Sociobiology 61(2): 189-197 (June, 2014) DOI: 10.13102/sociobiology.v61i2.189-197

Sociobiology
An international journal on social insects

RESEARCH ARTICLE - BEES

Pollinator Sharing in Specialized Bee Pollination Systems: a Test with the Synchronopatric Lip Flowers of *Centrosema* Benth (Fabaceae)

M Ramalho[1], M Silva[1,2], G Carvalho[2]

1 - Universidade Federal da Bahia (UFBA), Salvador, BA, Brazil.
2 - Faculdade de Tecnologia e Ciências, Salvador, BA, Brazil.

Article History
Edited by
Candida M L Aguiar, UEFS, Brazil
Received 30 December 2013
Initial acceptance 07 March 2014
Final acceptance 21 May 2014

Keywords
Nectar robber, size threshold , random interaction, bee pollinated flower

Corresponding author
Mauro Ramalho
Laboratório de Ecologia da Polinização
Instituto de Biologia
Universidade Federal da Bahia
Salvador, Bahia, Brazil
CEP: 40.210-730
E-mail: mrramauro@gmail.com

Abstract

Bee-pollinated lip flowers of two synchronopatric species of *Centrosema* were used as models to examine the influence of specialized pollination systems on the ecological mechanisms of pollinator sharing. Regression analysis of bee abundances in the habitat on bee abundances on *C. pubescens* flowers was significant ($r = 0.69$; $P = 0.001$) and became very consistent and highly significant ($r = 0.87$; $P = 0.00001$) using a size threshold of bee pollinators longer than 15mm. These same relationships were not significant ($P > 0.01$), however, for *C. brasilianum* flowers. The structures of the two pollination systems also sustained the hypothesis of a size threshold for pollinators, although only the *C. pubescens*-bees interactions sustained the hypothesis of random interactions proportional to species abundances in the habitat. The flower visitor pools of the two plant species shared the same four main bee guilds: the pollinators Centridini, *Xylocopa*, and Euglossini and the primary nectar robber *Oxaea*. However, a significant divergence ($P < 0.01$) was detected between the two systems when the abundances and behaviors (pollinators or cheaters) of the main shared flower visitors were incorporated into the overall quantitative analysis (NMDS). The flowers size differences are not significant ($P > 0.05$) and could not explain these divergences. Particularly, the concentrations of the largest pollinators *Eulaema* and *Xylocopa* on *C. pubescens* flowers and the behavior shift of Centridini bees that act as legitimate pollinators in *C. pubescens* and as nectar robbers in *C. brasilianum* are better understood as functional foraging responses triggered by the synchronopatry and by nectar volume differences ($P = 0.001$) between both lip flowers. Paradoxically, the robbery activity of Centridini bees arises as a supply side effect of smaller nectar volume in *C. brasilianum* flowers.

Introduction

Floral attributes have been protagonists in ample and controversial debates concerning adaptive changes and the mechanisms subjacent to flower-visitor interactions (e.g., Herrera, 1996; Ollerton et al., 2007). The roots of these debates reside, in large part, in the understanding that floral characteristics are adaptively flexible (Endress, 1994), even when they involve presumably specialized pollination modes (Tripp & Manos 2008), as flower-visitor interactions are often scale and ecological context dependent (Herrera, 2005; Ollerton et al., 2007). In comparing extensive floral data (on floral traits and pollinators) from six communities around the world, Ollerton et al. (2009) concluded that the “pollination syndrome hypothesis”, as traditionally stated, did not properly describe the diversity of floral phenotypes and only poorly predicted plant species pollinators. These authors therefore recommended a ‘fresh look at how the traits of flowers and pollinators relate to visitation and pollen transfer’ in searching for appropriate descriptions of functional floral diversity.

A question central to this debate is if other variables can be as important as morphological restrictions in structuring pollination systems. Recent analyses of partnerships and the structures of flower-visitor webs have both indicated that morphological constraints continue to play a central role in the general theory of the organization of pollination systems.

Open access journal: http://periodicos.uefs.br/ojs/index.php/sociobiology
ISSN: 0361-6525

The relationship between proboscis lengths and nectar chamber depth, for example, sustains the hypothesis that size thresholds are important in regulating those interactions (Stang et al., 2006). On the other hand, the pattern of "asymmetric specialization" that emerges from the webs (e.g., Vázquez, 2005) is also better explained when both size constraints and species abundance are incorporated into theoretical models (Stang et al., 2007). In this case, the following basic question directs the studies: are neutral mechanisms of random interactions proportional to species abundance sufficient to explain the asymmetric specialization (plants with specialized flowers frequently interacting with generalist visitors, and specialized floral visitors frequently interacting with generalized flowers) that has been observed in recent analyses of flower-visitor interaction webs (Vázquez & Aizen, 2004; Vázquez, 2005; Stang et al., 2007)?

Here we focus on the question of pollinator sharing in specialized pollination systems and have chosen as a model two synchronopatric species of *Centrosema* (Fabaceae) with specialized zygomorphic keel flowers. The highly zygomorphic keel flowers of Fabaceae are often pollinated by bees (Faegri & Van der Pijl, 1979; Westerkamp, 1996; Galloni et al., 2007). Bee-pollinated keel flowers have nectar guides and produce nectar as the main floral reward for those insects, but only large bees with appropriate nectar-searching behaviors are able to provoke the mechanical exposition of the fertile pollination organs (Van der Pijl, 1954; Gottsberger et al., 1988; Lopes & Machado 1996; Etcheverry et al., 2003; Ramalho & Rosa, 2010). A distinctive floral trait of bee pollinated keel flowers is protection of their pollen from bee consumption (which would presumably be more effective in lip-flowers, as their keel is dorsally positioned in relation to the landing petal) (Westerkamp, 1996).

Centrosema pubescens Benth and *Centrosema brasilianum* (L.) Benth have large and very similar lip-flowers with notable bee-pollination traits (Endress 1994). These two species show complete overlapping of their flowering periods and occur sympatrically along the tropical Atlantic coast of Brazil in herbaceous-shrub vegetation habitats (coastal "restingas" and sand dune areas). Their synchronopatric and specialized flowers should largely share pollinators according to the predictions of both the hypothesis of morphological constraints and that of random interactions proportional to species abundance. In cases such as this, pollinator sharing would favor the coexistence of species with subtle differences in their floral biology due to the potential loss of significant quantities of pollen to hetero-specific flowers (Jacobi et al., 2005). This study therefore assumed the following specific premises: first, the most shared groups of pollinators must be abundant in the habitat if divergences in floral biology of the two *Centrosema* species have only small influences on the functional differentiation of the two pollination systems; second, subtle morpho-functional differences in flower traits should produce greater differences among pollinating bees than among non-pollinating bees (flower visitors).

Material and Methods

Study area

The present study was undertaken in "restinga" vegetation along the eastern tropical Atlantic coast of Brazil. This coastal restinga vegetation develops under regional influences of the "The Tropical Atlantic Domain" (Por, 1992). The field study site was located in the Pituaçu Metropolitan Park (PMP) in the city of Salvador, Bahia State, Brazil (13°00' S; 38°30' W). The PMP is dominated by secondary growths of open shrubby-forests with patches of exposed sand dunes, covering an area of 450 ha, at approximately 50m above sea level. The regional climate is Af, according to Köppen classification system, with a mean annual rainfall of approximately 1500mm, and mean monthly temperatures vary between 18°C and 22°C. There is no marked dry season, although the vegetation, which grows on sandy soils, is exposed to sporadic water deficits throughout the year, and there are many areas with only thin vegetation covers.

Plant species. *Centrosema pubescens* Benth. and *Centrosema brasilianum* (L.) Benth. occupy open vegetation areas (dominated by herbs and small shrubs) in tropical restinga and coastal sand dune sites. *C. brasilianum* is a procumbent herb with glabrous inflorescences; its flowers are bluish-purple with white-yellowish pale stripes (nectar guides) on the banner petal. *C. pubescens* is a climbing vine with velutinous inflorescences; its flowers are bluish-pale lilac with pink and creamy white stripes on the banner. The flag (or banner) serves as a large landing platform for the bees on both lip flowers and the corolla base is completely surrounded by a robust tubular calyx. The robust keel (in a dorsal position) surrounds and protects the fertile verticils and it must be displaced upwards by bees moving towards the corolla base. Nectar is the main floral reward, and is well-protected at the base of the corolla by the tight juxtaposition of the petal bases (which form a rigid and very narrow passage to the nectar chamber). The nectar can be reached through the interiors of the flowers by robust bees with long proboscis (personal observation).

The two *Centrosema* species have complete overlapping flowering periods: *C. brasilianum* blooms year-round and *C. pubescens* from March to July (M. Ramalho & M. Silva unpublished). Permanent 20m X 20m plots were delimited in high density patches for field experiments, sampling, and bee visitation observations. The observed numbers of open flowers/day were relatively low (5-10 flowers/m^2/day and 10-15 flowers/m^2/day of *C.pubescens* and *C. brasilianum*, respectively; M.Silva & M. Ramalho unpublished) even in these dense patches where the flowers of both species were sometimes intermingled.

Floral Biology and morphology measures. The basic analyses of floral biology were performed following Dafni (1992). Stigma receptivity was tested with hydrogen peroxide, and nectar volumes were measured in ten flowers from five different individuals of each *Centrosema* species. Flowers (n = 10) were bagged one day before opening and the nectar was withdrawn using micropipettes soon after 08:00 h. This time was standardized based on a posteriori characterization of the period of highest floral visitor activity. Ten flowers from ten different individuals of each *Centrosema* species were measured with an electronic digital caliper (accuracy 0.1mm): the length and width of the banner, the length of the keel and the depth of the corolla were measured in fresh flowers. In this latter case, it was measured the distance between the point of insertion of the banner's spur and the internal base of the nectar chamber.

Bee Sampling Data. Floral visitors were captured using hand-held insect nets during the periods of overlapping flowering of the two *Centrosema* species (March to July) in two successive years. Based on observational field data on floral biology (principally anthesis and anther dehiscence), the bees were intensively sampled on the flowers of both species from 07:00 h to 12:00 h (most of the flowers are senescent after 12:00 h) on a daily basis for 15 minutes/species every hour for 24 days (30 hours of sampling efforts) along the overlapping flowering period. Visitor behavior on the flowers was observed simultaneously (and photographed for posterior analysis) during 15 minutes/h (totaling 15 hours) to characterize pollinators and non-pollinators. Bees that behaved in discordance with the keel morphology (i.e., making holes in the corolla and accessing nectar from the outside of the flower or biting the anthers to collect pollen) were considered flower robbers (see Inouye, 1980) and were separated into two categories (Inouye, 1980): primary or secondary. Primary flower robbers make perforations in the corolla in order to gain access to the nectar (or pollen), while secondary flower robbers take advantage of the perforations made by primary flower robbers. In general, visitors are called "cheaters" if they consume floral resources without entering into contact with the fertile verticils (Inouye, 1980).

The bee morpho-species were determined by consulting published keys and bee references in the Pollination Ecology Laboratory (ECOPOL) and the Bee Biology and Ecology Laboratory (LABEA) at the Biology Institute of the Universidade Federal da Bahia (UFBA). The identifications of the Centridini and Euglossini bee species were confirmed by Dr. Fernando Zanella (Universidade Federal da Integração Latino-Americana UNILA, Paraná, Brasil) and Dr. Ednaldo L. das Neves (Faculdade Jorge Amado, Bahia) respectively; the scientific names follow Moure et al. (2007). All specimens were deposited in the ECOPOL. The *Centrosema* species were identified by Dr. Luciano Paganucci de Queiroz of the Universidade Estadual de Feira de Santana - Bahia (UEFS), and those specimens were deposited in the Alexandre Leal Costa Herbarium at the Universidade Federalda Bahia (UFBA-ALCH).

Data Analysis. The Mann-Whitney non-parametric test ($\alpha = 0.05$) was used to compare the quantities of nectar produced by *C. pubescens* and *C. brasilianum*. The t-test was used to compare flower measures between both species (Gotelli & Ellison 2004).

In the global comparative analyses of the two pollination systems pseudo-species were created corresponding to two behavioral categories: robbers and pollinators. Data concerning the composition and abundance of 'pseudo-species' visiting the flowers of six individual plants of *C. pubescens* and *C. brasilianum* were used in non-metric multidimensional scaling (NMDS) based on a Bray-Curtis similarity matrix. Analyses of similarity (ANOSIM) of each plant species were performed to test the null hypothesis of equality in the composition and abundance of floral visitors of these pollination systems. Detailed analyses of the contributions of each bee group to the observed dissimilarity (SIMPER) were also performed. Rare pseudo-species with frequencies equal or less than one were excluded from the analysis.

The hypothesis of random interactions being proportional to species abundances (Vázquez & Aizen, 2004; Vázquez, 2005;) was evaluated comparing bee abundances in the local habitat. The bee abundances in the local habitat (PMP) were estimated by bee sampling during one year period. Using hand insect nets, the bees were captured on the flowers of all detected bee plants (Sakagami et al., 1967) in a transect of 2.5km length, from 07:00 h to 17:00 h, totalizing 240h of sampling effort. Linear regressions between the absolute abundance values of each bee species in the habitat and their respective 'relative abundance' on the flowers were estimated using GraphPad Instat 3 software (at a significance level of 0.01). The relative abundance of each bee species were estimated as follows: the abundance was transformed into a value between 0 and 1 (with 1= the maximum abundance observed on the flowers) by dividing the number of individuals on each flower by the total number of individuals of the most abundant bee species observed on both flowers. Using values of relative abundance between 0 and 1 facilitate interpretations of the graphs in terms of the probability of interactions being proportional to species abundance. A significance level of 0.01 was used.

As the flowers of *Centrosema* are quite large and their nectar is well-protected at the base of the corolla, we assumed an a posteriori size threshold of floral visitors to test for size constraints on random interactions proportional to species abundances (Stang et al., 2007). Body size was used as a surrogate for proboscis length, as all of the pollinators observed belonged to the general category of long-tongued bees (Michener, 2000). It is important to note that all members of the orchid bees or Euglossini have proboscis longer than the other bee groups with similar body size. The orchid bees have very long proboscis (longer than ¾ of their body length) that are more or less proportional to their body size (with several exceptions): being, for example, up to 10mm long in species whose bodies are approximately 10mm long, and up to 40mm long in species larger than 20mm (Roubik & Hanson

2004). Five individuals of each bee species were measured to estimate their body lengths using an electronic digital caliper (accuracy 0.1mm). The distance between the top of the head (at the height of middle ocellus) and the end extremity of abdomen was measured in order to obtain a rough estimate of relative size for insertion of bees in the following body length categories: small bees <9.9mm; medium bees ≥ 10 < 15mm; large bees ≥ 15 < 20mm; very large bees ≥ 20mm.

Results

The very similar zygomorphic lip-flowers of *C. brasilianum* and *C. pubescens* both offer nectar as the main reward to flower visitors. Nectar is produced from the start of flower anthesis (during the night) until pre-senescence (near 12:00 h) in both species, thus being available from sunrise until noon. In synchrony with nectar availability, the stamen is receptive from early morning until floral senescence in both species. *C. pubescens* produced greater quantities of nectar ($P < 0.01$) than *C. brasilianum* (Table 1), so that the availability of this resource to foragers is potentially greater on the flowers of the former species. Active pollen harvests were made by very few non-pollinators that often visited the flowers after the peak of activity of the pollinators – so that pollen protection by the keel structure appears to be quite effective considering the legitimate visitors.

The sizes of the flowers are very similar in the two species of *Centrosema* and there are no significant differences in width ($P = 0.45$) and length ($P = 0.67$) of the banner and, mainly, in the depth of the corolla ($P = 0.45$). The difference between the flowers is observed only on the dimensions of the keel ($P < 0.001$), more robust in *C. pubescens*. The keel size probably does not modify the nectar accessibility by large bees (≥ 15 < 19.9 mm) or very large bees (≥ 20 mm), however, it could affect the behavior of small and medium sized bees on the flowers.

Table 1. Floral biology of two synchronopatric species of *Centrosema* (Fabaceae) in an coastal tropical restinga (Brazil). Flower measures (N = 10) are described in methods.

Character		***Centrosema pubescens***	***Centrosema brasilianum***
Anthesis (start-end)		00:00 h – 05:00 h	00:00 h – 02:00 h
Stigma receptivity (start-end)		05:00 h until senescence	02:00 h until senescence
Nectar volume (ml)		26 ± 4.20	14 ± 5.50
Floral Reward		Nectar	Nectar
Flower color		bluish-pale lilac, with magenta and creamy-white stripes on the banner (nectar guide)	bluish-purple, with whitish-yellow pale stripes on the banner (nectar guide)
Flower Measures (mm)	Banner length	33.71 ± 2.16	36.4 ± 3.54
	Banner width	37.66 ± 2.79	38.85 ± 3.75
	Keel length	20.41 ± 1.75	16.34 ± 0.79
	Corolla depth	5.34 ± 0.31	5.46 ± 0.36

A total of 489 flower visitors were sampled on the flowers of the two *Centrosema* species, of which almost 98% and 27 species were bees (principally robust bees, with body sizes > 10mm; Table 2). Most of the bees collected nectar in the flowers of both *Centrosema* species, with the exception of few pollen robbers. Eighty-six percent of the observed bee species made the legitimate nectar harvests on *C. pubescens* flowers, as compared to only 46% on *C. brasilianum*. During legitimate visits in both flowers, pollinators typically landed on the ventral lip (banner) of the flower and forced their head and thorax towards the corolla base, displacing the keel upwards and triggering pollen deposition on their backs (nototribic pollination). They accessed the nectar by inserting their long tongues into the nectar chamber through a rigid and very narrow passage at the base of the corolla. This corolla structure therefore impedes legitimate nectar access by small bees (e.g., bees < 10mm).

In terms of both species richness and abundances on flowers, the major groups of pollinators were large (body length > 15mm < 20mm) or very large bees (body length ≥ 20mm), all with long (e.g., *Xylocopa*) or very long proboscis (*Eulaema*, Euglossini), as well as some medium-sized Euglossini bees with very long proboscis (*Euglossa*, Euglossini). The non-pollinators were medium-sized nectar robber bees (*Oxaea*) and small pollen robber bees (*Ceratina* and *Augochloropsis*). The roles of medium-size Centridini bees varied with *Centrosema* species (Table 2).

In contrast to legitimate visitors, nectar robbers always moved along the outside of the flower to the base of the perianth, where they would pierce the calyx to gain access to the nectar chamber. This type of behavior was often displayed by individuals of *Oxaea* species on *C. brasilianum* flowers (14% of total flower visitors) and on *C. pubescens* flowers (17% of total flower visitors), and by *Centris* and *Epicharis* bees on *C. brasilianum* flowers. *Oxaea* usually acted as a primary nectar robber, making holes in the calyx that could be used by secondary nectar robbers (*Centris*, *Epicharis*, *Ceratina*, *Pseudaugochlora*, and *Augochloropsis*). *Ceratina*, *Pseudaugochlora*, and *Augochloropsis* also acted as pollen robbers, harvesting it with their mouth parts directly from the anthers; *Ceratina* bees were often the primary pollen robbers, punching holes in the anthers inside the keel that the other two groups would later take advantage of.

A high similarity was seen between the two pollination systems, considering their sharing of higher taxa and functional bee groups. By contrast, considering the actual num-

Table 2. Abundance distributions (on the flowers and in the PMP habitat), size categories, and behaviors of the floral visitors to *Centrosema* flowers: *Centrosema pubescens* and *Centrosema brasilianum*. The behavioral categories follow Inouye (1980): R = Primary flower robbers; Rs = Secondary flower robbers; P = pollinators; n.r. = not recorded. The size categories of the bees were based on body lengths: (•) Small bees <9.9mm; (••) Medium sized bees ≥10 <14.9mm; (••••) Large bees ≥15 <19.9mm; (•••••••) Very Large bees ≥ 20mm.

BEE GROUPS	FLOWER VISITOR ABUNDANCE			BEE SIZE Categories
	Habitat PMP	*C.pubescens*	***C.brasilianum***	
EUGLOSSINI				
Euglossa cordata (Linnaeus, 1758)	87	17(P)	57(P)	••
Euglossa ignita Smith, 1874	0	1(P)	0	••
Euglossa securigera Dressler, 1982	0	2(P)	0	••
Eufriesia cf. *mussitans* Fabricius, 1787	26	0	1(P)	••••
Eulaema cingulata Moure 1950	13	12(P)	3(P)	••••
Eulaema flavescens Friese, 1899	6	6(P)	0	•••••••
Eulaema nigrita (Lepeletier, 1841)	71	45(P)	10(P)	••••
Eulaema bombiformis niveofasciata Friese, 1899	4	17(P)	0	•••••••
BOMBINI				
Bombus brevivillus Franklin,1913	26	10(P)	3(P)	••••
CENTRIDINI				
Centris (Hemisiella) tarsata (Smith, 1874)	15	1(P)	1(Rs)	••
Centris (Centris) flavifrons (Fabricius, 1775)	14	1(P)	1(Rs)	••••
Centris (Centris) leprieuri (Spinola, 1841)	48	0	8(Rs)	••
Centris (Trachina) fuscata (Lepeletier, 1841)	65	2(P)	2(Rs)	••
Epicharis (Xanthepicharis) bicolor Smith, 1854	2	1(P)	1(Rs)	••••
Epicharis (Epicharis) flava (Friese, 1900)	11	15(P)	1(Rs)	••••
XYLOCOPINI				
Xylocopa (Megaxylocopa) frontalis (Olivier, 1789)	131	85(P)	1(P)	•••••••
Xylocopa (Neoxylocopa) nigrocincta Smith,1854	15	3(P)	0	••••
Xylocopa (Neoxylocopa) suspecta Moure & Camargo, 1988	14	2(P)	1(P)	••••
Xylocopa (Neoxylocopa) cearensis Ducke, 1910	1	0	1(P)	••••
Xylocopa (Neoxylocopa) grisescens Lepeletier,1841	4	1(P)	0	•••••••
OXAEINI				
Oxaea flavescens Klung, 1807	18	2(R)	13(R)	••
Oxaea sp.1	48	57(R)	10(R)	••
CERATININI				
Ceratina (Crewella) sp.1		32(R)	24(R)	•
AUGOCHLORINI				
Pseudaugochlora pandora Smith, 1853	50	0(Rs)	6(Rs)	••
Augochloropsis callichroa Cockerell, 1900	37	4(Rs)	6(Rs)	•
EXOMALOPSINI				
Exomalopsis sp.1	13	0	2(R)	•
ERICROCIDINI				
Acanthopus sp.1	2	3(P)	0	•
OTHER INSECTS	(n.r.)	8 (n.r.)	10 (n.r.)	

bers of species, only 46% of the pollinators (12/26) and 67% of the non-pollinators (4/6) were shared by both *Centrosema* species. The percentage of shared bee species between the two flowers partially reflected sampling artifacts (the small numbers of sampled individuals of several species; Table 2). The main differences between the systems, however, are related to the distributions of abundances of the large pollinators and the shifting behavior of a shared bee group (Centridini).

Apparent size constraints can explain basic differences in the behaviors of bee visitors to the specialized lip flowers of *Centrosema* (Table 2) – with pollinators usually being larger than 15mm; non-pollinators were consistently less than 15mm long. Among the medium-sized bees (10-15mm), only some *Euglossa* species with very large proboscis (> 10mm) were abundant on flower and would legitimately access the nectar while performing pollination. The primary nectar flower robbers were medium-sized bees *Oxaea* (< 15mm). The behaviors of medium-size Centridini bees on the flowers were less predictable as they acted as pollinators of *C. pubescens* and as secondary nectar robbers of *C. brasilianum*. In this latter case, flower robbing was probably related to the lower nectar volume in *C. brasilianum* flowers. The nectar robber behavior of some larger Centridini bees (two species of *Epicharis*) probably was stimulated by easy access to nectar in *C. brasilianum* flowers that had been previously perforated by primary flower robbers (*Oxaea*).

The relationship between the relative abundance of bees on *Centrosema* flowers and their abundance in the habitat (Fig 1; see also Table 2) was very consistent and extremely significant (r = 0.87; $P < 0.00001$) when the analyses were restricted to large bees (≥ 15mm) visiting *C. pubescens* flowers (Fig 1a). Although significant, a loss of consistency was seen in the relationship when all floral visitors to *C. pubescens* flowers were included, independent of their sizes (r = 0.69; $P = 0.001$). The abundance of bees in the habitat, however, was not predictive of their abundance on *C. brasilianum* flowers under any circumstances – whether considering a minimum size constraint of 15mm (Fig 1b; $P = 0.49$; $P = 0.04$) or including all floral visitors in the analyses (r = 0.42; $P = 0.034$). The smaller nectar rewards and the activities of secondary flower robbers on *C. brasilianum* flowers seem to affect mainly large pollinators (e.g., *Eulaema nigrita* and *Xylocopa frontalis*) that probably shift to *C. pubescens* flowers with large nectar volume.

Considering the abundances and behaviors of floral visitors (pollinator or non-pollinator), the NMDS analysis revealed two distinct structural and functional organizations of the pollination systems of *C. brasilianum* and *C. pubescens* (Fig 2). Analysis of similarity (ANOSIM) confirmed significant differences between the pooled visiting bees (average dissimilarity = 83%; $P = 0.011$). Quantitative analyses of the contributions of particular bee groups to the observed dissimilarity indicated that the abundance distributions of the very large *Eulaema* and *Xylocopa* bees and the medium-sized *Euglossa* bees (with very large proboscides) were particularly important to the ecological differentiation between the two pollination systems. The pollinating bees *Xylocopa frontalis*, *Eulaema nigrita* and *Eulaema meriana* together, for example, were responsible for 27% of the observed dissimilarity between the two systems, while medium-sized pollinating bees *Euglossa* contributed 25%; medium-sized *Centris* bees contributed 4.2%, mainly due to the fact that they behave as pollinators or robbers depending on the *Centrosema* species. Altogether, the primary nectar or pollen robber bees *Oxaea* and *Ceratina* respectively, contributed for only 10% of the dissimilarity.

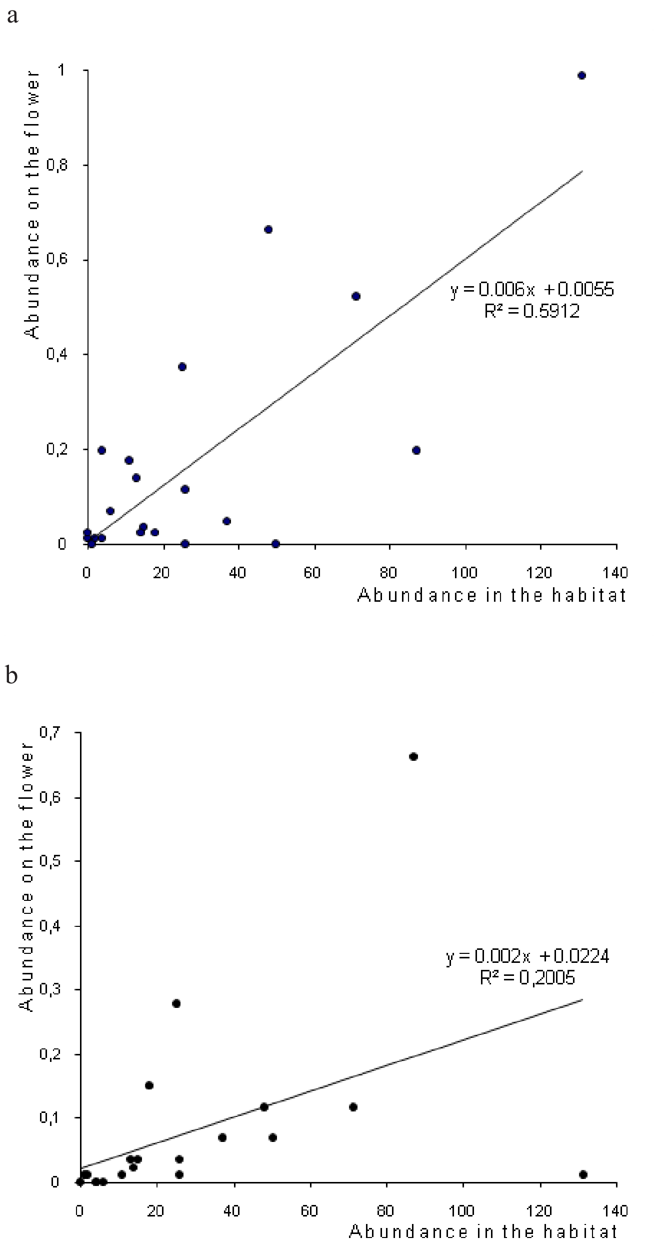

Fig. 1. Relationships between abundance of bees (pollinators + non-pollinators) in the habitat (PMP) and on the flowers of *Centrosema*: a) *Centrosema pubescens* (r = 0.87; $P < 0.00001$); b) *Centrosema brasilianum* (r = 0.49; $P = 0.04$). It is presented only the regression curves for the large bees + very large bees + Euglossini (bees with very long proboscis).

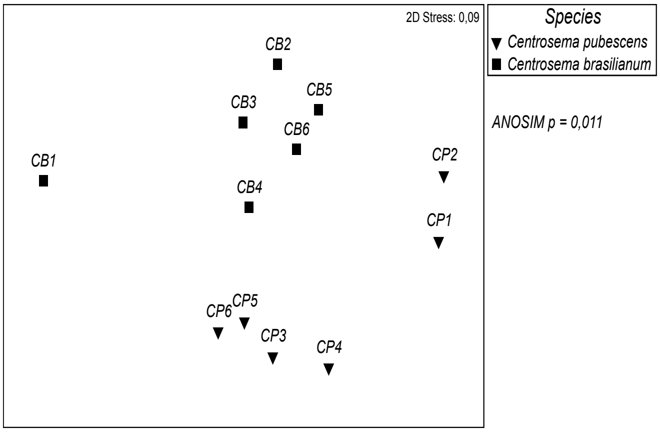

Fig. 2. Ordination diagram of the two pollination systems by non-metric multidimensional scaling (NMDS): CP = *Centrosema pubescens* and CB = *Centrosema brasilianum*.

Discussion

Similarity in floral biology and mainly synchronopatry expose the two *Centrosema* species to share the main visiting bees and functional bee groups (Camargo et al., 1984; Roubik, 1989; Michener, 2000): the huge carpenter bees of the genus *Xylocopa* and the very large-tongued Euglossini bees as the main pollinators; *Oxaea* primary nectar robbers; *Ceratina* primary pollen robbers; and the floral oil bees Centridini whose role is dependent on *Centrosema* species. In particular, the primary nectar and pollen robbers were bees evenly shared by both flowers, as expected, suggesting they are weakly responding to subtle floral differences between the *Centrosema* species (e.g., flower color and keel size). On the other hand, the significant differences in the abundances of shared pivotal pollinators, mainly the large bees *Xylocopa* and *Eulaema*, and secondary nectar robbers, cannot be attributed to random effects only.

The hypothesis of a size threshold modulating the interactions between nectar-flowers and consumers (Stang et al., 2006) is mainly supported by the predictable interactions of *C. pubescens* with large and very large bees that have large or very large proboscis (*Xylocopa* and *Eulaema* species). These relationships also corroborate the influence of random interactions proportional to bee species abundances in the habitat (Vázquez & Aizen, 2004, Vázquez, 2005; Stang et al., 2007).

The observed partnerships between pollinators and the very specialized *Centrosema* lip-flowers should therefore be attributed to the action of two basic mechanisms: neutral random interactions driven by species' abundances in the habitat, and interactions modulated by morphological constraints (i.e., bee size threshold). On the other hand, neither morphological constraints (e.g., proboscis x corolla length) nor neutral interactions (Vázquez, 2005; Stang et al., 2006) fully encompass the mechanisms responsible for the high concentrations of very large bees on *C. pubescens* flowers and the observed shifting of the behavior of Centridini bees from legitimate *C. pubescens* pollinators to *C. brasilianum* nectar robbers. Significant difference in keel size between both flowers doesn't explain adequately the exploitation modes and sharing of these two nectar sources by the bees. If the keel provides resistance to visitors, it was expected some difference in the frequency distribution of mid-sized bees between the two *Centrosema* species and a higher frequency of nectar thefts in *C. pubescens* flowers with the largest keel: only the first prediction is partially supported by the high abundance of *Euglossa cordata* on *C. brasilianum* flowers. The abundance distributions of primary and secondary nectar robbers on both *Centrosema* species are therefore not affected by the keel size.

Bees are notable for their ability to choose among nectar sources comparing foraging cost/benefit ratios (Waddington, 1980; Pyke, 1984). For instance, among coexisting *Bombus* species, the largest bees with the largest proboscis tend to choose flowers with the longest corollas, from which they can collect more nectar more efficiently than smaller bees (e.g., Harder, 1985; Heinrich, 2004). If the bees were mainly responding to size restrictions, therefore, the largest bees should visit the flowers of both *Centrosema* species with similar frequencies, in light of their similar conditions of nectar access (i.e., similar flower sizes and corolla lengths) and similar flower densities in the habitat (see methods). The same would not be true, however, in terms of floral nectar volume, and some large and very large-sized bees (e.g., *Xylocopa frontalis* and *Eulaema* species) likely choose *C. pubescens* flowers simply because they can obtain more nectar per visit. The difference in flower color between the *Centrosema* species must be used for recognition and choice of *C. pubescens* flowers, with more nectar, by these large bees.

Despite the low contribution of secondary nectar robbers to the structural divergences between *Centrosema* pollination systems (i.e., 4% dissimilarity), the Centridini bees deserve attention because of the distinctive roles of "cheaters"on structuring flower-visitor webs (Genini et al., 2010). Centridini is one of the most abundant bee group in tropical coastal restinga and sand dunes (Ramalho & Silva, 2002; Viana & Kleinert, 2006; Oliveira-Rebouças & Gimenes, 2011; Rosa & Ramalho, 2011), and therefore its low abundance on *Centrosema* flowers would not sustain the hypothesis of random interactions proportional to abundances in the habitat (Vázquez, 2005; Vázquez & Aizen, 2004). Behaving as secondary nectar robbers in *Centrosema* flowers is probably a response of Centridini bees to a contingent relationship: encounters facilitated by the abundance of these bees in the habitat (Ramalho & Silva, 2002; Rosa & Ramalho, 2011) and by the long flowering periods of *Centrosema* species.

In light of the intense activity of primary flower robbers (*Oxaea* spp) on both *Centrosema* species (14% to 17% of total flower visitors on flowers), and particularly on *C. pubescens*, if secondary flower robbers were responding to access opportunities to nectar by preexisting perforations in the corolla, it would be expected that they would rob the flowers of both species or that the secondary robbery activity should be slightly higher in *C. pubescens* flowers. As such, it must not be by chance that changes from legitimate (pollinator) to illegitimate (secondary nectar robbery) visiting behavior mainly involve medium-sized Centridini bees (=15mm) and *C. brasilianum* flowers with smaller nectar volumes.

The high observed frequency of medium-sized *Euglossa* bees, with very long tongues (proboscides >10mm) on *C. brasilianum* flowers provides indirect evidence that the body size/proboscis length ratio play a role in this relationship – as to maximize returns from the exploitation of the smaller nectar volumes in *C. brasilianum* flowers, medium-sized bees must have very long tongues (*Euglossa* species) or be primary flower robbers (*Oxaea*); being a secondary flower robber (Centridini) would be the "best thing to do with a worst thing", as they would be highly exposed to visit depleted flowers.

In some circumstances, nectar robbing (even from specialized flowers) can be a very rewarding strategy for bees, depending on their ability to make adjustments in their foraging behaviors (Zhang et al., 2011), and that is probably why cheaters are ubiquitous in mutualistic flower-visitors networks (Genini et al., 2010). Nectar robbing behavior could be stimulated by size restrictions triggered by subtle differences in corolla lengths among very similar zoophilous flowers and size differences between individuals visiting the same flower (Urcelay et al., 2006; Zhang et al., 2011) or by difference in nectar volume in *Centrosema* species. Paradoxically, in the relationship between the two *Centrosema* species and Centridini bees, secondary nectar robber seems to be more advantageous in the flowers with smaller nectar volume.

In synthesis, pollinators sharing by the two *Centrosema* species is potentialized by synchronopatry and modulated by pollinator choices between flower sources with different nectar volume and, apparently, by direct or indirect interactions among floral visitors, including flower robbers. The concentrations of the largest bee pollinators on *C. pubescens* flowers and the secondary nectar robber activity of medium-sized Centridini bees on *C. brasilianum* flowers are foraging responses better understood by nectar volume differences than by differences in floral morphology per se.

From the point of view of plant reproduction, nectar robbery has detrimental effects on maternal functions (e.g. seed set), depending on the species' reproductive system (e.g., Irwin et al., 2001). As such, by reducing the nectar volume, probably *C. brasilianum* is selecting for medium-sized bees with the largest proboscis (e.g. *Eulgossa*) as its major pollinators, and therefore it should present some reproductive adjustment to compensate for the loss of large-bodied pollinators and the parallel-paradoxical increase in robbers activities. Both *Centrosema* species invest principally in cross-pollination, although the ratios of seeds/ovules and seeds/fruits are both significantly smaller (P = 0.0001) in *C. brasilianum* than in *C. pubescens* (M. Ramalho & M. Silva unpublished), suggesting the first species is more adjusted to being visited and cross-pollinated mainly by a smaller number of large or very large bee species.

References

Camargo, J.M.F., Gottsberger, G. & Silberbauer-Gottsberger, I. 1984.On the phenology and flower visiting behavior of *Oxaea flavescens* (Klug) (Oxaeinae, Andrenidade, Hymenoptera) in São Paulo, Brazil. Beitrage zur Biologie der Pflanzen, 59: 159-179.

Dafni, A. (1992). Pollination Ecology: The practical Approach series. Ed. IRL Press. Oxford: Oxford University Press. 250p.

Endress, P.K. (1994). Diversity and evolutionary biology of flowers. Cambridge: Cambridge Univ. Press. 511p.

Etcheverry, A.V, Protomastro, J.J. & Westerkamp, C. (2003). Delayed autonomous self-pollination in the colonizer *Crotalaria micans* (Fabaceae: Papilionoideae): structural and functional aspects. Plant Systematics and Evolution, 239: 15-28. doi: 10.1007/s00606-002-0244-7

Faegri, K. & Van Der Pijl, L. (1979). The principles of pollination ecology. Oxford: Pergamon Press.

Galloni, M., Podda, L., Vivarelli & Cristofolini, G. (2007). Pollen presentation, pollen-ovule rations, and other reprodutive traits in Mediterranean Legumes (Fam. Fabaceae – Subfam.Faboideae). Plant Systematics and Evolution, 266: 147-164. doi: 10.1007/s00606-007-0526-1.

Genini, J., Morellato, P.C., Guimarães, P.R. & Olesen, J.M. (2010). Cheaters in mutualism networks. Biology Letters, 6: 494-497. doi: 10.1098/rsbl.2009.1021

Gotelli, N.J. & Ellison, A.M. (2004). A primer of ecological statistics. Massachusetts: Sinauer Associates, Inc.510p.

Gottsberger, G., Camargo, J.M.F. & Silberbauer-Gottsberger, I. (1988). A bee-pollinated tropical community: the beach dune vegetation of Ilha de São Luis, Maranhão, Brazil. Botanische Jahrbücher für Systematik, 109: 469-500.

Herrera, C.M. (1996). Floral traits and plant adaptation to insect pollinators: a devil advocate approach. In. Lloyd, D.G. & Barrett, S.C.H.(eds) Floral Biology (pp. 65-87). New York: Chapman and Hall.

Herrera, C.M. (2005). Plant generalization on pollinators: species property or local phenomenon? American Journal of Botany, 92: 13-20.

Harder L.D. (1985). Morphology as a predictor of flower choice by bumble bees. Ecology, 66: 198-209.

Heinrich, B. (2004). Bumblebee economics. Cambridge: Harvard University Press.

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

Irwin, R.E., Brody, A.K. & Waser, N.M. (2001). The impact of floral larceny on individuals, populations and communities.

Oecologia: 129, 161-168. doi: 10.1007/s004420100739

Jacobi, C,M., Ramalho, M. & Silva, M. (2005). Pollination biology of the exotic ratlleweed *Crotalaria retusa* L. (Fabaceae) in NE Brazil. Biotropica, 37: 357-363. doi: 10.1111/j.1744-7429.2005.00047.x

Lopes, A.V. De F. & Machado, I.C.S. (1996). Biologia floral de *Swartzia pickelii* Killipex Ducke (Leguminosae-Papilionoideae) e sua polinização por *Eulaema* spp. (Apidae-Euglossini). Revista Brasileira de Botânica, 19: 17-24.

Michener, C.D. (2000). The bees of the World. London: The Johns Hopkins Univ. Press. 912p.

Moure, J.S., Urban, D. & Melo, G.A.R. 2007. Catalogue of Bees (Hymenoptera, Apoidea) in the Neotropical Region. Sociedade Brasileira de Entomologia. 1058p.

Ollerton J., Killick, A., Lamborn, E., Watts, S. & Whiston, M. (2007). Multiple meanings and modes: on the many ways to a generalist flower. Taxon, 56: 717–728.

Ollerton, J., Alarco, R., Waser, N.M., Price, M.V., Watts, S., Cranmer, L., Hingston, A., Peter, C. I. & Rotenberry, J. (2009). A global test of the pollination syndrome hypothesis. Annals of Botany, 103: 1471-1480. doi:10.1093/aob/mcp031.

Por, F.D. (1992). Sooretama. The Atlantic Rain Forest of Brazil. The Nehterlands: SPB Academic Publish.

Pyke, G.H. (1984). Optimal foraging theory: a critical review. Annual Review of Entomology, 15: 523-75

Ramalho, M. & Silva, M. (2002). Flora oleífera e sua guilda de abelhas em uma comunidade de restinga tropical. Sitientibus: Série Ciências Biológicas, 2: 34-43.

Ramalho, M. & Rosa, J.F. (2010). Ecologia da Interação entre as pequenas flores de quilha de *Stylosanthes viscosa* (Faboideae) e as grandes abelhas *Xylocopa cearensis* (Apoidea, Hymenoptera), em duna tropical. Biota Neotropica, 10: 93-100.

Oliveira-Rebouças, P & Gimenes, M. (2011). Polinizadores potenciais de *Comolia ovalifolia* DC Triana (Melastomataceae) e *Chamaecrista ramosa* (Vog.) H.S. Irwin e Barneby var. ramosa (Leguminosae-Caesalpinioideae), na restinga, Bahia, Brasil. Brazilian Journal of Biology, 71: 343-351. doi: 10.1590/S1519-69842011000300002.

Rosa, J.F. & Ramalho, M. (2011). Spatial Dynamics of Diversity in Centridini Bees: The Abundance of Oil Producing Flowers as a Measure of Habitat Quality. Apidologie, 42: 150-158. doi: 10.1007/s13592-011-0075-z

Roubik, D.W. (1989). Ecology and natural history of tropical bees. Cambridge: Cambridge Univ. Press. 503p.

Roubik, D.W. & Hanson, P.E. (2004). Orchid bees of tropical America. Costa Rica: Editorial INBio. 370p.

Sakagami, S.F, Laroca, S. & Moure, J.S. (1967). Wild bee Biocoenotics in São José dos Pinhais (PR) South Brazil. Preliminary Report. Journal of the Faculty of Sciences. Hokkaido University. Ser.VI, Zool., 16: 253-291.

Stang, M., Klinkhamer, P.G.L. & van der Meijden, E. (2006). Size constraints and flower abundance determine the number of interactions in a plant–flower visitor web. Oikos, 112: 111-121. DOI: 10.1111/j.0030-1299.2006.14199.x.

Stang, M., Klinkhamer, P.G.L. & van der Meijden, E. (2007). Asymmetric specialization and extinction risk in plant–flower visitor webs: a matter of morphology or abundance? Oecologia, 151: 442–453. doi: 10.1007/s00442-006-0585-y

Tripp, E.A. & Manos, P.S. (2008). Is floral specialization an evolutionary dead-end? Pollination system transitions in *Ruella* (Acanthaceae). Evolution, 62: 1712-1737. doi: 10.1111/j.1558-5646.2008.00398.x

Urcelay, C., Morales, C. & Chalcoff, V.R. (2006). Relationship between corolla length and floral larceny in the South American hummingbird-pollinated *Campsidium valdivianum* (Bignoniaceae). Annales Botanici Fennici, 43: 205-201

Van Der Pijl, L. (1954). *Xylocopa* and flowers in the tropics. I. The bees as pollinators. Lists of flowers visited. Botany serie C. 57, 413-423.

Vázquez, D.P. (2005). Degree distribution in plant–animal mutualistic networks: forbidden links or random interactions? Oikos, 108: 421-426. doi: 10.1111/j.0030-1299.2005.13619.x

Vázquez, D.P. & Aizen, M.A. (2004). Asymmetric specialization: a pervasive feature of plant–pollinator interactions. Ecology, 85: 1251-1257. doi: 10.1890/03-3112.

Viana, B.F. & Kleinert, A.M.P. (2006). Structure of bee-flower system in the coastal sand dune of Abaeté, northeastern Brazil. Revista Brasileira de Entomologia, 50: 53-63. doi: 10.1590/S0085-56262006000100008.

Waddington, K.D. (1980). Flight patterns of foraging bees relative to density of artificial flowers and distribution of nectar. Oecologia, 44: 199-204.

Westerkamp, C. (1996). Pollen in bee-flower relations. Botanica Acta, 109: 325-332.

Zhang, Y. W., Zhao, J. M., Yang, C. F. & Gituru, W. R. (2011). Behavioural differences between male and female carpenter bees in nectar robbing and its effect on reproductive success in *Glechoma longituba* (Lamiaceae). Plant Biology, 13 (Suppl. 1): 25-32. doi: 10.1111/j.1438-8677.2009.00279.x.