominated the scent bouquet. Both substances are reported to occur in the floral odor of perfume-flowers (El-Sayed 2007, Hentrich *et al.* 2007, R. Kaiser, unpublished data) and 6,10,14-trimethylpentadecan-2-one was already found in large amounts in the hindtibiae of Panamanian euglossine bees (Eltz *et al.* 1999). Nevertheless, the attractiveness of these compounds for euglossine bees has never been tested and therefore we do not yet know if they play a role in the attraction of the flower visitors.

Eltz *et al.* (1999, 2006) and Zimmermann *et al.* (2006) showed that *Euglossa* and *Eulaema* store species-specific fragrance blends in the tibial pouches of their hindlegs. According to Eltz *et al.* (1999, 2006), euglossine bees are attracted to perfume flowers because these produce fragrance compounds, the bees seek for their specific blend collection. The emission of species-specific fragrance blends of the different Araceae species therefore leads to the attraction of distinct euglossine species. The perception of scents involves complex processes in the higher nervous centers of the bees and the occurrence of one compound has an impact on the perception of another (Eltz & Lunau 2005). Therefore we cannot conclude that the co-occurrence of a compound in the floral scent of different species attracts the same bee species although it might belong to its favored substances. Instead, the entire scent bouquet has to be regarded as one unit since the bees are confronted with a mixture of substances. The complexity of the floral scent further seems to influence the complexity of the visitor spectrum. *Spathiphyllum humboldtii*, which attracts a large number of euglossine species, emits quite a simple floral bouquet with only 5 to 6 principal compounds, while *Anthurium rubrinervium* with its complex floral bouquet attracts only three visitor species. *A. thrinax* seems to be intermediate between those two extremes. When we take a closer look at the distribution of the principal fragrance components among the different chemical classes, a similar tendency can be observed. The components of *A. rubrinervium* belong to three chemical classes, those of *S. humboldtii* are restricted to the group of terpenoids.

Differences in the composition of the scent samples of each species were predominantly quantitative and can be explained by the different climatic conditions during scent sampling. Striking contrasts occurred in the qualitative fragrance composition within *Anthurium rubrinervium*. Intraspecific variation in floral odor has also been observed in the euglossine-pollinated understory palm *Geonoma macrostachys* (Knudsen 2002). Additionally, qualitative differences in fragrance composition of euglossine-pollinated plants are frequently noticed when scent samples of more than one plant individual per species are analyzed

(Whitten & Williams 1992, Schwerdtfeger *et al.* 2002, H. Hentrich, unpublished data). In *A. rubrinervium*, intraspecific variation in floral scent composition did not alter the visitor spectrum of the sampled inflorescences. However, attractiveness of some inflorescences in the populations was higher than that of others. Maybe a certain degree of qualitative variability in scent composition is a means of year-round flowering euglossine-pollinated plants to remain attractive for the bees. Eltz *et al.* (1999) demonstrated that a saturation effect of the collected fragrances could lead to a decrease in the attractiveness of these fragrances. Plant populations flowering throughout the year could avoid this saturation effect by producing slightly altered odor bouquets in different individuals. Obviously, intraspecific floral scent variability should remain within a certain range. Otherwise, different visitor species might be attracted and this could lead to an interruption of intraspecific pollen flow. In geographically isolated populations such development could result in reproductive isolation and ultimately in the rise of new plant species.

#### *Visitor spectra*

The visitor spectrum of each of the co-occurring Araceae was strongly dominated by two euglossine species, which did not visit the spadices of the other two plant species or only appeared there sporadically. Random differences in the visitor spectra, caused by temporal or spatial variation in bee abundance are unlikely since euglossine bees are known to be strong flyers that may cover distances up to 23 km a day (Janzen 1971, Roubik & Hanson 2004). As intergeneric hybrids in the Araceae have not been discovered in the field yet, the occasional sharing of pollinators does not seem to significantly affect the reproductive biology of the plants. Although artificial hybridization in the genus *Anthurium* is successful in intrasectional and in few cases also in intersectional crosses, natural interspecific hybrids do only occur in the section *Porphyrochitonium* (T. Croat, pers. comm.). The investigated species belong to different sections (*A. rubrinervium*: sect. *Cardiolonchium*; *A. thrinax*: sect. *Dactylophyllum*; Croat & Sheffer 1983). Intermediate *Anthurium* or *Spathiphyllum* individuals, which are an indicator for natural hybridization, have never been found in the study area so far.

Considering the qualitative composition of the visitor spectrum, *Spathiphyllum humboldtii* attracted more bee species than the two *Anthurium* species. Armbruster *et al.* (1992) observed a similar phenomenon in two sympatric euglossine-pollinated *Dalechampia* species. They explained the differences in the visitor spectra by their flowering phenology and distinguish ‘generalists’ from ‘specialists’. The ‘specialist’, *Dalechampia brownsbergensis*, flowers all year round and is visited by few bee species while the ‘generalist’, *Dalechampia fragrans*, flowers seasonally and has a large spectrum of visiting bee species. Armbruster *et*

*al.* (1992) suggested that *D. fragrans* could not afford to specialize on only one or a few pollinator species because it highly depended on the pollination service of those bee species occurring during its short flowering period. This may also be true for the Araceae studied. While the two *Anthurium* species flower throughout the whole year, *S. humboldtii* only produces flowers in the late dry and early rainy season. This is the time of year where abundance and number of euglossine bee species are greatest in Neotropical rainforests (Ackerman 1983, Pearson & Dressler 1985). Since we did not observe the *Anthurium* species over the entire year, it is feasible that in other months additional bee species may visit the inflorescences.

The distribution of flower visits in the daily course suggests that phenological adaptations might have evolved, which additionally support the reproductive separation of the Araceae species. While *Spathiphyllum humboldtii* was predominantly visited in the early morning hours, visitors of *Anthurium thrinax* arrived during the entire morning and in *A. rubrinervium* not before 0930 h. This distinctive visiting behavior was probably caused by differences in the timing of floral scent production. Temporal differences in scent emission have been observed in several *Anthurium* species and are supposed to be related to the foraging times of their pollinators (Croat 1980, Kuanprasert *et al.* 1998). Armbruster & McCormick (1990) discussed the influence of male euglossine bee diel activity patterns on the flowering behavior of perfume-flowered plants and suggest that temporal patterns in anthesis and fragrance secretion match the period of highest male-euglossine activity. Perfume-flowered plants should therefore differ in their time of floral activity if they were pollinated by different euglossine species with distinctive foraging times. Following Armbruster & McCormick (1990), the specialization to specific pollinator species could have led to differences in the flowering phenology of the investigated Araceae species.

In conclusion, we suggest that the Araceae genera *Anthurium* and *Spathiphyllum* evolved isolation mechanisms analogous to those of euglossine-pollinated Orchidaceae. The results of our study support the initial hypothesis that the emission of species-specific floral fragrance bouquets assures each sympatric plant species an individual euglossine pollinator spectrum within an environment where dozens of euglossine species occur. This reproductive barrier enabled different *Anthurium* and *Spathiphyllum* species to grow in the same habitat without interfering in the pollination biology of the other species and guaranteeing a directed pollen flow. Subsequently to the publication of many models about the reproductive isolation in perfume-flowered plants by other authors (Dodson *et al.* 1969, Williams & Dodson 1971, Hills *et al.* 1972, Williams & Dressler 1976, Whitten & Williams 1992, Schwerdtfeger *et al.*



2002), our study has demonstrated that the sympatric occurrence of different euglossine-pollinated species through the reproductive isolation by floral scent really works in nature. Moreover, there are indications that also phenological barriers have evolved between the investigated Araceae, and that considerable intraspecific variations in floral fragrance composition exist, which might play a role in the pollination biology and speciation processes of the plants. Future studies should focus on the perception of complex fragrance mixtures by euglossine-bees and the impact of intraspecific floral scent variability on the visitor spectrum of perfume-flowered plants. Further, there are many compounds produced by perfume-flowers which have not been tested on their attractiveness or repellence to male euglossine bees yet. Maybe the variability of certain substances does not have any impact on the visitor attraction because the bees cannot perceive them? New baiting experiments coupled with electroantennography using these unstudied scent compounds would broadly contribute to our understanding of speciation in these plants.

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Male *Euglossa* cf. *imperialis* during territorial display ('scent spraying') at the trunc of a tree.



Male *Euglossa* sp. during territorial display ('scent spraying') at the branch of a tree.

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## FLORAL SCENT COLLECTION AT THE PERFUME FLOWERS OF *ANTHURIUM RUBRINERVIUM* (ARACEAE) BY THE KLEPTOPARASITIC ORCHID BEE *AGLAE CAERULEA* (EUGLOSSINI)

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### INTRODUCTION

Orchid bees (Hymenoptera: Apidae: Euglossini) are known to be important pollinators in the tropics of the New World (Dodson 1966, Vogel 1966, Dressler 1982, Roubik & Hanson 2004). They became particularly famous for the scent-collecting behavior of the males on the perfume flowers of various orchid species and of many other plant families (Vogel 1966, Dodson *et al.* 1969). The tribe Euglossini is divided into five genera (*Aglae*, *Eufriesea*, *Euglossa*, *Eulaema*, *Exaerete*; Kimsey 1987), with a similar distribution for all but one genus (*Aglae*), ranging from northern Mexico to Argentina (Michener 2000). Whilst the ecology of the largest genera (*Eufriesea*, *Euglossa*, *Eulaema*) has been well documented during the past 40 years, information on the kleptoparasitic euglossines (*Aglae*, *Exaerete*), especially on the genus *Aglae*, remains scarce. Bees of the monotypic genus *Aglae* are restricted to moist forests from Colombia to Bolivia with an unverified finding in Panama (Michener 2000) and seem to be rare even in their natural habitat (Cameron 2004). For this reason very few observations on their ecology exist. In contrast to the other parasitic genus in the tribe, *Exaerete*, *A. caerulea* was never before observed collecting floral scent on perfume flowers, although there is a series of clues which leads to the presumption that they do so. Morphologically, the males are provided with the same features for scent collection (foretarsal brushes, inflated hind tibia) as their relatives in the other Euglossini genera (Sakagami 1965, Vogel 1966, Kimsey 1987, Michener 2000). Moreover, they are attracted to artificial fragrance compounds (eugenol, methyl cinnamate, p-cresol, skatole; Williams & Dodson

1972, Morato 2001, Ramírez *et al.* 2002), which are components of natural floral scent (Williams & Whitten 1983, Gerlach & Schill 1991, Knudsen *et al.* 1993).

This is the first documentation of the scent-collecting behavior of *Aglae caerulea* at flower organs, viz. the inflorescences of *Anthurium rubrinervium* (Araceae) in the reserve Nouragues, French Guiana. Also, the floral odor composition of *A. rubrinervium* is analyzed and discussed.

## MATERIAL & METHODS

### *Study site*

The study was conducted in the early rainy season (January-April 2006) around the Inselbergstation of the reserve 'Les Nouragues' in French Guiana (04°05'N, 52°41'W, 120 m A.S.L.). The area is located in primary lowland tropical rainforest with an annual rainfall of 2990 mm and a mean annual temperature of 26.3°C (Grimaldi & Riéra 2001). A large population of *A. rubrinervium* (about 125 individuals) was found growing on a large rock near trail F-XIIX on the 'Petit Plateau'. Twenty individuals were flowering simultaneously in the study period, creating a sweet pleasant smell of floral perfume in the surroundings. Vouchers of *A. rubrinervium* investigated are deposited in the herbaria CAY and ULM.

### *Visitor observations*

Flower visitors to 15 individuals of *A. rubrinervium*, belonging to one population, were observed and recorded during 09:30-15:00 h on eight days between 9 and 29 March 2006. Minimum observation distance to the flowers was 2 m. The climate on 9 March (visit of *Aglae caerulea*) and the days before was very sunny and hot with a little rain in the afternoon (9 March: temperature 21-32°C, precipitation 2.2 mm). Voucher specimens of the bee species were collected and determined using the reference collection of G. Gerlach in Munich. They are deposited in the collection of the first author and the collection of the herbarium ULM (Hentrich FGIC25, FGIC26, FGIC28, FGIC31, FGIC32, FGIC47).

### *Scent sampling*

Five scent samples from four individuals of different populations were collected in the field by headspace method. The inflorescences were covered by an inert oven-bag during collection. The scented air was drawn through a sampling cartridge by a battery operated membrane pump for four hours with a flow rate of 150 ml/min. The sampling cartridge was prepared with 25 mg of Tenax TA (mesh 80-100, Macherey Nagel, Düren, Germany) and 40 mg of Carbopack X (mesh 20-40, Supelco, Bellefonte, Pennsylvania, USA). The adsorbents



were conditioned by washing with methanol (purity 99.8%, Merck, Darmstadt, Germany) and acetone (purity 99.9%, Merck, Darmstadt, Germany) and heated out for 30 min at 220°C before sampling. Subsequently, the adsorbed scent was recovered by elution with 200  $\mu$ l acetone into glass vials. The samples were investigated by GC/MS using a Thermo Finnigen Voyager Mass Spectrometer combined with a Trace GC 2000 Series and the Xcalibur software. The analyses were made on a DB-WAX column (J&W Scientific) 30 m x 0.32 mm i.d., film thickness 0.25  $\mu$ m; splitless injection, temperature program was 50°C-2'iso-2.5°C/min-230°C-40'iso. Compounds were identified by comparison of their mass spectra and retention times with those of authentic reference samples available from the Givaudan reference compound collection.

Differences in the qualitative similarity of the scent samples were calculated using Sørensen's index of similarity (Magurran 1988), considering all compounds detected.

## RESULTS

### *Visitor observations*

Principal visitors to *Anthurium rubrinervium* were male bees of *Euglossa piliventris* and *E. cyanura*. They showed typical odor-collecting behavior. Visits began between 09:30 h and 10:15 h and ceased between 14:00 h and 15:00 h. In the whole study period, *Aglae caerulea* was observed only once visiting the plants, viz. on 9 March 2006. At about 09:55 h, the bee approached the population in wide turns with a strong rattling buzz. Finally, it landed on the spadix of an *A. rubrinervium* individual and started to brush the surface of the spadix with its foretarsal brushes (Fig.1). After some seconds, it flew up, hovered in the air and landed on the same spadix to continue brushing. This behavior was repeated twice. Then the bee flew away quickly in a straight line. The duration of the visit was quite short. The bee barely stayed 1 min at the spadix. At the same time no other bees were observed in the surroundings of the *Anthurium* plants.

### *Scent Composition*

*Anthurium rubrinervium* has a strong pleasant floral perfume. Emission of scent was tested olfactorily ('sniffing' by the first author) and could be perceived between 09:30 h and dusk. The floral fragrance contained a large row of monoterpenes followed by benzenoids and three lipid-derived compounds (Tab. 1). The main components were (Z)-8-heptadecene, methyl salicylate, 1,8-cineole, benzyl benzoate, (E)-ocimene and linalool.

Qualitative similarity of the scent samples varied between 74% and 98%. Sample 6 differs from other samples in having (E)-ocimene and 2,6-dimethyl-2,5,7-octatrien-1-ol, while methyl (Z)-cinnamate and methyl (E)-cinnamate was only detected in sample 2. Quantitative differences in the composition of the scent samples also occurred ((Z)-8-heptadecene: 1.55-43%, methyl salicylate: 4.67-44.93%, 1,8-cineole: 4.66-33.32%, benzyl benzoate: 1.90-19.86%).



FIG. 1. Fragrance collection of an *Aglae caerulea* male on the spadix of *Anthurium rubrinervium*.

TABLE 1. Floral fragrance compounds of four different *Anthurium rubrinervium* individuals (in %). Samples AR3 and AR4 are taken from the spadix of the same individual in the female (AR3) and the male phase (AR4). Substances are divided into chemical classes and within each class in the order of their GC retention time. Values are percent of total fragrance.

Sample No.	AR2	AR3	AR4	AR5	AR6
<b>Terpenoids</b>					
$\alpha$ -Pinene	2.09	0.17	0.04	1.20	0.30
$\beta$ -Pinene	0.74	0.17	0.05	0.60	0.20
Sabinene	0.61	0.14	0.04	1.30	1.70
Myrcene	0.61	0.69	0.41	0.60	0.20
Limonene	3.81	1.72	0.55	1.30	1.10
$\beta$ -Phellandrene	0.37	0.14	0.04	-	-
<b>1,8-Cineole</b>	<b>33.32</b>	<b>19.45</b>	<b>4.66</b>	<b>18.50</b>	<b>15.00</b>
(Z)-Ocimene	-	-	-	-	1.10
<b>(E)-Ocimene</b>	-	-	-	-	<b>9.50</b>
Terpinolene	1.48	0.34	0.14	0.60	0.50
6-Methyl-5-hepten-2-one	0.05	0.10	0.05	0.01	0.01
trans-Sabinene hydrate	-	-	-	0.05	0.06
(E)-Ocimene epoxide	-	-	-	-	0.10
<b>Linalool</b>	<b>4.55</b>	<b>8.44</b>	<b>5.34</b>	<b>0.05</b>	<b>0.04</b>
Terpinen-4-ol	0.12	-	-	0.05	0.05
$\delta$ -Terpineol	0.25	0.01	-	0.01	0.04
$\alpha$ -Terpineol	3.44	0.14	0.02	0.20	0.70
2,6-Dimethyl-2,5,7-octatrien-1-ol (exact isomer not yet known)	-	-	-	-	5.80
<b>Benzenoids</b>					
Styrene	0.06	0.03	0.07	0.01	0.02
Benzaldehyde	0.07	0.10	0.41	0.02	0.20
<b>Methyl salicylate</b>	<b>4.67</b>	<b>44.93</b>	<b>42.87</b>	<b>23.90</b>	<b>13.30</b>
Methyl benzoate	0.49	3.44	3.15	0.10	0.50
Benzyl alcohol	0.06	0.17	0.96	0.05	0.10
Amyl benzoate	-	-	-	0.02	0.03
Methyl (Z)-cinnamate	0.49	-	-	-	-
Methyl 2-methoxybenzoate	0.04	0.52	0.55	0.02	0.08
Methyl (E)-cinnamate	5.29	-	-	-	-
p-Cresol	-	-	-	0.01	0.01
Eugenol	-	-	-	0.03	0.03
(Z)-Isoeugenol	-	-	-	0.02	0.02
(E)-Isoeugenol	0.12	-	-	1.20	0.80
<b>Benzyl benzoate</b>	<b>1.90</b>	<b>8.95</b>	<b>19.86</b>	<b>8.40</b>	<b>4.00</b>
Benzyl salicylate	0.25	0.69	0.96	0.10	0.20
<b>Lipid-derived compounds</b>					
Nonanal	0.01	0.17	0.07	0.01	0.01
Acetic acid	0.37	0.52	0.55	-	-
<b>(Z)-8-Heptadecene</b>	<b>31.48</b>	<b>1.55</b>	<b>14.79</b>	<b>35.00</b>	<b>43.00</b>
<b>Total</b>	<b>96.74</b>	<b>92.58</b>	<b>95.58</b>	<b>93.36</b>	<b>98.70</b>

## DISCUSSION

Floral scent seems to play an important role not only in the life of the non-parasitic-, but also in the parasitic genera of the Euglossini. Although the morphology of their collecting organs is very rudimentarily developed (Sakagami 1965, Dressler 1968, Kimsey 1987, Michener 2000), they have been observed gathering floral scent from perfume flowers (Dressler 1968, Williams & Dressler 1976, Ackerman 1983, Ramírez *et al.* 2002). Current investigations show that collected scents are of great importance in the territorial display of male euglossine bees and might also be involved in their mating (Bembé 2004, Eltz *et al.* 2005, Zimmermann *et al.* 2006). Parasitic euglossines have so far never been observed at territorial display nor at mating. But since they also collect natural scents, we can presume that they must do so.

In the pollination of perfume flowers, the parasitic euglossines probably play a lesser role (Dressler 1968, Ackerman 1983). Even if there are records of *Exaerete frontalis* and *Exaerete smaragdina* visiting perfume flowers, their visits are documented as very irregular and short (Dressler 1968). Besides this, even if they have a vast distribution and are proved to fly long distances they are not as abundant as their non-parasitic relatives and therefore appear in lower numbers at the perfume flowers. Another aspect is the pollination mechanism of perfume flowers. Especially in the orchid family, the pollination mechanism is highly specific and only matches with a certain size-group of pollinators (Dodson & Frymire 1961, Dressler 1968, Dodson *et al.* 1969). Therefore the parasitic euglossine bees, with their large and slender bodies, often do not fit the pollination mechanism of these flowers (Dressler 1968). In another plant family, where parasitic euglossines have been observed at perfume flowers, the aroids, pollination mechanisms are not as specific as orchids in selecting pollinators of a particular size. *Exaerete* is known to visit the spadices of the perfume-flowered genera *Spathiphyllum* (Dressler 1967, Williams & Dressler 1976, Montalvo & Ackerman 1986) and *Anthurium* (Hentrich, pers. obs.) and is able to pollinate the flowers at least as well as the smaller bees of the genus *Euglossa*, which also visit spadices of these genera (Williams & Dressler 1976, Croat 1980, Schwerdtfeger *et al.* 2002).

In our case, *Aglae caerulea* visited the spadix of *Anthurium rubrinervium*. Since *A. caerulea* was observed only once in the whole study period, and is not as abundant as the other two euglossine visitors which quantitatively dominated the flower visits, we estimate that its importance as a pollinator of *A. rubrinervium* is small. Regarding an objective for the flower visit of *A. caerulea*, the bee showed the typical scent-collecting behavior, known from the other genera in the tribe Euglossini (Vogel 1966, Evoy & Jones 1971). Further, the analysis of the floral scent of *A. rubrinervium* demonstrated that its composition resembles

that of other species of the Araceae and Orchidaceae belonging to the perfume flowers (Williams & Whitten 1983, Gerlach & Schill 1991, Knudsen *et al.* 1993, Schwerdtfeger *et al.* 2002). Methyl salicylate, 1,8-cineole, and benzyl benzoate can be found in large quantities in the floral scent of several *Stanhopea* species (Orchidaceae) (Williams & Whitten 1992). Linalool, (E)-ocimene, and methyl (E)-cinnamate are important compounds in the floral scent of *Gongora* (Orchidaceae) (Williams & Whitten 1983). 1,8-Cineole is the major compound in the floral scent of many perfume-flowered Araceae (Gerlach & Schill 1991, Schwerdtfeger *et al.* 2002) and was also a main constituent of the floral scent of *A. rubrinervium*. These compounds are known to attract different species of euglossine bees to artificial chemical baits as well as to perfume flowers and play an important role in the pollination and speciation of these plants (Dodson *et al.* 1969, Williams & Dodson 1972, Ackerman 1983). Latest research results show that the variety of compounds gathered by euglossine bees is specific for each bee species (Eltz *et al.* 1999). Data on the content of the hind tibia of the parasitic euglossine genera are not yet documented but it is likely that they also have a compound spectrum which is species-specific.

In the odor-driven pollination system of euglossine pollination, variability of floral scent between the populations of a perfume-flowered species needs to be low because attraction of the pollinating species is highly specific and small changes in the odor bouquet could attract different visitors (Williams and Whitten 1992, Knudsen 2002). In our case, visitor observations at all sampled inflorescences showed no differences in the pollinator spectrum, although the scent composition differed between individuals. Qualitative and quantitative differences in the scent samples may be due to intraspecific variation of floral scents between the individuals of different populations. In addition, the different size of the inflorescences and environmental conditions (e.g., temperature, light conditions, humidity) during sampling may also have affected chemical composition of the samples.

In conclusion, we document that *A. caerulea* collected floral fragrance on the spadix of *A. rubrinervium* and presume that the floral fragrance of *A. rubrinervium* contains one or several compounds important for the scent spectrum of this bee. Eugenol, methyl cinnamate, and p-cresol, which are minor compounds of *A. rubrinervium* floral fragrance, proved to be attractive for *A. caerulea* (Williams & Dodson 1972, Morato 2001, Ramírez *et al.* 2002). For the other compounds no attractiveness for *A. caerulea* in baiting experiments is documented. (Z)-8-heptadecene has not yet been described as a compound in floral fragrances of other euglossine-visited flowers, although it is found in the floral fragrance of other plants (Knudsen *et al.* 2006). Interestingly, (Z)-8-heptadecene was also identified as semiochemical

in mites (Nazzi *et al.* 2002, Milani *et al.* 2004, El-Sayed 2003-2006). Nazzi *et al.* (2002) described the significant reduction of *Varroa destructor* offspring in the brood cells of infested *Apis mellifera* colonies by (Z)-8-heptadecene. Even if male euglossine bees do not participate in brood care, this compound could be important in mating. Therefore the attractiveness of (Z)-8-heptadecene for euglossine bees should be tested.

Although *A. caerulea* was observed only once collecting floral scent, the bee was quite common and often seen flying in the surroundings of the *A. rubrinervium* population. A male individual of *A. caerulea* was collected at the nectar-flowers of *Psychotria carapichea* (Rubiaceae). The bee had a strong smell of butyric acid, which has been observed by Vogel (1966) and Williams & Whitten (1983) in individuals of *Eulaema*. Williams & Whitten (1983) suggest that the released odor may represent a defensive secretion or alarm pheromone. But perhaps butyric acid also plays a role in pathogen prevention in the brood cells of *Eulaema* and the newly emerged bees still wear the smell of the nest? Little seems to be known about that.

Another remarkable observation about parasitic euglossines is their reserved and shy behavior. The short stay and the sole observed visit of *A. caeruleas* at a perfume-flower was perhaps due to disturbance either by the presence of the observer or by other bees. Other studies on the biology of the parasitic euglossine bees also mention their shy behavior (Bennett 1972, Garófalo & Rozen 2001). The parasitic euglossines only enter the host's nest when the host is not present. Inside the nest they act very carefully and they immediately leave the nest when the host arrives. No aggressive action by a parasitic euglossine against its host is known. At chemical baits they act similarly. They spend an unusually long time inspecting the situation before landing on the bait, in contrast to other euglossine bees. If one tries to catch them and fails, they are not seen at the same bait for several days (Hentrich, pers. obs.). Obviously the shy and careful nature of these parasitic bees is also reflected in their fragrance collection.

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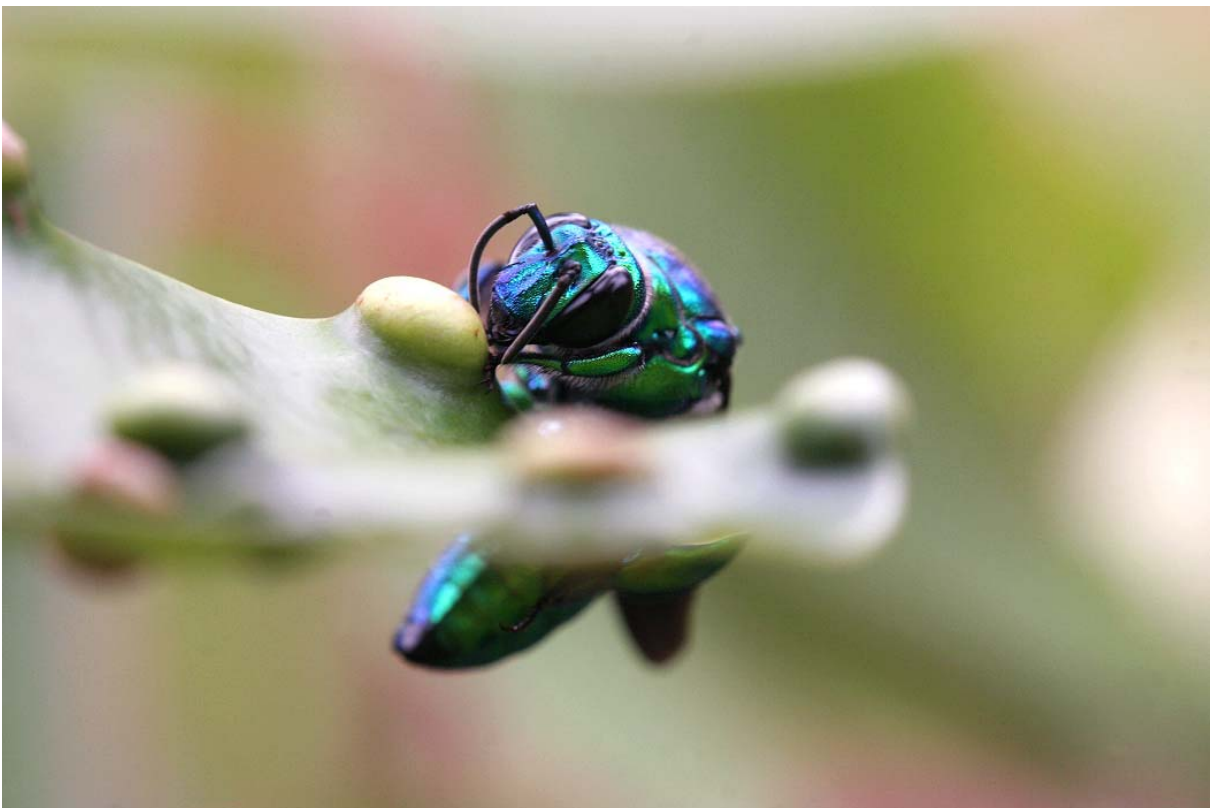
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Male *Euglossa piliventris* gnawing the bark of *Croton guianensis* (Euphorbiaceae) with its mandibles probably to collect leaking fragrance substances.



Male *Exaerete frontalis* during scent collection at a gall of a leaf of *Clusia* sp. (Clusiaceae).

# DOES SELECTION FAVOR SELFING IN MYCO-HETEROTROPHIC PLANTS? - THE REPRODUCTIVE BIOLOGY OF *VOYRIA* (GENTIANACEAE) SPECIES IN FRENCH GUIANA

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## ABSTRACT

The reproductive biology of the myco-heterotrophic species *Voyria caerulea*, *V. clavata* and *V. rosea* (Gentianaceae) was studied in French Guiana. The three species possess large, conspicuously-colored flowers that produced a sucrose-rich nectar with sugar concentrations between 16.4 and 23.9% and that emitted a rosy floral perfume with a citrus-related aspect, which was dominated by terpenoids. Principal scent compounds of *V. caerulea* and *V. rosea* were (E)-nerolidol and geraniol, and (E)-8-oxo- $\beta$ -farnesene and (E,E)-farnesyl acetate in *V. clavata*. Germinating pollen grains were observed in the anthers of all species, forming large interweaved clumps that were transferred by the pollinators as a unit. In *V. caerulea* and *V. rosea*, stamens and stigmas were close together, enabling the plants to emergency self-pollinate. This was not the case in *V. clavata* since here the reproductive organs were widely separated from each other. P/O ratios of all species were low (33-78), indicating that pollen transfer (either by selfing or by outcrossing) is very efficient. Flower visitors were butterflies (*V. caerulea*, *V. rosea*) and/or long-tongued bees of the genus *Euglossa* (*V. clavata*, *V. rosea*). The floral characteristics and our visitor observations indicate that the studied species have adapted to butterfly and euglossine pollinators. Nevertheless, *Voyria caerulea* and *V. rosea* were visited only rarely and irregularly and were able to self-pollinate through germinating self-pollen. In contrast, the non-selfing *V. clavata* showed a higher degree of specialization towards euglossine-pollinators, which were frequent visitors. The reliability of pollinators appears to be an important factor in the establishment of xenogamy in this genus. Recruitment of highly reliable and frequent pollinators that guarantee fertilization of the ovules enabled the plants to evolve obligate xenogamy, whereas plants with unreliable pollinators required emergency mechanisms like competing self-pollination to assure seed-production. Although many myco-heterotrophic plants are able to self-pollinate, the requirements for outcrossing

are preserved, indicating that outcrossing is still important for them. Average seed set in the studied plants ranged between 36-49% and was very variable in all species, probably due to resource-limitation. The capsules of *V. caerulea* and *V. clavata* showed characteristics of endozoochorous dispersal and were frequently found gnawn. It appears that the seeds are primarily dispersed by rodents.

Key words: *Voyria*, Gentianaceae, myco-heterotrophic plants, pollination biology, Euglossini, butterflies, French Guiana.

## INTRODUCTION

More than 400 species of angiosperms belong to the group of myco-heterotrophic plants ('saprophytes'; Furman & Trappe, 1971). These organisms are achlorophyllous and probably receive all required carbon from associated mycorrhizal fungi (Leake 1994). Most myco-heterotrophic plants have evolved convergent adaptations in their morphology and anatomy (Maas 1986, Leake 1994). As a rule, they are very small, herbaceous, and possess reduced vegetative organs, e.g., the leaves are scale-like, the overground organs often lack stomata, the vascular tissue of stems and roots is reduced and the subterranean parts are strongly modified for the housing of the fungi (Leake 1994).

Analogously, strong modifications are also observed in the reproductive structures. Despite the fact that vegetative reproduction is very common, sexual reproduction seems to be the prevailing form of propagation. In comparison to their autotrophic relatives, however, their inflorescences, flowers and floral organs are usually reduced in size and complexity. Their fruits contain large amounts of tiny seeds, which belong to the smallest in the plant kingdom. Thus, embryo and endosperm are strongly reduced, which has led to the assumption that many of these plants depend upon their fungal partner for germination (Maas 1979, 1986, Leake 1994).

While our knowledge about the interaction between fungi and plants is quite proficient, the reproductive biology of myco-heterotrophic plants has been poorly studied, with the result that information about the pollination of whole families and genera is completely lacking (Maas *et al.* 1986, Maas & Rübsamen 1986, Maas & Ruyters 1986). Most earlier reports consider myco-heterotrophic species as being exclusively autogamous, giving the impression that all of them follow a strategy of selfing. In these studies, scientists examined the morphology and anatomy of alcohol-preserved flowers and discovered that the stamens are closely arranged to the pistil and that pollen often germinates within the anthers

or that pollen tubes sometimes even penetrate the pistil (Warming 1901, Schoch 1920, Oehler 1927). Nevertheless, the majority of these early researchers have not seen the plants in their natural habitat and mostly did not take into consideration that many produce showy flowers and even secrete nectar. In obligately autogamous plants, structural adaptations for the attraction of pollinators are usually reduced or completely lost because selection for the maintenance of these adaptations is normally absent (Ornduff 1969). Our knowledge on the ecology of myco-heterotrophic plants has strongly increased over the last decades and some recent publications also deal with their reproductive biology. These studies confirm that many of these plants are able to self-pollinate, but they also give evidence that flower visitors still might play a role in their reproduction. In some species, flower visitors are suggested to be mediators for self-pollination (Takahashi *et al.* 1993, Lehnebach *et al.* 2005), while other species seem to have a mixed selfing-outcrossing pattern (Zhang & Saunders 2000, Lehnebach *et al.* 2005). In such cases self-pollination is performed when pollen transfer by pollinators has failed (Zhang & Saunders 2000, Lehnebach *et al.* 2005). In addition, several species of strictly xenogamous myco-heterotrophic plants were discovered, whose reproduction completely depends on flower visitations by insects (Tanaka 1978, Wallace 1977, Ushimaru & Imamura 2002).

The genus *Voyria* Aubl. (Gentianaceae) comprises 19 species, which show different degrees of specialization towards myco-heterotrophy. The plants have a vast distribution in tropical and subtropical America with its center in the Guianas and with one species occurring in western tropical Africa (Maas & Ruyters 1986, Albert & Struwe 1997). Most taxa possess brightly-colored flowers that emit scent and offer nectar. Consequently, they are generally considered to be cross-pollinated (Maas & Ruyters 1986). However, only two studies explore the reproductive biology of the plants and, adding to the uncertainty, they show ambiguous results. While Oehler (1927) demonstrated on preserved material that *Leiphaimos* sp. (= *V. rosea*) is strongly autogamous, Imhof *et al.* (1994) observed flower visitors at *V. tenella* and considered these to contribute to the cross-pollination of the plants. Unfortunately, both studies are incomplete in their description of the plants' reproduction; either by lacking visitor observations or by not testing on autogamy. Thus, flower visitors as well as selfing might play a role in both cases. In order to evaluate the importance of autogamy and xenogamy in the genus *Voyria*, we studied the reproductive biology of *V. caerulea*, *V. clavata*, and *V. rosea* in the Natural Reserve Nouragues in French Guiana. Due to their conspicuous and rewarding flowers with deep corolla tubes, our working hypothesis was that the plants are not obligately autogamous and that their flowers are visited by long-tongued insects. To test our hypothesis,

we studied visitor spectrum, floral morphology with regard to pollination, P/O ratios, nectar sugar composition, floral scent composition, and seed set of the three species. We present further data on the dispersal of seeds, and on floral morphology and distribution of additional *Voyria* species occurring in the reserve.

## MATERIAL & METHODS

### *Study site*

The study was conducted during the early to midrainy season (January-June) between 2004 and 2006 in the environs of the Inselberg station of the Nouragues Natural Reserve in French Guiana (04°05' N, 52°41' W, 120 m asl.). Nouragues is located in primary lowland tropical rainforest with an annual rainfall of 2990 mm and a mean annual temperature of 26.3°C (Grimaldi & Riéra 2001).

### *Plant Material*

*Voyria caerulea* Aubl., *V. clavata* Splitg. and *V. rosea* Aubl. (Gentianaceae) are myco-heterotrophic plants that grow in lowland tropical rainforests up to 600 m and produce large, showy, and highly fragrant flowers (Maas & Ruyter, 1986). Based on their morphological characters, Albert & Struwe (1997) divide the *Voyria* genus into two subgenera, *Voyria* and *Leiphaimos*. *Voyria caerulea* and *V. clavata* belong to the subgenus *Voyria*, which is characterized by a rather unspecialized morphology, resembling that of other autotrophic Gentianaceae. The second subgenus, *Leiphaimos*, includes various species that show strong adaptations to myco-heterotrophy. Although the study species *V. rosea* is incorporated in the subgenus *Leiphaimos*, its morphological characters are intermediate between low and highly specialized species (Maas & Ruyters 1986). Besides *V. rosea*, data of further species of the subgenus *Leiphaimos* [*V. aphylla* (Jacq.) Pers., *V. aurantiaca* Splitg., *V. corymbosa* Splitg., *V. tenella* Hook., *V. tenuiflora* Griseb.] and of *Voyriella parviflora* (Miq.) Miq., which all occur in Nouragues, were included in the study. While most of these species occur throughout the Neotropics, *V. caerulea*, *V. clavata*, and *V. tenuiflora* are only known from the Guianas and Amazonian Brazil; moreover, *V. rosea* is restricted to the Guianas. The studied species flower the year round with a flowering peak in the early rainy season (Maas & Ruyters 1986, Maas-van de Kamer & Maas 1997). Although the focus species produce large and conspicuously colored flowers, pollinators have not been reported yet (Maas & Ruyter 1986). Vouchers of all species studied are deposited in the herbaria CAY, French Guiana (Hentrich, FGIC 212, 213, 214) and ULM, Germany (Hentrich, FGIC 86, 87, 91, 97, 99, 100, 101, 123, 212).

### *Distribution of the plants at the study site*

In 2004, inflorescences of all *Voyria* species growing along, and within a distance of 5 m from the trails at the Inselberg station were recorded weekly between 8 March and 8 April (at Petit Plateau, Grand Plateau, Crique Cascade, and Inselberg, as well as on trails from the campsite to these places; total trail length: ca. 20 km).

### *Floral morphology and flowering phenology*

The duration of the flowering time of individual flowers was recorded for several plants of *Voyria caerulea*, *V. clavata*, and *V. rosea* (Tab. 1).

Corolla tube length and corolla limb diameter (platform, built by the corolla lobes) of fresh flowers of *Voyria aurantiaca*, *V. caerulea*, *V. clavata*, *V. corymbosa*, *V. rosea*, and *V. tenuiflora* were measured with a caliper (0.1 mm accuracy). Corolla tube length was defined as the distance between the ovary base and the corolla limb, except for *V. clavata*, where the distance between ovary base and the union point of anther filaments and corolla tube was measured since this reflects the nectar bearing part the visitor penetrates with its tongue. Corolla limb diameter of *V. clavata* was not measured since it does not develop any.

Floral microstructures of *Voyria caerulea*, *V. clavata*, and *V. rosea* were studied under a Zeiss DSM 942 scanning electron microscope (SEM; Zeiss, Oberkochen, Germany). For the SEM analyses, alcohol-preserved plant material was prepared by gradual dehydration in propanol, critical point drying, and sputtercoating with gold (Balzers Union Sputter Coater, Balzers, Liechtenstein).

UV-absorption photos of flowers were made in the field at fresh inflorescences under direct sunlight on an Ilford HP 5 Plus (ISO 400) film using a Canon 50E SLR camera with a 100 mm macro lens and a UV-transmission filter (55E, B+W Filter, Bad Kreuznach, Germany).

Pollen/ovule ratios (P/O ratios) of alcohol-preserved material were determined after Cruden (1977). Ovules were counted using a stereomicroscope. Pollen number of one anther per flower was determined using a microscope or a CASY cell counter (SCHÄRFE System GmbH, Reutlingen, Germany). P/O ratio was calculated by multiplying the pollen number by five and dividing the result through the ovule number of the same flower. Sample sizes are given in Tab. 1.

### *Floral nectar sugar composition*

The floral nectar sugar concentration and the sugar composition of several inflorescences of *Voyria caerulea*, *V. clavata*, and *V. rosea* (Tab. 2) were analyzed by high-performance liquid chromatography (HPLC). Samples were taken from the flowers between the first and the fourth day of anthesis. The flowers were protected from floral visitors and rain by nylon nettings and a small transparent plastic umbrella (30 x 30 cm) that was positioned ca. 10 cm above them. Microcapillary pipettes of different sizes (2 µl, 3 µl, and 5 µl microcaps; Hirschmann, Germany) were used to withdraw the nectar. The samples were immediately transferred into undenaturated ethanol (70%) and stored at –27°C until analysis. Before the analysis, samples were dried in a vacuum-centrifuge (Savant Speed Vac SC 110) at 65°C, subsequently redissolved in ultrapure water (HPLC-grade), and filtered (syringe nylon filter Acrodisk, Pall, USA; diameter: 4 mm, poresize: 0.45 µm,). HPLC was equipped with a Waters 717 plus autosampler, Waters 510 HPLC pump, and a Waters 410 RI-detector with Waters CHM column-heater. Separation was achieved using a 72:28% acetonitril-water mixture as solvent system on a Waters High Performance Carbohydrate Column (4.6 x 250 mm, 60Å 4 µm). The system was controlled by the Waters Millennium Software 3.01 with the following parameters: sample volume: 10 µl, flow rate: 1.4 ml/min, temperature: 35°C, runtime: 12 min. Identification of sugar types and quantitative determination of sugar contents was achieved by comparing chromatograms and peak areas to standards (fructose, glucose, sucrose).

### *Floral scent composition*

Four scent samples from four different inflorescences of each of the focus species were collected in the field by the headspace method (Williams & Whitten 1983, Hills & Schutzman 1990, Raguso & Pellmyr 1998). The flowers were covered by an inert oven-bag during collection. The scented air was drawn through a sampling cartridge by a battery-operated membrane pump for four hours with a flow rate of 150 ml/min. The sampling cartridge was prepared with 25 mg of Tenax TA (mesh 80-100, Macherey Nagel, Düren, Germany) and 40 mg of Carbopack X (mesh 20-40, Supelco, Bellefonte, Pennsylvania, USA). The adsorbents were conditioned by washing with methanol (purity 99.8%, Merck, Darmstadt, Germany) and acetone (purity 99.9%, Merck, Darmstadt, Germany) and heated out for 30 min at 220°C before sampling. The adsorbed scent was recovered by elution with 200 µl acetone into glass vials. The samples were investigated by GC/MS using a Thermo Finnigen Voyager Mass Spectrometer combined with a Trace GC 2000 Series and the Xcalibur software. The analyses were made on a DB-WAX column (J&W Scientific) 30 m x 0.32 mm i.d., film thickness 0.25



µm; splitless injection, temperature program was 50°C-2'iso-2.5°C/min-230°C-40'iso. Compounds were identified by comparison of their mass spectra and retention times with those of authentic reference samples available from the Givaudan reference compound collection. Substances that had a mean value of > 1% of all volatiles in the samples of the respective species were classified as major compounds.

#### *Visitor observations*

Flower visitors to *Voyria caerulea*, *V. clavata*, and *V. rosea* were recorded on six days for *V. caerulea*, on 21 days for *V. clavata*, and on 15 days for *V. rosea* between 08:30 h and 15:00 h, respectively.

#### *Seed set*

Three inflorescences of *Voyria rosea* were bagged with nylon nettings in bud stage to test them on their potential to self-pollinate. Unfortunately, this experiment could not be done for *V. caerulea* and *V. clavata* since we did not find enough flowering individuals. The seeds of alcohol-preserved mature fruits from unbagged flowers were counted under a stereomicroscope and the proportion of developed seeds in relation to total ovule number was calculated. In the capsules of *V. caerulea* and *V. clavata*, unfertilized or aborted ovules were strongly degenerated and could not be counted. Instead, seed set per fruit was compared with the average ovule number of the flowers.

## RESULTS

#### *Distribution of the plants at the study site*

The distribution of all *Voyria* species occurring at the study site was almost completely limited to the area west of the Nouragues creek (Campsite, Inselberg, Petit Plateau, Crique Cascade). Very few inflorescences (*Voyria aphylla*, n = 1; *V. caerulea*, n = 5; *V. corymbosa*, n = 3) were found at the Grand Plateau, which is situated in the east of the Nouragues creek. The most common species at the study site was *V. rosea*, while *V. tenella*, *V. tenuiflora*, and *Voyriella parviflora* were scarce (Fig. 1). The preferred habitat of the plants differed among species. *Voyria aurantiaca* and *V. tenuiflora* grew exclusively on sand and granite rock dominated soils, whereas *V. caerulea*, *V. clavata*, *V. corymbosa*, *V. rosea*, and *V. tenella* were usually found on strongly decayed wood. *Voyria aphylla* and *Voyriella parviflora* predominantly occurred at the edge of low forests adjacent to granite rock savannas where they grew on a thin humus layer. Most habitats of all studied species were light-flooded and the plants were exposed to indirect sunlight for several hours a day. We found both single and

grouped inflorescences (e.g., near decaying trunks). Especially *V. rosea* often grew in large populations ( $> 5$  inflorescences/m<sup>2</sup>) together with individuals of *V. clavata* and *V. corymbosa*. *Voyria aurantiaca* sometimes shared its habitat with *V. tenuiflora*, *V. caerulea*, or *V. corymbosa*. Although their distribution was only recorded in 2004, most plants were seen at the same sites in 2005 as well as in 2006 and the proportional distribution of the different species remained approximately the same.

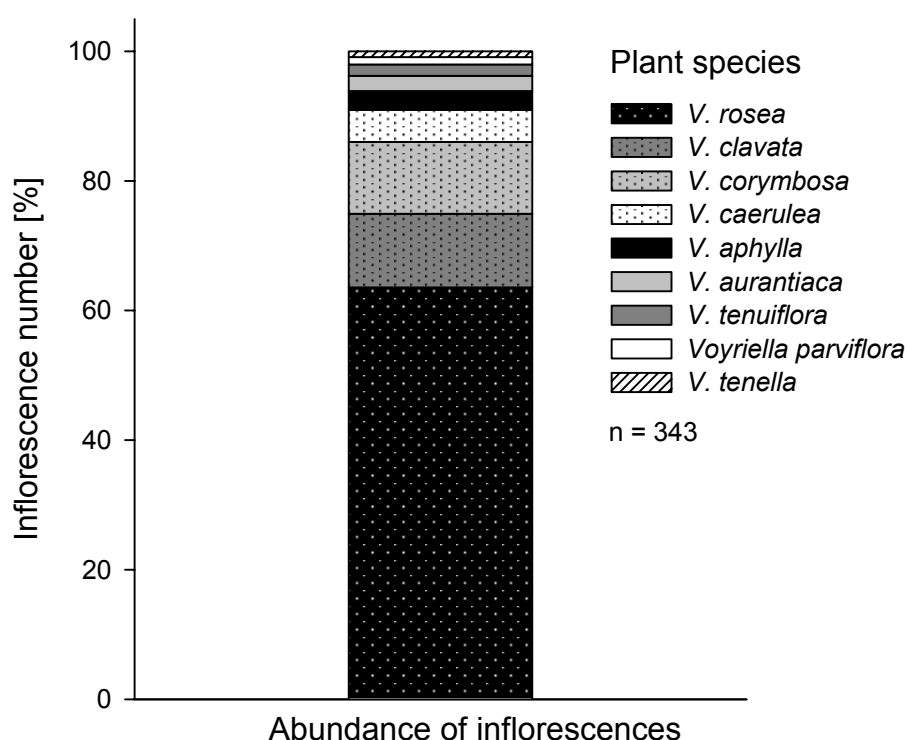


FIG. 1. Proportional distribution of the *Voyria* and *Voyriella* inflorescences at the study site in the year 2004 (values are percentage of all recorded inflorescences).

#### Floral morphology and flowering phenology

*Voyria* species possess brightly colored tubular flowers that consist of five fused sepals and petals, respectively. Generally, the petals form a corolla limb (platform, built by the corolla lobes) at the flower entrance, which facilitates the landing of visiting insects. *Voyria caerulea*, *V. clavata*, and *V. rosea* represented the species with the largest and most conspicuous flowers at the study site (Fig. 2A, B, C). Flower color was purple/white in *V. caerulea*, reddish/yellow/white (transition from the bottom to the top of the flower) in *V. clavata*, and pink in *V. rosea*. Strong UV absorption by the entire flower was common in all studied species (Fig. 6A, B). Further, especially in *V. caerulea*, UV absorption increased towards the

center of the flower entrance (Fig. 6C, D). *Voyria clavata* usually produced one single flower per inflorescence, while *V. rosea* had 2-3 and *V. caerulea* 3-4 flowers (Tab. 1). Rarely, we also found inflorescences with a dozen or more flowers in the latter two species.

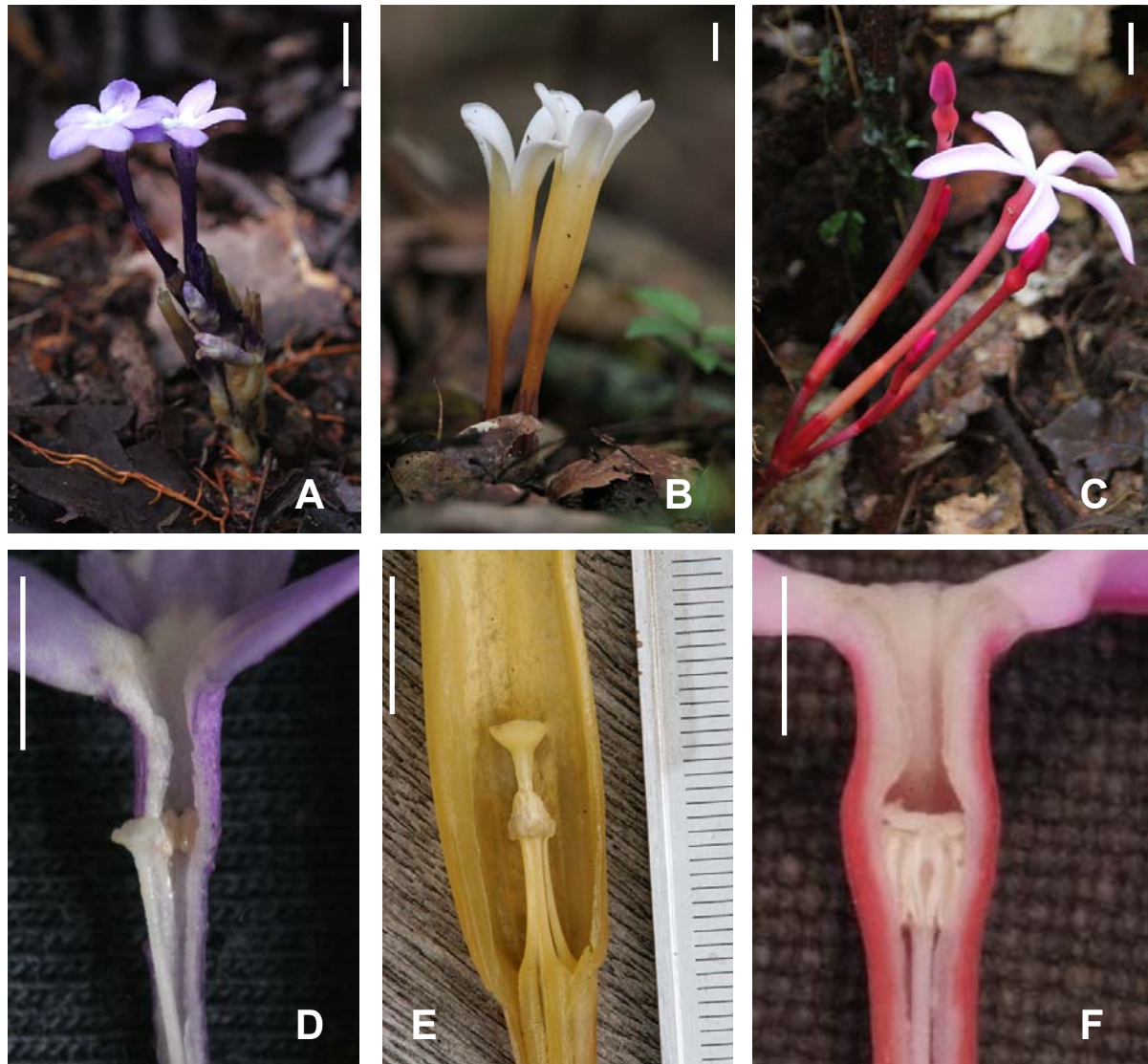


FIG. 2. Flowers of *Voyria caerulea* (A, D), *V. clavata* (B, E) and *V. rosea* (C, F) at the study site (first row) and longitudinal sections of the flowers (second row). Scale bar = 1 cm.

The anthesis of individual flowers took six days in *Voyria caerulea* and nine days in *V. clavata* and *V. rosea* (Tab. 1). Flowers usually lasted longer (1-4 days) under dry and sunny conditions than under rainy weather. Inflorescences with several flowers showed a ‘steady state’ flowering behavior, which is characterized by a prolonged flowering period resulting from the opening of only one or few flowers a day (Gentry 1974, Bawa 1983). In the studied plants one or two flowers of an inflorescence had their anthesis at a time. Succeeding flowers began to open 1-2 days before the previous ones started to wilt. Thereby, a flowering period of several weeks per inflorescence was attained. In *V. rosea*, it was frequently observed that

older inflorescences, which were already in the stage of fruit development, produced new flowers that were smaller than the former ones. Opening of the flowers was not fixed to a certain time of day. Under sunny conditions, flowers opened early in the morning, whereas on rainy days opening could be delayed until midday.

With an average of 59 mm, *Voyria rosea* had the longest corolla tube, followed by *V. clavata* (45 mm) and *V. caerulea* (31 mm; Fig. 3A). *Voyria rosea* also produced the corolla limb with the largest diameter (32 mm; Fig. 3B). In flowers of *V. clavata*, the petals were only slightly reflexed and did not provide a good landing platform (Fig. 2B).

Stamens and stigma of *Voyria caerulea* and *V. rosea* are located closely below the flower entrance in an inflated segment of the corolla tube (Fig. 2D, F). In *V. clavata* they are deeply hidden in the corolla tube (Fig. 2E). All three species possess a very broad conical to disc-shaped stigma, whose apical surface is flattened (*V. clavata*, *V. rosea*) or slightly funnel-shaped (*V. caerulea*) and covered by many papillae, which stands in contrast to the smooth basal stigma surface. (Fig 4A, E, F; Fig. 5A-D). The stigma margin is slightly lobed in *V. caerulea* and *V. rosea* (Fig. 5A, C) and has a sharp denticulate edge in *V. clavata* (Fig. 4E). Clumps of germinated pollen were found on the stigma surface (often close to the margin) of several collected flowers (Fig. 4F, 5 B, D).

All investigated species possess five coherent anthers, which are tubularly arranged around the style. In *Voyria caerulea* and *V. rosea* the anther apex is adjacent to the stigma margin (Fig. 2F), while the anthers of *V. clavata* are located at a distance of ca. 3 mm below the stigma (Fig. 2B) and are covered by a jelly-like substance. In *V. clavata*, the anthers never open autonomously during anthesis, but one can easily tear up the thin anther-wall mechanically. As a result, the pollen clumps in the jelly and forms large masses (Fig. 4D). SEM investigations showed that the anther surface is densely packed with a papillous tissue (Fig. 4B, C), which might secrete the jelly-like substance. The anthers of the other two species have a smooth surface and do not secrete any such substance. They open transversally at the beginning of anthesis (intrors: *V. caerulea*; extrors: *V. rosea*). We observed in all three focus species that small amounts of pollen already germinated in the anthers one or two days before anthesis. These pollen grains were interwoven with non-germinated pollen and formed large pollen-bundles.

The ovules of the focus species were always located in two placentas, each divided into two branches. Pollen and ovule numbers per flower were very high and increased with flower size (Tab. 1). The resulting P/O ratio for the focus species was low (Tab. 1) and is similar to P/O ratios of facultative autogamous species (Cruden 1977).

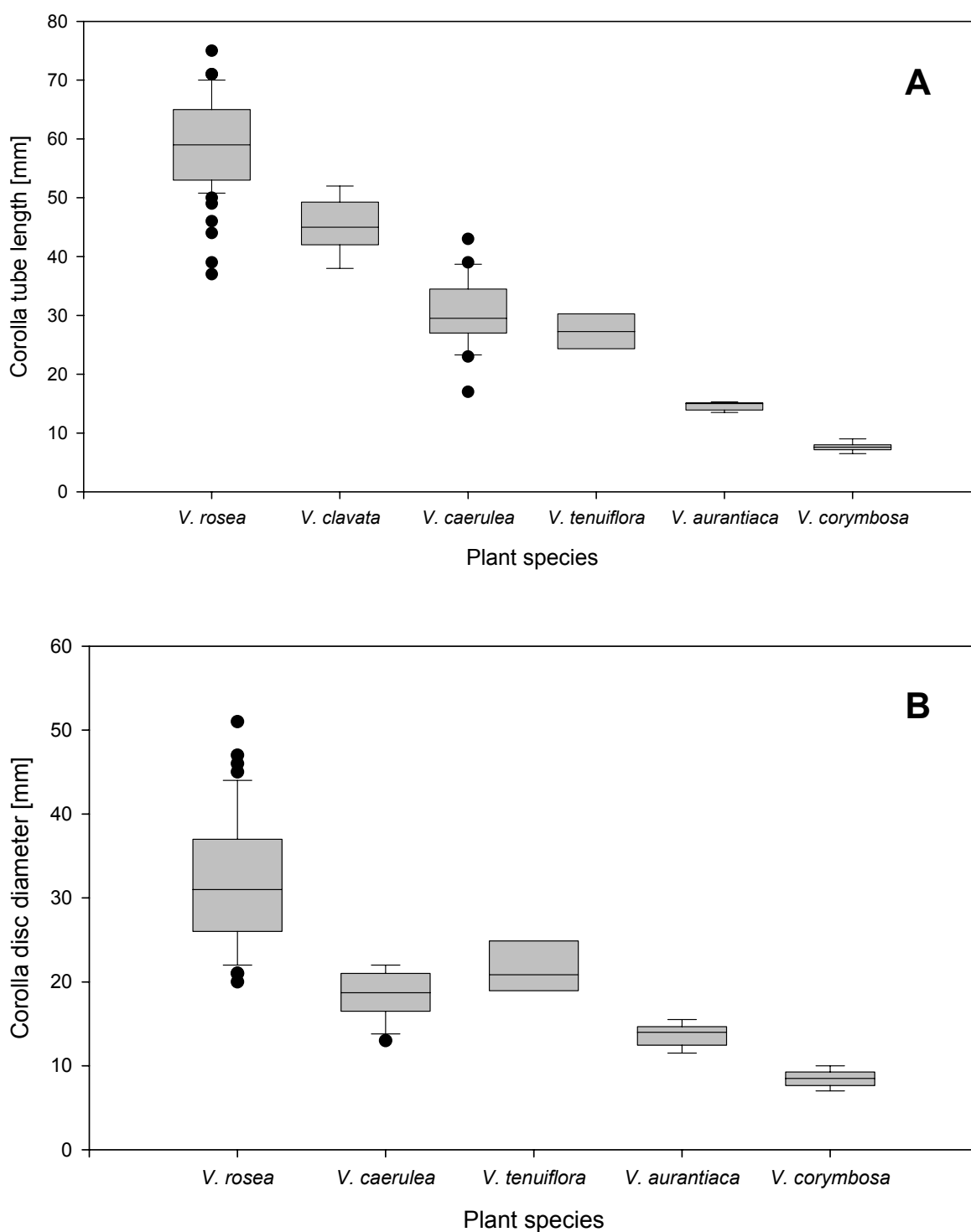


FIG. 3. A: comparison of the corolla tube length of six *Voyria* species (*V. rosea*, n = 67; *V. clavata*, n = 18; *V. caerulea*, n = 17; *V. tenuiflora*, n = 8; *V. aurantiaca*, n = 9; *V. corymbosa*, n = 9). B: comparison of the corolla limb diameter of five *Voyria* species (*V. rosea*, n = 59; *V. caerulea*, n = 14; *V. tenuiflora*, n = 7; *V. aurantiaca*, n = 9; *V. corymbosa*, n = 9).

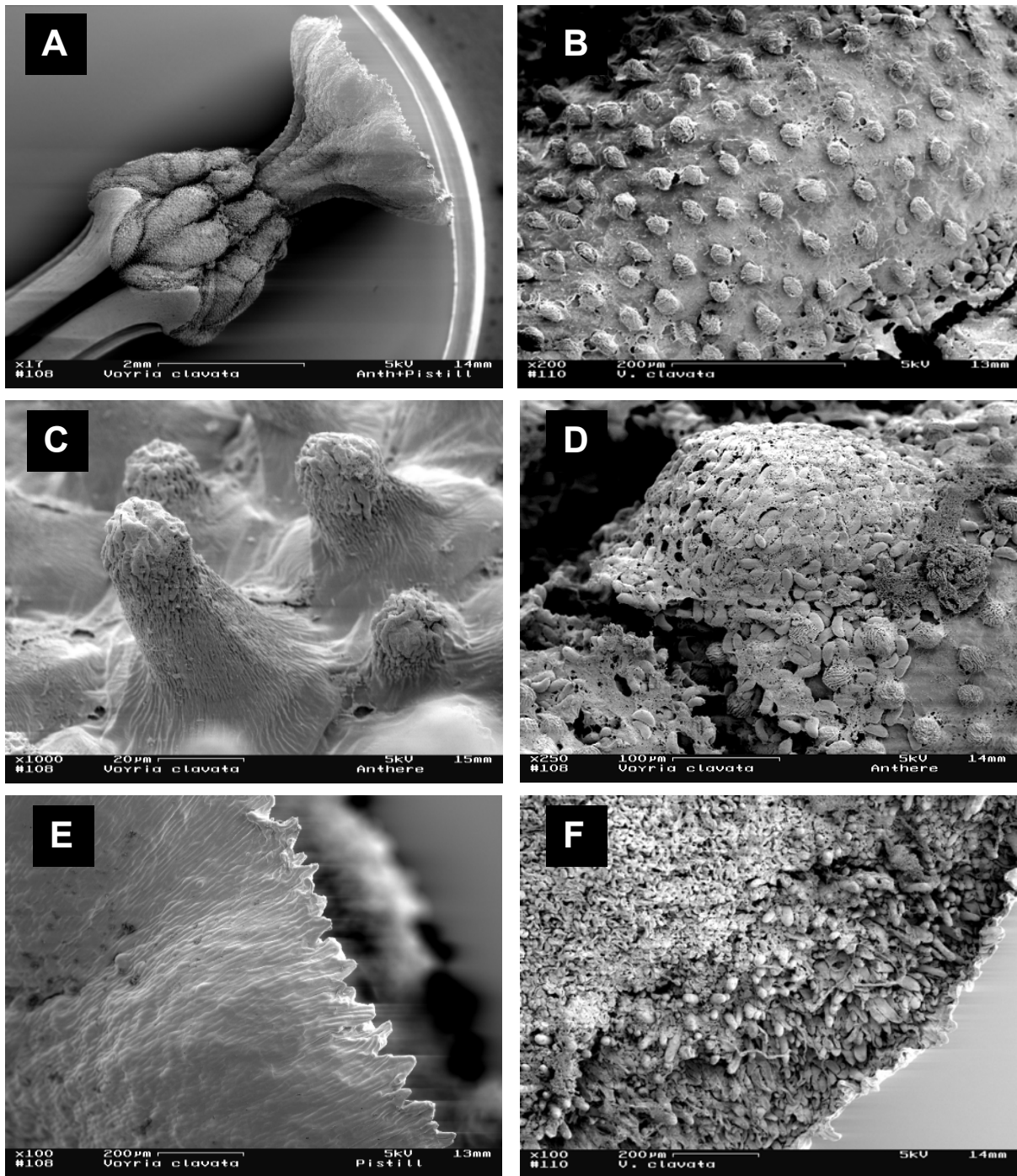


FIG. 4. SEM-pictures of *Voyria clavata* floral microstructures. A: androeceum and pistil; B: papillous anther surface covered by jelly-like substance; C: detail of secretory tissue of the anthers; D: clumped pollen mass on the anther surface; E: denticulate stigma margin; F: apical surface of the stigma showing papillae (right) and pollen mass (left).

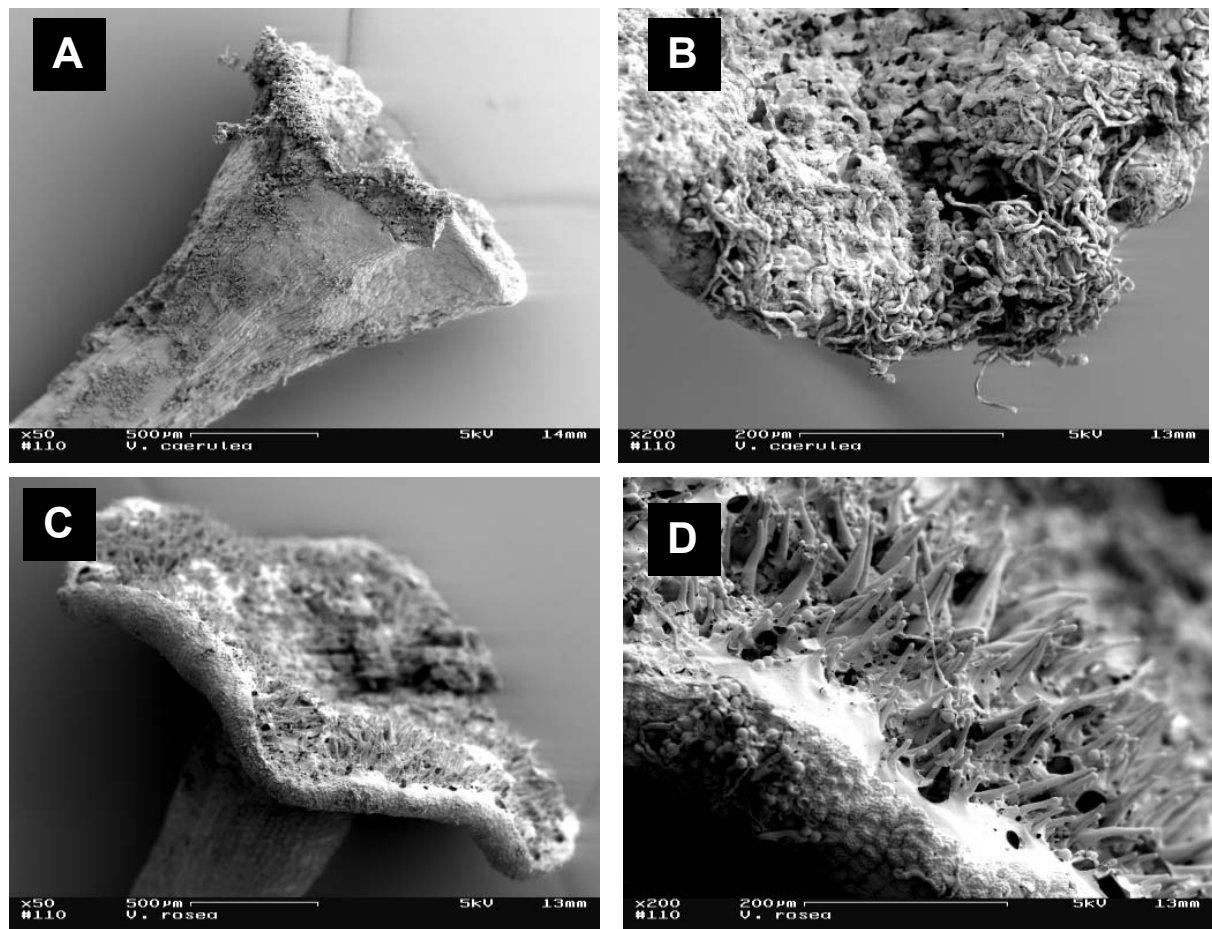


FIG. 5. SEM-pictures of *Voyria caerulea* (A, B) and *V. rosea* (C, D) floral micro-structures. A: pistil; B: apical surface of the stigma with germinated pollen; C: pistil; D: apical surface of the stigma with papillae and pollen.

TABLE 1. Pollen and ovule numbers per flower, seed number and amount of fertilized ovules per fruit, P/O ratios, flower number per inflorescence and flowering time of individual flowers of *Voyria caerulea*, *V. clavata* and *V. rosea*. In *V. caerulea* and *V. clavata* values of seed set are the ratio between mean seed and mean ovule number since unfertilized or aborted ovules in the capsules were strongly degenerated and could not be counted. Values are presented as average  $\pm$  S.D. (n).

	Pollen grains per flower	Ovules per flower	Seeds per fruit	Seed set per fruit (%)	P/O ratio	Flowers per inflorescence	Anthesis of individual flowers (d)
<i>Voyria caerulea</i>	95,129 $\pm$ 8,521 (7)	2,866 $\pm$ 964 (12)	1,612 $\pm$ 832 (13)	49.2	33:1	2.9 $\pm$ 1.4 (13)	5.8 $\pm$ 2.2 (10)
<i>Voyria clavata</i>	370,608 $\pm$ 66,324 (13)	4,728 $\pm$ 2,666 (14)	2,150 $\pm$ 1,248 (11)	45.5	78:1	1.3 $\pm$ 0.5 (18)	8.7 $\pm$ 1.4 (6)
<i>Voyria rosea</i>	255,556 $\pm$ 24,575 (9)	4,131 $\pm$ 1,572 (20)	1,343 $\pm$ 1,112 (20)	35.8 $\pm$ 30.2	62:1	1.8 $\pm$ 1.6 (100)	9.1 $\pm$ 2.9 (29)

TABLE 2. Nectar sugar concentration and composition (mean  $\pm$  S.D.) of *Voyria caerulea* (n = 8 samples from 6 individuals), *V. clavata* (n = 13 samples from 6 individuals), and *V. rosea* (n = 16 samples from 9 individuals; S/H-ratio: [%sucrose]/[%glucose + %fructose]).

	Nectar sugar concentration (% w/w)	Sucrose (%)	Glucose (%)	Fructose (%)	S/H-ratio
<i>Voyria caerulea</i>	16.4 $\pm$ 2.0	100	0	0	-
<i>Voyria clavata</i>	20.9 $\pm$ 5.2	100	0	0	-
<i>Voyria rosea</i>	23.9 $\pm$ 3.2	93.6 $\pm$ 8.4	1.1 $\pm$ 1.7	5.2 $\pm$ 7.2	11.6 $\pm$ 15.2



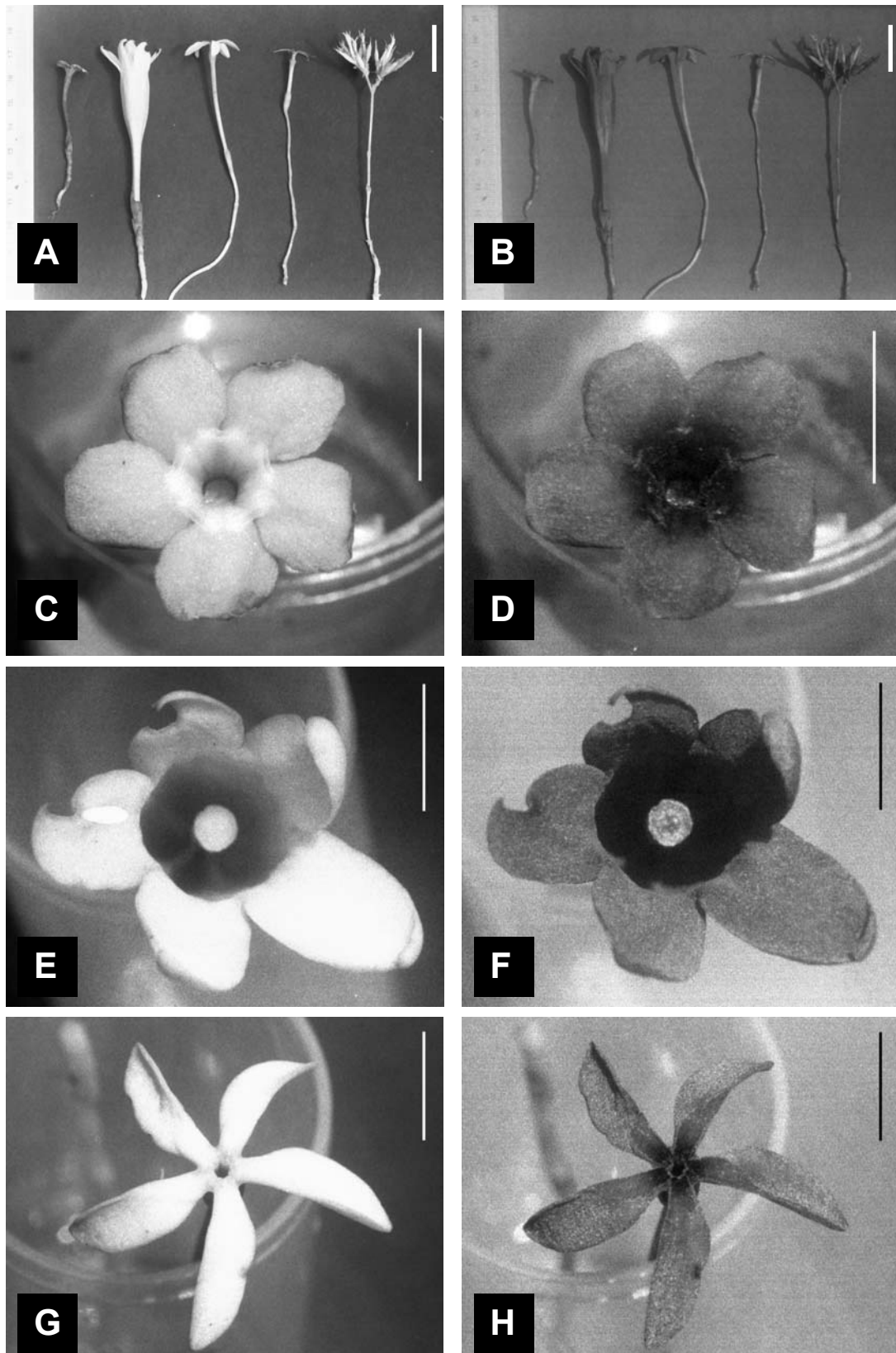


FIG. 6. Photographs of *Voyria* flowers taken with (right column) and without (left column) UV-transmission filter. A, B: lateral view of entire flowers of *V. caerulea*, *V. clavata*, *V. rosea*, *V. tenuiflora* and *V. corymbosa* (from left to right) absorbing UV; C-H: apical view on the flowers of *V. caerulea* (C, D), *V. clavata* (E, F) and *V. rosea* (G, H). All flowers strongly absorb UV-light. *V. caerulea* and *V. rosea* show higher UV-absorption in the center than in the outer regions of the flower. Scale bar: A, B = 2 cm; C-H = 1 cm.

#### *Floral nectar sugar composition*

Mean sugar concentrations of the nectar ranged from 16.4% (*V. caerulea*) to 23.9% (*V. rosea*; Tab. 2) and nectar of all investigated samples was strongly sucrose-dominant (Tab. 2). Glucose and fructose could only be detected in seven out of 16 nectar samples of *V. rosea*. In the nectar of *V. caerulea* and *V. clavata* no hexose-sugars were detected at all.

Nectar accumulated during the flowering period and rose up to 1-2 cm above the ovary base within two days in *Voyria rosea* and *V. clavata*. In *V. caerulea*, the nectar sometimes even reached the height of the stigma.

In the large-flowered *V. clavata*, floral nectar was often diluted by rain-water after strong rainfalls. Water-filled corolla tubes were not visited by bees anymore and began to wilt soon. In the other two species, the large stigma and the close arrangement of the anthers in the corolla tube prevented an oozing of rain-water.

#### *Floral scent composition*

The flowers of *V. caerulea*, *V. clavata*, and *V. rosea* produced a rosy floral perfume with a citrus-related aspect, which could be perceived from the early morning until dusk. Flowers of *V. rosea* were very fragrant. In direct comparison, the floral fragrance of *V. clavata* was less strong and characterized by an unpleasantly acrid note in higher concentrations. Fragrance emission in *V. caerulea* was very weak so that the concentration of the scent samples in this species was below the detection threshold for the GC/MS analysis.

A total of 48 compounds was detected in the analyzed scent samples (Tab. 3). The floral scent of *V. clavata* and *V. rosea* contained a large number of terpenoids, while benzenoids and lipid-derived compounds were only present in minor amounts. Quantitatively, sesquiterpenes dominated the floral scent of both species. The major scent compounds of *V. rosea* were (E)-nerolidol (mean: 62.87%), geraniol (mean: 11.57%), linalool (mean: 8.46%), (E-E)-farnesol (mean: 5.51%), and methyl geranate (mean: 4.20%). The major compounds of *V. clavata* were (E)-8-oxo- $\beta$ -farnesene (mean: 57.88%), (E,E)-farnesyl acetate (mean: 19.75%), (E)- $\beta$ -farnesene (mean: 8.77%), limonene (mean: 1.87%), and 1,8-cineole (mean: 1.35%). The scent samples were quite uniform for each species. Minor differences only occurred in the proportional amount of single substances.

TABLE 3. Floral fragrance compounds of *Voyria rosea* and *V. clavata*. Four samples per plant species are presented. Sample numbers refer to samples from different plant individuals. Substances are divided into chemical classes and within each class in the order of their GC retention time. Values are percent of total volatiles and major compound values are printed in bold face.

Plant species	<i>Voyria rosea</i>				<i>Voyria clavata</i>			
Compound/Sample No.	VR10	VR11	VR12	VR13	VK10	VK12	VK13	VK14
<b>Hemiterpenes</b>								
Prenol	0.02	0.02	0.02	0.01				
<b>Monoterpenes</b>								
$\alpha$ -Thujene	0.11	0.11	0.24	0.06	0.51	0.82	0.79	1.10
$\alpha$ -Pinene			0.24	0.06				
$\beta$ -Pinene	0.03	0.11	0.05		0.04	0.02	0.03	0.03
Sabinene	0.06	0.07	0.09	0.01				
Myrcene	0.04	0.04	0.06	0.02	0.25	0.71	1.97	0.11
Limonene	0.07	0.08	0.08	0.02	<b>1.26</b>	<b>2.47</b>	<b>1.97</b>	<b>1.77</b>
1,8-Cineole	0.32	0.33	0.47	0.23	<b>1.01</b>	<b>1.88</b>	<b>1.18</b>	<b>1.32</b>
$\gamma$ -Terpinene	0.32		0.24	0.08	0.38		0.39	0.22
(E)-Ocimene	0.06	0.07	0.06	0.02				
p-Cymene					0.05	0.06	0.04	0.02
Terpinolene	0.04	0.04	0.04	0.01	0.06	0.06	0.07	0.02
6-Methyl-5-hepten-2-one	0.32	0.33	0.35	0.23	0.38	0.12	0.07	0.04
trans-Sabinene hydrate			0.02		0.05	0.04		
6-Methyl-5-hepten-2-ol	0.08	0.09	0.06	0.01	0.04	0.01	0.03	0.02
cis-Sabinene hydrate	0.06	0.07	0.07	0.02	0.08	0.06	0.13	0.06
Linalool	<b>6.34</b>	<b>6.69</b>	<b>9.93</b>	<b>10.86</b>	0.03	0.02	0.04	0.02
Ethyl 3,7-dimethyl-6-octenoate	0.01	0.01						
Bornyl acetate	0.01	0.01		0.01				
Terpinen-4-ol	0.02	0.02	0.02	0.01				
Neral	0.07	0.08	0.08	0.06				
Methyl geranate	<b>4.75</b>	<b>4.46</b>	<b>4.02</b>	<b>4.23</b>				
$\alpha$ -Terpineole		0.56	0.59	0.34	0.38	0.59	0.26	0.44
Geranial	0.21	0.22	0.24					
Geranyl acetate	0.01	0.01			0.13	0.06	0.07	0.06
Citronellol	0.06	0.07	0.04					
Nerol	0.11	0.11	0.12	0.06				
Geraniol	<b>12.14</b>	<b>12.83</b>	<b>13.12</b>	<b>11.20</b>				
Methyl (E,E)-3,7,11-trimethyl-2,6,10-dodecan	0.63	0.67	0.71	0.34				
Geranic Acid	0.01	0.01						
<b>Sesquiterpenes</b>								
(E)- $\beta$ -Farnesene	0.01	0.01		0.01	<b>8.21</b>	<b>8.71</b>	<b>12.31</b>	<b>5.85</b>
(Z,E)- $\alpha$ -Farnesene	0.32	0.33	0.24	0.11				
(E,E)- $\alpha$ -Farnesene	0.03	0.03	0.04	0.02				
(E)-Geranylacetone					0.25	0.12	0.07	0.11
(E)-Nerolidol	<b>62.30</b>	<b>65.83</b>	<b>61.82</b>	<b>64.03</b>				
(E)-8-Oxo- $\beta$ -Farnese					<b>46.11</b>	<b>64.15</b>	<b>53.97</b>	<b>67.30</b>
(E,E)-Farnesyl acetate					<b>31.58</b>	<b>14.12</b>	<b>18.08</b>	<b>15.23</b>
(Z,E)-Farnesal	0.01	0.01	0.08					

(E,E)-Farnesal	0.05	0.06		0.02				
(E,E)-Farnesol	<b>5.49</b>	<b>5.80</b>	<b>5.44</b>	<b>5.15</b>	0.09	0.06	0.07	0.06
<b>Benzenoids</b>								
Styrene					0.13	0.06	0.07	0.06
Benzaldehyde					0.04	0.01	0.01	0.01
Acetophenone					0.05	0.02	0.01	0.01
<b>Lipid-derived compounds</b>								
(Z)-3-Hexenyl acetate	0.01	0.01	0.02	0.01				
Octanal					0.05	0.02	0.03	0.02
Nonanal	0.02	0.02	0.02	0.01	0.25	0.12	0.03	0.06
Acetic acid	0.05	0.06	0.02	0.02	0.13	0.06	0.03	0.06
Decanal	0.03	0.03	0.04	0.01	0.13	0.06	0.03	0.02
<b>Total</b>	94.26	99.33	98.68	97.31	91.65	94.43	91.70	94.01

### Visitor observations

*Voyria clavata* was visited by medium-sized, long-tongued bees of the tribe Euglossini (Hymenoptera: Apidae). Visits were rare but frequent. In nine out of 21 days, a total of twelve bees (*Euglossa chalybeata* agg., n = 8; *E. intersecta*, n = 4) were observed entering the flowers. Eight additional bees were recorded that inspected the flowers and flew away without landing (*Euglossa chalybeata* agg., n = 6; *E. intersecta*, n = 2). Main visitation time was between 10:00 and 12:00 h. The bees landed on the tip of the petals, raised their heads to extend the long proboscis, and crawled down the corolla tube. Since the tube was very narrow, the bees had to push aside the large stigma with their body in order to pass downwards (Fig. 6A). This close contact ensured pollen transfer from the bees' dorsal mesosomata to the papillous stigma surface. Inside the flower, the bees' further advances were stopped by a grating formed by the elongated filaments (Fig. 2E). However, they were able to reach the nectar with their long proboscides. While sucking nectar, the bees' dorsal mesosomata came in contact with the anthers and picked up the sticky pollen mass (Fig. 7B). Finally, a bee left a flower by moving backwards until it reached the entrance and then flew away. The smooth and cuneiform basal surface of the stigma prevented the pollen to be wiped off (Fig. 4E). A flower visit took between 5-23 seconds.

Although *Voyria rosea* represented the most widespread *Voyria* species at the study site, flower visits occurred less often than in *V. clavata* and were very irregular. Floral visitors were only observed on two days out of 15, mainly between 10:00 and 13:00 h. A total of seven flower visits were made by Lepidoptera (Hesperiidae, n = 2; Heliconiidae, n = 1; Fig. 7E, F) and Euglossini (*Euglossa chalybeata* agg., n = 3; *E. decorata*, n = 1; Fig. 7C, D). Three small green *Euglossa* sp. inspected the flowers without landing.

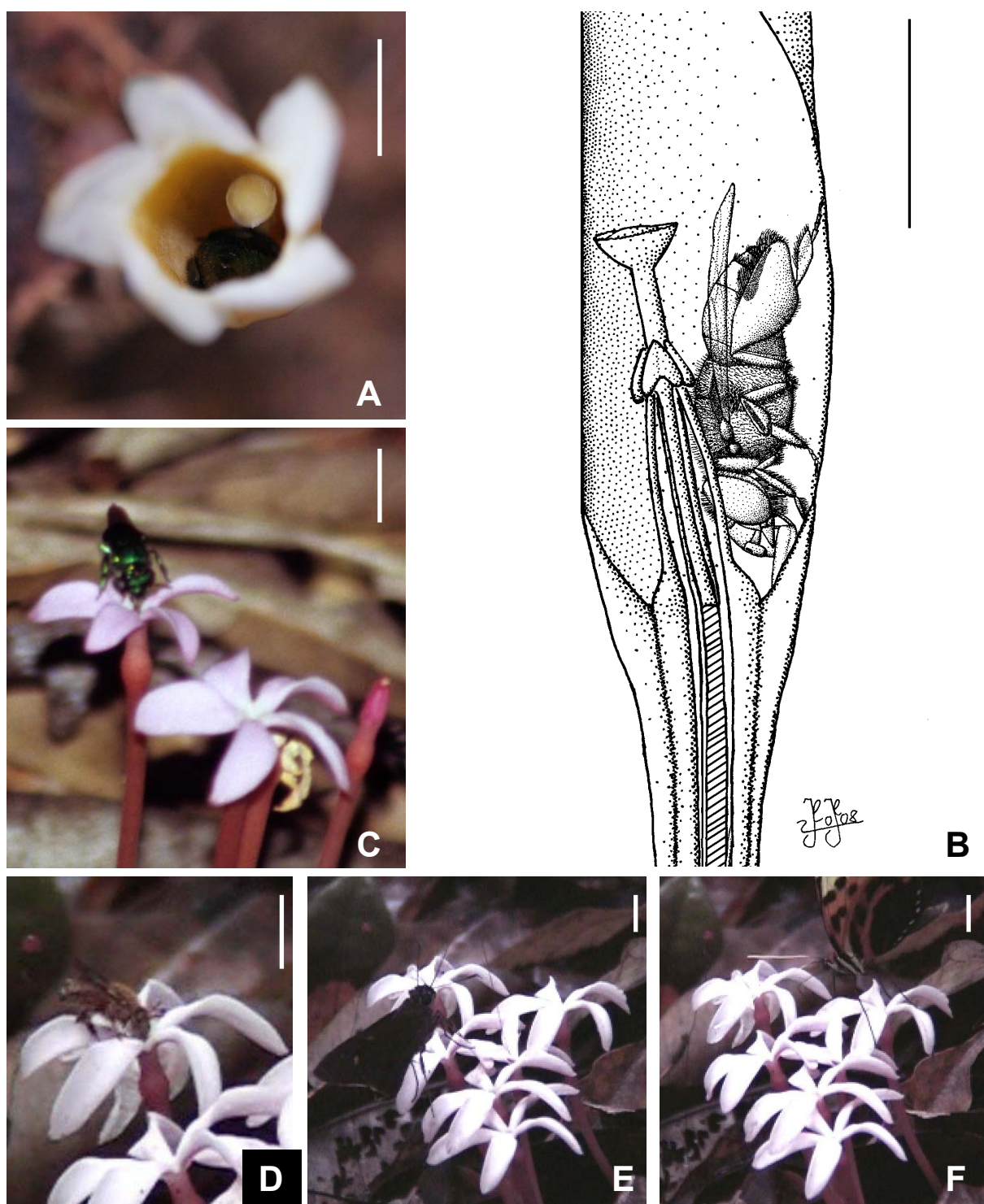


FIG. 6. Flower visitors to *Voyria clavata* (A, B) and *Voyria rosea* (C-F). A: *Euglossa intersecta* inside of the corolla tube; B: drawing of flower visit by *E. imperialis* agg. showing the bee sucking nectar and touching the anthers with its back; C: *E. imperialis* agg. sucking nectar; D: *E. despecta* introducing its proboscis into the corolla tube; E: Hesperidae probing at the corolla tube; F: Heliconiidae probing at the corolla tube. Scale bar = 1 cm.

The visitors alighted on the large corolla limb and usually needed several seconds of probing at the corolla tube until they reached the nectar. In the process, they were touching the stigma with their proboscides. In one instance, a clump of pollen mass was observed sticking at the proboscis of an hesperiid butterfly after its flower visit. The visits took between 24-208 seconds (butterflies) and 5-10 seconds (*E. chalybeata* agg.). *Euglossa decorata* stayed only for a very short time at the flower (ca. 3 seconds), probably because its tongue was too short to reach the nectar.

*Voyria caerulea* was visited once in six observation days by a butterfly. The butterfly stayed for 5 seconds and did not succeed in entering its proboscis into the flower.

In all investigated *Voyria* species, thrips were frequently observed crawling inside of the corolla tube and on the reproductive organs. Furthermore, meliponine bees occasionally landed on the flowers of *V. rosea* and *V. clavata* and moved around on the petals but never entered the corolla tube.

#### *Seed set*

With the exception of flowers that wilted at the beginning of the anthesis due to heavy rainfall, most flowers at the study site, including the bagged ones of *V. rosea*, developed fruits. The fruits of the focus species are fleshy, indehiscent capsules, which stand close to the ground. During fruit maturation, which took between 2-3 months, the ovaries of *V. caerulea* and *V. clavata* strongly increased in size and became bulb-shaped (Fig. 8A, B), while the ovary of *V. rosea* did not change much in shape. In ripe fruits of *V. caerulea* and *V. clavata*, the seeds were immersed in the swollen, spongy placenta tissue (Fig. 8C). Unfertilized or aborted ovules were strongly degenerated. The placenta-tissue of *V. rosea* did not increase. Instead, the fruits became hard and the tissue of the capsule wall became thinner. Unfertilized or aborted ovules developed to paraphyses (Oehler 1927), which stood between the dense packed seeds (Fig. 8F).

The harvested mature capsules all contained seeds. Average seed number was high (Tab. 1), resulting in an average seed set of 49% in *V. caerulea*, 45% in *V. clavata*, and 36% in *V. rosea*. However, the amount of developed seeds per capsule was highly variable in the three species (between 1% and nearly 100%; Fig. 8D-G).

At the study site, many mature capsules of *V. caerulea* and *V. clavata* showed large bite marks or were sometimes completely eaten (Fig. 8H, I).



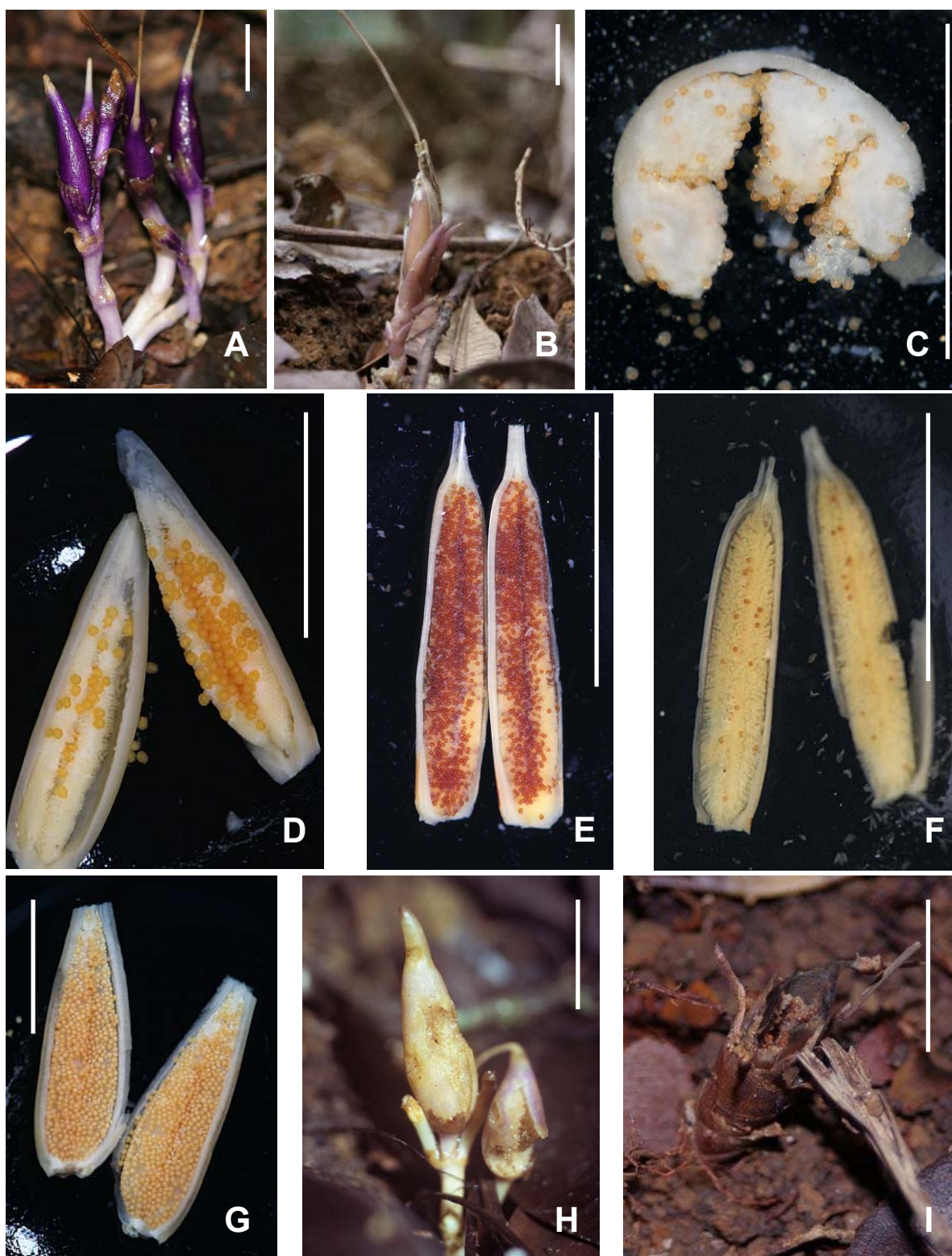


FIG. 1. Mature fruits of the focus species. Bulb-shaped fruits of *Voyria caerulea* (A) and *V. clavata* (B) at the study site; C: transversal section through the fruit of *V. caerulea*, showing the swollen spongy placenta tissue and the immersed seeds; D, G: longitudinal section through fruits of *V. clavata* with few (D) and many developed seeds (G); E, F: longitudinal section through fruits of *V. rosea* with few (F) and many developed seeds (E); H: fruits of *V. caerulea* at the study site with bite marks from rodents; I: completely eaten fruit of *V. clavata* at the study site. Scale bar = 1 cm (A, B, D-I) and 0.5 cm (C).

## DISCUSSION

### *Distribution of the plants at the study site*

The limited distribution of *Voyria* species to the region located in the west of the Nouragues creek might be caused by the edaphic conditions at the study site. Grimaldi & Riéra (2001) found that the Nouragues creek forms the natural boundary for two soil types of different origin. In the east, soils are descendants of metavolcanic rocks of the Paramaca Formation, which are composed of Ferralsols with plenty of ferruginous nodules. On the other hand, soils in the west are of ‘Caribbean’ type granite origin and represent very sandy organic-rich Cambisols, which pass laterally into Ferralsols. Ferruginous nodules are uncommon in this type of soil. *Voyria* species preferably grow on brown to black clayey to loamy soils (Maas & Ruyters 1986) and the availability of water further seems to be an important feature for all myco-heterotrophic plants (Leake 1994). Probably, the sandy organic-rich Cambisols offer better life conditions for the plants and/or for the associated fungi than the Ferralsols.

Most inflorescences were found clustered and at the same sites over the three year study period. Since we do not have much information about the life cycle of these plants [several *Voyria* species are supposed to be annual (Imhof *et al.* 1994) or hapaxanth (Franke 2002)], this phenomenon could be attributed to various factors. The plants might remain in the ground for several years, regularly producing reproductive shoots. On the other hand, the seeds might germinate near the mother plant, where they find good living conditions since the fungus partner is already present. A sharing of habitats by certain *Voyria* species could be a hint that these species are associated with the same fungus partner.

### *Floral nectar sugar composition*

The nectar of the three focus species was sucrose-dominant, which is characteristic for flowers visited by butterflies and long-tongued bees (Percival 1961, Baker & Baker 1983). Optimal nectar sugar concentration for long-tongued nectar-sucking insects are between 35-40% (Borrell 2004, 2007). Although our measurements yielded much lower values, they lie within the range of the results of other studies. In these, nectar sugar concentration of butterfly-pollinated flowers was between 15-25% (Proctor *et al.* 1996) and between 22-48% in euglossine-pollinated flowers (Roubik *et al.* 1995).

### *Floral scent composition*

As the dense vegetation in the understory limits long distance attraction by visual cues, floral scent plays an important role in the attraction of pollinators. Flowers of the studied focus species were highly fragrant and produced compounds that are common components of floral



perfumes (Knudsen *et al.* 2006). Kaiser (2006) analyzed the floral scent of *Voyria caerulea* in an earlier study. In contrast to the present study, the specimens investigated by him had a very intensive rosy-floral scent, characterized by a citrus-related note. His scent samples were dominated by (E)-nerolidol, geraniol, (E-E)-farnesol, and nerol. Thus, his results almost exactly match the qualitative and quantitative scent composition of *V. rosea*. The major compounds of *V. rosea* (e.g., (E)-nerolidol and geraniol) are also found in butterfly-pollinated flowers (Knudsen *et al.* 2006) and Andersson & Dobson (2003) demonstrated that sesquiterpenes are preferred by *Heliconius melpomene* (Heliconiidae)-butterflies. However, these major compounds are also reported from bee-pollinated flowers (Knudsen *et al.* 2006) since the floral fragrance spectrum of bee-visited flowers is very large and also contains compounds that attract butterflies (Proctor *et al.* 1996). Thus, the floral fragrance composition of the studied species might be able to attract both butterflies and euglossine bees.

#### *Flower visitors, floral morphology and flowering phenology*

Our observations confirm our initial hypothesis that flowers of the studied *Voyria* species are visited by long-tongued insects. *V. clavata* is specialized on visits by middle-sized euglossine bees with long tongues. These are the only insects that are able to crawl down into the flower and to reach the nectar with their long proboscides. The floral morphology automatically excludes butterflies and larger Euglossini (e.g., *Eulaema* spp.) that both possess long proboscides but are not able to enter the narrow corolla tube. The corolla tube length and its upper diameter perfectly fit to the body of the observed visitors (average tongue length of *Euglossa imperialis*: 32 mm; Roubik & Hanson 2004). The plant benefits strongly from the bees, as Euglossini are able to fly long distances and are supposed to be trapliners (Janzen 1971, Ackerman *et al.* 1982, Roubik & Hanson 2004). With these attributes, they represent reliable pollinators that transfer pollen from widely dispersed plant individuals.

The corolla tube length of *Voyria rosea* exceeds the proboscis length of Euglossini. This species rather seems to be adapted to butterfly species, which possess much longer tongues. The pinkish flower color is preferred by butterflies in behavioral studies (Proctor *et al.* 1996) and many pinkish flowers are reported to be associated with butterflies (Kevan 1983, Burr 1992). Moreover, *V. rosea* produces large corolla limbs, which are ideal landing platforms for these animals. Individual butterflies probably do not cover the same flight distances as euglossine bees do and are not supposed to be trapliners (Goulson 1997). However, *V. rosea* is very common at the study side and different inflorescences and populations stand closer together than in other *Voyria* species, so the visitors do not have to fly very far to encounter new flowers. The observed euglossine bees might play a minor role

as pollinators of this species because most corolla tubes are too long for them to receive nectar and unsuccessful flower visits would result in the avoidance of the flowers. Nevertheless, in smaller flowers they might reach the nectar when its level has risen in the course of the anthesis.

In *Voyria caerulea* the only observed flower visitor was a butterfly. However, the flower color, the corolla tube length, and the small corolla limb are characteristics that rather seem to be adaptations towards long-tongued bees, viz. Euglossini. Due to the large distances between single inflorescences (at least in our study area) it would be an advantage for this species to be pollinated by a long-distance flyer. Further observations are needed to test this hypothesis. The other studied *Voyria* species have a similar floral morphology to *V. caerulea* (long corolla tube, small corolla limb) and also show a scattered distribution. This leads to our suggestion that they are also pollinated by long-tongued insects that are strong flyers.

Thrips, which have been observed in the flowers of all focus species, probably play a lesser role in the cross-pollination of the plants. Nevertheless, they moved over the anthers and the stigmas as well, so they might be mediators for self-pollination.

The flowering phenology of the studied species is characterized by a pattern of ‘steady state’ (Gentry 1974, Bawa 1983). This pattern has the advantage that flower-visiting insects have a reliable source of nectar, which they can include in their daily flights. For the plants again, the extended blooming period increases the probability of simultaneous flowering individuals and thus the likelihood of pollen transfer by pollinators.

In contrast to humans, most insect groups are optically sensitive to ultraviolet light (Kevan 1983). The flowers of several *Voyria* species distinctly absorbed ultraviolet light, while the sandy soils the plants grow on slightly reflect it (Kevan 1979, Burr *et al.* 1995, Kevan *et al.* 1996). In this way, the optical contrast between flowers and their background increased, which might facilitate flower detection by pollinators. The stronger UV adsorption in the center of the flower could act as a mark, that guides the floral visitor to the nectar (Proctor *et al.* 1996). Studies on the floral UV reflective characteristics of other myco-heterotrophic plants show that UV absorption is more common than UV reflection (Takahashi *et al.* 1993, Burr *et al.* 1995). Since most myco-heterotrophic plants share similar environmental conditions (Leake 1994), UV absorption could be an adaptation to their habitats.

The focus species possessed P/O ratios smaller than 100. According to Cruden (1977), P/O ratios reflect the efficiency of pollen transfer from the anthers to the stigma. Low P/O ratios, like the ones observed, stand for a very efficient transfer. This is the case in facultative

autogamous plants as well as in obligately xenogamous plants that disperse their pollen in small packages ('sweepstake reproduction'; Cruden 1977). Cruden (1981) further showed that large stigmatic areas in relation to pollen-bearing areas on the pollinating agent additionally increase the efficiency of pollen transfer. In the studied species, pollen was packed together in larger clumps, either by being interweaved through a net of pollen tubes (*Voyria caerulea*, *V. rosea*) or by being stuck together through a jelly-like mass (*V. clavata*). Moreover, all plants possessed a very large stigmatic area in relation to the small pollen-bearing area on the insect. As a consequence, pollen transfer in the studied plants should be very efficient and one flower visit would probably be enough to fertilize a large number of ovules. On the other hand, stamens and stigmas of *V. caerulea* and *V. rosea* stand close together and pollen grains were observed to germinate within the anthers of mature flower buds. Thus, self-pollination is very likely in these species. Oehler (1927) already demonstrated for *V. rosea* that pollen tubes of self-pollen penetrate the margin of the stigma, proceed to the ovary, and fertilize the ovules. The fruit-production of the bagged *V. rosea* inflorescences in our experiment confirm Oehler's results. Nevertheless, even if self-pollination is possible, the pollen tubes of self-pollen need some time to germinate and to reach the ovary. During this phase, cross-pollen from a pollinating agent may arrive at first at the stigma and successfully fertilize the ovules ('competing self-pollination'; Lloyd 1992). Self-pollination might thus in *Voyria* just represent a kind of emergency mechanism in the absence of pollinators. Therefore, we suggest that *V. caerulea* and *V. rosea* are facultative autogamous. However, in *V. clavata*, anthers are widely separated from the stigma surface and self-pollination by pollen-tube growth is very unlikely. This species hence should be considered obligately xenogamous.

#### *Seed set and dispersal*

Since *Voyria caerulea* and *V. rosea* were facultative autogamous and autogamous plants usually have very high fertilization rates of nearly 100% (Ornduff 1969, Takahashi *et al.* 1993, Zhang & Saunders 2000, Lehnebach *et al.* 2005), high seed sets were expected in the two species. This was not the case. Average seed set was between 36-49% and varied strongly. On the other hand, myco-heterotrophic species largely depend on the energy supply of their fungus partner and seed set also seems to be strongly resource-limited, in xenogamous as well as in autogamous plants (Oehler 1927, Ushimaru & Imamura 2002). As resource limitation probably also had an impact on the seed production of the studied plants we cannot conclude that the observed seed set represents the real amount of fertilized ovules. Average seed set and its strong variation rather have to be considered a result of both fertilized ovules

and available resources. This is probably also true for the xenogamous *V. clavata*, which showed strong variation in seed set as well.

The seeds of *Voyria caerulea* and *V. clavata* were supposed to be dispersed by water when the indehiscent fruits begin to decay (Maas & Ruyters 1986, Leake 1994). Zoochorous seed dispersal by ants, earthworms, and birds was discussed for other *Voyria* species (Beccari 1880, Maas 1979, Imhof *et al.* 1994). Our observations of gnawn fruits indicate that rodents might be involved in the seed dispersal of these species. Many morphological characteristics of the capsules stress the possibility that endozoochorous dispersal plays a bigger role than dispersal by water: the fruits develop close to the ground, never open, and the seeds are immersed in a fleshy placental tissue. Of course, after being gnawn, some seeds remain within the decaying capsule and are probably washed out by rain. Wagner (1979) noted that rodents graze the berries of the myco-heterotrophic genera *Pityopus* and *Hemitomes* (Monotropaceae). The same mammals are supposed to dig up and eat the fruit bodies of ectomycorrhizal fungi that are associated with these plants (Leake 1994). Leake (1994) suggests that these rodents might act as dispersal vectors for both organisms, ensuring a co-dispersal of seeds and spores. This kind of co-dispersal would offer the plants the opportunity to colonize new areas where the fungus-partner has not been present before. Although we have not observed rodents eating the fruit bodies of *Glomus* sp., which is the fungus-partner of *Voyria* (Bidartondo *et al.* 2002), unknown fruiting fungi were common in the habitat of the investigated plants.

In contrast to the other species, the capsules of *Voyria rosea* were very hard, never fleshy, and their wall became thinner with the course of fruit-maturation. Dispersal by water seems to be more likely in this species although open fruits were never observed.

### Conclusion

As it is intrinsic to myco-heterotrophic plants, *Voyria* species are confronted with a number of ecological factors that affect the selection of their breeding systems. *Voyria* species depend on their fungus partners in the choice of a habitat (Furman & Trappe 1971) and therefore often grow in pollinator-unfavorable environments. The population density is low and flowering individuals are often separated by several hundreds of meters. Additionally, the density of potential insect pollinators in the understory is low, which entails a strong competition for these among the plants flowering in this habitat. Several *Voyria* species are supposed to be annual (Imhof *et al.* 1994) or hapaxanth (Franke 2002). This implies that strong pressure is exerted on each individual for a successful seed production to assure the survival of the species. All of these factors lead to the suggestion that theoretically, the appropriate reproductive system for the genus *Voyria* should be self-fertilization (Solbrig 1976, Lloyd

1992). Selfing assures seed production when cross-fertilization is unavailable. Further, due to the genetic similarity to the adapted parental generation, progeny-fitness is high if the offspring grows in the same environment. In addition, energy that is normally expended on the attraction of pollination vectors can be saved to support seed production (Solbrig 1976, Catling 1990, Lloyd 1992). However, relying on inbreeding can lead to serious disadvantages. Gene flow between individuals is interrupted and can result in inbreeding depression (Ornduff 1969, Lloyd 1992). The adaptive capability to react on changing environmental conditions is limited due to a lowered genetic variability and might additionally restrain the plants from colonizing new habitats (Lloyd 1992). Therefore, a mixed strategy, ensuring both seed set and gene flow, appears to be the most advantageous system for myco-heterotrophic plants.

Our results confirm the initial hypothesis and show that the studied species have evolved mechanisms that ensure both seed set and gene flow. The flowers of the three focus species were visited by long-tongued insects that were attracted by means of bright colors, floral scent and nectar as a resource. *Voyria clavata* was highly adapted to pollination by certain euglossine species. These bees were reliable visitors since they perform trap-lining and fly long distances (Roubik & Hanson 2004). Therefore, seed set was almost always guaranteed by their visits and *V. clavata* was able to evolve obligate xenogamy. In contrast, *V. caerulea* and *V. rosea* were less specialized towards a certain pollinator group. The visitors of these plants were irregular (at least in the rainy season) and more unreliable pollinating agents. To ensure seed production if pollen transfer by visitors fails, *V. caerulea* and *V. rosea* have conserved autogamy as a means of emergency pollination. Since other myco-heterotrophic species are confronted with similar selection factors, a mixed selfing-outcrossing strategy might be quite abundant in this lifeform.

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Female *Euglossa stilbonota* hanging with her mandibles on a leaf of *Calathea propingua* (Marantaceae) to stow the pollen loads of *Episcia sphalera* (Gesneriaceae) from its dorsal mesosoma into the corbiculae.



Female *Euglossa* sp. during resin-collection at the trunc of a *Protium* sp. (Burseraceae).

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## THE REPRODUCTIVE BIOLOGY OF EUGLOSSINE-POLLINATED UNDERSTORY PLANTS IN A LOWLAND RAINFOREST IN FRENCH GUIANA – A COMPARATIVE STUDY

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### ABSTRACT

The reproductive biology of seven euglossine-pollinated, non-perfume-flowered understory species was studied in French Guiana. The plants, namely *Bonaifousia disticha*, *Mandevilla rugelosa* (Apocynaceae), *Costus congestiflorus* (Costaceae), *Episcia sphalera* (Gesneriaceae), *Calathea erecta*, *Ischnosiphon martianus* (Marantaceae), and *Rapatea paludosa* (Rapateaceae), were visited by the bees for their nectar, pollen, or both. All species had a flowering phenology of ‘steady state’ and either flowered the year round or only during the rainy season. Pollen grain numbers per flower were much higher in pollen than in nectar hosts. With the exception of *R. paludosa*, P/O ratios were very low (35.8 – 185.8), which is usually an indicator for autogamy. However, it is suggested that in the majority of the studied species this is related to a highly efficient outcrossing, since autonomous self-pollination is mostly ruled out by their floral morphology. The nectar-producing flowers had large nectar tubes, whose length slightly correlated with the tongue-length of the visiting bees. Floral nectar was sucrose-dominant and showed average concentrations between 25.5% and 29.7%. Mean daily nectar amounts were large (3.7 – 51.1 µl) and correlated with the body length of the visiting bees. In most plants, nectar production was highest in the morning and strongly decreased at the beginning of noon. Euglossine bees were the major visitors of the flowers and pollinator specificity of the plants was high. Usually one bee species or a bee subgenus dominated the visits of one plant species. Differences in the visitor spectrum were probably related to floral morphology and to significantly different daily floral nectar amounts between plant species. The most common flower visitors were bees of the *Euglossa* subgenus *Glossura*, a fact that could be related to the high abundance of these species. Large-bodied euglossines were generally only observed at flowers that produced high amounts of nectar and grew at open sites in the forest. This might further indicate that these bee species prefer

foraging at the forest edge. While pollinator specificity of the plants was high, each bee species visited a large spectrum of nectar hosts. As flowers of most plant species placed pollen on the proboscis of the visitors, mixing of heterospecific pollen occurred. All euglossine species were present at nectar flowers between 9:00-16:00 h. However, activity in the afternoon was slightly lower than in the morning, which is probably due to the decreased nectar availability in the afternoon. Visitor activity at the pollen flowers of *R. paludosa* was highest shortly after sunrise and had usually ceased until noon. The behavior during foraging bouts of all observed euglossine species, led to the assumption that the bees learn to discriminate between rewarding and non-rewarding flowers and that they are able to detect odor marks of con- and heterospecifics. High fruit and seed set further indicate that euglossine bees are effective pollinators of the plant species investigated.

Key words: Euglossini, butterflies, pollination biology, French Guiana, lowland rainforests, understory, *Bonaifousia*, *Calathea*, *Costus*, *Episcia*, *Ischnosiphon*, *Mandevilla*, *Rapatea*.

## INTRODUCTION

Bees represent the world's most important pollinator group of zoophilous plant species (Michener 2000). Besides the pollination of many agricultural crops, they ensure the sexual reproduction of a large part of natural vegetation. Therefore, they do not only have an immense economical impact but, even more important, represent essential organisms in environmental concerns and conservatory efforts. In the lowland and mid-elevated rain forests of the Neotropics, flower visits of most melittophilous plants are quantitatively dominated by four large bee tribes, i.e., the Centridini, Euglossini, Meliponini, and Xylocopini (Roubik *et al.* 2003). Bees of the tribe Euglossini (Hymenoptera: Apidae) clearly differ from the other bee groups in having a unique combination of morphological and ecological peculiarities. Euglossini particularly became famous for the scent-collecting behavior of the males, which gather fragrances from floral and other resources and store them in large tibial pouches of their hindlegs (e.g., Vogel 1966, Dodson *et al.* 1969, Roubik & Hanson 2004). The purpose of scent-collection can probably be seen in the territorial behavior of the bees and might further play a role in their mating (Roubik & Hanson 2004, Zimmermann *et al.* 2006, Eltz *et al.* 2007). In their morphology, the most conspicuous feature of both sexes is the extremely prolonged tongue (12-43 mm; Roubik & Hanson 2004), which in some species even exceeds the body length. Males and females are known to be strong flyers, covering large distances during foraging (Williams & Dodson 1972, Roubik & Hanson 2004). Furthermore, they are

expected to trap-line, which means that each individual follows an established foraging route; new flowers are included in the original track probably without considering optimal energetics of flight routes (Janzen 1971, Ackerman *et al.* 1982). Euglossines have extended life spans of ca. two months in some *Euglossa* spp. and ca. 4-5 months in *Eulaema* spp. and *Exaerete frontalis* (Dodson 1966, Ackerman 1981, Zimmerman & Madriñan 1988).

The food hosts of these bees are predominantly found in the forest understory (Ackerman 1985). This habitat is characterized by low solar radiation, high relative humidity and diurnal stability of air temperature (Kato 1996). Similar to canopy species, population densities are low in the understory, and pollinators have to cross wide distances to accomplish successful outcrossing between conspecifics (Janzen 1971, Bawa *et al.* 1985a, Bawa 1990). While canopy plants generally attract a broad species spectrum of foraging insects by offering high numbers of flowers in a short time (Frankie *et al.* 1983, Bawa *et al.* 1985b, Bawa 1990, Kress & Beach 1994, Momose *et al.* 1998), the understory does not provide enough energy to make large floral displays possible. Instead, plants produce few flowers daily over an extended period of time ('steady state flowering'; Gentry 1974, Bawa 1983) and restrict access to the floral resources by the development of long corolla tubes (Roubik 1989). Most euglossine nectar hosts are therefore supposed to be exclusively pollinated by bees of this tribe and benefit from their trap-lining behavior (Roubik & Hanson 2004). Since the flowers of one plant do not supply enough nectar to compensate the energy requirements of the foragers, the bees are obliged to visit various plants and thereby contribute to cross-pollination and large-distance gene flow (Janzen 1971, Heinrich & Raven 1972).

Although extended lists of euglossine-pollinated nectar and pollen hosts exist (Dodson 1966, Ackerman 1985, Ramírez *et al.* 2002, Roubik & Hanson 2004), in-depth studies are scarce and generally focus on single plant taxa or genera (Kennedy 1978, Schemske 1981, Ackerman *et al.* 1982, Schemske & Horvitz 1984, Kay & Schemske 2003, Borrell 2005, Almeida Barreto & Freitas 2007, Leite & Machado 2007). Therefore, our knowledge about the reproductive biology of these plants and the importance of pollinator specificity for reproductive isolation and directed pollen flow in euglossine-pollinated plant communities is poor. Studies on understory communities of hummingbird-pollinated *Heliconia* species have shown, that the plants possess a high pollinator specificity, while each visitor species uses a broad spectrum of nectar hosts (Stiles 1975, 1978). Reproductive isolation is attained by spatial and temporal flowering patterns, different corolla tube lengths, contrasts in offered daily nectar amounts, and the species-specific pollen placement at different spots on the birds bill (Stiles 1975). Euglossine pollination systems share several traits with hummingbird

pollination systems. Especially hermit-hummingbirds (Phaethorninae) are also known to trap-line and to cross large distances during foraging (Feinsinger 1983). Population densities of *Heliconia* species visited by them are low (Stiles 1975). The plants show a steady state flowering pattern and the access to floral nectar is limited by long corolla tubes (Stiles 1975). Due to the high resemblance of the two systems, similar isolation mechanisms might have evolved.

We studied the reproductive biology of seven euglossine-pollinated plant species in the almost pristine lowland rainforest site Nouragues, French Guiana. Among these, five species offered nectar: *Bonaifousia disticha*, *Mandevilla rugelosa* (Apocynaceae), *Costus congestiflorus* (Costaceae), *Calathea erecta*, and *Ischnosiphon martianus* (Marantaceae). One species offered both pollen and nectar, i.e., *Episcia sphenoloba* (Gesneriaceae), and one exclusively pollen, i.e., *Rapatea paludosa* (Rapateaceae). The investigated plants were among the most frequent euglossine-pollinated understory taxa at the study site, which flower during the rainy season. They belonged to different life forms and occurred in different habitats (Tab. 1). Additionally, pollinator data of 29 further taxa were collected. Altogether, the studied plants represent ca. 70-80% of all euglossine-pollinated understory species at the study site, which flower during the rainy season. Our working hypothesis was that similar isolation mechanisms as they were found in hummingbird-pollinated plants can also be found in euglossine-pollinated food hosts. In order to test our hypothesis, we studied a large spectrum of reproductive traits, including floral morphology, P/O ratio, floral nectar, flowering phenology, flower visitors, and reproductive success.

## MATERIAL & METHODS

### *Study site*

The study was conducted during the early to midrainy season (January-June) of the years 2005 and 2006 in the environs of the Inselberg station of the Nouragues Natural Reserve in French Guiana (04°05' N, 52°41' W, 120 m asl.). Nouragues is located in primary lowland tropical rainforest with an annual rainfall of 2990 mm and a mean annual temperature of 26.3°C (Grimaldi & Riéra, 2001).

TABLE 1. General overview of the studied species' characteristics. Information about the species' distribution was obtained from TROPICOS (2008). Notes on the plants' habitat and values of population sizes, distances between populations and flower height above the ground are observed characteristics of the plants at the study site.

	Life form	Habitat	Distribution	Flowering plants per population	Distance between populations (m)	Height of flowers above ground (cm)
<b>Apocynaceae</b>						
<i>Bonaifousia disticha</i> (A. DC.) Boiteau & L. Allorge	Understory treelet	Forest interior, along trails, forest edges and gaps	French Guiana	15-100	> 600	50-400
<i>Mandevilla rugellosa</i> (Rich.) L. Allorge	Liana	Forest edges, rock savannas	Bolivia, French Guiana, Peru, Surinam, Venezuela	1-30	> 900	100-700
<b>Costaceae</b>						
<i>Costus congestiflorus</i> Gagnepain	Erect herb	Forest interior, usually epilithic on large granite rocks	Guianas, Venezuela, Amazonian Brazil	3-15	200-300	50-70
<b>Gesneriaceae</b>						
<i>Episcia sphenoloba</i> Leeuwenb.	Creeping herb	Low forests near rock savannas, epilithic	French Guiana	10-200	> 500	5-8
<b>Marantaceae</b>						
<i>Calathea erecta</i> L. Andersson & H. Kenn.	Erect herb	Forest interior	French Guiana, Venezuela, Amazonian Brazil	3-5	100-200	10-20
<i>Ischnosiphon martianus</i> A.W. Eichler ex Petersen	Erect herb	Along creeks and trails	Guiana, Brazil	1-5	100-200	40-130
<b>Rapateaceae</b>						
<i>Rapatea paludosa</i> Aubl.	Erect herb	Along creeks	Guianas, Colombia, Ecuador, Amazonian Brazil, Peru, Venezuela	3-10	100-200	50-70

### *Floral morphology and flowering phenology*

The floral morphology of the studied species was examined under a stereomicroscope. Flower number per inflorescence was counted and the nectar tube length of the flowers was measured (distance from the bottom of the nectar bearing part of the flower to the most inferior point where the pollinator's head can enter; caliper: 0.1 mm accuracy; Tab. 2).

Pollen/ovule ratios (P/O ratios) of alcohol-preserved material were determined after Cruden (1977). Ovules were counted under a stereomicroscope. Pollen number of one anther per flower was either counted under a stereomicroscope or determined by a CASY cell counter (SCHÄRFE System GmbH, Reutlingen, Germany). P/O ratio was calculated by multiplying the pollen number with the number of anthers per flower and dividing the result through the flower's ovule number. Pollen size was measured under a Zeiss DSM 942 scanning electron microscope (SEM; Zeiss, Oberkochen, Germany). For SEM observations, alcohol-preserved plant material was prepared by gradual dehydration in propanol, critical point drying, and sputtercoating with gold (Balzers Union Sputter Coater, Balzers, Liechtenstein; sample sizes are given in Tab. 3).

The flowering phenology was daily recorded for several plant individuals of each of the studied species (Tab. 2). Inflorescences of *Mandevilla rugellosa* were checked for flowers between 11 March and 5 June 2005, those of *Bonaifousia disticha* between 14 March and 9 May 2005, *Costus congestiflorus* between 11 April and 19 May 2005, *Episcia sphalera* between 27 April and 5 June 2005, *Rapatea paludosa* between 24 January and 9 April 2006, *Calathea erecta* between 3 February and 9 April 2006, and *Ischnosiphon martianus* between 11 February and 9 April 2006.

### *Floral nectar*

Daily nectar production, nectar sugar concentration, and nectar sugar composition were studied on flowers that were bagged with nylon netting to exclude flower visitors (Corbet 2003). Microcapillary pipettes of different sizes (1 µl, 2 µl, 3 µl, and 5 µl microcaps; Hirschmann, Germany) were used to withdraw the nectar. Flowers of *Mandevilla rugellosa*, *Bonaifousia disticha*, *Calathea erecta*, and *Ischnosiphon martianus* were sampled several times a day to measure the daily nectar production (between 8:00-16:00 h). In *Costus congestiflorus* and *Episcia sphalera* the nectar was only extracted once a day (between 14:00-16:00 h) since the flowers were seriously damaged at sampling. Nectar sugar analysis was carried out by high-performance liquid chromatography (HPLC). Samples were extracted from the flowers between 13:00-14:00 h, immediately transferred into undenaturated ethanol (70%), and stored at -27°C until analysis. Before the analysis, samples were dried in a



vacuum-centrifuge (Savant Speed Vac SC 110) at 65°C, subsequently redissolved in ultrapure water (HPLC-grade), and filtered (syringe nylon filter Acrodisk, Pall, USA; diameter: 4 mm, poresize: 0.45 µm). HPLC was equipped with a Waters 717 plus autosampler, Waters 510 HPLC pump, and a Waters 410 RI-detector with Waters CHM column-heater. Separation was achieved using a 72:28% acetonitril-water mixture as solvent system on a Waters High Performance Carbohydrate Column (4.6 x 250 mm, 60Å 4 µm). The system was monitored by the Waters Millennium Software 3.01 (sample volume: 10 µl, flow rate: 1.4 ml/min, temperature: 35°C, runtime: 12 min). Identification of sugar types and quantitative determination of sugar contents was achieved by comparing chromatograms and peak areas to standards (fructose, glucose, sucrose).

#### *Visitor observations*

Flower visitors of *Bonafousia disticha*, *Mandevilla rugellosa*, and *Episcia sphalera* were recorded from 9:00-16:00 h; *Costus congestiflorus* was observed from 9:30-15:30 h, *Calathea erecta* and *Ischnosiphon martianus* from 8:00-14:30 h, and *Rapatea paludosa* from 7:30-11:00 h. In each species, visitor observations were repeated for several days and at different plant individuals (*Bonafousia disticha*: seven days, four plants; *Mandevilla rugellosa*: 14 days, six plants; *Episcia sphalera*: six days, large population of > 50 flowering plants; *Costus congestiflorus*: four days, two populations of twelve flowering plants each; *Calathea erecta*: five days, two plants; *Ischnosiphon martianus*: five days, four plants; *Rapatea paludosa*: eight days, population of ten flowering plants). Minimum observation distance to the flowers was 2 m. Voucher specimens of the visiting bees were collected from each plant species on separate days to avoid disturbance to pollinator activities. The bees were identified using the reference collection of G. Gerlach (Botanical Garden Nymphenburg, Munich) and are deposited in the collection of the first author (Hentrich FGIC 19, 27, 29, 42, 49, 50-65).

#### *Fruit and seed production*

Fruiting and non-fruited plants were counted in all investigated plant populations at the study site. The number of developed fruits and non-fruited flowers as well as the seed number per fruit were determined for several mature infructescences in each of the studied species (Tab. 6).

#### *Statistical analysis*

All statistics but  $\chi^2$ -tests were conducted in SigmaStat (v. 3.5, Systat Software Inc.). Pearson product moment correlation was used to calculate if there were significant correlations among

floral characteristics of the plant species and between floral characteristics and flower visitor morphology. Analysis of variance (ANOVA) was performed to calculate if there were significant differences in the nectar amounts and concentrations, and the nectar tube length of the studied plant species. In addition, a  $\chi^2$ -test was used to calculate if there were significant differences in visitor spectrum and visiting time between and within plant species (Zar 1998). Body sizes and tongue lengths of euglossine bees were taken from Roubik & Hanson (2004), and from own measurements with a caliper (accuracy: 0.1 mm) of fresh bees collected at the study site. Average values were: *Euglossa chalybeata*: body length (bl): 14.0 mm, extended tongue length (tl): 30.0 mm (n = 3); *Euglossa intersecta*: bl: 17.6 mm, tl: 33.0 mm (n = 2); *Euglossa piliventris*: bl: 15.7 mm, tl: 32.0 mm (n = 3); *Euglossa* sp. 3: bl: 11.0 mm, tl: 19.4 mm (n = 3). For Pearson product moment correlations measured values of an average visitor were used. These values were obtained by multiplying the body lengths or tongue sizes with the visiting frequency of the respective euglossine species at each plant species and summing up the values of all euglossine visitors of one plant species.

## RESULTS

### *Floral morphology and flowering phenology*

The flowering phenology of all studied species followed the pattern of ‘steady state’ (Gentry 1974, Bawa 1983), which is characterized by large inflorescences that flower over a long period of time, but have only one or few flowers open a day. With the exception of *Episcia sphalera*, the flowers of all plants lasted only 24 hours or less. In *E. sphalera* the flowers were protandrous with the male phase at the first and the female phase at the second day. In the female phase, the pistil was elongated, bringing the stigma close to the corolla entrance while the wilted anthers were retracted. Opening of the flowers in all species usually started at sunrise (ca. 6:15 h), but could be delayed until 9:00 h if the weather was cloudy or the plants were standing in shaded places. Closing time of the flowers was quite uniform among the species studied. Normally, they lasted the entire day until the night (two days in *E. sphalera*, respectively). At night or the next morning, the corolla lobes turned inward and the flower fell off the day after, or two days after anthesis. The exceptions were *Costus congestiflorus*, whose flowers usually started wilting at 14:30 h and nearly completely closed until 17:00 h, and *Rapatea paludosa*, whose petals already wilted at noon. Daily weather conditions also influenced flowering. On dry, sunny days, the flowers of *Mandevilla rugellosa* already withered at noon and strong rainfalls could easily destroy the delicate corollas of *M. rugellosa*, *C. congestiflorus*, *Ischnosiphon martianus*, and *R. paludosa* earlier the day. Within

an inflorescence, flower opening proceeded from the base to the top in most species studied. In the capitate inflorescences of *R. paludosa* no direction pattern of flower maturation could be recognized. Usually one single flower opened a day, seldom two (sometimes three in *R. paludosa*) and there were regular periods of 1-3 days without any new open flowers. In the inflorescences of *Calathea erecta*, always several flowers (2-7), belonging to different flower groups, opened each day.

The flowering time of entire inflorescences depended on their flower number and could take few days (*Bonafousia disticha*, *Episcia sphalera*), 2-3 weeks (*Mandevilla rugellosa*, *Costus congestiflorus*, *Ischnosiphon martianus*) or even some months (*Calathea erecta*, *Rapatea paludosa*; Tab. 2). The blooming period of *B. disticha*, *C. congestiflorus*, and *E. sphalera* was restricted to few weeks in the rainy season. In each of these species, all observed mature plants at the study site flowered within one and a half months and produced large flowering peaks. The other studied species probably flowered the year round or at least for the entire rainy season, since they regularly developed new inflorescences. In these species, the blooming period of a new inflorescence usually had already started when flowering of another inflorescence ended.

With respect to floral morphology, *Rapatea paludosa* was the only studied species whose flowers exclusively offered pollen as a resource for visitors. The erect inflorescence of the plant is a capitulum, which stands on a long peduncle 50-70 cm above the ground and is subtended by two foliaceous green bracts (Fig. 1H). It bears several dozens of short pedicellate actinomorphic flowers (Tab. 2), which consist of 16 brownish bracts, three small white inconspicuous sepals, three large delicate yellow petals, and six anthers that are arranged in a cone around a single pistil with a small pointing stigma emerging at its top. Each anther dehisces by a single apical pore, which leans against the style.

The zygomorphic flowers of *Episcia sphalera* are located on a few-flowered cymose inflorescence (Tab. 2), close to the ground (Fig. 1D, F). They were visited for nectar and also for pollen, although they do not represent typical pollen flowers. The nectar is hidden in a short spur behind the ovary, which is surrounded by five green free sepals (Fig. 1D). The anthers are pairwise arranged above the flower entrance and deposit their pollen on the visitors' dorsal heads and mesosomata by small slitlike openings. In the center of the two filament pairs the papillous stigma arises on an elongated style. The lower portion of the filaments is sheath-like and channels the tongue of the visitor to the spur. Among the populations studied, ca. one third of the flowers had purple nectar guides in the corolla entrance, while the remaining flowers were entirely white (n = 69).

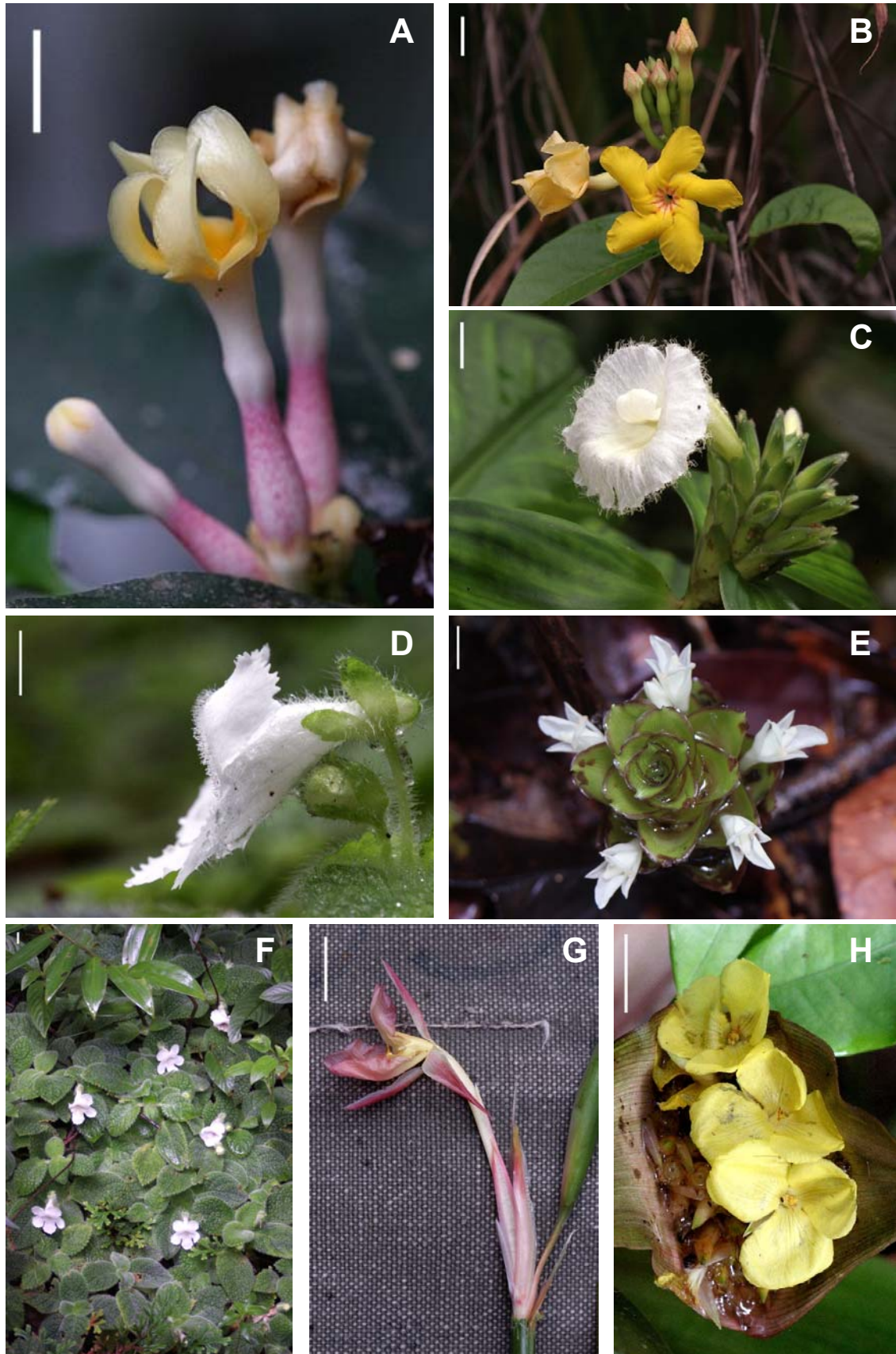


FIG. 1. Inflorescences of *Bonafousia disticha* (A), *Mandevilla rugellosa* (B), *Costus congestiflorus* (C), *Episcia sphalera* (D, F), *Calathea erecta* (E), *Ischnosiphon martianus* (G, flower-protecting bract removed), and *Rapatea paludosa* (H) at the study site. Scale bar = 1 cm.

All of the five corolla lobes but the lip are densely covered by long glandular trichomes at the height of the flower entrance. The purpose of the sticky liquid they produce was probably to keep off pollen robbers from the anthers. A covering with the liquid of the visitors' back for a better pollen adhesion did not occur, since the trichomes were not long enough to come in contact with the insects that regularly entered the flower.

Flowers of the other species studied were exclusively visited for their nectar, which is hidden in long corolla tubes (Tab. 2) and can only be gathered by long-tongued insects. Nectar tubes of these species were significantly longer than those of *Episcia sphalera* and there were also significant differences in the nectar tube length between species with extended corolla tubes (Kruskal-Wallis ANOVA on ranks;  $H = 223.463$ ;  $df = 5$ ;  $P < 0.001$ ; Dunn's post-hoc test;  $P < 0.05$ ; Tab. 2). Pollination mechanisms in the species with extended corollas were highly complex, with the flowers often having secondary pollen presentation (Apocynaceae, Marantaceae) and a floral morphology that was outlined to fit to a certain pollinator group. The inflorescences of *Calathea erecta* (Marantaceae) arise on separate shoots, stand only few centimeters above the ground, and contain more than hundred white flowers (Tab. 2), which are spirally arranged in groups of several pairs (2-7; Fig. 1E). The flower number of *Ischnosiphon martianus* (Marantaceae) is much lower (Tab. 2). About one dozen reddish flowers are located pairwise and distichously on an elongated inflorescence axis that arises from a leaf axil at the height of 40-130 cm (Fig. 1G). *Ca. erecta* and *I. martianus* use a special trigger mechanism for pollination. The asymmetric flowers consist of three inconspicuous sepals and three fused petals that end in separate corolla lobes. Three of the four stamens are modified to large petaloid staminodes that surround the voluminous style. Male and female organs are fused with the corolla tube. One staminode serves as a lip-like landing platform for the visitor. During flower maturation, the single fertile anther deposits its pollen on a stylar depression below the cup-shaped stigma. At flower-opening, the tensed style is held downward by a hood-shaped staminode. When the visiting bee introduces its proboscis, a 'trigger'-called appendage (Kennedy 1978) at the hood-shaped staminode is pushed, and the style is released, bending explosively upwards. Thereby, the stigmatic cavity scrapes off previously deposited pollen from the bees proboscis and pollen from the stylar depression together with a sticky secretion produced at the edge of the stylar depression is placed at the same position. The explosive mechanism can only be released once and takes a split of a second. Therefore, pollination of the flower is usually restricted to the first visitor. The movement of the style is stopped by a fleshy staminode, located directly above the hood-shaped one. The long nectar tubes of the flowers are well protected against nectar robbers by

several bracts and bracteoles. Additionally, in *Ca. erecta*, the space between the outer bract and the grouped flowers is filled with a mucilaginous liquid.

The studied Apocynaceae, *Bonafousia disticha* and *Mandevilla rugellosa*, have actinomorphic flowers with five small green free sepals and a long nectar tube ending in a turned five-lobed corolla. In *M. rugellosa*, several dozen yellow-colored flowers (Tab. 2) are arranged on terminal racemes, usually at a height of one to several meters above the ground. The center of the corolla entrance has red guide marks and its lobes are a broad landing platform for visitors (Fig. 1B). The corolla lobes of the purple/white/yellow-colored (from base to top) flowers of *B. disticha* do not completely open at anthesis and stay turned above the flower entrance. They represent a bad landing platform for insects (Fig. 1A). The inflorescences of this species are few flowered cymes (Tab. 2), which arise from the axils of young leaves at a height of 50-400 cm above the ground. In both species, the anthers are basally adnate to the corolla tube and form a stigma-surrounding cone right below the corolla entrance. At anthesis, the pollen grains are released into a chamber in the center of the cone that lies above the stigma. The stigma itself has a complex structure and is divided into different functional parts. Only the basal section, which has no contact to the self-pollen, is receptive. The stigma apex encloses the pollen chamber, while the lateral regions secrete a sticky liquid (Schick 1980). At a flower visit, the insect's tongue has to pass through one of five openings at the base of the cone to reach the nectar. When the tongue is withdrawn after drinking, it is directed to the center of the cone, and foreign pollen, already sticking at the visitor's tongue from another flower, is scraped off by stiff hairs at the stigma base. Few millimeters upwards, the tongue gets covered by the sticky liquid of the stigma's secretory region and passes the pollen chamber, where it picks up pollen before the insect leaves (Schick 1982, Fallen 1986).

The zygomorphic flowers of *Costus congestiflorus* are spirally arranged on a spike, which terminates the leafy stem 50-70 cm above the ground (Fig. 1C). About one dozen flowers can be found in each inflorescence (Tab. 2). Three green fused bracts and three green basally fused sepals and petals surround the long nectar tube, which is made up of five united staminodes and a sole petaloid stamen. The staminodes terminate in a broad white labellum with the large stamen standing above the flower entrance. The elongated pistil is embraced by the two thecae and terminates in a large cup-shaped papillous stigma at the top of the anther. When a flower visitor crawls into the corolla entrance, it has to push the petaloid stamen upwards to advance. Thereby, it passes the brush-like stigma first and subsequently touches the anther with its dorsal head and mesosoma.

Many flowers of the studied species were highly fragrant during anthesis. The floral perfume was throughout pleasant to the human nose. Flowers of *Bonafousia disticha* had a fruity, apple-like perfume, flowers of *Costus congestiflorus* smelled very sweet, like vanilla, while the scent of *Ischnosiphon martianus* resembled that of raspberry fizzy lemonade and that of *Rapatea paludosa* was similar to honey, sometimes slightly alcoholic. In *Mandevilla rugellosa*, *Episcia sphalera*, and *Calathea erecta* no floral scent could be olfactorily perceived.

Considering the number of pollen grains produced by each flower, pollen offering flowers had much higher amounts of pollen than nectar flowers (Tab. 3). In *Episcia sphalera* and *Rapatea paludosa*, pollen numbers were at least ten times as high as in the nectar flowers of the other species studied. The lowest pollen numbers were counted in *Calathea erecta* and *Ischnosiphon martianus*. There was a tendency to a negative correlation between pollen number and pollen size ( $r = -0.740$  ;  $P = 0.0572$ ). *E. sphalera* possessed the smallest, *Ca. erecta* the largest pollen grains (Tab. 3). Ovule numbers were highest for *E. sphalera*, with several hundred ovules per flower, and lowest for *R. paludosa* and the two Marantaceae, bearing only 1-3 ovules (Tab. 3). *Bonafousia disticha*, *Mandevilla rugellosa*, and *Costus congestiflorus* possessed intermediate ovule numbers between 24-112. The resulting P/O ratios were very low, ranging between  $35.8 \pm 8.2$  in *B. disticha* and  $185.8 \pm 41.7$  in *I. martianus*. The only exception was *R. paludosa*, which showed a high P/O ratio of  $15,190 \pm 1,649$ .

TABLE 2. Floral nectar tube length, flower number per inflorescence, duration of flowering of individual flowers, entire inflorescence, and plant individuals of the species studied. Values are presented as means with standard deviations. Sample sizes are given in brackets. Small letters behind the values of average floral tube lengths indicate if they are significantly different from the other values (Kruskal-Wallis ANOVA on ranks; Dunn's post-hoc test).

Species	Length of nectar tube (mm)	Flowers per inflorescence	Anthesis of flower (d)	Flowering time of inflorescence (d)	Flowering time of plant individual(d)
<i>Bonafousia disticha</i>	29.5 ± 1.6 (53) <sup>b</sup>	2.8 ± 2.1 (575)	1	4.5 ± 3.6 (231)	10.3 ± 7.5 (36)
<i>Mandevilla rugelosa</i>	38.7 ± 2.1 (89) <sup>a</sup>	25.4 ± 16.1 (100)	1	19.0 ± 11.4 (44)	probably year round (8)
<i>Costus congestiflorus</i>	34.2 ± 1.7 (7) <sup>a, b</sup>	11.7 ± 6.3 (48)	1	15.0 ± 7.5 (45)	15.0 ± 7.5 (45)
<i>Episcia sphalera</i>	22.8 ± 0.8 (55) <sup>c</sup>	2.4 ± 1.2 (103)	2	5.1 ± 4.2 (57)	5.1 ± 4.2 (57)
<i>Calathea erecta</i>	33.0 ± 5.4 (67) <sup>b</sup>	152.8 ± 36.2 (10)	1	48.0 ± 8.6 (7)	probably year round (7)
<i>Ischnosiphon martianus</i>	38.4 ± 4.0 (32) <sup>a</sup>	15.5 ± 3.3 (19)	1	19.9 ± 6.6 (9)	probably year round (9)
<i>Rapatea paludosa</i>	-	63.7 ± 23.9 (15)	1	> 65 (11)	probably year round (11)



TABLE 3. Number of pollen grains per flower, pollen grain size, number of ovules per flower, and pollen/ovule ratio (P/O ratio) of the species studied. Pollen grain sizes refer to the diameter if pollen was spherical. Otherwise, length (first row) and width (second row) of pollen grains is presented. Values are means with standard deviations. Sample size is twelve for all characteristics studied.

Species	Pollen grains per flower	Pollen grain size (µm)	Ovules per flower	P/O ratio
<i>Bonafousia disticha</i>	4,118 ± 915	40.5 ± 1.8 27.5 ± 1.3	112 ± 17	35.8 ± 8.2
<i>Mandevilla rugellosa</i>	2,326 ± 292	91.0 ± 3.1	51 ± 5	46.2 ± 6.4
<i>Costus congestiflorus</i>	2,335 ± 272	67.5 ± 8.1	26 ± 4	91.7 ± 19.0
<i>Episcia sphenoloba</i>	62,347 ± 5,384	23.3 ± 1.0	680 ± 117	94.1 ± 15.5
<i>Calathea erecta</i>	255 ± 18	111.9 ± 5.1	3 ± 0.3	83.3 ± 6.3
<i>Ischnosiphon martianus</i>	192 ± 31	88.9 ± 3.9	1 ± 0.3	185.8 ± 41.7
<i>Rapatea paludosa</i>	45,570 ± 4,947	43.6 ± 1.5 28.0 ± 1.9	3 ± 0	15,190.0 ± 1,649.0

TABLE 4. Means and standard deviations for daily floral nectar production, nectar sugar concentration, and nectar sugar composition of the species studied. Sample sizes are given in brackets. Small letters behind the values indicate if they are significantly different from others in the same column (Daily floral nectar production: Kruskal-Wallis ANOVA on ranks; Dunn's post-hoc test; nectar sugar concentration: ANOVA; Holm-Sidak post-hoc test). S/H-ratio: [%sucrose]/[%glucose + %fructose].

Species	Daily floral nectar production (µl)	Nectar sugar concentration (% w/w)	Sucrose (%)	Glucose (%)	Fructose (%)	S/H-ratio
<i>Bonafousia disticha</i>	21.9 ± 10.5 (19) <sup>a, b</sup>	26.5 ± 2.8 (18) <sup>a</sup>	96.6 ± 8.0	1.5 ± 3.9	1.9 ± 4.1	126.8 ± 223.6
<i>Mandevilla rugelosa</i>	51.1 ± 16.6 (17) <sup>a</sup>	29.4 ± 3.0 (13) <sup>b</sup>	41.3 ± 14.5	29.7 ± 7.0	29.1 ± 7.4	0.9 ± 0.9
<i>Costus congestiflorus</i>	22.7 ± 6.9 (8) <sup>a, b</sup>	25.5 ± 4.9 (10) <sup>a, b</sup>	100.0 ± 0.0	-	-	-
<i>Episcia sphalera</i>	3.7 ± 0.9 (50) <sup>c</sup>	29.7 ± 3.9 (13) <sup>b</sup>	83.8 ± 2.6	0.6 ± 1.3	11.7 ± 10.2	5.3 ± 1.0
<i>Calathea erecta</i>	6.6 ± 1.8 (8) <sup>b, c</sup>	25.7 ± 4.2 (4) <sup>a, b</sup>	97.2 ± 3.0	1.1 ± 1.9	1.8 ± 1.3	42.1 ± 22.7
<i>Ischnosiphon martianus</i>	11.0 ± 2.4 (15) <sup>b</sup>	28.3 ± 1.8 (5) <sup>a, b</sup>	92.0 ± 1.5	4.1 ± 0.6	3.9 ± 1.0	11.9 ± 2.4

### Floral nectar

Average daily nectar production ranged between 3.7-51.1  $\mu\text{l}$  and differed significantly between species (Kruskal-Wallis ANOVA on ranks;  $H = 104.744$ ;  $df = 5$ ;  $P < 0.001$ ; Dunn's post-hoc test;  $P < 0.05$ ; Tab. 4). The highest amounts of 51.1  $\mu\text{l}$  were measured in the large-flowered *Mandevilla rugellose*, whereas the other species showed much lower values of 22  $\mu\text{l}$  (*Costus congestiflorus*, *Bonaifousia disticha*), 11  $\mu\text{l}$  (*Ischnosiphon martianus*), 6.6  $\mu\text{l}$  (*Calathea erecta*) or 3.7  $\mu\text{l}$  (*Episcia sphalera*). While in *M. rugellose* and *B. disticha* the nectar level of bagged flowers rose in the course of day, sometimes even reaching the corolla entrance, nectar amounts in *Ca. erecta* and *I. martianus* flowers did not increase much and remained at a height of 0.8-1.4 cm above the ovary (mean: 0.96 cm,  $n = 45$ ). Nectar production in *M. rugellose* and *B. disticha* was highest in the morning and strongly decreased until noon (Fig. 2A, B). Flowers of *Ca. erecta* and *I. martianus* did only secrete small nectar amounts after the first extraction.

Average nectar sugar concentration ranged between 25.5-29.7%. Significant differences could be found between the flowers of *Bonaifousia disticha* and *Episcia sphalera*/*Mandevilla rugellose* (ANOVA;  $P = 0.007$ ; Holm-Sidak post-hoc test;  $P < 0.05$ ; Tab. 4). The highest sugar concentrations were detected in the nectar of *E. sphalera* ( $29.7 \pm 3.9\%$ ) and *M. rugellose* ( $29.4 \pm 3.0\%$ ). Sugar concentrations in *Costus congestiflorus* ( $25.5 \pm 4.9\%$ ) and *Calathea erecta* ( $25.7 \pm 4.2\%$ ) were lowest but showed higher deviations. With the exception of *M. rugellose*, the nectar of all species was sucrose-dominant (Tab. 4). Glucose and fructose were only present in small amounts (Glucose: 0.6-4.1%; Fructose: 1.8-11.7%) and the nectar of *Costus congestiflorus* contained no hexose sugars at all. In the nectar of *M. rugellose* the ratio of the three major sugars was nearly balanced (Tab. 4). However, samples of this species extracted at 9:00 h in the morning showed higher sucrose and lower hexose concentrations than samples extracted at noon.

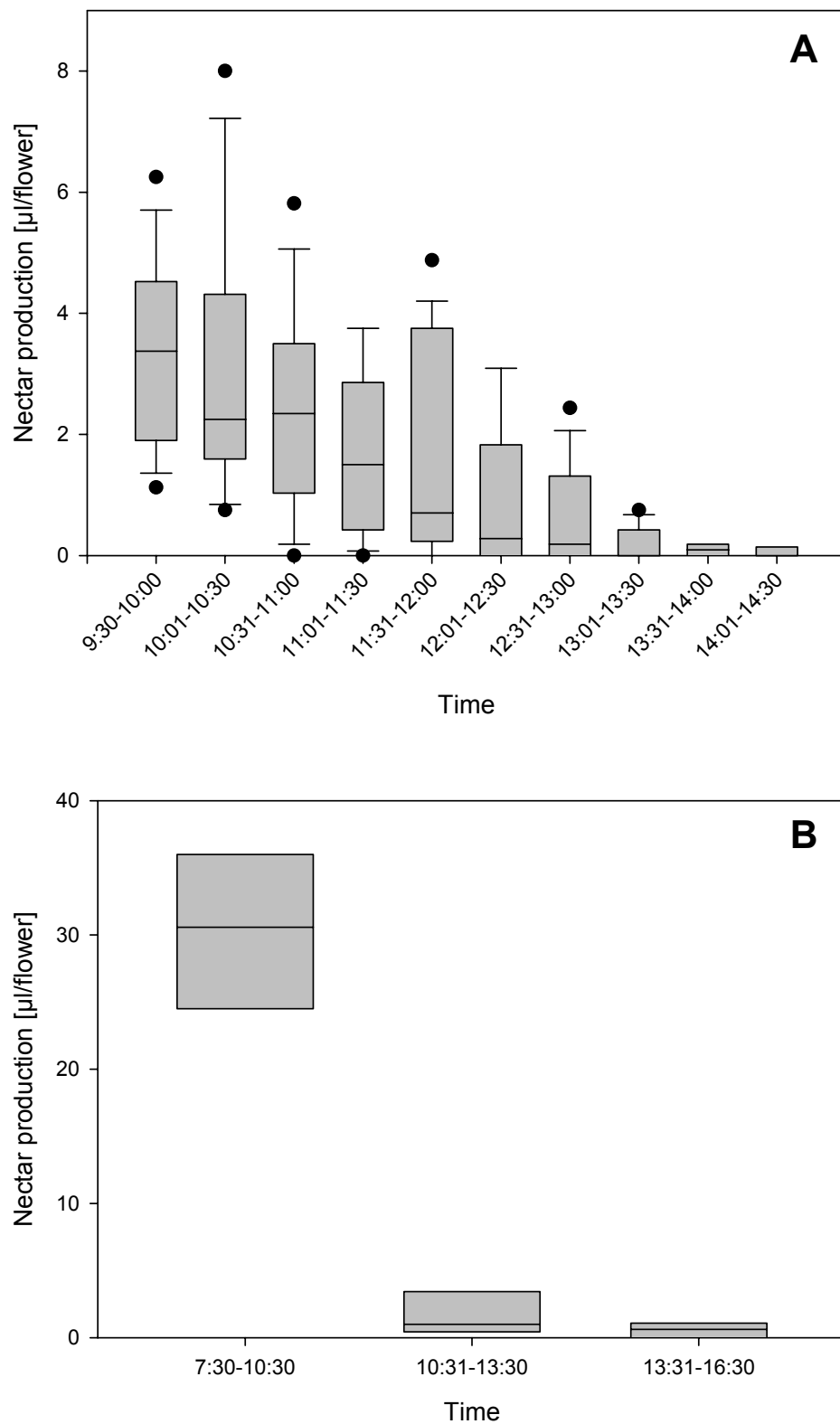


FIG. 2. Means and standard deviations for the course of daily floral nectar production of *Mandevilla rugellosa* (A; n = 17), and *Bonafousia disticha* (B; n = 5). Nectar in *M. rugellosa* was measured every 30 min, that of *B. disticha* was measured the first time after flower opening at 10:30 h and afterwards every 180 min.

### *Visitor observations*

Euglossine bees were the principal flower visitors to all plant species studied (Fig. 4). With the exception of *Calathea erecta*, they made up at least 80% of each plants' flower visits. Other common visitors were butterflies of the family Hesperiidae, which were frequently observed at the flowers of *Ca. erecta*, *Bonafousia disticha*, and *Mandevilla rugellosa* (Fig. 3B). Furthermore, long-tongued Bombyliidae, Meliponinae, and Lepidoptera of other families were observed. While the Euglossini were seeking nectar and pollen at the flowers (dependent on the resources offered), the Lepidoptera and Bombyliidae only drank nectar and the Meliponinae only collected pollen.

Within the Euglossini, the medium-sized long-tongued bees of the *Euglossa* subgenus *Glossura* were the most frequently observed species (Fig. 4). They visited the flowers of all plant species but *Episcia sphalera*. The collected bees of this subgenus belonged to the species *Euglossa piliventris* and *Euglossa chalybeata*. However, other *Glossura* species look very similar and also occur in French Guiana (Roubik 2004). Since we did not collect all visiting bees it cannot be excluded that these were among the flower visitors of the studied plants, too. Other common Euglossini at the study site were the large and long-tongued *Eulaema* subgenus *Eulaema* s.s., which were observed at the flowers of all plant species but *E. sphalera* and *Calathea erecta*. The most frequently collected *Eulaema* individuals belong to the species *Eulaema bombiformis*, but analogously to *Euglossa* subgenus *Glossura*, other morphologically similar species, which have the same distribution (Dressler 1979, Cameron 2004) might as well have been among the observed visitors. Although further euglossine species were less frequent, they sometimes represented the principal visitors of a plant species (Fig. 4). Average body length of the euglossine foragers showed a strong positive correlation with the daily produced nectar amounts of the visited plants ( $r = 0.841$ ;  $P = 0.0361$ ) and the bees' average tongue length was slightly positively correlated with the average nectar tube length of the visited plants ( $r = 0.742$ ;  $P = 0.0910$ ). Conversely, neither average tongue length ( $r = -0.363$ ;  $P = 0.479$ ) nor average body length of the bees ( $r = 0.0562$ ;  $P = 0.916$ ) were correlated with average nectar sugar concentrations of the visited plants. Sex ratios of euglossine bees were not determined, but both females and males were collected at the nectar flowers of *Bonafousia disticha*, *Mandevilla rugellosa*, *Costus congestiflorus*, *Ca. erecta*, and *Ischnosiphon martianus*. At the pollen/nectar flowers of *E. sphalera* and the pollen flowers of *Rapatea paludosa*, however, only females were captured.

One euglossine species (or one subgenus) usually dominated a plant species' flower visits or was even its only visitor as in *Episcia sphalera* (Fig. 4).

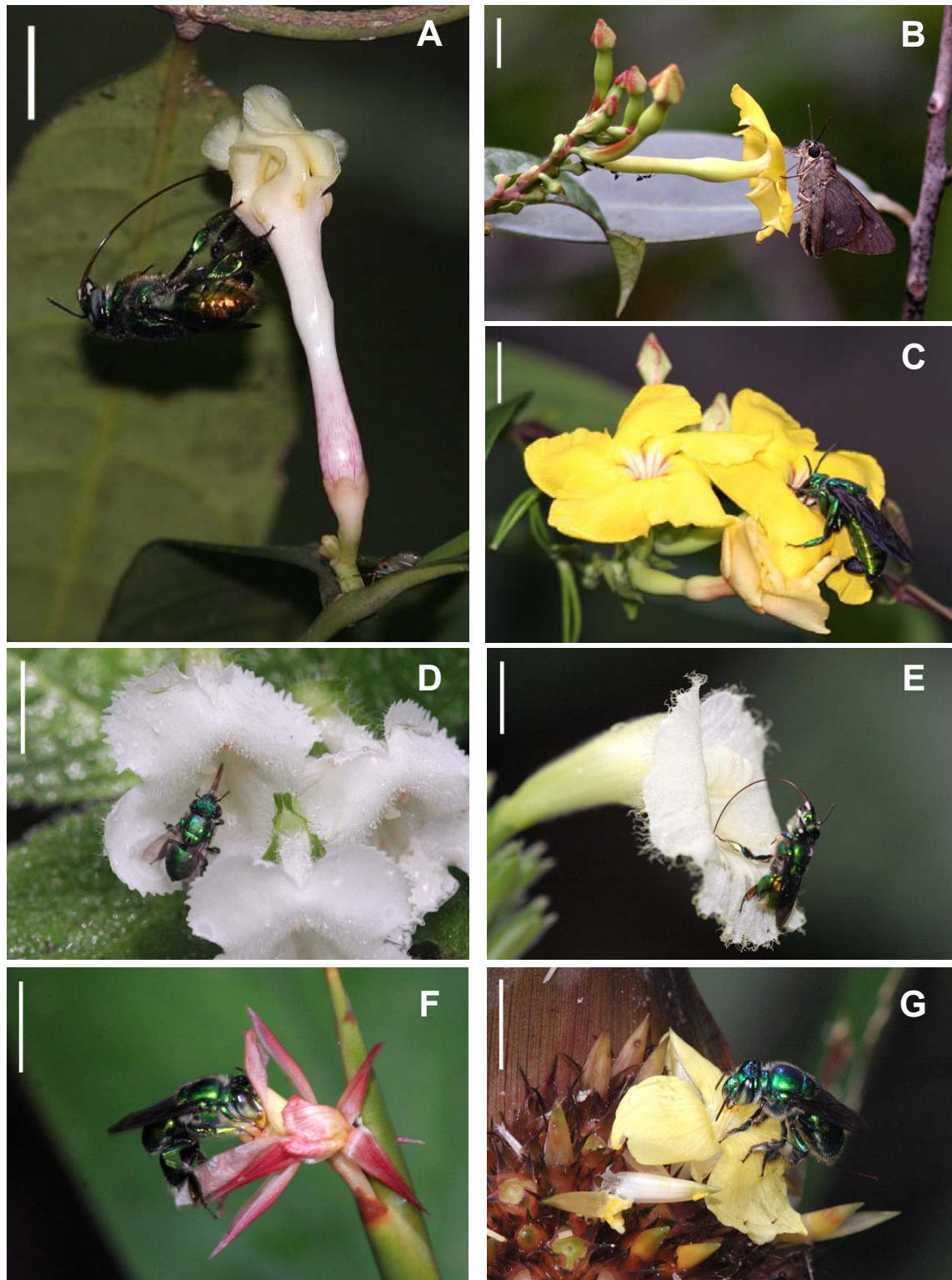


FIG. 3. Flower visitors to the species studied. A: Female *Euglossa* aff. *chalybeata* introducing its proboscis into the corolla tube of a *Bonafousia disticha* flower. B: Hesperidae sucking nectar at flower of *Mandevilla rugelosa*. C: *Exaerete frontalis* sucking nectar at flower of *Mandevilla rugelosa*. D: Female *Euglossa* sp. 3 entering the flower of *Episcia sphalera*. E: Male *Euglossa piliventris* introducing its proboscis into the corolla tube of *Costus congestiflorus*. F: Female *Euglossa* aff. *chalybeata* sucking nectar at flower of *Ischnosiphon martianus*. G: Female *Euglossa* aff. *chalybeata* collecting pollen at flower of *Rapatea paludosa*. Scale bar = 1 cm.

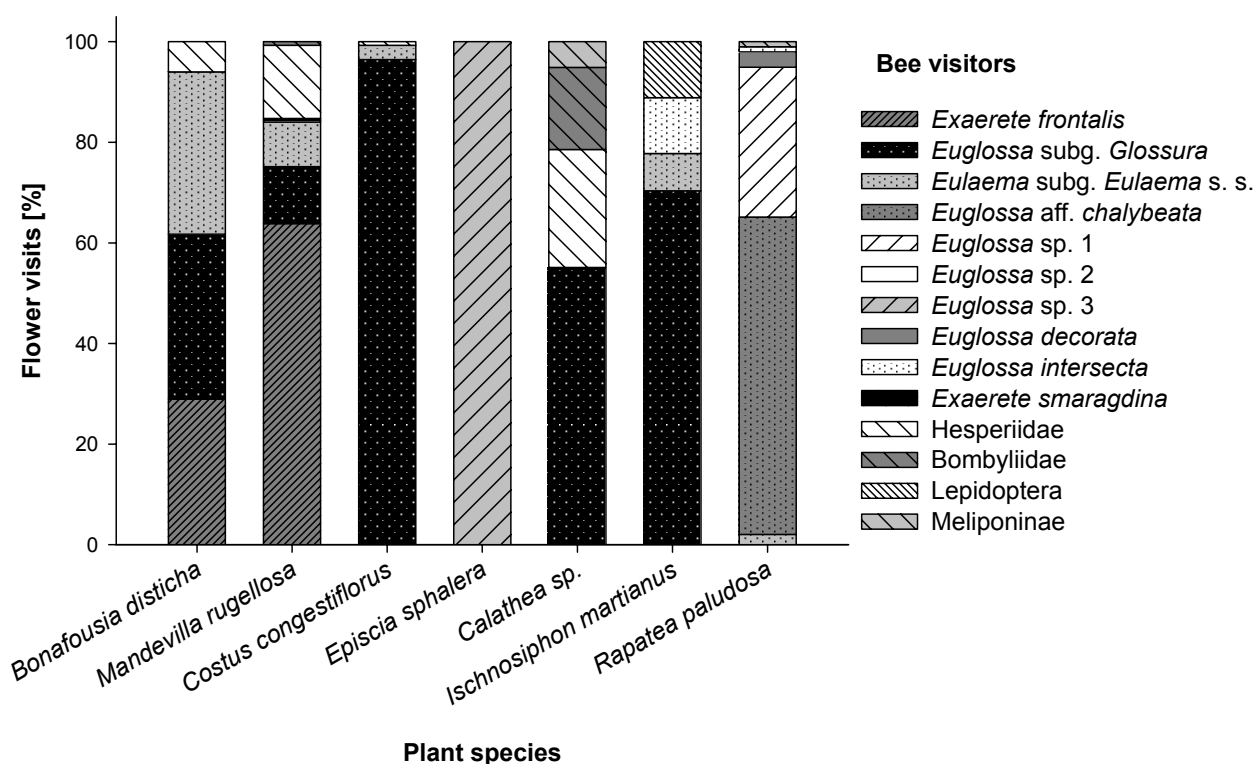


FIG. 4. Visitor spectra of the species studied. The total number of observed visits per plant species was: *Mandevilla rugellosa*: 282; *Bonafousia disticha*: 183; *Costus congestiflorus*: 141; *Episcia sphalera*: 138; *Calathea erecta*: 98; *Ischnosiphon martianus*: 27; *Rapatea paludosa*: 198. Values presented are percent of all flower visits.

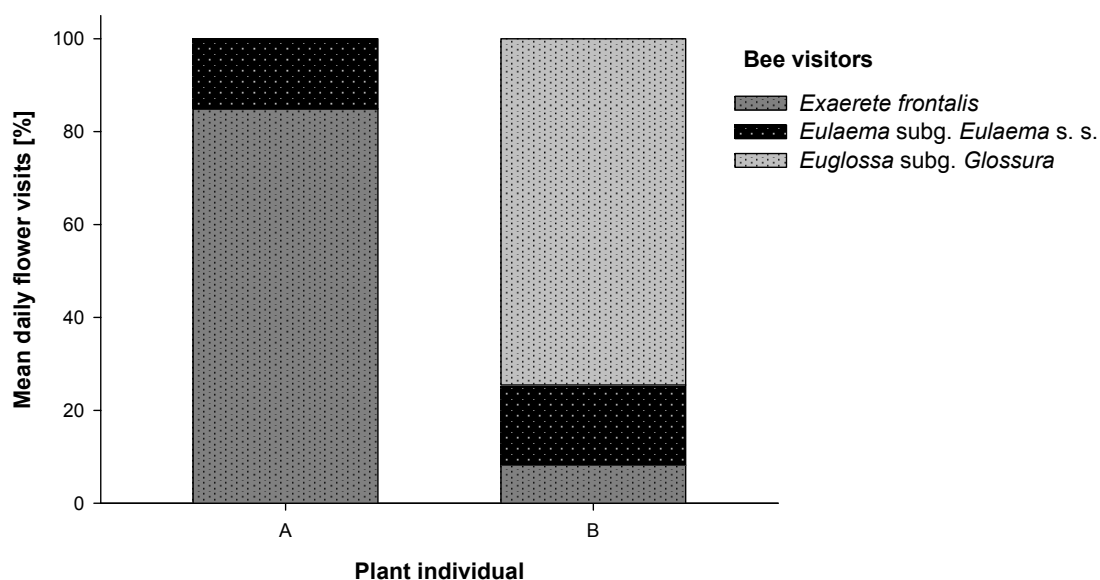
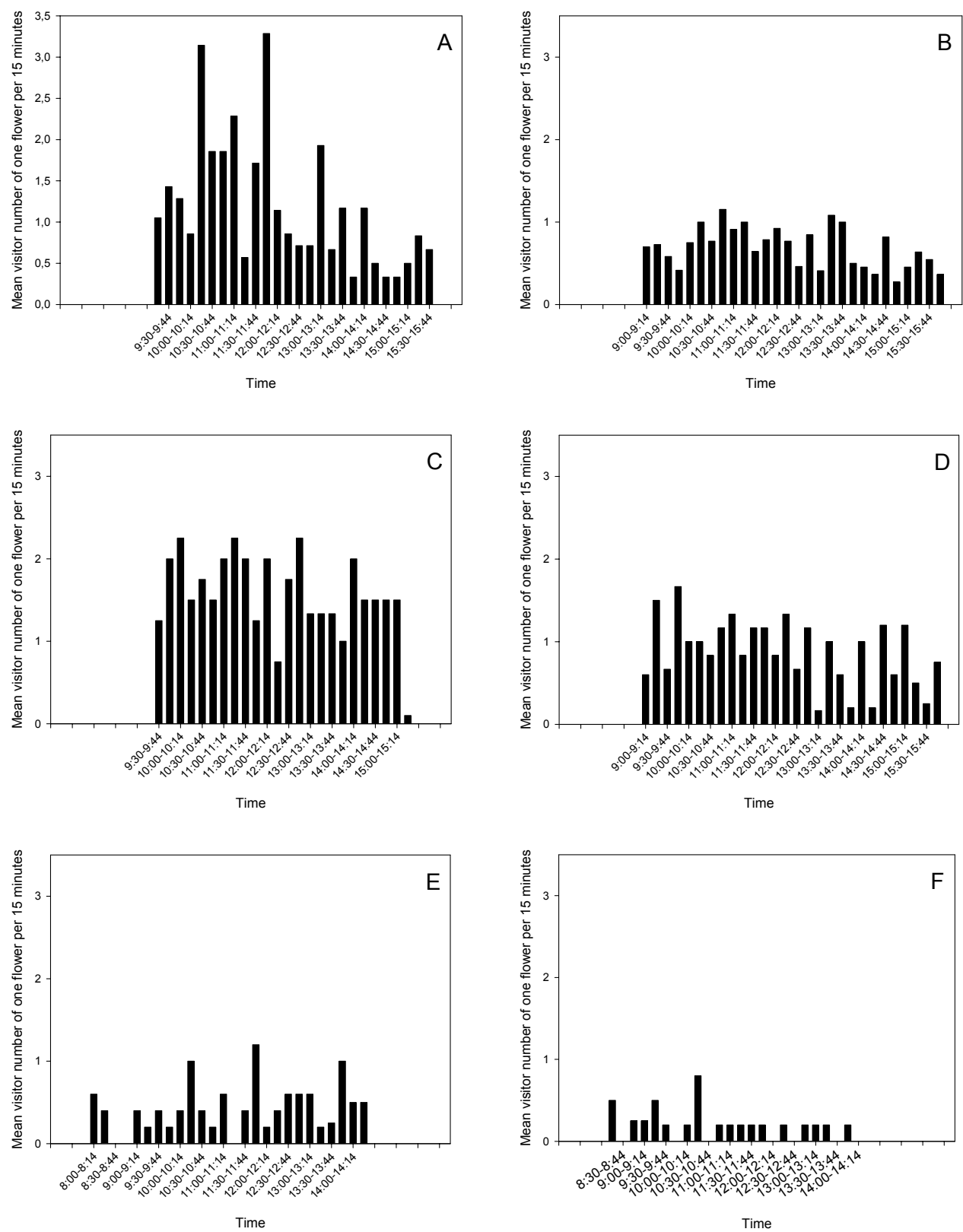


FIG. 5. Daily visitor spectrum of two *Bonafousia disticha* plant individuals. Plant A stood exposed at the forest edge and possessed ca. three times as many flowers at the observations as plant B, which was growing in the forest interior. Values are means of two (plant A) and three (plant B) observation days (9:00-16:00 h).





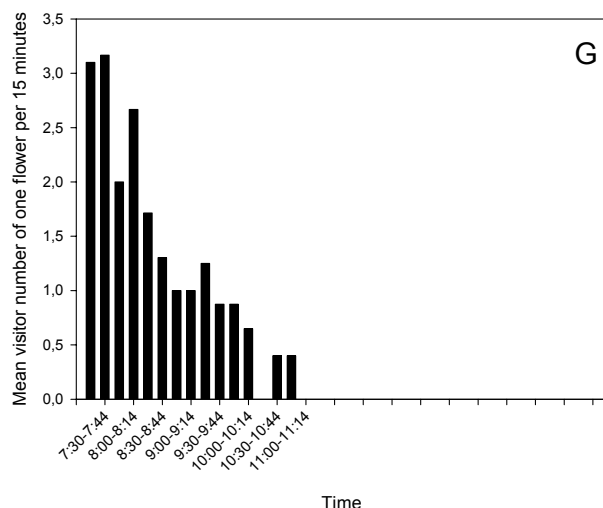


FIG. 6. Visitor activity of euglossine bees (all species and sexes) at the inflorescence of *Bonafousia disticha* (A; n = 7 observation days), *Mandevilla rugellosa* (B; n = 14 observation days), *Costus congestiflorus* (C; n = 4 observation days), *Episcia sphalera* (D; n = 6 observation days), *Calathea erecta* (E; n = 5 observation days), *Ischnosiphon martianus* (F; n = 5 observation days), and *Rapatea paludosa* (G; n = 8 observation days). The results are presented as mean visits of one inflorescence in groups of 15 minutes.

In most plant species studied (*Costus congestiflorus*, *Calathea erecta*, *Ischnosiphon martianus*, and *Rapatea paludosa*), *Euglossa* subgenus *Glossura* were the major visitors while other euglossine species were recorded in minor quantities (Fig. 4). The only exception was *Mandevilla rugellosa*, whose principal visitor was the large cleptoparasitic *Exaerete frontalis*. At first sight, *Bonafousia disticha* had balanced proportions of different visitors (*Exaerete frontalis*, *Eulaema* subgenus *Eulaema* s.s., and *Euglossa* subgenus *Glossura*). However, this species showed highly significant differences ( $\chi^2 = 95.0$ ; df = 2;  $P < 0.001$ ) in the visitor spectrum between plant individuals growing at the forest edge and individuals growing in the forest interior (Fig. 5). Plants from the forest edge usually produced higher numbers of flowers and were exclusively visited by the large bees *Exaerete frontalis* and *Eulaema* subgenus *Eulaema* s.s. On the other hand, the major visitor of the few-flowered plants in the forest interior was *Euglossa* subgenus *Glossura*, which did not appear at all at plants of the forest edge.

Foraging euglossine bees could be observed at the nectar and nectar/pollen flowers between ca. 7:00 h in the morning and 18:00 h in the afternoon (Fig. 6A-F). There were no significant differences in the visitation time of euglossine bees between plant species ( $\chi^2 = 1.4$ ; df = 10;  $P > 0.05$ ). Neither were there significant differences in the visitation time between different euglossine species at *Bonafousia disticha* ( $\chi^2 = 5.8$ ; df = 4;  $P > 0.05$ ) and at *Mandevilla rugellosa* ( $\chi^2 = 2.5$ ; df = 4;  $P > 0.05$ ). In contrast to the nectar hosts, the pollen

flowers of *Rapatea paludosa* were only visited in the morning (Fig. 6G). Here, the first bees already arrived at sunrise (ca. 6:15 h); visitor activity was highest between 7:00-8:00 h and decreased constantly until noon. In the majority of the nectar and nectar/pollen flowers, bee numbers were greatest between 10:00-12:00 h and declined in the afternoon. Slight rain did not detain the bees from foraging. Large *Exaerete* spp. and *Eulaema* spp. as well as the smaller *Euglossa* subgenus *Glossura* were observed at flower visits during rain. The highest visitor frequencies over the day were recorded at the flowers of *Costus congestiflorus* ( $1.56 \pm 0.51$  visits/15 min) and *B. disticha* ( $1.17 \pm 0.82$  visits/15 min) while the flowers of *Ischnosiphon martianus* ( $0.20 \pm 0.19$  visits/15 min) and *Calathea erecta* ( $0.43 \pm 0.29$  visits/15 min) received very few visits. Frequencies of the other species lay in between those two extremes (*M. rugellosa*:  $0.69 \pm 0.24$  visits/15 min ; *Episcia sphalera*:  $0.88 \pm 0.39$  visits/15 min ; *R. paludosa*  $1.07 \pm 1.01$  visits/15 min). In *R. paludosa* and *B. disticha* large visitation peaks occurred in the morning ( $> 3$  visits/15 min), which were responsible for the high standard deviations in these species.

During flower visits, all euglossine bees showed a similar behavior. They passed the flowering plant or plant population several times at a high velocity when approaching. Then they stopped in front of an inflorescence, hovering for a short moment to inspect the flowers, and alighted on the corolla limb (platform, built by the corolla lobes) or the lip. In *Bonaifousia disticha* the bees landed laterally at the semi-closed corolla and clung to the upright petals (Fig. 3A). After landing, the bees proceeded by raising their heads and rearing away from the flower in order to extend their long tongues (Fig. 3A, E). Then they introduced their tongues into the corolla tube (*B. disticha*, *Mandevilla rugellosa*, *Calathea erecta*, *Ischnosiphon martianus*) or crawled into the flower entrance with the tongues extended (*Costus congestiflorus*, *Episcia sphalera*, Fig. 3D, E). The flowers of *Ca. erecta*, *I. martianus*, and *Rapatea paludosa* were sometimes still closed at bee arrival. In this case, the bees opened the flowers by gnawing the tip of the flowers with their mandibles and pulling away the lateral petals with their foretarsi. During nectar sucking, the bees usually forced their proboscides deeper into the flower entrance, sometimes introducing their entire heads or even the upper parts of their bodies into the corolla tube. In *B. disticha* and *M. rugellosa* pollen was placed at the proboscides, in *C. congestiflorus* and *E. sphalera* at the dorsal head and mesosoma of the bees. In *Ca. erecta* and *I. martianus* only the first flower visitors received pollen on their proboscidal fossae. When leaving, the bees usually crawled backwards and pushed themselves up into the air, flying backwards and thereby withdrawing their proboscides off the flower. After the flower visit, the bees approached another flower or flew

away quickly in a straight direction. Some observations were made of bees that grasped a leaf edge with their mandibles after the flower visit and started grooming. This behavior was very frequently observed at the flowers of *E. sphalera*. The mesosomata of the visiting females were often densely pollen-covered after the visit. During grooming, they stowed away the pollen in their corbiculae. The flowers of *R. paludosa* were visited exclusively for pollen. Therefore, the bees did not extend their proboscides after alighting. Instead, they curled over the anther cone, grasped the stamens, and began vibrating with their bodies. In doing so, the pollen was expelled out of the anthers and accumulated at the bees' bodies (see also, Michener *et al.* 1978, Buchmann 1983). Subsequently, the bees flew upwards in order to transfer the pollen to the corbiculae and either landed one or two more times at the same flower or headed away to visit another inflorescence.

When several bees foraged at the same plant or population, usually no aggressive behavior occurred between individuals, as it is sometimes observed during perfume-gathering. Most bees appeared to be avoiding the contact with other bees during their visits. The only case of aggressive behavior was observed at the flowers of *Mandevilla rugellosa*. Here, *Exaerete frontalis* and *Euglossa* subgenus *Glossura* were seen rapidly waving their hind- and midlegs up and down when another bee approached during the flower visit. However, a direct contact between bees was never observed. Some *Exaerete frontalis* individuals foraged in close distance to each other and gave the impression that they were competing to reach the next flower. Large ants, which were collecting nectar at the sepals' extrafloral nectaries of *Bonaifousia disticha* and *M. rugellosa* often defeated flowers and occasionally prevented bees from landing.

Comparing the foraging bouts of individual bees, striking differences occurred irrespectively of the observed bee species. Some bees landed on every flower offered by the plant and made control flights before leaving, thereby assuring that they did not leave any flower unvisited. These bees sometimes even alighted on wilted flowers or flower buds. Other bees, however, only visited few flowers of a plant or a population and approached them in a straight manner without inspecting other inflorescences. In addition, many bees were observed that arrived at a plant, inspected one or two flowers without landing and then flew away quickly. Some flowers obviously were preferred by some visitors, while others were neglected. As a consequence, strongly differing visiting rates of single inflorescences arose (e.g., mean daily visits per flower: *Bonaifousia disticha*:  $6.7 \pm 4.8$ ;  $n = 45$ ; *Mandevilla rugellosa*:  $9.0 \pm 4.6$ ;  $n = 55$ ).

TABLE 5. Flowers of further plant species visited by euglossine bees at the study site. Resources foraged at the flowers are indicated in the last three columns.

	Bee visitor	Nectar	Pollen	Floral scent
<b>Annonaceae</b>				
<i>Unonopsis stipitata</i>	<i>Euglossa</i> subg. <i>Glossura</i> , <i>Eulaema bombiformis</i>			x
<b>Apocynaceae</b>				
<i>Bonafousia undulata</i>	<i>Euglossa</i> subg. <i>Glossura</i>	x		
<b>Araceae</b>				
<i>Anthurium rubrinervium</i>	<i>Aglae caerulea</i> , <i>Euglossa piliventris</i> , <i>Euglossa viridis</i>			x
<i>Anthurium thrinax</i>	<i>Euglossa intersecta</i> , <i>Euglossa piliventris</i> , <i>Euglossa prasina</i> , <i>Euglossa variabilis</i> , <i>Eulaema bombiformis</i> , <i>Exaerete frontalis</i>			x
<i>Spathiphyllum humboldtii</i>	<i>Euglossa chalybeata</i> , <i>Euglossa decorata</i> , <i>Euglossa retroviridis</i> , <i>Euglossa stilbonota</i> , <i>Euglossa townsendi</i> , <i>Eulaema bombiformis</i> , <i>Exaerete smaragdina</i>			x
<b>Convolvulaceae</b>				
<i>Ipomoea batatoides</i>	<i>Euglossa</i> subg. <i>Glossura</i> , <i>Eulaema</i> subg. <i>Eulaema</i> s.s., <i>Exaerete frontalis</i> , <i>Exaerete smaragdina</i>	x		
<b>Costaceae</b>				
<i>Costus claviger</i>	<i>Euglossa</i> subg. <i>Glossura</i> , <i>Eulaema</i> subg. <i>Eulaema</i> s.s.	x		
<b>Gentianaceae</b>				
<i>Voyria clavata</i>	<i>Euglossa</i> subg. <i>Glossura</i> , <i>Euglossa intersecta</i>	x		
<i>Voyria rosea</i>	<i>Euglossa</i> subg. <i>Glossura</i>	x		
<b>Gesneriaceae</b>				
<i>Lembocarpus amoena</i>	<i>Euglossa decorata</i> , <i>Euglossa</i> sp. 1 (green, small, short-tongued)	x		
<i>Paradrymonia campostyla</i>	<i>Euglossa decorata</i> , <i>Euglossa</i> sp. 1 (green, small, short-tongued)	x		
<i>Paradrymonia maculata</i>	<i>Eulaema</i> subg. <i>Eulaema</i> s.s.	x		
<b>Lecythidaceae</b>				
<i>Eschweilera grandiflora</i>	<i>Eulaema</i> subg. <i>Eulaema</i> s.s.	x		

<b>Marantaceae</b>	
<i>Calathea propingua</i>	<i>Euglossa</i> subg. <i>Glossura</i> , <i>Eulaema</i> subg. <i>Eulaema</i> s.s., <i>Eulaema cingulata</i> x
<i>Monophyllante oligophylla</i>	<i>Euglossa decorata</i> x
<b>Melastomataceae</b>	
<i>Ernestia granvillei</i>	<i>Euglossa</i> sp. 2 (green, medium-sized, short-tongued) x
<i>Maieta guianensis</i>	<i>Euglossa</i> subg. <i>Glossura</i> x
<i>Topobea parasitica</i>	<i>Eulaema</i> subg. <i>Eulaema</i> s.s. x
<b>Orchidaceae</b>	
<i>Catasetum</i> sp.	<i>Euglossa piliventris</i> x
<i>Chaubardiella tigrina</i>	<i>Euglossa mixta</i> , <i>Euglossa piliventris</i> , <i>Euglossa viridis</i> x
<i>Ornithocephalus</i> sp.	<i>Euglossa mixta</i> x
<i>Paphinia cristata</i>	<i>Euglossa analis</i> , <i>Euglossa crassipunctata</i> , <i>Euglossa decorata</i> , <i>Euglossa intersecta</i> , <i>Euglossa variabilis</i> x
<i>Paphinia</i> sp.	<i>Euglossa piliventris</i> x
<i>Stanhopea grandiflora</i>	<i>Eulaema bombiformis</i> x
<b>Polygalaceae</b>	
<i>Securidaca</i> sp. 1	<i>Exaerete frontalis</i> x
<i>Polygala</i> sp. 2	<i>Euglossa</i> sp. 1 (green, small, short-tongued) x
<b>Rubiaceae</b>	
<i>Psychotria carapichea</i>	<i>Euglossa decorata</i> , <i>Euglossa</i> sp. 1 (green, small, short-tongued) x x
<i>Psychotria medusula</i>	<i>Aglae caerulea</i> , <i>Eulaema</i> subg. <i>Eulaema</i> s.s. x
<b>Solanaceae</b>	
<i>Markea sessiliflora</i>	<i>Euglossa</i> sp. 2 (green, medium-sized, short-tongued) x x

We had the impression that many bee individuals sought for the same plants day after day and that they even returned several times a day. For example, in *B. disticha* a male *Euglossa piliventris* individual, which could easily be distinguished from other individuals through the natural marking of two different orchid pollinaria, was observed at 9:29 h, 10:31 h, 10:52 h, 11:14 h, 12:18 h, and 13:13 h on the first day and at 9:17 h, 10:21 h, 10:46 h, and 11:07 h on the second day at the same plant. Evidently, visiting times and visiting intervals of this particular bee were quite similar on the two days.

TABLE 6. Proportion of fruiting individuals of all observed flowering plants, proportion of fruiting flowers per inflorescence, and number of developed seeds per fruit. In *Bonafousia disticha*, *Costus congestiflorus*, and *Episcia sphalera* values of fruiting flowers relate to all flowers of a plant individual. Values are presented as means with standard deviations. Sample sizes are given in brackets.

Species	Fruiting individuals (%)	Fruiting flowers per inflorescence/plant (%)	Seeds per fruit
<i>Bonafousia disticha</i>	24 (166)	11.7 ± 9.4 (7)	51.7 ± 23.9 (43)
<i>Mandevilla rugellose</i>	100 (8)	9.2 ± 8.5 (57)	29.3 ± 8.5 (113)
<i>Costus congestiflorus</i>	100 (15)	43.4 ± 19.4 (15)	8.9 ± 3.6 (93)
<i>Episcia sphalera</i>	100 (37)	65.5 ± 30.1 (37)	333.1 ± 149.4 (35)
<i>Calathea erecta</i>	100 (10)	1.8 ± 1.2 (10)	3 ± 0 (27)
<i>Ischnosiphon martianus</i>	100 (17)	24.3 ± 7.2 (17)	1 ± 0 (62)
<i>Rapatea paludosa</i>	100 (16)	33.7 ± 14.6 (16)	2.5 ± 0.7 (250)

#### Fruit production

With the exception of *Bonafousia disticha*, all flowering plant individuals of the studied species developed fruits (Tab. 6). In *B. disticha* only 24% of the observed treelets produced mature fruits. Fruit maturation took between 50-60 days in *Costus congestiflorus*, *Episcia sphalera*, *Calathea erecta*, *Ischnosiphon martianus*, and *Rapatea paludosa*, and between 80-90 days in *B. disticha* and *Mandevilla rugellose*. Fruits of the two Apocynaceae species were dehiscent follicles. The other species studied developed dehiscent capsules. Fruit number in relation to flower number per inflorescence was highest in *E. sphalera* (65.5 ± 30.1% ) and *C.*

*congestiflorus* ( $43.4 \pm 19.4\%$ ). *Calathea erecta* achieved the lowest reproductive success by developing only  $1.8 \pm 1.2\%$  of its flowers into fruits. The two Apocynaceae, *B. disticha* ( $11.7 \pm 9.4\%$  per plant) and *M. rugelosa* ( $9.2 \pm 8.5\%$ ), also showed a very low fruit set. Many flowers of these two species were indeed pollinated and started to develop fruits, but fruit development often stopped after a few days and the follicles were dropped. The inflorescences of *R. paludosa* seriously suffered from herbivory. On average, more than one third ( $41.4 \pm 18.4\%$ ;  $n = 16$ ) of the flowers of each inflorescence was destroyed by herbivores. As a result, reproductive success was only  $33.7 \pm 14.6\%$ . Average seed number in relation to ovule number was high in the fruits of all observed plant species (Tab. 6). Highest seed sets were achieved by species that only possessed few ovules per flower (*Ca. erecta*, *I. martianus*, *R. paludosa*; Tab. 6). In the other species seed set ranged between 46-61%. Lowest seed numbers were counted in the fruits of *E. sphenoloba*.

## DISCUSSION

Our results show that the investigated plants possess similar traits in their reproductive biology as hummingbird-pollinated heliconias (Stiles 1975). All species displayed a 'steady state' flowering pattern, which is a frequently observed characteristic of tropical understory plants (Gentry 1974, Bawa 1983). Although most inflorescences had periods of several days, in which no open flowers were present, at least on the plant or population level some flowers were always available for visitors. Some authors suggest that this flowering pattern and the foraging behavior might have coevolved with each other (Feinsinger 1983, Frankie *et al.* 1983). By the daily presentation of few flowers over a long period of time, flower visitors have a reliable nectar source, they can include in their flight route. Thus, they save energy, which otherwise would be spent for the search of new nectar sources (Ackerman *et al.* 1982). The plants benefit from the visitors' behavior by increased gene flows since the bees have to collect resources from several widely spaced individuals to get satiated (Janzen 1971). While female euglossine bees have been demonstrated to be trap-liners (Janzen 1971), males were for a long time thought to be very transient because they do not return to their nesting site after emergence (Dodson 1966, Dodson 1970). However, Kroodsmas (1975) and Ackerman *et al.* (1982) showed that males are quite local and also display a behavior of trap-lining. In our study, male bees that carried orchid pollinaria were observed returning to flowers of the same plant over several days. This supports the hypothesis that males are trap-liners, too. However, contrary to the results of Ackerman *et al.* (1982), we observed similar time schedules of one bee individual for two days with resembling interval lengths between visits. Although our

sampling size was minimal, this could be a hint that the bees follow their trap-lining sequence very precisely.

While some of the study species flowered the year round, others had a flowering period that was restricted to few weeks in the rainy season. Ackerman (1985) showed, that long-tongued euglossines were most abundant in the rainy season. Therefore, the flowering of the latter species can be interpreted as an adaptation to the increased abundance of bees, whereas the former represent keystone species that maintain the long-tongued bee population over the year. Interestingly, most 'steady state' flowering plant species of tropical lowland forests, including heliconias, are known to have their major blooming peak during the rainy season (Stiles 1978, Frankie *et al.* 1983, Ackerman 1985). In contrast to this, mass-flowering plants of the canopy generally bloom throughout the dry season (Frankie *et al.* 1983). Different blooming peaks between forest strata might have evolved through the competition for pollinators.

In regard to floral visitors, euglossine bees dominated the flower-visits of all studied species. They can be considered as their principal pollinators since they had contact with pollen and stigma and consecutively foraged at the flowers of several individuals of each plant species. In the nectar-flowered plants, a restriction within the visitor spectrum was achieved by the development of extended corolla tubes, which excluded most foragers from access to the nectar. Nevertheless, also other long-tongued insects, predominantly butterflies, were observed at the flowers. Since these possessed proboscides that were even longer than those of euglossines, a contact with the reproductive organs of the flowers usually did not take place. The only two cases, where butterflies can be considered as co-pollinators were *Bonaifousia disticha* and *Mandevilla rugellosa*. Altogether, pollen transfer by euglossine bees was probably much more efficient, since their foraging was more directed, and since they visited a larger number of flowers within the same time. The observed Bombyliidae do probably not play a role as pollinators. Their proboscides were very short and it is therefore questionable if they actually reached the nectar and the reproductive organs of the flower. Even the open pollen flowers of *Rapatea paludosa* were almost exclusively visited by euglossine bees. One issue remained unclear: studies about euglossine pollen hosts are very scarce, but in the few existing publications, the flowers were usually shared with other bee groups (Frankie & Coville 1979, Buchmann 1980, Roubik *et al.* 1982). Maybe alternative bee visitors were not present during the studying period, or *R. paludosa* was not in their focus as this species grew in low densities and offered only small amounts of pollen per inflorescence. The only non-euglossine bees observed at *R. paludosa* were Meliponinae, which cannot be



considered as pollinators as they arrived very late in the morning and confined themselves to collecting fallen pollen grains from the petals. In the case of *Calathea erecta*, they can also be regarded as pollen thieves.

All plant species were characterized by a high pollinator specificity. Although the flowers of each plant species were visited by several euglossine species, usually one or two bee species that belonged to the same subguild dominated the visits. The following subguilds could be distinguished: large bees with long tongues, medium-sized bees with long tongues, and small bees with medium-sized tongues. Principal visitors to most of the studied plant species and to further nectar-, pollen-, and perfume-flowered plants that grow in the reserve (Tab. 5) were medium-sized bees with long tongues of the *Euglossa* subgenus *Glossura*. Several long-term baiting studies have shown that bees of this subgenus are the most abundant euglossines in Neotropical lowland rainforests and that their populations are very stable over the year (Ackerman 1983a, Pearson & Dressler 1985, Roubik & Ackerman 1987, Powell & Powell 1987, Roubik 1989, Roubik & Hanson 2004). This is attributed to the fact that they possess the same tongue length as the large *Eulaema* species but have much smaller bodies (Roubik & Hanson 2004). Thus, they are able to exploit the same nectar resources, while having a lower energy consumption. In addition, they usually establish large colonies, (Roubik & Hanson 2004). Bees of the *Euglossa* subgenus *Glossura*, above all the species *Euglossa chalybeata* and *Euglossa piliventris*, were probably also the most individual-rich euglossine group at the study site. Their large and stable populations make them good and reliable pollinators and many plant species might have evolved and specialized under their influence.

Plants growing at low densities are supposed to be often highly specialized towards its pollinators in order to assure pollen transfer between widely-spaced plant individuals (Heinrich & Raven 1972, Feinsinger 1978, Feinsinger 1983). However, high specialization also brings about many disadvantages. Plants have to provide sufficient nectar to maintain the pollinator population. Moreover, both species could run the risk of extinction if one partner is not available anymore. Close 1:1 relationships are thus very seldom observed in pollinator-plant relationships (Roubik 1989, Bawa 1990, Roubik 1992). More common are associations like the ones observed, in which entire subguilds of pollinators are involved. These relationships have probably emerged through diffuse coevolution where several species came into interaction (Roubik 1989). To a certain degree, these systems are rather loose and the importance of different partner species may change over space and time (Roubik et al. 2003). In this context, the fact that *E. sphalera* was visited by only one euglossine species is rather

surprising. Usually, euglossine-pollinated flowers with short corolla tubes are supposed to be less specific than the ones with extended corollas (Borrell 2005). However, in *E. sphalera* a closer relationship might be explained by the fact that the visitors were females, which, beside nectar, also collected pollen. Euglossine bees are known to have much fewer pollen hosts than nectar hosts, and in some cases pollen collection at single host-species were reported (Janzen 1971, Roubik 1989, Roubik 1992, Peruquetti *et al.* 1999). Maybe the bees have specialized on the pollen of *E. sphalera*. On the other hand, the relationship might not be as close as suspected since we cannot rule out that the flowers were also visited by other bee species that were indistinguishable from the ones collected during observations.

Differences in the euglossine visitor spectrum can be attributed to floral and ecological characteristics of the studied plant species. It was conspicuous that larger bees were rarely observed at plants growing in the forest interior, while medium-sized and small bees visited flowers of any habitat. This phenomenon might have arisen from contrasting ‘preferences’ of foraging territories. Stratum ‘preferences’ amongst the Euglossini have often been discussed in the context of floral biology (Ducke 1901, Roubik 1989, Roubik 1993, Oliveira & Oliveira Campos 1996, Rincón *et al.* 1999, Vélez & Pulido-Barrios 2005). Similar to our observations, large bees were predominantly found in the canopy and at forest edges in these studies, while small *Euglossa* species seemed to ‘prefer’ the shady understory. Some authors relate this to stratum fidelity of certain species (Oliveira & Oliveira Campos 1996). Roubik (1989) argues that differences in the strata foraged by bees could depend on where they find rewarding resources. This hypothesis might also explain habitat ‘preferences’ observed in our study. Daily produced floral nectar amounts significantly differed between species and were strongly correlated with the body size of the flower visitors. *Mandevilla rugelosa* and some individuals of *Bonafousia disticha* occurred at forest edges, produced large nectar totals and were predominantly visited by euglossines. On the contrary, nectar amounts of the other plant species studied and of *B. disticha* individuals that were growing in the forest interior were significantly smaller. Consequently, these plants were visited by small and medium-sized bees. Since foragers cross large distances between plant populations, probably a minimum caloric reward exists, the flowers have to offer in order to be included in the bees’ foraging route (Heinrich & Raven 1972, Schemske 1981). Larger euglossine species have a higher energy consumption than smaller ones (Casey *et al.* 1985). Therefore, the minimal threshold of nectar amounts should also be higher for them. Low nectar amounts are probably also one of the reasons why the medium-sized *Euglossa* subgenus *Glossura* did not visit the flowers of *Episcia sphalera* and other plant species that were exploited by short-tongued *Euglossa*

species (Tab. 5). Pyke (1978) already demonstrated on bumblebees that there should be a positive correlation between the size of a bee and the average amount of nectar it obtained per flower. Our study let to suppose that this is also true for the Euglossini.

In contrast to nectar volumes, differences in nectar sugar concentrations probably had no impact on the euglossine visitor spectrum. Values of all species but *Bonafousia disticha* did not significantly differ from the others and no correlation between body length and nectar sugar concentration was found. Moreover, according to Borrell (2007) optimal nectar sugar concentrations for euglossine bees should be largely independent of body size. Sample values of the studied plants were 5-10% below the optimum of 35-40%, which could be a cause of dilution through very high relative humidities during the rainy season (Corbet *et al.* 1979, Bertsch 1983). Nevertheless, nectar sugar concentrations measured in our study are within the range of values detected in other euglossine nectar hosts (22-48%; Roubik *et al.* 1995).

Another important trait that led to specific visitor spectra was the floral morphology. Nectar tube length restricted access to the nectar to euglossine bees with a corresponding proboscis length. Large bees, which theoretically had the capacity to exploit a broad spectrum of nectar hosts were often excluded from flowers by the development of narrow corolla tubes or small corolla lobes. On the other hand, in some species flower entrances were blocked by floral organs that could only be pushed aside by strong large bees.

While the studied plant species had a small spectrum of major pollinators, the bees collected nectar from several plant species and did not possess a principal nectar host. This phenomenon was already observed in hummingbird-pollinated plants (Stiles 1975, Feinsinger 1983) as well as in euglossine nectar hosts (Schemske 1981, Kay & Schemske 2003). The authors explained it by the fact that the lifetime of the animals often exceeds the flowering period of a plant and that a single plant species is not able to attract and to feed a pollinator group. When several species are pollinated by the same visitor, selection might favor divergence in the placement of pollen on the flower visitor (Feinsinger 1983). Indeed, this was reported from several hummingbird-pollinated *Heliconia* species (Stiles 1975) and also from euglossine-pollinated perfume orchids (Ackerman 1983b). In our study, plants placed the pollen either on the proboscis (Apocynaceae, Marantaceae), the dorsal mesosoma (*Costus congestiflorus*, *Episcia sphalera*) or the ventral mesosoma (*Rapatea paludosa*). Since the same bees visited the flowers of four species that all deposited pollen on the proboscis, heterospecific pollen was inevitably mixed. This overlap might particularly be critical in closely related taxa. Here, selection for strong barriers to hybridization may occur (Schemske 1981). The absence of different pollen placement could be explained by the size of the

visitors. In contrast to hummingbirds, euglossine bees are quite small and the exact placement of pollen at different positions on the bee might be impossible. Perfume orchids on the other hand possess pollen-packages that are attached to the pollinator's body by a small adhesive disc, which enables orchids to place the pollen at determined spots, also on small bees (Ackerman 1983b).

With regard to the sex of the principal flower visitors, nectar hosts were visited by bees of both sexes, while at nectar/pollen and pollen hosts only female bees were observed. The sharing of nectar hosts by male and female euglossine bees is reported by several other studies (Dodson 1966, Ackerman 1985, Zimmerman & Madriñan 1988, Roubik 1993, SanMartin-Gajardo & Sazima 2004) and the exclusive visit of pollen flowers by females is reasonable since male euglossines usually do not collect pollen. However, the fact that only females were observed at the nectar/pollen flowers of *Episcia sphalera* is rather surprising. Maybe the offered nectar amount was too small to attract males, whereas in combination with pollen-collection the visits were profitable for the females. Another possible explanation is that the number of females in the population of this species was temporarily higher than that of males due to a decrease in the population-size of short-tongued euglossines during the rainy season (Roubik & Ackerman 1987).

In their foraging bouts, the observed euglossines showed a variety of different behaviors. While some individuals used to visit all offered flowers, others showed a more directed foraging, landed on few flowers, and headed forward to the next plant. As nectar production considerably differed among individual flowers of a plant, flower choice might be very important to decrease foraging costs. Single inflorescences flowered over an extended period of time. Therefore, nectar-rich blossoms might be memorized by the bees and are approached directly in the next foraging flight. We suggest that the observed behavior could be related to age and foraging experience of individual bees. While experienced euglossines already know the most profitable flowers of a population, younger bees still have to establish a trap-lining route and learn to discriminate between 'good' and 'bad' inflorescences. Besides the directed approaching of blossoms, the bees were also frequently observed inspecting several flowers in flight, choosing only one to exploit. Other bee groups that show a similar behavior, namely *Apis mellifera*, *Bombus* spp., or *Xylocopa virginica texana*, are known to leave odor marks on the flowers they visit. These marks are used by con- and heterospecifics to assess potential foraging profits at the flowers without the need to land and can thereby save energy (Frankie & Vinson 1977, Stout *et al.* 1998, Goulson *et al.* 1998, Stout & Goulson 2001). Amongst the Euglossini, the use of odor marks has not been studied yet. However, the

tribe is closely related to the Bombini (Michener 2000, Cameron 2004) and our observations could be a hint that they also employ this strategy. Furthermore, the plants might also benefit from this behavior by having higher gene flows.

Diel activity patterns of euglossine bees have previously been studied at perfume flowers and scent baits for the males (Dodson *et al.* 1969, Janzen *et al.* 1982, Whitten 1985, Armbruster & McCormick 1990, Armbruster & Berg 1994, Oliveira 1999, Hentrich *et al.* in prep.), and at euglossine nests for the females (Zucchi *et al.* 1969, Bennett 1972, Cameron & Ramírez 2001). These studies demonstrated that both sexes are predominantly active in the morning hours until noon. Numbers of fragrance-foraging males usually peak at ca. 11:00 h, start to decrease at noon, and cease at ca. 14:30 h (Armbruster & McCormick 1990), while females tend to collect pollen and nectar in the morning, and construction material for the brood cells in the afternoon (Cameron & Ramírez 2001). In our study, bees were mainly active at the nectar flowers between 9:00-16:00 h, but their number was slightly higher in the morning. Unfortunately, it was not possible to distinguish between males and females during observations. Therefore, we cannot state whether there were differences in the visiting time at flowers visited by both sexes. *Episcia sphalera* was pollinated by female bees only and the visitation frequencies were similar to those of the other plant species. Armbruster & Berg (1994) tried to explain the varying frequencies during the day. They showed that the activity of male *Euglossa* spp. in the morning was positively correlated with environmental temperatures. However, in the afternoon visitor frequency did only correlate with time of day or an unmeasured variable. Nectar evaluations of our study demonstrated that most species produced the largest nectar amounts in the morning or even directly after flower opening. These results are in accordance with the measurements done in other studies (Madrigal & Girón 1982, Torres & Galetto 1998, Leite & Machado 2007, Almeida Barreto & Freitas 2007). We suggest that the decreasing nectar production of many flowers in the afternoon could be an important factor affecting the activity of the bees. Morning-based nectar secretion might be common, as strong rainfall and high temperatures mostly occur in the afternoon and do negatively influence the longevity of the flowers. Additionally, the bees' foraging during strong rainfall usually stops (Janzen *et al.* 1982, Armbruster & Berg 1994). Therefore, average visitor numbers and successful outcrossing are probably lower during rainfall.

In regard to visitation time of small and large euglossine species, we expected clear differences between bees of different sizes. Since large bees of the genus *Eulaema* and *Exaerete* have better cooling capacities than the smaller *Euglossa* spp. (May & Casey 1983), they should be less affected by high temperatures and direct exposition to the sun. This

hypothesis was not substantiated. Maybe our sampling size was too low to find clear differences. On the other hand, overheating might not play a significant role for bees foraging in the understory since temperatures are lower than in the canopy and more stable throughout the day.

In contrast to the nectar-offering species, visitor activity of female bees at the pollen flowers of *Rapatea paludosa* was highest in the early morning. This is probably related to the limited daily pollen number offered by each plant, which leads to strong competition among the bees. Therefore, most bees already arrived immediately after flower opening. Later in the morning, most anthers were exhausted and visitor numbers strongly declined. The limited resources in pollen flowers could explain why female euglossine bees predominantly forage for food in the morning. In contrast to pollen, material for nest construction is an unlimited commodity and can be collected at any time (Cameron & Ramírez 2001). Nevertheless, the pollen/nectar flowers of *Episcia sphalera* were visited throughout the day, probably because this species offered nectar as an additional resource and the pollen could not be actively collected by buzzing. In effect, only limited amounts of pollen were removed at each flower visit and sufficient pollen was available for all visitors that arrived in the course of a day.

Pollen characteristics of nectar- and pollen-offering flowers contrasted considerably in pollen grain numbers and pollen grain sizes. High pollen grain numbers and small pollen sizes are common properties of pollen flowers and are generally related to a trade-off between number and size (Charnov 1982, Vohnhof & Harder 1995, Cruden 2000). The poricidally opening anthers of most of these flowers may select for small pollen grains. Higher pollen numbers have probably evolved because flower visitors collect a large proportion of the pollen offered, which then is not available for pollination anymore.

P/O ratios of all studied species but *Rapatea paludosa* were very low. According to Cruden (1977), P/O ratios reflect the efficiency of pollen transfer from the anthers to the stigma. While autogamous plants usually have low P/O ratios, they are high in xenogamous plants. Accordingly, *R. paludosa* should have a xenogamous breeding system, while the other species tend towards autogamy. However, low P/O ratios have also been reported from several other taxa of the Apocynaceae, Costaceae, and Marantaceae, and seem to be a common feature in these families (Ramírez & Seres 1994, Torres & Galetto 1999, Lopes & Machado 1999, Löhne *et al.* 2004, Leite & Machado 2007). It is known that most of these species did not or only very seldom produce fruits by autonomous self-pollination (Schemske 1981, Ramírez & Seres 1994, Torres & Galetto 1999, Lopes & Machado 1999, Löhne *et al.* 2004, Leite & Machado 2007, Almeida Barreto & Freitas 2007). Also, the contact between

self-pollen and the stigma is usually excluded by their special floral morphology. Cruden (1981) showed that exceptions to the rule exist, where also flowers with low P/O ratios are xenogamous. These flowers produce large pollen grains and have a large stigma area. Thereby, pollen transfer is much more efficient than in flowers with small stigmas, and the larger pollen grains are more successful in fertilizing the ovules. Consequently, fewer pollen grains are required for the reproduction of the plant. Since the studied Apocynaceae, Costaceae and Marantaceae correspond in their floral characteristics with these features, we suppose that they are xenogamous. In contrast, *Episcia sphalera*, which also showed a very low P/O ratio, has to be considered facultative autogamous, since plants in the greenhouse, where natural pollinators do not exist, developed fruits. Here, self-pollination probably occurs in an intermediate reproductive phase (in the succession of male to female phase), which was already described for some Gesneriaceae species of the genus *Sinningia* (SanMartin-Gajardo & Sazima 2004).

Fruit production widely ranged in the studied species: while *Costus congestiflorus*, *Episcia sphalera*, and *Rapatea paludosa* showed high average fruit sets, they were very low in the two Apocynaceae species and *Calathea erecta*. Self-pollination probably only played a role in *Episcia sphalera*. In this species, we cannot exclude that high fruit and seed numbers are partially attributed to self-pollination. Low fruit production in *Bonafousia disticha* and *Mandevilla rugeliosa* was probably not due to a low pollination success, as abortion of fruits was recorded in both species. Indeed, this phenomenon has been observed in several other Apocynaceae species (Torres & Galetto 1999, Lopes & Machado 1999). In these studies, also most fruits of hand-pollinated inflorescences dropped before reaching maturity. The authors suggest that this could be a widespread phenomenon within the family (Lopes & Machado 1999) that is probably related to a limitation of resources (Torres & Galetto 1999). Instead of producing many few-seeded fruits, these plants probably save resources for the development of few fruits that bear many seeds. A high seed set in the plants of the mentioned studies and in the Apocynaceae species of our study confirm this hypothesis. Conversely, *C. congestiflorus* showed a high fruit set, but had the lowest seed set of all species studied. Fruit set in *Ca. erecta* was very low, although its visitor frequency was higher than in *Ischnosiphon martianus*, which had a fruit set of 24%. Schemske & Horvitz (1984) showed in *Ca. ovadensis* that butterfly visitors had a negative effect on the fruit-set by tripping the flowers without coming into contact with the stigma. The large amount of other flower visitors than euglossines in the studied *Ca. erecta* might therefore conduce to the low fruit set observed. If we take into consideration that the low fruit set of some species was not related to a poor

pollen transfer, euglossine bees can be considered as very successful pollinators of the studied species.

In conclusion, our results confirmed the initial hypothesis that the studied euglossine food hosts evolved similar isolation mechanisms as hummingbird-pollinated plants. We showed that Euglossini were the major flower visitors and that specialization was greater for plants than for pollinators. In the nectar plants, small pollinator spectra were attained by differences in daily produced nectar amounts, contrasting corolla tube lengths and additional morphological traits. These characteristics contribute to a directed pollen flow and might further be important for the reproductive isolation of the plants. Since our study was restricted to the rainy season, we cannot exclude, that moreover temporal flowering patterns have evolved. In contrast to hummingbird-pollinated plants, species-specific pollen placement within a plant genus was not observed, which is probably attributed to the small size of the bees. Therefore, closely related species, which are visited by the same bee species should have developed strong post-pollination barriers to avoid hybridization. Besides nectar plants, also the pollen hosts showed a very limited visitor spectrum. This observation was rather surprising since the plants produced open accessible flowers. However, the results of our study let to suppose that some euglossine species were oligolectic, at least temporally or/and spatially.

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## PUBLICATIONS FROM THIS THESIS AND CONTRIBUTIONS OF CO-AUTHORS

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The present thesis is based on the following four manuscripts, which have been published (manuscript 2) or will be submitted to scientific journals:

1. Hentrich, H., Kaiser, R., & G. Gottsberger. (in prep.). Reproductive isolation by floral scent in three sympatric euglossine-pollinated *Anthurium* and *Spathiphyllum* (Araceae) species in French Guiana.
2. Hentrich, H., Kaiser, R., & G. Gottsberger. 2007. Floral scent collection at the perfume flowers of *Anthurium rubrinervium* (Araceae) by the kleptoparasitic orchid bee *Aglae caerulea* (Euglossini). *Ecotropica* 13: 149-155.
3. Hentrich, H., Kaiser, R., & G. Gottsberger. (in prep.). Does selection favor selfing in myco-heterotrophic plants? – The reproductive biology of *Voyria* (Gentianaceae) species in French Guiana.
4. Hentrich, H., & G. Gottsberger. (in prep.). The reproductive biology of euglossine-pollinated understory plants in a lowland rainforest in French Guiana – a comparative study.

**Gerhard Gottsberger** is co-author on all publications resulting from this thesis as he provided many valuable comments on the manuscript and gave important guidance to my work.

**Roman Kaiser** is co-author on the manuscripts I-III as he analyzed the floral scent samples and provided valuable comments and informations on the fragrance substances.

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Hentrich, H., Kaiser, R., Gottsberger, G. (2007). Floral scent collection at the perfume flowers of *Anthurium rubrinervium* (Araceae) by the kleptoparasitic orchid bee *Aglae caerulea* (Euglossini). – *Ecotropica* 13: 149-155.

Hentrich, H., Kaiser, R., Gottsberger, G. (in prep.). Reproductive isolation by floral scent in three sympatric euglossine-pollinated *Anthurium* and *Spathiphyllum* (Araceae) species in French Guiana.

Hentrich, H., Kaiser, R., Gottsberger, G. (in prep.). Does selection favor selfing in myco-heterotrophic plants? – The reproductive biology of *Voyria* (Gentianaceae) species in French Guiana.

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## CONFERENCE CONTRIBUTIONS

Hentrich, H., Gottsberger, G. (2008). Reproductive success in the euglossine-pollinated *Anthurium rubrinervium* (Araceae) in French Guiana. Poster presented at the 21<sup>st</sup> annual meeting of the Society for Tropical Ecology, Hohenheim, Germany.

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## EIDESSTATTLICHE ERKLÄRUNG

Hiermit erkläre ich, die vorliegende Dissertationsarbeit selbständig angefertigt und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet zu haben. Alle wörtlich oder sinngemäß übernommenen Stellen wurden als solche kenntlich gemacht.

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