Journal of Pollination Ecology, 4(6), 2011, pp 39-47

POTENTIAL POLLINATORS AND ROBBERS: A STUDY OF THE FLORAL VISITORS OF *HELICONIA ANGUSTA* (HELICONIACEAE) AND THEIR BEHAVIOUR

Katharina Stein* and Isabell Hensen

Martin-Luther-University Halle-Wittenberg, Institute for Biology/Geobotany and Botanical Garden, Am Kirchtor 1, 06108 Halle/Saale, Germany

Abstract—Floral syndromes are traditionally thought to be associated with particular pollinator groups. Ornithophilous flowers tend to have traits that facilitate bird pollination such as having long, narrow, tubular corollas, often vivid coloration and diluted, sucrose-rich nectar. However, recent studies have shown that flowers attract a broader spectrum of visitors than might be expected. Furthermore, the classification of floral visitors as 'robbers' or 'pollinators' often is not as simple as it seems, as pollinators can at times act as robbers and vice versa. We studied the species composition, behaviour and ecology of floral visitors, including potential pollinators and robbers, of *Heliconia angusta* (Heliconiaceae), an endemic understory herb of the Atlantic Rainforest of Brazil. In addition, the impact of the plant inflorescence attractiveness and of weather and light conditions on visitor abundance and frequency was investigated.

Flower visitors were found to be scarce with a total of only 151 visits being observed during 120 h of field observations. A stingless bee species *(Trigona* sp.) appeared to be the most abundant visitor to the ornithophilous flowers of *H. angusta*, along with four different species of hummingbirds and two species of butterflies. We consider *Trigona* sp. rather as pollen robber, but which still has the potential to be a secondary pollinator, whereas the hummingbirds were the principle legitimate visitors. Most flower visitors were recorded between 9.00 am and 1.00 pm with a higher number visiting under semi-shaded conditions than in full shade. Hummingbird numbers increased with flower abundance while the other visitor group numbers were not affected.

Keywords: *hummingbirds, stingless bees, Trigona, pollen robbers, nectar, inflorescence attractiveness*

INTRODUCTION

Tropical flowering plants depend overwhelmingly on animals as vectors of pollen transfer. The vast majority of pollinators are represented by insects, such as bees and butterflies, as well as by some vertebrate groups including mainly birds and bats (Proctor et al. 1996). Despite the great variety of plant-pollinator systems, it is possible to associate floral traits with particular pollinator groups as a series of pollination syndromes (e.g. Campbell et al. 1996; Galetto 1998). As an example, ornithophilous flowers tend to have traits that facilitate bird pollination (Smith et al. 1996), such as long, narrow, tubular corollas and often vivid coloration (Willmott & Burquez 1996). Neotropical *Heliconia* species (Heliconiaceae) are associated with "bird-flower" pollination syndromes in respect to their long, white, tubular flowers and intensive red coloured bracts, attributes which may otherwise infer that they have no other types of visitors (Stiles 1975).

However, the pollination syndrome concept has recently been criticized with researchers finding that flowers often attract a broader spectrum of visitors than that predicted from their respective syndromes (Fenster et al. 2004; Dias da Cruz 2006; Ollerton et al. 2009; Schmid et al. 2011) and that many plants have more than one type of pollinator (Waser et al. 1996; Waser & Ollerton 2006). Ollerton et al. (2003) reported that generalist insects often visit more specialized plants, a situation that was also found for bird-adapted mistletoe flowers being visited by bees (Robertson et al. 2005). In addition to representing secondary or alternative pollinators for more specialized plants (Canela & Sazima 2005; Schmid et al. 2011) other studies have shown that more generalist insects may frequently be regarded as pollen and nectar robbers, in particular to flowers of many long-tubed, nectar rich, hummingbird pollinated species (McDade & Kinsman 1980; Renner 1983). The interaction of plants with robbers and pollinators may be influenced by factors such as the morphology of the visitors, the availability and quality of rewards, and spatio-temporal variation in the abundance of flowers, robbers and pollinators (Horvitz & Schemske 1990; Thompson & Pellmyr 1992). This complexity and interdependence of factors can lead to the assumption, that the classification of floral visitors as either 'robbers' or 'pollinators' may be simplistic (Arizmendi et al. 1996), as some robbers can act as pollinators (e.g. Graves 1982, Slaa et al. 2006) and pollinators sometimes can act as robbers (e.g. Willmer & Corbet 1981; Roubik 1989)

The principle reward for most flower visitors is nectar (Proctor et al. 1996), and the particular nectar sugar

Received 21 January 2011, accepted 13 May 2011

*Corresponding author; email: steinkatharina@hotmail.com, Telephone: +49 345 5526264, Fax: +49 345 5527228

composition has often been related to the pollinators of a plant. Flowers pollinated by hummingbirds, butterflies or long-tongued bees often secrete sucrose-rich nectar (Baker & Baker 1990; Perret et al. 2001; Krömer et al. 2008). In addition to the floral reward, in terms of number of flowers displayed or open, flower attractiveness may have a positive influence on the abundance and frequency of the flower visitors (Bosch & Waser 2001; Steven et al. 2003; Harder et al. 2004). In addition, weather and light conditions influence the flower visitors´ behaviour. Studies by Renner (1983), Stone and Jenkins (2008), Vicens and Bosch (2000) and Döll et al. (2007) revealed impacts of temperature, solar radiation and time of day on the number of floral visitors and their frequency.

This study focuses on *H. angusta* Vell. (Heliconiaceae), an understory herb endemic to the Atlantic Rainforests of Brazil. Wild populations are classified as vulnerable by the World Conservation Union, mostly because of the conversion of their diminishing habitats into agricultural land. Studies have been carried out concerning the morphology and anatomy of the species (Simão & Scatena 2001), or the role of the species as a nectar source for hummingbirds from an ornithological point of view (Sazima et al. 1995; De Castro & Araujo 2004). However, knowledge on its ecology is still lacking. This study focuses on the pollination biology of *H. angusta*. Thus we address the following questions:

1. What are the floral visitors of *H. angusta* and what is their behaviour in terms of being potential pollinators or flower robbers?

2. Does the floral attractiveness (number of bracts, flowers) have an impact on the number of visitors or their frequency?

3. Is the flower visitation rate influenced by the time of day, weather and/or light conditions?

4. What is the sugar composition of the nectar produced and is it related to the preferences of the observed flower visitors?

MATERIAL AND METHODS

Species and study region

The family Heliconiaceae comprises a single genus, *Heliconia* L., with 250-300 species distributed mainly throughout neotropical areas from northern Mexico to southern Brazil (Dahlgren et al. 1985; Kress 1990). In Brazil, there are approximately 40 species covering two primary areas of distribution: the Amazon basin and the Atlantic coastal rainforest (Kress 1990).

H. angusta is an ornithophilous, perennial, clonally growing, understory herb endemic to the Atlantic Rainforest in south-eastern Brazil. Like other *Heliconia* spp., it is a common component of the understory of Neotropical forests and has a patchy distribution. *H. angusta* displays a "steady state" flowering strategy (Gentry 1974b; Stiles 1975) producing flowers from April to October, making it a crucial nectar resource for hummingbirds (De Castro & Araujo 2004). The single-terminal inflorescence consists of five to eight red coloured bracts and about five hermaphroditic flowers per cincinnus. The approximately 4 cm long tubular corolla of the flowers is white, while the ovary and pedicel are orange in colour. Flower anthesis usually lasts for only one day (Stein, pers. obs.); flowers open in the early morning and are abscised in the evening, or at the latest, the next morning. Over a period of 2 – 4 months (between June and October), each ramet produces one or two new flowers roughly every 2nd day. In the Atlantic Rainforest, *H. angusta* is found mainly in shaded, moist places inside the forest, sometimes at the forest edge close to rivers (Simão & Scatena 2001; Stein, pers. obs.).

The study was conducted in the Atlantic Rainforest ("Mata Atlântica") of the state of Rio de Janeiro, Brazil in the private reserve "Reserva Ecológica de Guapiaçu" (REGUA – 22°25'53"S, 42°45'20"W) in the municipality of Cachoeiras de Macacu. The 5500 ha reserve is located on the south-facing slopes of the Serra dos Órgãos Mountain range, about 100 km from the city of Rio de Janeiro. The mean annual temperature for this region is about 23 °C with a mean annual rainfall of about 2560 mm. There is a hot and rainy season from October to March and a cooler and drier season from April to September (Kurtz & de Araújo 2000). The vegetation can be classified as "evergreen dense ombrophilous forest" (Veloso et al. 1991), which is typical for the lower and medium elevations of the coastal mountain range (Morellato & Haddad 2000; Oliveira-Filho & Fontes 2000).

Data collection

Data collection was carried out from July to September 2009 and from June to August 2010. Flower visitor observations were carried out at three different 2 x 2m plots at elevations of 50 m to 200 m a.s.l. The three plots were chosen to have replication. The distance between observation plots ranged between 800 m (minimum) and 4 km (maximum) as a result of the patchy distribution of the plants. The mean number of open flowers per plot and observation day was 2.08 (standard deviation SD: 1.56), with a minimum of 1 and a maximum of 6 open flowers. Mean density of ramets per observation plot and day was 2.58 (SD: 1.44). Each ramet was considered an individual due to the fact that an assignment of ramets to a certain individual was not possible in the field. Flower visitors were observed on a total of twelve days (6 d in 2009, 6 d in 2010, 2 d per plot and year), each continuously from 7:00 am to 5:00 pm, resulting in a total observation period of 120 hours. Care was taken to ensure that weather conditions on all observation days were comparable (sunny to cloudy). In order to test for any potential impact on the number/frequency of flower visitors, the data recorded on each observation day included the number of flowering conspecifics within the 2 x 2m observation plot, the total number of displayed and open flowers respectively and the total number of bracts per plot.

Young flowers tend to be hidden among the bracts and, shortly before anthesis, the flowers (still closed) become erect and visible. Open flowers show a lower lip that curls downwards and provides access to the corolla interior with the reproductive organs and the nectar reward. In addition to the flowers, the bracts of the inflorescences might play an important role in attracting flower visitors due to their bright reddish colour (assumed to be very attractive to hummingbirds, e.g. Waser 2006), which makes them highly visible. We counted flowers and bracts separately because young flowers remain hidden in the bracts and potentially do not contribute to the attractiveness of an inflorescence, whereas the bracts appear to be very "showy". Open flowers were counted separately because they offer floral rewards such as nectar and pollen. The number of displayed flowers includes the number of open flowers, thus being the total number of visible flowers to visitors. Flower visitors entering an open flower or touching the anthers/stigma respectively were counted as legitimate flower visitors (potential pollinators). Only one "nectar thieve" species (see Inouye 1980) was observed during the whole observation period and not included in this study: a butterfly species that stole nectar eight times without touching the flowers' reproductive organs. Flower visitors that only collected pollen without touching reproductive parts of the flower were counted as "pollen robbers" and included in this study. Each time a flower visitor entered the observation plot, it was counted, with any visitor leaving the plot and entering it again being counted as a "new" visitor. Marking individuals was not possible, thus some visits may have been repeat visits by the same individual. Flower visitors were counted in observation units of 20 min each. Three observation units per hour were conducted, resulting in 30 units per observation day of 10 hours (7:00 am-5:00 pm) and in 360 observation units in total for all twelve observation days. In addition, for each unit, weather (sunny, bright, cloudy, overcast) and light conditions (half shade, shade) were recorded as well as the guild of visitor (Trochilidae, Hymenoptera, Lepidoptera) and the number of visited flowers in a row per plot. Hummingbird species were identified using a fieldguide and confirmed by ornithologists from the University of Rio de Janeiro. Insect visitors were caught by sweep netting. Whereas the butterfly species remained unidentified, the genus of the stingless bee species could be identified with the help of a binocular microscope and an identification key for neotropical insects.

Nectar sugar composition

Twenty nectar samples of *H. angusta* were collected in the late afternoon from flowers bagged before anthesis using micro-pipettes (Drummond "Microcaps" 10 µl). The nectar was transferred onto small strips of filter paper, which were then dried and stored in silica gel (Schwerdtfeger 1996). The laboratory analysis was carried out using a "Merck Hitachi-HPLC" with RI-Detector. The solvent used was 30 % water and 70 % acetonitril (ACN); the column was a Macherey-Nagel Nucleodur 100-5 NH2, 250 x 4.6 mm. S/H ratio was calculated as [sucrose] / [fructose + glucose].

Statistical analyses

In order to test the homogeneity of the recorded number of total visits and number of visits of each visitor group for all days and sites we performed a Two-Way-ANOVA.

The impact of plant inflorescence attractiveness (number of open and displayed flowers, number of bracts) on the number of visitors per group and visitation frequency (number of visitors / flower / hour) per plot was analysed by way of a Best Subsets Regression. Best Subsets Regression is a technique for selecting variables in a multiple linear regression by systematically searching through the different combinations of the independent variables (number of flowers displayed, number of flowers open, number of bracts) and selecting the subsets of variables that best contribute to predicting the dependent variable (number of visitors; visitation frequency, respectively).

To statistically determine the potential influence of the time of day, light- and weather-conditions on the number and frequency of all visits, a Kruskall-Wallis-ANOVA on ranks with Student-Newman-Keuls multiple comparison procedure was used, as the data were not normally distributed. To test for differences between the number of visits of each visitor group in respect to the time of day, a One-Way-ANOVA was carried out. The levels of significance are as followed: $p < 0.05$ = *; $p < 0.01$ = ** and $p < 0.001$ = ***. All analyses were performed using SigmaStat 3.0.1, SPSS Inc. 2003 and SigmaPlot 2000, SPSS Inc. 2000.

RESULTS

Visitor species and their behaviour

The numbers of overall visits for each observation day and site were comparable (total visits vs. observation day, $F = 0.16$, $p = 0.965$; total visits vs. site, $F = 0.33$, $p = 0.67$). Furthermore, no influence of the observation day or site could be observed on the number of visits of each visitor group (Hymenoptera: day vs. visits $F = 0.04$, $p = 0.99$; site vs. visits $F = 0.28$, $p = 0.69$; Trochilidae: day vs. visits $F = 5.37$, $p = 0.32$; site vs. visits $F = 0.64$, $p = 0.57$; Lepidoptera: day vs. visits $F = 0.02$, $p = 0.88$; site vs. visits $F = 0.16$, $p = 0.76$).

TAB. 1. Total number of floral visits to the three 2 x 2 m observation plots of *H. angusta* based on 360 20 min observation periods in the Atlantic Rainforest of Brazil.

visitor species	number of visits
Meliponini (stingless bees) *Trigona* sp.	81
Trochilidae (hummingbirds) in total	60
Phaethornis ruber	*41*
Phaethornis squalidus	*10*
Aphantochroa cirrochloris	*8*
Thalurania glaucopis	*1*
Lepidoptera (butterflies), 2 species	10
Total number of visits	151

During the 360 observation units of 20 minutes each we recorded a total of 151 flower visits to the flowers of *H. angusta*, with a total of seven different visitor species being noted (Tab. 1). The most frequent visitor to *H. angusta* was *Trigona* sp., a small black stingless bee (Hymenoptera, Apidae, Apinae, Meliponini), which accounted for 54 % of all visits and collected only pollen. Four different species of nectar feeding hummingbirds accounted for 40 % of all visits, of which two species of traplining hermits (*Phaethornis ruber* and *Phaethornis squalidus*) visited *H. angusta* most frequently

(Tab. 1). All hummingbird species made legitimate visits to the flowers. Pollen was deposited on the forehead of the birds and they touched the stigma each time when visiting a flower. Therefore the observed hummingbird species can be considered as potential pollinators. *Trigona* sp. was observed collecting pollen 81 times in contrast to the hummingbirds that only were observed 60 times visiting the flowers during the whole observation period. Out of all visits of *Trigona* sp. only 16.05 % were legitimate visits, meaning that in 13 visits out of a total of 81 visits the stingless bees touched the stigmas and thus might have pollinated the flowers of *H. angusta*. 83.95 % of all visits can be considered as illegitimate, because the stingless bees were only collecting pollen without touching the stigmas and thus were robbing the flowers. Two unidentified species of butterfly were rare flower visitors, together accounting for only 6 % of all visits. When visiting the flowers they touched the anthers and pollen got deposited to their body.

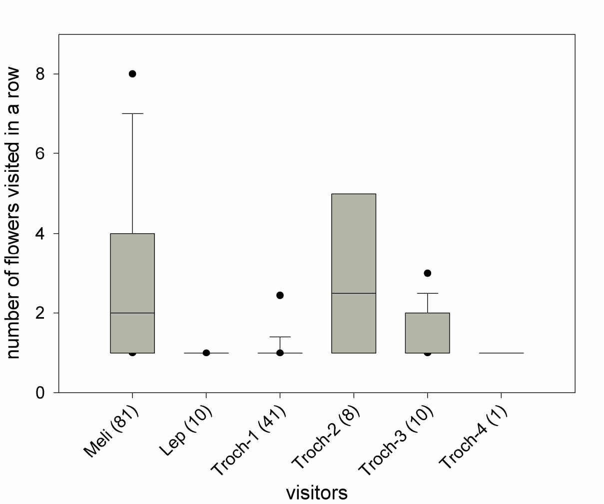

FIG. 1. Box-plots showing the number of *H. angusta* flowers visited in a row by each visitor species per 20min observation period for a total of 120 h of observation (figures in parentheses indicate number of visits; the black line is the median; the upper/lower lines of the box are the 1st and 3rd quartiles; the vertical lines are the 5th and 95th percentiles and the dots represent outliers within the 5th and 95th percentiles). Meli = stingless bees (Meliponini), single species: *Trigona sp.*; Lep = butterflies (Lepidoptera), two unidentified species; Troch = hummingbirds, Troch-1: *Phaethornis ruber*, Troch-2: *Aphantochroa cirrochloris*, Troch-3: *Phaethornis squalidus*, Troch-4: *Thalurania glaucopis*

Trigona sp. visited the flowers per plot up to 12 times in a row (mean: two flowers, Fig. 1). The Sombre Hummingbird (*Aphantochroa cirrochloris*) visited an average of three flowers in a row, with *P. ruber* and *P. squalidus* visiting one and two, respectively. The Violet-capped Woodnymph (*Thalurania glaucopis*) was recorded only once visiting one flower (Fig. 1).

Influence of plant inflorescence´ attractiveness on visitor abundance

The mean number of open flowers per plot was 2.08 (SD: 1.56); mean number of flowers displayed per plot 21.16 (SD: 13.45), and mean number of bracts per plot and day 17.00 (SD: 9.74). Inflorescence attractiveness (parameters: number of open and displayed flowers, number of bracts) had an effect on the number of floral visitors for hummingbirds (Tab. 2), but not for *Trigona* sp. or butterflies. Hummingbird visits per plot significantly increased with more flowers displayed ($p < 0.001$, $R^2 = 0.68$, Tab. 2). The same positive interrelation was found when the two parameters of flowers displayed and number of bracts were taken together ($p < 0.01$, $R^2 = 0.71$), and when all three parameters (plus number of open flowers) were included in the analysis ($p < 0.05$, $R^2 = 0.71$, Tab. 2). There was no influence of inflorescence attractiveness on visitor frequency in any of the three visitor groups ($p \geq 0.19$, $R^2 \leq 0.20$).

TAB. 2. Best Subsets Regression of the influence of the plant inflorescence attractiveness on the number of visits. Each visitor group was tested separately (R^2 = coefficient of determination, p = level of significance; p < 0.05 = *; p < 0.01 = **; p < 0.001 = ***)

	P number of flowers displayed	P number of bracts	P number of open flowers	R^2
Meliponini	-	-	0.13	0.21
	-	0.53	0.12	0.25
	0.86	0.63	0.18	0.25
Lepidoptera	-	-	0.45	0.06
	0.97	-	0.55	0.06
	0.99	0.99	0.58	0.06
Trochilidae	<0.001 ***	-	-	0.68
	0.01 **	0.4	-	0.71
	0.03 *	0.43	0.83	0.71

Influence of time of day, weather and light conditions on number and frequency of visits

Most total visits were recorded in the morning between 9:00 am and 11:00 am (morning vs. early morning: 7:00 am to 9:00 am, $q = 4.06$, $p < 0.05$, Fig. 2) and between 11:00 am and 1:00 pm (Fig. 2). The total number of visits decreased throughout the course of the day and was lowest in the late afternoon between 3:00 pm and 5:00 pm (morning vs. late afternoon, $q = 7.51$ $p < 0.05$, Fig. 2). Stingless bees (Meliponini) responded strongest to the time of day with a clear peak around noon (Fig. 2, Tab. 3). Significantly more bees than hummingbirds ($q = 3.27$, $p < 0.05$) and butterflies ($q = 6.18$, $p < 0.001$) were observed between 9:00 am and 11:00 am. The number of visits of hummingbirds and bees within the time intervals of 11:00 am to 1:00 pm and 1:00 pm to 3:00 pm did not differ significantly from each other. No differences in the number of visits of all visitor groups could be found for the time intervals 7:00 am - 9:00 am and 3:00 pm - 5:00 pm (Tab. 3).

Light conditions had a significant impact on visitor frequency, but not on visitor number. The number of visits per flower increased under half-shade conditions in comparison to full shade at the observation plots (half-shade vs. shade, $q = 4.28$, $p < 0.05$). There was no significant influence of weather conditions on number and frequency of visits.

Nectar sugar composition

The one-day flowers of *H. angusta* produced 44.4 µl (SD: 18.4) of nectar on average per day (maximum 80 µl). The nectar was sucrose-rich with an average of 10 % sucrose (SD: 2.1), but the hexose level was rather low with 3 % fructose (SD: 0.8) and 3 % glucose (SD: 0.9). The total nectar concentration accounted for 16 % (SD: 3.5) and the sugar ratio (sucrose/hexose) was 1.63 (SD: 0.33).

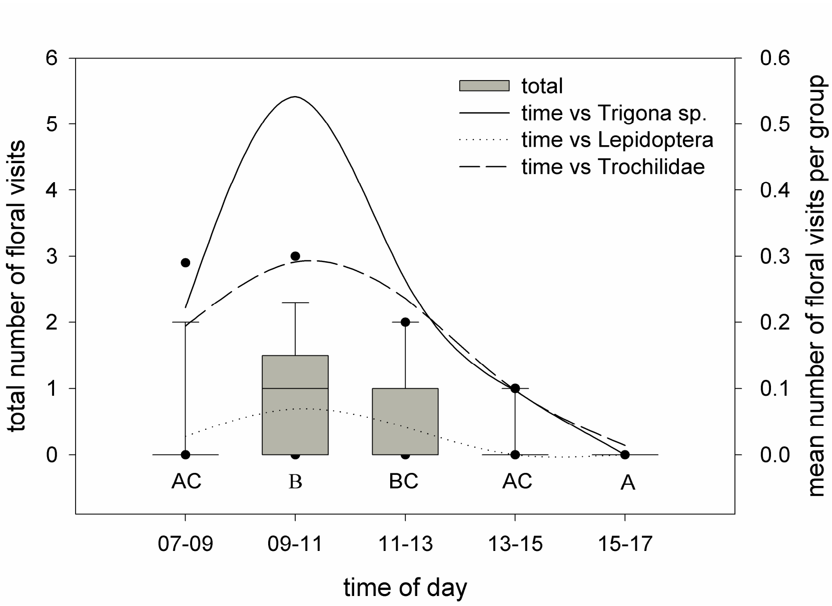

FIG. 2. Boxplots (corresponding to left y-axis) showing the total number of floral visits per flower per 2 x 2m observation plot to *H. angusta* at different times of day (number of 20 min observation units was 72 for each time span; dots, outliers within 5th and 95th percentiles). Box-plots with equal letters do not differ significantly from each other (ANOVA testing). Curves (corresponding to right y-axis) show the mean number of floral visitors per group. *Trigona* = stingless bees (Meliponini, one species); Lepidoptera = butterflies; Trochilidae = hummingbirds.

TAB. 3. Mean number of visits (± SD) of each visitor group during the course of day (different time intervals of 2 hours). ns = not significant.

time period (hrs)	7 – 9	9 – 11	11 – 13	13 – 15	15 – 17
visitor group:					
Hymenoptera	0.22 ± 0.75	0.54 ± 0.93	0.26 ± 0.63	0.1 ± 0.30	-
Trochilidae	0.19 ± 0.49	0.29 ± 0.57	0.24 ± 0.52	0.1 ± 0.34	0.01 ± 0.12
Lepidoptera	0.03 ± 0.45	0.07 ± 0.26	0.04 ± 0.20	-	-
F*	2.84	9.57	4.5	3.3	1.0
p*	0.06 ns	< 0.001	< 0.05	< 0.05	0.37 ns

* One-Way-ANOVA

DISCUSSION

The investigated plant species was found to be visited by two dominant flower visitor species (a stingless bee *Trigona* sp. and a hermit hummingbird *P. ruber*), which is in accordance with the results of other studies in tropical and temperate regions. De Castro and De Oliveira (2002) also reported visitation by one or two dominant flower-visitor species for several species of Rubiaceae while Gao et al. (2004) revealed different flower visitors for *Curcumorpha longiflora* (Zingiberaceae), Mitchell et al. (2004) for *Mimulus ringens* (Phrymaceae) and Tomimatsu & Ohara (2003) for *Trillium camschatcense* (Trilliaceae). However, care has to be taken when interpreting the most common visitor as the most important pollinator (Fenster et al. 2004). Schmid et al. (2011) reported that although bees most frequently visited the ornithophilous flowers of *Aechmea nudicaulis* (Bromeliaceae), a rare visiting hummingbird had the highest relative pollination effectiveness and was significantly more effective than all the bees combined. Only experimental tests can reveal the pollination effectiveness of each visitor species (Freitas & Paxton 1998; Botes et al. 2009). As this study only deals with the flower visitor species of *H. angusta* and their behaviour, our data do not allow for conclusions to be drawn on the effectiveness of the visitors as pollinators. Further reports on many tropical long-tubed, nectar rich, hummingbird-pollinated species suggest that a more complicated relationship frequently exists involving floral parasitism, e.g. by pollen and nectar collecting insects (Gentry 1974a, Janzen 1975, McDade & Kinsman 1980). Many species of stingless bees, in particular of the genus *Trigona* are known to rob flowers (Almeda 1977; Roubik 1982; Renner 1983) by collecting nectar and pollen. A study of McDade and Kinsman (1980) revealed that species of the genus *Trigona* took pollen from the ornithophilous *Aphelandra golfodulcensis* (Acanthaceae) flowers in Costa Rica without contributing to pollination. Roubik (1982) postulated that robbing *Trigona* sp. may present a reproductive hazard to many species of flowering plants throughout the tropics (see also Hubbel & Johnson 1978; Michener 1979). On the other hand stingless bees are considered important pollinators of the native flora in tropical and subtropical parts of the world, and they have been found to contribute to the effective pollination of 18 crops and many wild plants (Heard 1999; Slaa et al. 2006). Especially in the Neotropics stingless bees were the only bees for a long time (before the introduction of European honey bees) and often make up half of all flower visitors (Biesmeijer JC, 2011, pers. comm.). The review of Slaa et al. (2006) on the importance of stingless bees in applied pollination specifies ten different species of *Trigona* alone, which effectively pollinated different crops. Clearly the objective of bees (and all other flower visitors) is not to pollinate but to get reward, but in the process they might pollinate (Biesmeijer JC, 2011, pers. comm.). In our study, *Trigona* sp. observed at flowers of *H. angusta* only collected pollen, and the typical robbing behaviour of piercing of the corolla to steal nectar was not observed. *Trigona* sp. was observed at times touching the stigmas of *H. angusta* flowers when collecting pollen. Although these legitimate visits only account for 16.05 % of all visits of the bees, they might have resulted in a transfer of pollen to the stigma and the pollination of flowers on occasion. However, 83.95 % of all bee visits were illegitimate, when *Trigona* sp. only collected pollen without touching the stigma. Thus, *Trigona* sp. may rather be considered a robber to *H. angusta* due to the frequent removal of pollen that otherwise would have been transferred to stigmas by more-effective pollinators. Nevertheless species of the genus *Trigona* still have the potential to be secondary pollinators, even if pollination may happen occasionally and rare during pollen collection. In contrast, all visits of hummingbirds were legitimate. Pollen was deposited to the forehead of the birds and they touched the stigmas each time they visited a flower. Thus the observed hummingbird species are considered as principle potential pollinators of *H. angusta*.

Although the stingless bees and the main hummingbird visitors visited several flowers in a row, the mean visitation rate of all visitor groups was very low at 0.7 visits per flower per hour. Similar results were found by Bruna et al. (2004) for *Heliconia acuminata* (Heliconiaceae) from Amazonia and Singer and Sazima (2000) also recorded between zero and four visits per day to the flowers of *Stenorrhynchos lanceolatus* (Orchidaceae), a hummingbird pollinated orchid from the Atlantic rainforest.

Our study revealed a significant correlation between plant inflorescence attractiveness and the number of hummingbird visits per plot. Hummingbird visits increased with the number of flowers displayed and the influence of this parameter on the number of hummingbird visits was statistically the strongest. The number of bracts or that of open flowers had no individual influence on the number of hummingbird visits; however, when they were assessed together with the number of displayed flowers, a significantly higher number of hummingbird visits were recorded. The contrast of the red bracts with the long white flowers (ornithophilous flowering syndrome, see Vogel 1996; Waser 2006) seems to be very attractive to hummingbirds, as this colour display is highly visible in the tropical forest vegetation environment. A positive correlation between number of flower visitors and increasing attractiveness of the plant (e.g. number of flowers, inflorescence display size) was also reported by Brody and Mitchell (1997) for the hummingbird-pollinated plant *Ipomopsis aggregata* (Polemoniaceae), by Rodriguez-Robles et al. (1992) for the ornithophilous orchid *Comparettia falcate* (Orchidaceae), and by Bosch and Waser (2001) for *Aconitum columbianum* (Ranunculaceae).

The time of day had a significant influence on the number of visitors of each floral visitor group, with most visitors appearing between 9:00 am and 1:00 pm (Fig. 2, Tab. 3). Similar results were reported by Renner (1983) for *Trigona* spec. at Melastomataceae and by Stone and Jenkins (2008) for a solanaceous shrub in Costa Rica with a concentration of pollinator visits in the late morning, while Singer and Sazima (2001) observed most visitors appearing between 10:00 am and 2:00 pm for three orchid species from south-eastern Brazil. In addition, better light conditions promoted higher visitor frequencies and significantly more visitors per flower per hour were observed under half shade conditions than under full shade. Our results are in accordance with Vicens and Bosch (2000), who found bee activity to be positively related to temperature and light conditions, and with Singer and Sazima (2001), who recorded more visitors during sunnier weather conditions. A feasible explanation for these results might be the activity dependence of ectothermic insects on external heat. Furthermore the body colour and body size influence a bee´s thermoregulation (Biesmeijer et al. 1999). The observed species of *Trigona* has a thorax width of > 2 mm, thus it can be considered as rather large in comparison to other stingless bee species. Larger insects gain and lose heat more slowly but attain higher temperature excesses than smaller insects (Pereboom & Biesmeijer 2003). This might explain the clear activity peak around noon, when temperatures are highest. More visits of these bees were recorded under half-shade conditions than under full shade, which might be due to a better avoidance of temperature excesses under half-shade conditions. Higher temperatures, co-correlated with time of day, and sunlight also increased the visitation rates of the Florida scrub, belonging to the family of Lamiaceae, *Dicerandra frutescens* (Deyrup & Menges 1997) and for the understory herb *Justicia rusbyi* (Acanthaceae) in eastern Bolivia (Döll et al. 2007).

The number of visits by hummingbirds showed a clear peak between 9:00 am and 11:00 am and then decreased during the day. A similar pattern of foraging activity was reported by Garrison and Gass (1999) for the traplining hermit hummingbird *Phaethornis longirostris* that uses highly productive flowers such as *Heliconia pogonantha* (Heliconiaceae) in Costa Rica. The birds visited the flowers less in early morning and late afternoon than in mid-morning with a peak at 9:00 am. Because nectar production rates of many flowers used by traplining hummingbirds (including *H. pogonantha*) are high in the early morning and declines rapidly during the day (Stiles 1975; Stiles & Freeman 1993) the hummingbirds decrease their feeding frequency during the day to maximize net energy intake (Gass & Garrison 1999) rather than attempting to maintain constant net intake (Hainsworth et al. 1981; Stiles & Wolf 1979). The low visitation rate during the early morning hours may reflect satiation due to an abundance of food (Gill 1988). This early morning surplus of food could affect visitation rates in two ways: birds would be full after visiting few flowers and would not have to visit many others to meet their energy requirements. After the morning surplus is depleted, foraging effort should increase because birds must visit more flowers to take the same amounts of nectar. As nectar production rates continue to decrease and standing crop is diminished, hermits should decrease feeding efforts to save energy (Garrison & Gass 1999). This foraging pattern was also the case in our study with two traplining hermits being the most abundant hummingbird visitors to *H. angusta*. Tiebout (1992) and Stiles (1995) also reported that total observed foraging activity was lower in the afternoon than in the morning for several species of hermit and nonhermit hummingbirds in Costa Rica. These tropical species also feed primarily on flowers that have a decreasing nectar production rate over the day (Feinsinger 1976). This suggests that unlike in temperate systems, where foraging effort is bimodal with peaks of visitation rates in the morning and afternoon (Gass & Montgomerie 1981), tropical hummingbirds decrease foraging efforts after mid-morning to conserve energy because nectar production and standing crop are quite low in the afternoon (Stiles 1975, Stiles & Wolf 1979).

The nectar of *H. angusta* is rich in sucrose, whereas hexoses (glucose and fructose) are rare. The total sugar concentration is 16 %. Nectar sugar composition is claimed to be a useful predictor of pollinators, with sucrose-dominant nectar being indicative of butterflies, hummingbirds and large bees (Stiles & Freeman 1993; Baker et al. 1998). Stingless bees are usually included in the sucrose-preferring group (Schwerdtfeger 1996, Biesmeijer et al. 1999a,b). Our data are in accordance with these findings and all observed floral visitor groups fit well with the nectar sugar characteristics. The sucrose-hexose ratio of 1.63 is typical of hummingbird and large bee flowers (Baker & Baker 1990). Similar results were found by Perret et al. (2001) for ornithophilous neotropical Gesneriaceae and by Schmid et al. (2011) for the ornithophilous bromeliad *Aechmea nudicaulis* (Bromeliaceae). According to Roubik et al. (1995), stingless bee foragers tend to use all nectar concentrations, even as low as 5 % or as high as 67 %, although they prefer higher sugar concentrations of about ≤ 45 % (e.g. Roubik & Buchmann 1984; Fidalgo & Kleinert 2010). They are true generalists, collecting nectar and pollen from a vast array of plants (Ramalho et al. 1990; Biesmeijer et al. 2005). Pollen is used for larval development and nectar as the energy source. Since we did not observe *Trigona* bees collecting nectar, because they could not enter the narrow corolla tube nor did they pierce the corolla, we consider them visiting the flowers only to collect pollen. Thus the nectar characteristics fit to stingless bee foragers, but the nectar reward seems not to be the reason why *Trigona* bees visit the flowers of *H. angusta*.

We conclude that the specialized flowers of *H. angusta* not only attract hummingbirds, but as well generalistic insects, that have a certain potential to be pollinators and that the classification of floral visitors as either 'robbers' or 'pollinators' may be simplistic as some robbers can at times be considered potential pollinators.

The study confirms the importance of direct field observations to investigate the visitor spectrum of a particular plant species and to determine potential pollinators and antagonistic floral visitors. Further studies should focus on the pollination effectiveness of each visitor group separately in terms of e.g., fruit set, seed set, pollen deposition rate and the role of *Trigona* sp. in particular as pollen robber and/or secondary alternative pollinator of the flowers of *H. angusta*.

ACKNOWLEDGEMENTS

We would like to thank Nickolas and Raquel Locke from the NGO "Regua" for logistical support, Martin Hoepke and Jens Rabenstein for help in the field as well as Anja Wodak from the Institute of Pharmacy for analysing the nectar samples. Special thanks to Koos Biesmeijer for rich information on stingless bees and Josef Settele for valuable hints. We also thank three anonymous reviewers for their valuable comments on the manuscript. KS is funded by Studienstiftung des Deutschen Volkes.

REFERENCES

Almeda F (1977) Systematics of the neotropical genus *Centradenia* (Melastomataceae). Journal of the Arnold Arboretum 58:73-108.

Arizmendi MC, Domínguez CA, Dirzo R (1996) The role of an avian nectar robber and of hummingbird pollinators in the reproduction of two plant species. Functional Ecology 10:119-127.

Baker HG, Baker I, Hodges SA (1998) Sugar composition of nectar and fruits consumed by birds and bats in the tropics and subtropics. Biotropica 30:559-586.

Baker HG, Baker I (1990) The predictive value of nectar chemistry to the recognition of pollinator types. Israel Journal of Botany 39:157-166..

Bawa KS (1990) Plant-pollinator interactions in tropical rain forests. Annual Review of Ecology, Evolution, and Systematics 21:399-422.

Bawa KS, Perry DR, Bullock SH, Coville RE, Grayum MH (1985) Reproductive biology of tropical lowland rain forest trees. II. Pollination mechanisms. American Journal of Botany 72:346-356.

Biesmeijer JC, Slaa EJ, Siqueira de Castro M, Viana BF, Kleinert A, Imperatriz-Fonseca VL (2005) Connectance of Brazilian social bee – food plant networks is influenced by habitat, but not by latitude, altitude or network size. Biota Neotropica 5:1-9.

Biesmeijer JC, Richter JAP, Smeets MAJP, Sommeijer MJ (1999a) Niche differentiation in nectar-collecting stingless bees: the influence of morphology, floral choice and interference competition. Ecological Entomology 24:380-388.

Biesmeijer JC, Smeets MAJP, Richter JAP, , Sommeijer MJ (1999b) Nectar foraging by stingless bees in Costa Rica: botanical and climatological influences on sugar concentration of nectar collected by *Melipona*. Apidologie 30:43-55.

Bosch M, Waser NM (2001) Experimental manipulation of plant density and its effect on pollination and reproduction of two confamilial montane herbs. Oecologia 126:76-83.

Botes C, Johnson SD, Cowling RM (2009) The birds and the bees: using selective exclusion to identify effective pollinators of African tree *Aloes*. International Journal of Plant Sciences 170: 151-156.

Brody AK, Mitchell RJ (1997) Effects of experimental manipulation of inflorescence size on pollination and pre-dispersal seed predation in the hummingbird-pollinated plant *Ipomopsis aggregata*. Oecologia 110:86-93.

Bruna EM, Kress WJ, Marques F, da Silva OF (2004) *Heliconia acuminata* reproductive success is independent of local floral density. Acta Amazonica 34:467-471.

Campbell DR, Waser NM, Price MV (1996) Mechanisms of hummingbird-mediated selection for flower width in *Ipomopsis aggregata*. Ecology 77:1463-1472.

Canela MBF, Sazima M (2005) The pollination of *Bromelia antiacantha* (Bromeliaceae) in southeastern Brazil: ornithophilous *versus* melittophilous features. Plant Biology 7:411-416.

Dahlgren RMT, Clifford HT, Yeo PF (1985) The families of the monocotyledons. Springer-Verlag, Berlin.

De Castro CC, De Oliveira PE (2002) Pollination biology of distylous Rubiaceae in the Atlantic rain forest, SE Brazil. Plant Biology 4:640-646.

De Castro CC, Araujo AC (2004) Distyly and sequential pollinators of *Psychotria nuda* (Rubiaceae) in the Atlantic rain forest, Brazil. Plant Systematics and Evolution 244:131-139.

Deyrup M, Menges E (1997) Pollination ecology of the rare scrub mint *Dicerandra frutescens* (Lamiaceae). Florida Scientist 60:143-157.

Dias da Cruz D, Mello MAR, Van Sluys M (2006) Phenology and floral visitors of two sympatric *Heliconia* species in the Brazilian Atlantic forest. Flora 201:519-527.

Döll S, Hensen I, Schmidt-Lebuhn AN, Kessler M (2007) Pollination ecology of *Justicia rusbyi* (Acanthaceae), a common understory plant in a tropical mountain forest in eastern Bolivia. Plant Species Biology 22:211-216.

Feinsinger P (1976) Organization of a tropical guild of nectarivorous birds. Ecological monographs 46:257-291.

Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD (2004) Pollination syndromes and floral specialization. Annual Review of Ecology, Evolution, and Systematics 35:375-403.

Fidalgo A, Kleinert A (2010) Floral preferences and climate influence in nectar and pollen foraging by *Melipona rufiventris* Lepeletier (Hymenoptera: Meliponini) in Ubatuba, Sao Paulo State, Brazil. Neotropical Entomology 39:879-884.

Freitas BM, Paxton RJ (1998) A comparison of two pollinators: the introduced honey bee *Apis mellifera* and an indigenous bee *Centris tarsata* on cashew *Anacardium occidentale* in its native range of NE Brazil. Journal of Applied Ecology 35:109-121.

Galetto L (1998) Estructura floral y composición química del néctar en tres especies de Rubiaceae. Kurtziana 26:83-98.

Gao JY, Zhang L, Deng XB, Ren PY, Kong JJ, Li QJ (2004) The floral biology of *Curcumorpha longiflora* (Zingiberaceae): a ginger with two day flowers. American Journal of Botany 91:289-293.

Garrison JSE, Gass CL (1999) Response of a traplining hummingbird to changes in nectar availability. Behavioural Ecology 6:714-725.

Gass CL, Garrison JSE (1999) Energy regulation by traplining hummingbirds. Functional Ecology 13:483-492.

Gass CL, Montgomerie RD (1981) Hummingbird foraging behaviour: decision-making and energy regulation. In: Karnil AC, Sargent TD (eds.) Foraging behaviour: ecological, ethological, and psychological approaches. Garland STPM Press, New York, pp 157-194.

Gentry AH (1974a) Coevolutionary patterns in Central American Bignoniaceae. Annals of the Missouri Botanical Garden 61:728-759.

Gentry AH (1974b) Flowering phenology and diversity in tropical Bignoniaceae. Biotropica 6:54-68.

Gill FB (1988) Trapline foraging by hermit hummingbirds: competition for an undefended, renewable resource. Ecology 69:1933-1942.

Graves GR (1982) Pollination of *Tristerix* mistletoe (Loranthaceae) by *Diglossa* (Aves, Thraupidae). Biotropica 14:316-317.

Gross C (2005) Pollination efficiency and pollinator effectiveness. In: Dafni A, Kevan P, Husband B (eds). Practical Pollination Biology. Ontario Enviroquest, Cambridge, pp 354–363 .

Hainsworth FR, Tardiff M, Wolf LL (1981) Proportional control for daily energy regulation in hummingbirds. Physiological Zoology 54:452-462.

Harder LD, Jordan CY, Gross WE, Routley MB (2004) Beyond floricentrism: The pollination function of inflorescences. Plant Species Biology 19:137-148.

Horvitz CC, Schemske DW (1990) Spatio temporal variation in insect mutualism of a neotropical herb. Ecology 71:1085-1097.

Hubbell SP, Johnson LK (1978) Comparative foraging behaviour of six stingless bee species exploiting a standardized resource. Ecology 59:1123-1136.

Inouye DW (1980) The terminology of floral larceny. Ecology 61:1251-1253.

Janzen DH (1975) Ecology of plants in the tropics. Edward Arnold, London.

Kress WJ (1990) The diversity and distribution of *Heliconia* (Heliconiaceae) in Brazil. Acta Botanica Brasilica 4:159-167..

Krömer T, Kessler M, Lohaus G, Schmidt-Lebuhn AN (2008) Nectar sugar composition and concentration in relation to pollination syndromes in Bromeliaceae. Plant Biology 10:502-511.

Kurtz BC, de Araújo DSD (2000) Composição florística e estrutura do componente arbóreo de um trecho de Mata Atlântica na Estaoção Ecológica Estadual do Paraíso, Cachoeiras de Macacu, Rio de Janeiro, Brasil. Rodriguésia 51:69-112.

McDade LA, Kinsman S (1980) The impact of floral parasitism in two neotropical hummingbird-pollinated plant species. Evolution 34:944-958.

Michener CD (1979) Biogeography of the bees. Annals of the Missouri Botanical Garden 66:277-347.

Mitchell RJ, Karron JD, Holmquist KG, Bell JM (2004) The influence of *Mimulus ringens* floral display size on pollinator visitation patterns. Functional Ecology 18:116-124.

Morellato LPC, Haddad CFP (2000) Introduction: The Brazilian Atlantic Forest. Biotropica 32:786-792.

Oliveira-Filho AT, Fontes MAL (2000) Patterns of floristic differentiation among Atlantic Forests in southeastern Brazil and influence of climate. Biotropica 32:793-810.

Ollerton J, Alarcón R, Waser NM, Price MV, Watts S, Cranmer L, Hingston A, Peter CI, Rottenberry J (2009) A global test of the pollination syndrome hypothesis. Annals of Botany 103:1471-1480.

Ollerton J, Johnson SD, Cranmer L, Kellie S (2003) The pollination ecology of an assemblage of grassland Asclepiads in South Africa. Annals of Botany 92:807-834.

Pereboom JJM, Biesmeijer JC (2003) Thermal constraints for stingless bee foragers: the importance of body size and coloration. Oecologia 137:42-50.

Perret M, Chautems A, Spichiger R, Peixoto M, Savolainen V (2001) Nectar sugar composition in relation to pollination syndromes in Sinningieae (Gesneriaceae). Annals of Botany 87:267-273.

Proctor M, Yeo P, Lack A (1996) The natural history of pollination. Timber Press, Portland.

Ramalho M, Kleinert-Giovannini A, Imperatriz-Fonseca VL (1990) Important bee plants for stingless bees (Melipona and Trigonini) and Africanised honeybees (Apis mellifera) in neotropical habitats: a review. Apidologie 21:469-488.

Renner S (1983) The widespread occurrence of anther destruction by *Trigona* bees in Melastomataceae. Biotropica 15:251-256.

Robertson AW, Ladley JI, Kelly D (2005) Effectiveness of short-tongued bees as pollinators of apparently ornithophilous New Zealand mistletoes. Austral Ecology 30:298-309.

Rodriguez-Robles JA, Melendez EJ, Ackermann JD (1992) Effects of display size, flowering phenology, and nectar availability on effective visitation frequency in *Comparettia falcata* (Orchidaceae). American Journal of Botany 79:1009-1017.

Roubik DW, Yanega D, Aluja SM, Buchmann SL, Inouye DW (1995) On optimal nectar foraging by some tropical bees (Hymenoptera: Apidae). Apidologie 26:197-211.

Roubik DW (1989) Ecology and Natural History of Tropical Bees. Cambridge University Press, Cambridge.

Roubik DW, Buchmann SL (1984) Nectar selection by *Melipona* and *Apis mellifera* (Hymenoptera: Apidae) and the ecology of nectar intake by bee colonies in a tropical forest. Oecologia 61:1-10.

Roubik DW (1982) The ecological impact of nectar-robbing bees and pollinating hummingbirds on a tropical shrub. Ecology 63:354-360.

Sazima I, Buzato S, Sazima M (1995) The Saw-billed Hermit *Ramphodon naevius* and its flowers in southeastern Brazil. Journal of Ornithology 136:195-206.

Schmid S, Schmid VS, Zillikens A, Harter-Marques B., Steiner J (2011) Bimodal pollination system of the bromeliad *Aechmea nudicaulis* involving hummingbirds and bees. Plant Biology 13:41-50.

Schwerdtfeger, M (1996). Die Nektarzusammensetzung der Asteridae und ihre Beziehung zu Blütenökologie und Systematik. Dissertationes Botanicae 264..

Singer RB, Sazima M (2001) Flower morphology and pollination mechanism in three sympatric Goodyerinae Orchids from Southeastern Brazil. Annals of Botany 88:989-997.

Singer RB, Sazima M (2000) The pollination of *Stenorrhynchos lanceolatus* (Aublet) L.C. Rich. (Orchidaceae: Spiranthinae) by hummingbirds in southeastern Brazil. Plant Systematics and Evolution 223:221-227.

Simão DG, Scatena VL (2001) Morphology and anatomy in *Heliconia angusta* Vell. and *H. velloziana* L. Emygd. (Zingiberales: Heliconiaceae) from the Atlantic forest of southeastern Brazil. Revista Brasileira de Botânica 24:415-424.

Slaa EJ, Sánchez Chaves LA, Malagodi-Braga KS, Hofstede FE (2006) Stingless bees in applied pollination: practice and perspectives. Apidologie 37:293-315.

Smith CE, Stevens JT, Temeles EJ, Ewald PW, Hebert RJ, Bonkovsky RL (1996) Effect of floral orifice width and shape on hummingbird-flower interactions. Oecologia 106:482-492.

Steven JC, Rooney TP, Boyle OD, Waller DM (2003) Density-dependent pollinator visitation and self-incompatibility in upper Great Lakes populations of *Trillium grandiflorum*. Journal of the Torrey Botanical Society 130:23-29.

Stiles FG, Freeman CE (1993) Patterns in floral nectar characteristics of some bird-visited plant species from Costa Rica. Biotropica 25:191-205.

Stiles FG, Wolf LL (1979) Ecology and evolution of lek mating behaviour in the long-tailed hermit hummingbird. Ornithological Monographs 27:1-78.

Stiles FG (1975) Ecology, flowering phenology, and hummingbird pollination of some Costa Rican *Heliconia* Species. Ecology 56:285-301.

Stone JL, Jenkins EG (2008) Pollinator abundance and pollen limitation of a solanaceous shrub at premontane and lower montane sites. Biotropica 40:55-61.

Thompson JN, Pellmyr O (1992) Mutualism with pollinating seed parasites amid co-pollinators: constraints on specialization. Ecology 73:1780-1791.

Tiebout HM (1992) Comparative energetics of divergent foraging modes: a doubly labelled water experiment on hummingbird competition. Animal Behaviour 44:895-906.

Tomimatsu H, Ohara M (2003) Floral visitors of *Trillium camschatcense* (Trilliaceae) in fragmented forests. Plant Species Biology 18:123-127.

Veloso HP, Rangel-Filho AL, Lima JCA (1991) Classificação da vegetação brasileira, adaptada a um sistema universal. IBGE/CDDI, Departamento de Documentação e Biblioteca, Rio de Janeiro.

Vicens N, Bosch J (2000) Weather-dependent pollinator activity in an apple orchard, with special reference to *Osmia cornuta* and *Apis mellifera* (Hymenoptera: Megachilidae and Apidae). Environmental Entomology 29:413-420.

Vogel S (1996) Christian Konrad Sprengel´s theory of the flower: The cradle of floral ecology. In: Lloyd DG, Barrett SCH (eds). Floral ecology, studies on floral evolution in animal-pollinated plants. Chapman and Hall, New York, pp 44-62.

Waser NM (2006) Specialization and Generalization in Plant-Pollinator Interactions: A Historical Perspective. In: Waser NM, Ollerton J (eds). Plant-Pollinator Interactions: from Specialization to Generalization. The University of Chicago Press, Chicago, pp 3-17.

Waser NM, Ollerton J (2006) Plant-pollinator interactions: from specialization to generalization. The University of Chicago Press, Chicago.

Waser NM, Chittka L, Price MV, Williams NM, Ollerton J (1996) Generalization in pollination systems, and why it matters. Ecology 77:1043-1056.

Waser NM, Price MV (1990) Pollination efficiency and effectiveness of bumble bees and hummingbirds visiting *Delphinium nelsonii*. Collectanea Botanica 19:9-20.

Willmer PG, Corbet SA (1981) Temporal and microclimatic partitioning of the floral resources of *Justicia aurea* amongst a concourse of pollen vectors and nectar robbers. Oecologia 51:67-78.

Willmott AP, Búrquez A (1996) The pollination of *Merremia palmeri* (Convolvulaceae): can hawk moths be trusted? American Journal of Botany 83:1050-1056.