Sociobiology
An international journal on social insects

RESEARCH ARTICLE - BEES

Comparing the Structure and Robustness of Passifloraceae - Floral Visitor and True Pollinator Networks in a Lowland Atlantic Forest

CR Benevides[1], DM Evans[2], MC Gaglianone[1]

1 - Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, Rio de Janeiro, Brazil
2 - University of Hull, Cottingham Road, Hull, United Kingdom

Corresponding author
Maria Cristina Gaglianone
Programa de Pós-Graduação em Ecologia e Recursos Naturais
Univ. Estadual do Norte Fluminense
Av. Alberto Lamego, 2000
Campos dos Goytacazes, RJ, Brazil
E-Mail: mcrisgag@gmail.com

Abstract

We investigated the plant-pollinator interactions of Passifloraceae occurring in fragments of lowland semi-deciduous Atlantic forest. We described floral biology, pollination syndromes and the pollinators of *Passiflora alata*, *Passiflora kermesina*, *Passiflora suberosa*, *Passiflora malacophylla* and *Mitostemma glaziovii*. We examined the robustness of the interaction networks to species loss, a plausible scenario resulting from forest fragmentation. The effects of pollination syndrome (flower size) on network robustness was also examined. *Passiflora alata*, *P.malacophylla* and *P.suberosa* were pollinated by bees of different corporal sizes. *P.kermesina* and *M.glaziovii* presented the highest diversity of visitors and were pollinated mainly by hummingbirds and butterflies, respectively. Through the analysis of the networks we differentiate the structures of the flower-visitor network with the 'true' plant-pollinator network. The robustness of the flower-visitor network to animal loss was generally high, but it declined when only true pollinators were included in the network. The sequential loss of plants from the flower-visitor network resulted in low robustness: the loss of key plants could have significant cascading effects on the animals feeding on them within the forest fragment. Future studies should consider the interactions between all flowering plants and animals in this habitat in order to guide conservation and management plans for these forest fragments.

Introduction

According to the concept of pollination syndromes, the characteristics of flowers, such as colour, odour, shape, rewards, position of reproductive structures and flowering strategies are some of the attributes that can determine the group of pollinators visiting different plant species (Gentry, 1974; Faegri & Pijl, 1979). On the other hand, the foraging behaviour of floral visitors, frequency of visits and the movements between flowers of the same species influence their potential to promote pollination (Waddington, 1983) and, consequently, the reproductive success and genetic diversity of plants (Vogel, 1983; Richards, 1986).

Ecological networks describe the interactions between species, the underlying structure of communities and the function and stability of ecosystems (Montoya et al., 2006). They are an important tool for understanding the complex interactions between communities such as plants and pollinators and have the potential to quantify the effects of environmental changes, such as habitat fragmentation (Memmott et al., 2007; Tylianakis et al., 2008). In analyses of multiple plant-animal mutualistic networks, Bascompte et al. (2003) and Jordano et al. (2003) reported high levels of generalization and highly nested networks (although networks with high level of generalization not always will be nested (see Almeida-Neto et al. 2008). Nested interaction networks are thought to be highly cohesive, because generalist plants and animals tend to interact with each other. As a result, the concept of pollination syndromes has been widely questioned, since plant-pollinator interactions have been shown to be more generalist than was previously thought (Hingston & McQuillan, 2000; Ollerton et al., 2009). However, a recent study of a plant-pollinator network showed that 69% of the total interactions resulted from the functional group of pollinators predicted by the plant

syndrome (Danieli-Silva et al., 2012). Of the numerous ecological network properties, network 'robustness' [a measure of the tolerance of the network to species extinctions (Dunne et al., 2002; Memmott et al., 2004)] has received particular attention, partly driven by advances in computational modelling (Kaiser-Bunbury et al., 2010; Staniczenko et al., 2010), but mostly by the desire to understand the real threat of biodiversity loss to ecosystem services and functioning (see Santos et al., 2012). Recent work suggested that plant-pollinator networks are less robust to species extinction than other plant-animal interaction networks such as invertebrate-parasitoid and bird-seed feeder networks (Pocock et al., 2012), which is pertinent given the current decline of pollinator populations in many parts of the world (e.g. Biesmeijer et al., 2006). However, to our knowledge, no study has considered the importance of floral syndromes in robustness analysis of plant-pollinator networks.

The flowers of Passifloraceae are considered primarily nectar sources for pollinators, and these plants may depend on these agents for their reproduction, since many species are self-incompatible (Sazima & Sazima, 1978; Koschnitzke & Sazima, 1997). Interspecific variation in floral morphology, relative to the perianth and corona orientation, size and colour of the sepals, petals and filaments, and volume and sugar concentration on nectar (Varassin et al., 2001), can distinguish species that attract different pollinators. In this case, the identification of pollination syndromes appears to be a valuable tool for prediction of the main pollinators of Passifloraceae species. However, the association between plants and pollinators may not be so evident, and analysis of the composition of visiting animals, their body size, behaviour and frequency of visits are essential to better understand pollination processes.

Passifloraceae are distributed in the tropics and subtropics, and among the 600 species, 150 occur in Brazil (Souza & Lorenzi, 2005), represented mostly by vines and scandent shrubs. Studies about their pollinators indicate different functional groups in several biomes. Moths and bats are the main pollinators of species with nocturnal anthesis (Sazima & Sazima, 1978; Buzato & Franco, 1992; Varassin et al., 2001), while bees (Koschnitzke & Sazima, 1997; Varassin et al., 2001), hummingbirds (Vitta, 1997; Fischer & Leal, 2006; Varassin et al., 2001), wasps and butterflies (Koschnitzke & Sazima, 1997; Varassin et al., 2001) pollinate species with diurnal flowers. For some Passifloraceae however, plant-pollinator interactions are poorly understood, which may be important given the ecosystem service provided by animal pollinators for cultivated species such as *P. alata* (Gaglianone et al., 2010) and *P. edulis* (Benevides et al., 2009; Yamamoto et al., 2012).

In this study, we examine the plant-pollinator interactions of Passifloraceae occurring in fragments of Atlantic forest. The lowland seasonal Atlantic forests are biodiversity hotspots but have been severely devastated and fragmented over the last century. The remnants in the southern part of its

distribution are small and isolated fragments subjected to a range of anthropogenic pressures, such as proximity to urban areas, intensive cultivation and pasture. Investigating the robustness of these plant-animal interactions to species loss in these forest fragments is thus to plan possible management actions. By examining whether the body parts of animals contact the anthers and stigmas of the flowers, we use our data to construct and compare the structures of the 'true' plant-pollinator network with the general plant-flower visitor network, the latter widely (and wrongly) termed in the literature as 'plant-pollinator' networks despite lack of evidence of actual pollination. In this study our aims are: 1) to describe the floral biology and pollinators of sympatric species of Passifloraceae in a remnant of lowland seasonal Atlantic forest, including new pollination data for two species not previously studied; 2) to compare the structure of quantitative plant-pollinator and plant-flower visitor networks; 3) to examine the robustness of the networks to simulated plant and animal extinctions. Our expectation is that the true plant-pollinator network is less robust than the more complex plant-flower visitor network; 4) to examine the importance of pollination syndrome on network robustness.

Material and Methods

Study sites

The study was conducted in two fragments of lowland seasonal semi-deciduous forest - Guaxindiba Ecological Station (21°24'S and 41°04' W, circa 1200 ha) and Funil Forest (21°33'S and 41°02' W, 130 ha), in Rio de Janeiro state, Brazil. The average annual rainfall in the region was 1023 mm. This vegetation physiognomy also known as "tabuleiro" forest occupies a large tertiary plain area near the coast with plant species distributed along a coastal-inland climate gradient (Rizzini, 1979). The sclerophylly is also a distinguishing feature of these forests, where in general, epiphytic species are rare (Rizzini, 1979; Silva & Nascimento, 2001). The Ecological Station represents the largest remnant of this forest type in the state of Rio de Janeiro, which suffered high impact in the past due to deforestation for plantation crops, pasture, charcoal production, and logging of commercial timber species (Villela et al., 2006).

Five species of Passifloraceae with diurnal flowers occurred in the area: *Passiflora alata* Curtis, *Passiflora kermesina* Link & Otto, *Passiflora malacophylla* Mast., *Passiflora suberosa* L. and *Mitostemma glaziovii* Mast., and we studied aspects of their floral biology as well as animal visitors. The floral biology and pollinators of *Passiflora malacophylla* and *M. glaziovii* have not been previously described.

Sampling and Data collection

The flowering period of the five species was monitored

monthly and blooming plants were monitored weekly from May 2004 to October 2005. Morphological features including colour, shape and size, and also odour were obtained from fresh material. The time and duration of anthesis was determined by monitoring marked flowers from pre-anthesis to the closing of the petals.

To check the volume and solute concentration in the nectar throughout the day, we isolated buds (*n*=5) in pre-anthesis and monitored the same flowers during one day. In 1-hour intervals, the entire content of nectar was collected, using graduated microcapillary (± 5μl) or syringes (± 0.3 ml). The solute concentration in the nectar was measured using the Brix scale with a manual refractometer (BS Eclipse model). The nectar was collected until the closing of the petals or until the total absence of this resource.

We tested the self-pollination through the bagging of flowers (*n*=4 to 19) in pre-anthesis phase, without manipulation (spontaneous self-pollination) and after they were manually pollinated with their own pollen (hand self-pollination). Tested flowers were monitored until fruit maturation or flower senescence. The fruit set was calculated from the number of tested flowers (*n*=4 to 19) and formed fruits in each treatment.

We attributed categories of pollination syndromes using the following floral traits: size, height of stigmas, colours, presence of odour, anthesis time, nectar volume and nectar concentration (Faegri & Pijl, 1979). The melittophily was considered according to the functional groups of bees in pollination by large (height of the thorax more than 6mm), medium sized (between 3 and 6mm) or small (less than 3mm) bees (scale adapted by the authors).

We captured the floral visitors with entomological nets on the flowers during their visits for taxonomic identification. The vouchers of plant and pollinator species were deposited at the 'Universidade Estadual do Norte Fluminense Darcy Ribeiro', in Campos dos Goytacazes, RJ, Brazil, in the Herbarium (HUENF) and Zoology Collection of Laboratory of Environmental Sciences, respectively.

In order to analyse the frequency of visits we counted all visitors during timed observation sessions totaling at least 6 hours in one to four observation-days for each plant species. Besides the frequency of visits, behaviour features such as landing site, intra-floral behaviour and time spent on the flower were recorded by focal observations throughout the day (Dafni, 1992). Visitors were considered legitimate pollinators when they contacted the reproductive parts of the plants during the visits for nectar collecting; visitors whose size did not permit contact with the reproductive parts were considered robbers, as well as floral visitors arriving on the flower illegitimately or damaging parts of the plant.

Analysis

We tested the differences among the values of volume and concentration of nectar along the day through analysis of variance (ANOVA) in R 3.0.1 (R Core Team, 2013). For each Passifloraceae species, we calculated the diversity of visitors using the Shannon index (Magurran, 1988).

For the network analysis, we pooled all plant-flower visitor interaction data into a single matrix incorporating plants, animals and the total number of interactions observed in the field. We created a separate plant-pollinator matrix by excluding the animals observed visiting the flowers, rather than pollinating them (e.g. some nectar feeders). We visualized the quantitative networks and examined the robustness (R) of the two networks to simulated species extinctions using package 'bipartite' in R 3.0.1 (R Core Team, 2013). First, we simulated the sequential loss of pollinating animals and recorded the proportion of plants still remaining, calculating robustness as the area under the curve (Burgos et al., 2007). If R → 1, this is consistent with a very robust system in which, for instance, most of the plant species survive even if a large fraction of the animal species go extinct. Conversely, if R → 0, this is consistent with a fragile system in which, for instance, even if a very small fraction of the animal species are eliminated, most of the plants loose all their interactions and go extinct.The order of extinction was based on the most-to-least connected animals. This is the most extreme case, where the most generalist species goes extinct first (see Memmott et al., 2004). Second, we simulated the sequential loss of flower-visitors and recorded the proportion of plants still remaining. This was to compare the robustness of the two networks. Third, we simulated the sequential loss of plants (most-to-least connected) to examine the cascading effects on the interacting animals. In all cases robustness values were compared with null models (*n* = 100) using t-tests to determine whether robustness values were significantly different to random. Finally, to examine the importance of pollination syndromes, we calculated robustness values for the networks based on the sequential loss of large-to-small flowers and large-to-small body-size animals.

Results

Flowering period and plant reproductive systems

Mitostemma glaziovii flowered only in the dry-season, for approximately six weeks, with simultaneous production of a large number (averaging 53) of open flowers per individual. *Passiflora alata*, *P. malacophylla* and *P. suberosa* flowered exclusively in the rainy season (during one to three months). *Passiflora alata* opened only a few flowers per day and presented low synchrony between individuals. In contrast, *P. kermesina* flowered throughout the year with few open flowers per plant (on average 2 flowers) and few flowering individuals simultaneously. The flowers opened up to 5am and lasted until one day. *Passiflora malacophylla* had the shortest anthesis (six hours), while *P. kermesina* had the longest one (24 hours) (Table 1).

Passiflora alata, *P. kermesina* and *P. malacophylla*

produced no fruit in selfing experiments, whereas *P. suberosa* and *M. glaziovii* produced fruits in hand self-pollination experiments (Table 2), in different rates.

Pollinators attraction

Passiflora kermesina and *P. alata* have the largest flowers considering the diameter and height of the stigmas (Table 2, Fig 1). The flowers have four (*M. glaziovii*) or five (the others) petals, the ovary is elevated on an androgynophore. The flowers of *P. alata* have petals and sepals purplish-red and corona with long filaments striped violet and white. Flowers of *P. malacophylla* have white petals, sepals and filaments. The petals and sepals of *P. kermesina* are dark pink and short filaments of corona are purplish-violet, densely arranged. *Passiflora suberosa* has yellow-green sepals and filaments. The flowers of *M. glaziovii* have sepals and petals white and orange filaments of corona (Table 2, Fig 1). Flowers of *Passiflora alata*, *P. kermesina* and *P. malacopylla* are axillary and solitary; *M. glaziovii* presents axillary or terminal inflorescences, whereas *P. suberosa* has axillary flowers solitary or in pairs.

The volume of nectar produced per flower during the day did not change significantly for *P. alata* (p> 0.05), although slightly higher values were observed at 11am, when it reached 42 µl in one hour. The average concentration of solute in the nectar for this species also did not vary during anthesis (p> 0.05) and reached 45% (Fig 2). Nectar production of *P. kerme-*

sina occurred throughout the day, peaking between 10am and 12pm reaching 44 µl per hour at 11am. After this time the production decreased until 4pm, when only about 4 µl were produced in a flower during one hour. The total concentration of solutes present in the nectar was kept constant during anthesis (Fig 2). For *M. glaziovii*, nectar production was higher at the beginning of anthesis, between 5 and 6.30am (Fig2), 8µl per flower on average, decreasing continuously (p <0.05) up to 12pm when the average production was 0.2 µl per flower per hour. The concentration of solutes in the nectar of this species did not differ (p> 0.05) along the day.

Based on the analysed features, the Passifloraceae species were closer to the patterns described for species melittophilous, psicophyllous, ornithophilous and pollination by small insects (Table 2).

Floral visitors and Pollinators

Mitostemma glaziovii was visited more frequently by Hesperiidae butterflies (Table 3, Fig 1F). They land on the corona and insert the proboscis between the corona and androgynophore in search of nectar, and after that pollen grains were observed in the proboscis. Large bees such as *Eulaema nigrita* Lepetelier, *Eulaema cingulata* (Fabricius), *Xylocopa ordinaria* Smith and *Xylocopa frontalis* Oliver were rarely observed visiting flowers of *M. glaziovii*, and in those cases always contacted the reproductive parts of the flower with the thoracic and metasomal sterna (Fig 1G). Medium-sized bees

Table 1. Flowering of Passifloraceae species studied in lowland seasonal semi-deciduous forest, RJ, Brazil. FP: flowering period; AN: beginning of anthesis; DA: duration of anthesis in hours; NF: numbers of flowers per plant; n = number of flowers analysed; ni= number of plants analysed (M= mean e SD = standard deviation).

	FP	AN (n)	DA (n)	NF M ± SD (ni)
Passiflora kermesina	Jan to Dec	5 to 5:30h (10)	24 (10)	2 ± 0.8 (6)
Passiflora malacophylla	Jan	5 to 6:00h (14)	6 (14)	12 ± 7 (3)
Passiflora alata	Feb to Apr	5 to 6:00h (20)	10 (20)	2.5 ± 1.3 (11)
Passiflora suberosa	Mar to May	5 to 7:30h (19)	12 (19)	8 ± 4.4 (3)
Mitostemma glaziovii	Jul to Aug	5 to 6:30h (27)	12 (27)	53 ± 8 (3)

Table 2. Floral biology characteristics and pollination syndrome of Passifloraceae species in lowland seasonal semi-deciduous forest (Guaxindiba Ecological Station and Funil Forest) in Rio de Janeiro state, Brazil. n= total number of tested flowers in each reproductive experiment, NM= not measured.

|	Floral diameter (mm)	Hight stigmas (cm)	Colour petals	Colour corona	Odour	Maximal nectar volume (µl)	Nectar concentration	Pollination syndrome	% self pollination	% hand self pollination
Passiflora kermesina	8.3	2.2	pink	purple	yes	44 µl (11am)	34 to 30%	Ornitophily	0 (n=4)	0 (n=4)
Passiflora alata	8.2	1.9	purplish	purple/white	yes	42 µl (6am)	45 to 36%	Melittophily/large bees	0 (n=10)	0 (n=10)
Passiflora malacophylla	5.4	0.8	white	white	yes	NM	NM	Melittophily/medium to large bees	0 (n=5)	0 (n=5)
Mitostemma glaziovii	4.1	0.8	white	orange	yes	8 µl (6am)	22 to 10%	Psycophily	NM	10 (n=19)
Passiflora suberosa	1.7	0.4	greenish	purple	yes	NM	NM	Small insects	0 (n=9)	50 (n=8)
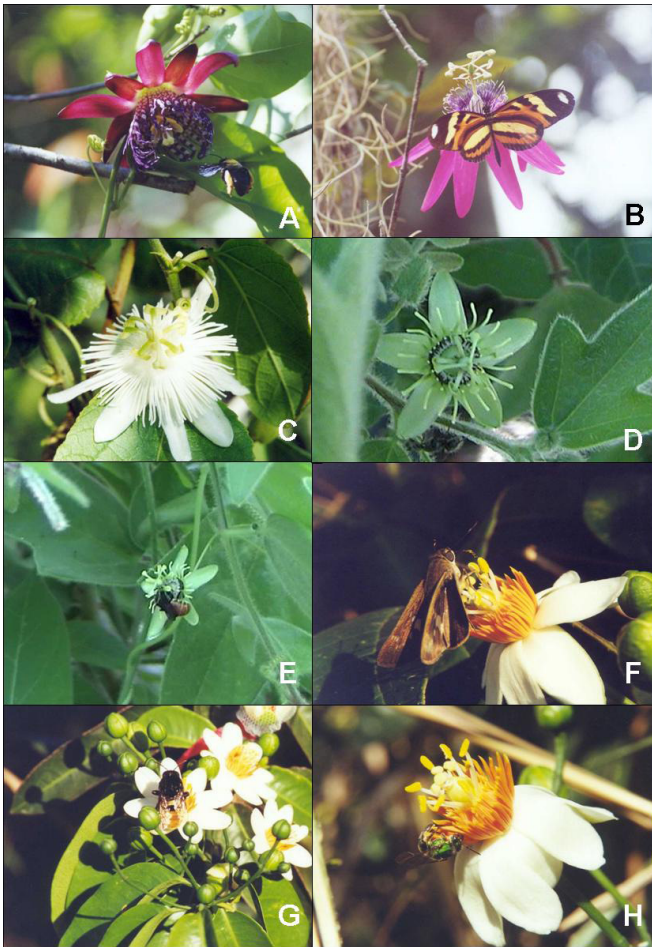
Fig 1. Passifloraceae flowers and pollinators / robbers. A: *Epicharis flava* visiting flower of *Passiflora alata*; B: *Heliconius ethila narcaea* on *Passiflora kermesina*; C: flower of *P. malacophylla*; D and E: *Passiflora suberosa*: flower and visit by *Hypanthidium foveolatum*; F, G and H: *Mitostemma glaziovii* visited by Hesperiidae, Augochlorini (Halictidae) and *Eulaema cingulata*, respectively (Photographs by Paulo Augusto Ferreira).

such as *Augochloropsis patens* (Vachal) visited these flowers in search of nectar (Fig1H).

The flowers of *P. alata* were visited exclusively by bees, and *Epicharis flava* (Friese) was the most frequent visitor (Table 3, Fig1A); it searched for nectar, remaining on average 16 seconds in each flower. Except for *Plebeia* sp. that landed directly on the anthers in search of pollen, the other visitors (Table 3) entered the flower between the corona and androgynophore to the nectary ring. We considered *Epicharis flava* to be a pollinator as we observed contact between floral reproductive parts and thoracic terga, whereas no such contact by *Euglossa cordata* was observed.

Butterflies (Lepidoptera) and hummingbirds (Trochilidae) were the most frequent visitors of *P. kermesina* (Table 3, Fig1B). The hummingbirds performed quick visits (about 7 seconds on each flower) and always contacted the reproductive parts of the flower with their head. *Heliconius ethilla narcaea* remained on the flowers for 40 seconds on average and the contact, less frequent, could occur via both antennae and wings (Table 3, Fig 1B).

Apis mellifera L. was the most frequent visitor of *P. malacophylla*, collecting pollen and nectar from flowers in visits that lasted on average 23 seconds (Table 3). These bees landed directly on the anthers to collect pollen and they could empty their content during one visit. Sporadically *Xylocopa frontalis* and *Xylocopa ordinaria* visited flowers in search of nectar and always contacted the reproductive parts with the dorsal thorax.

The most frequent visitors of *P. suberosa* were small bees, such as *Plebeia* sp. (Table 3) that visited flowers in search of pollen and nectar. These bees landed directly on the anthers and collected large amounts of pollen. When seeking nectar, they landed on the sepals and walked to the ring nectary. Individuals of medium-sized bees *Hypanthidium foveolatum* (Alfken) visited the flowers of *P. suberosa* less frequently, contacting the reproductive parts on thoracic terga while feeding on the ring nectary (Table 3, Fig 1E).

The highest richness of visitors was observed for *Mitostemma glaziovii*, while the highest diversity was observed for *P. kermesina* and *M. glaziovii* (Table 3).
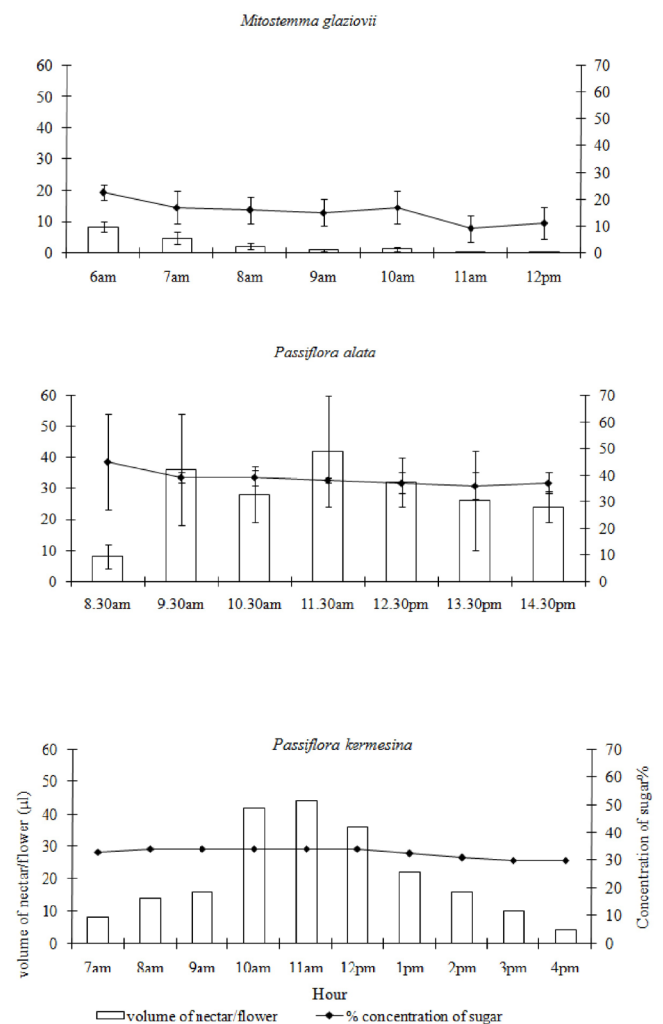

Fig 2. Volume and concentration of nectar (mean and standard deviation) in flowers of *Passiflora alata* (n=5), *Passiflora kermesina* (n=1), and *Mitostemma glaziovii* (n=5), taken at intervals of one hour.
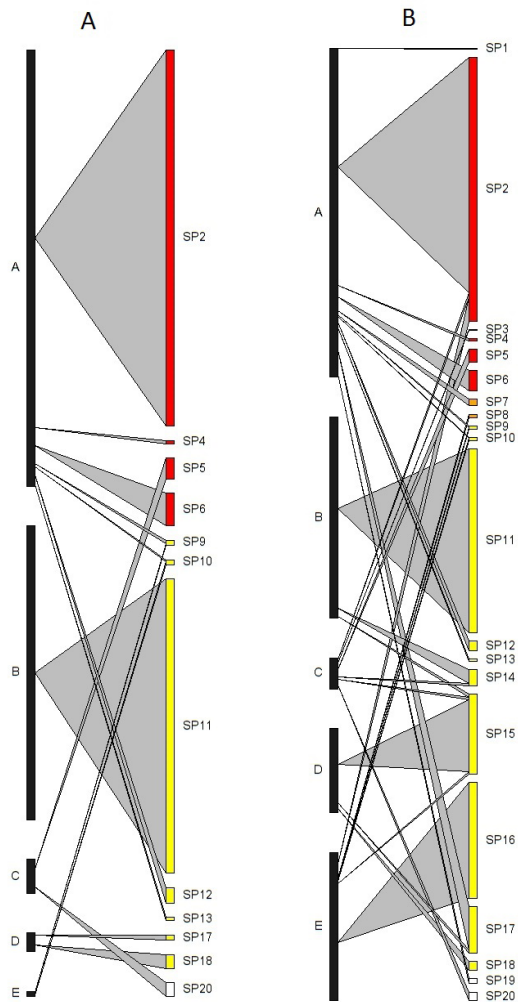
Fig 3. Quantitative networks for (A) the plant-pollinators and (B) plant-flower visitors of a lowland Atlantic forest fragment, Brazil. Black rectangles, left represent the Passifloraceae species (A= *M.glaziovii*; B= *P.alata*; C= *P.kermesina*; D= *P.suberosa*; E= *P.malacophylla*) interacting with animal species (colour rectangles, right), with the gray triangles representing the frequency of interactions. For animal species rectangles, red represents Lepidoptera, orange represents Diptera, yellow represents Hymenoptera and white represents Aves. Including species only observed contacting the plant reproductive parts resulted in a loss of 8 species from the network (A), including all Diptera, leading to lower network complexity.

Network plants-pollinators

The flower-visitor network (Fig3B) consisted of 20 animals with 1.16 links per species (*l*/*s*) and connectance (*l*/*s²*) and interaction evenness values of 0.29 and 0.47 respectively. The plant-pollinator network (Fig3A) had 8 less animals, leading to lower complexity and structure values (0.82 links per species and connectance and interaction values of 0.23 and 0.32 respectively). The robustness of the flower-visitor network to animal loss was generally high (R = 0.77, Table 4), although robustness declined when only true pollinators were included in the network (R = 0.68). The robustness of the flower-visitor net-

work to plant extinctions was low (R = 0.38), suggesting that the network was particularly fragile (Fig4). In all cases the robustness values were significantly different to the null models (P < 0.001, Table 4). Sequentially removing the plants based on flower size resulted in higher robustness values than removing plants based on their number of interactions (R = 0.60 and 0.64 for the flower-visitor and plant-pollinator networks respectively). When considering the loss of animals based on body size, the flower-visitor network was highly robust (R = 0.84). However, when examining the plant-pollinator network, robustness to animal loss was considerably lower (R = 0.67) as many insects such as *Apis mellifera* and *Plebeia* sp. although visiting the plants, did not pollinate them.

Discussion

Flowering period, floral biology and pollinators

Species of Passifloraceae in the studied semi-deciduous forest fragments differ in their flowering strategies and morphological features, such as colour, size, orientation and position of the corona and perianth and consequently vary in the main visitor groups associated with them. The importance of these animals for the five species was confirmed through pollination experiments showing that these plants could not self-pollinate.

Different flowering strategies were observed among the plant species. The high intensity of flowering of *Mitostemma glaziovii* over a period of several weeks was attractive to numerous groups of visitors, including opportunistic species, a phenomenon observed by others when multiple plants concurrently offer of flowers (Gentry, 1974; Ratchcke & Lacey, 1985). This may explain the greater richness and diversity of visitors, including different groups of animals, to the flowers of *M. glaziovii*. Although the floral characteristics of this species point to pollination by Lepidoptera, the offer of abundant resources by intense flowering associated with a sweet odour and exposed nectary facilitates the exploitation of nectar by other insects too, as observed in our work. The flowering of *M. glaziovii* restricted to the dry season possibly also contributes to the high species richness of visitors, since this is the season of lower availability of flowers (Morellato et al., 2000), indicating the high importance of *M. glaziovii* for numerous groups of insects. This is probably the case for large bees of the genus *Xylocopa* and *Eulaema*, whose adults are active throughout the year in the region (Aguiar & Gaglianone, 2008; Bernardino & Gaglianone, 2013). Despite the low concentration of nectar, when compared to a typical melittophilous species such as *P. alata*, the flowers of *M. glaziovii* must be important for these bees by intense flowering in a period of reduced availability of floral resources in the environment more generally. *Mitostemma glaziovii* should be considered in future studies because of this relevant ecological role in a seasonal forest and also because of their geographical

Table 3. Visitors/pollinators of Passifloraceae flowers in lowland seasonal semi-deciduous forest in Rio de Janeiro state, Brazil, and features of their behaviour. VT = average visit time; SD = standard deviation; FV = average relative frequency of visits; FR = floral resource collected; BC = body parts that contact anthers and stigmas; B = behaviour, N = nectar; P = pollen; Po = Pollinator; Ro: Robber. H' = Shannon diversity index. NM= not measured.

Species (H')	Visitors		VT±SD	FV %	FR	BC	B
Passiflora alata	HYMENOPTERA						
(H´= 0.33)	Apidae	*Epicharis flava* (Fr)	16±6	91.5	N	Thorax dorsal	Po
		Euglossa cordata (L.)	9±3	6.9	N	No contact	Ro
		Plebeia sp.	20±5	1.6	P/N	No contact	Ro
Passiflora kermesina	LEPIDOPTERA						
(H´= 1.44)	Heliconiidae	*Heliconius ethila narcaea* Gordat	43±14	41.3	N	Wings and antennae	Po
	Hesperiidae	Hesperiidae sp.	53±8	17	N	No contact	Ro
	Pieriidae	*Phoebis sennae* L.	22	3.4	N	No contact	Ro
	HYMENOPTERA						
	Apidae	*Euglossa cordata* (L.)	10	3.4	N	No contact	Ro
		Plebeia sp.	42±7	6.9	P	No contact	Ro
	AVES						
	Trochilidae	Trochilidae sp.	7±1	28	N	Head	Po
Passiflora malacophylla	HYMENOPTERA						
(H´= 0.73)	Apidae	*Apis mellifera* L.	23±5	78	P/N	No contact	Ro
		Plebeia sp.	25	1.4	P/N	No contact	Ro
		Xylocopa frontalis Ol.	5	1	N	Thorax dorsal	Po
		Xylocopa ordinaria Sm.	4	1.4	N	Thorax dorsal	Po
	DIPTERA						
	Syrphidae	Syrphidae sp.	42±29	2.2	N	No contact	Ro
	LEPIDOPTERA						
	Hesperiidae	Hesperiidae sp.	46±30	16	N	No contact	Ro
Passiflora suberosa	HYMENOPTERA						
(H´= 0.49)	Apidae	*Plebeia* sp.	72±49	85.9	P/N	No contact	Ro
	Halictidae	*Augochloropsis patens* (Vachal)	15	3.8	N	Thorax dorsal	Po
	Megachilidae	*Hypanthidium foveolatum* (Alfken)	18±7	10.3	N	Thorax dorsal	Po
Mitostemma glaziovii	LEPIDOPTERA						
(H´= 1.32)	Arctiidae	*Utheteisa ornatrix* L.	NM	0.5	N	No contact	Ro
	Hesperiidae	Hesperiidae spp	40±12	71.8	N	Proboscis	Po
	Nymphalidae	*Dione juno* Stoll	39±21	6.2	N	Proboscis	Po
	Pieriidae	Pieriidae sp.	14	0.5	N	Proboscis	Po
	DIPTERA						
	Syrphidae	*Ordinia obesa* Fab.	10±6	1.9	N	No contact	Ro
	HYMENOPTERA						
	Apidae	*Eulaema cingulata* (Fab)	4	3	N	Ventral side	Po
		Eulaema nigrita Lep.	4±1	0.7	N	Ventral side	Po
		Xylocopa frontalis Ol.	10±0.7	0.5	N	Ventral side	Po
		Xylocopa ordinaria Sm.	4	0.2	N	Ventral side	Po
	Halictidae	*Augochloropsis patens* (Vachal)	25±11	13	N	No contact	Ro
	AVES						
	Trochilidae	Trochilidae sp.	3.3±0.5	1.7	N	No contact	Ro

Table 4. The robustness (R) of a) the pollinator network to animal extinctions (M1) and b) the flower-visitor network to animal and plant extinctions (M2 and M3 respectively). If R → 1, this is consistent with a very robust system in which, for instance, most of the plant species survive even if a large fraction of the animal species go extinct. Conversely, if R → 0, this is consistent with a fragile system in which, for instance, even if a very small fraction of the animal species are eliminated, most of the plants loose all their interactions and go extinct.

	Observed R	Null mean	Lower CI	Upper CI	t	P
M1	0.679	0.562	0.549	0.574	-18.813	< 0.001
M2	0.765	0.788	0.778	0.797	4.648	< 0.001
M3	0.378	0.660	0.651	0.668	64.716	< 0.001

distribution restricted to the Atlantic forest (Bernacci et al., 2013).

The flowering pattern of *P. kermesina*, in contrast to *M. glaziovii*, presents shorter flowering periods during the year and produces fewer flowers per day in plants sparsely distributed. This strategy is associated with trapline behaviour of pollinators such as hummingbirds and orchid bees (Janzen, 1971), also observed in our study. The floral morphology, however, with petals and filaments pink and red, high volume of nectar, reduced corona and long distances between nectaries and reproductive organs indicate the ornithophilous pollination syndrome (Faegri & Pijl, 1979). Hummingbirds were undoubtedly the most efficient pollinators, by their behaviour and body size, although Lepidoptera were the most frequent

Fig 4. The robustness of the Passifloraceae-animal interaction networks to simulated species extinction (based on the losing the most-to-least connected species). The robustness of the plant-pollinator (A) and plant-flower visitor network (B) to sequential animal loss is relatively high, whereas the sequential loss of Passifloraceae species in the plant-flower visitor (C) leads to low robustness.

visitors. These insects are very abundant, especially in the late morning, when the volume of nectar produced by the flowers of *P. kermesina* reached the highest values. This high frequency of visits, associated with the possible pollination behaviour through the movement of their wings or the touch of antennae in floral reproductive parts, suggests that *Heliconius* butterflies may be important pollinators, especially in the low frequency of visits or absence of hummingbirds. This suggestion has already been made by Benson et al. (1976) and our observations confirm these insects as potential pollinators, although less efficient than the hummingbirds.

The flowering of *Passiflora alata* differs from the previous species because of the opening of only a few flowers on each plant per day, but in higher numbers of flowering plants simultaneously. Beyond flowering, floral traits are compatible with the description of the melittophily with pollination by large bees: large flowers with great volume (especially early in the morning) and high concentration of the nectar and sweet odour. *Epicharis flava* was the main pollinator due to its large body. As noted in other studies with natural populations (Varassin & Silva, 1999) or even in cultivations of *P. alata* (Gaglianone et al., 2010), large oil bees of the tribe Centridini are their main pollinators. The use of Passifloraceae flowers as nectar sources by these bees had been highlighted by Gaglianone (2006).

Some features of *P. malacophylla* such as the size of flowers and short period of anthesis (only in the morning), suggested it as primarily melittophilous with pollination by medium or large sized bees. Based only on body size, honey bees could be considered potential pollinators. However, the behaviour of these bees on the flowers indicated them as robbers. This behaviour is similar to that observed in flowers of *Passiflora edulis flavicarpa*, the yellow passion fruit, cultivated in the study region (Benevides et al., 2009). In flowers of both species, honey bees seek the pollen, which is taken directly from the anthers, without contact with the stigmas.

The intense pollen removal directly from the anthers without promoting pollination was also observed by *Plebeia* sp in flowers of *Passiflora suberosa*. Flowers of this plant, the smallest among the studied species, present pollination syndromes by small insects. However, only bees visited them in the study area and medium sized bees were the pollinators. Unlike other species, *P. suberosa* showed high self-compatibility, which had already been described in other study (Varassin & Silva, 1999). Our observations suggest that pollen removal made by *Plebeia* can even prevent self-pollination, by pollen removal mainly in the period in which the stigmas were still curving themselves, before they were receptive.

Network plants-pollinators

The construction and analysis of our relatively simple plant-animal interaction networks within Atlantic forest fragments enabled us to consider how robust they are to simulated
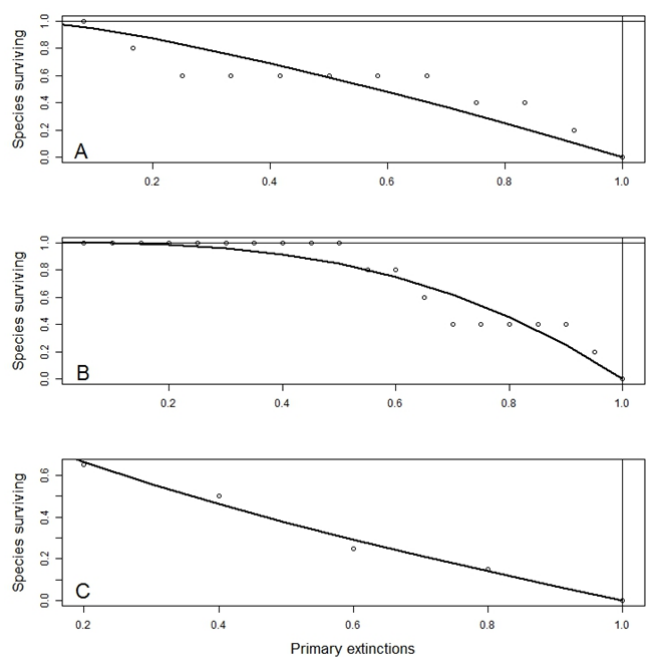
species extinction. Moreover, by examining which animals contacted the reproductive parts of the plants we were able to differentiate the structures of the flower-visitor network with the 'true' plant-pollinator network. We found differences in network structure and complexity. Although the robustness of the flower-visitor network to animal loss was generally high, robustness declined when only true pollinators were included in the network. This has implications for studies of ecological networks that in the past have considered flower-visitor networks as pollination networks and have used the terms interchangeably (e.g. Pocock et al., 2012). We found that the flower-visitor network had low robustness when plants were sequentially lost (based on the most-to-least connected) and was particularly fragile. Although body size can predict degree in plant-animal mutualistic networks (e.g.Chamberlain & Holland, 2009), we found that the frequency of interaction of key plants was more important to network integrity than plant and animal size, although we concede that our network was too small and incomplete to test this conclusively. Considering the possible effects of the forest fragmentation on the ecological interactions (e.g. Hagen et al., 2012), our results suggest that the loss of Passifloraceae could have considerable cascading effects on the animals feeding on them within the forest fragment. Future studies should consider the wider interactions between all flowering plants and animals in this habitat.

Acknowledgments

References

Aguiar, W.M. & Gaglianone, M.C. (2008). Comunidade de abelhas Euglossina (Hymenoptera: Apidae) em remanescentes de mata estacional semidecidual sobre tabuleiro no estado do Rio de Janeiro. Neotrop. Entomol., 37: 118-125.

Almeida-Neto, M. Guimarães, P.R., Guimarães, P.R. Jr, Loyola R.D.,Ulrich, W. (2008). A consistent metric for nestedness analysis in ecological systems: reconciling concept and quantification. Oikos, 117: 1227-1239.

Bascompte, J., Jordano, P., Melián, C.J. & Olesen, J.M. (2003). The nested assembly of plan-animal mutualistic net-

works. Proc. Natl. Acad. Sci. USA, 100: 9383-9387.

Benevides, C.R., Gaglianone, M.C. & Hoffmann, M. (2009). Visitantes Florais do Maracujá-amarelo (Passiflora edulis f. flavicarpa Deg. Passifloraceae) em Áreas de Cultivo com Diferentes Proximidades a Fragmentos Florestais na Região Norte Fluminense, RJ. Rev. Bras. Entomol., 53: 415-421.

Benson, W.W., Brown, J.R. & Gilbert, L.E. (1976). Coevolution of plants and herbivores: passion flower butterflies. Evolution, 29: 650-680.

Bernacci, L.C., Cervi, A.C., Milward-de-Azevedo, M.A., Nunes, T.S., Imig, D.C., Mezzonato, A.C. (2013). Passifloraceae in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. (http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB12505).

Bernardino, A.S. & Gaglianone, M.C. (2013). Comparisons in nesting biology of two sympatric carpenter bee species (Apidae: Xylocopini), J. Nat. Hist., 47: 1481-1499. doi :10.1080/00222933.2012.763054.

Biesmeijer, J. C., Roberts, S.P.M., Reemer, M., Ohlemuller, R., Edwards, M., Peeters, T., Schaffer, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., Settele, J., Kunin, W.E. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science, 313(5785): 351-354.

Burgos, E., Ceva, H. R., Perazzo P.J., Devoto M., Medan, D. Zimmermann, M., & Delbue, A.M. (2007). Why nestedness in mutualistic networks? J. Theor. Biol., 249: 307-313.

Buzato, S. & Franco, A.L.M. (1992). Tetrastylis ovalis: a second case of bat-pollinated passsion flower (Passifloraceae). Plant Syst. Evol., 181: 261-267.

Chamberlain, S.A. & Holland, J.N. (2009). Body size predicts degree in ant-plant mutualistic networks. Funct. Ecol., 23: 196-202.

Dafni, A. (1992). Pollination Ecology: a practical approach. New York: Oxford University Press.

Danieli-Silva, A., Souza J.M.T., Donatti, A.J., Campos, R.P., Vicente-Silva.J, Freitas L. & Varassin, I.G. (2012). Do pollination syndromes cause modularity and predict interactions in a pollination network in tropical high-altitude grasslands? Oikos, 121: 35-43.

Dunne, J.A., Williams, R.J. & Martinez, N.D. (2002). Network structure and biodiversity loss in food webs: robustness increases with connectance. Ecol. Lett., 5: 558-567.

Faegri, K. & Pijl, L.V.D. (1979).The Principles of Pollination Ecology.Third Revised Edition. London: Pergamon Press.

Fischer, E. & Leal, I.R. (2006). Effect of nectar secretion rate on pollination success of Passiflora coccinea (Passifloraceae) in the Central Amazon. Braz. J. Biol., 66: 747-754.

Gaglianone, M.C. (2006). Centridini em remanescentes de

Mata Atlântica: diversidade e interações com flores. In: VII Encontro sobre Abelhas, 2006, Ribeirão Preto. Anais do VII Encontro sobre abelhas, 1, 6-11.

Gaglianone, M.C., Rocha, H.H.S., Benevides, C.R., Junqueira C.N. & Augusto, S.C. (2010). Importância de Centridini (Apidae) na polinização de plantas de interesse agrícola: o maracujá-doce (*Passiflora alata* Curtis) como estudo de caso na região sudeste do Brasil. Oecololgia Australis, 14: 152-164.

Gentry, A.H. (1974). Flowering phenology and diversity in tropical Bignoniaceae. Biotropica, 6: 64-68.

Hagen, M., Kissling, W.D., Rasmussen, C., De Aguiar, M.A.M., Brown, L.E., Carstensen, D.W. et al. (2012). Biodiversity, species interactions and ecological networks in a fragmented world. Adv. Ecol. Res., 46: 89-210.

Hingston, A.B. & McQuillan, P.B. (2000). Are pollination syndromes useful predictors of floral visitors in Tasmania? Austral Ecol, 25: 600-609.

Janzen, D.H. (1971). Euglossine bees as long-distance pollinators of tropical plants. Science, 171: 203-205.

Jordano, P., Bascompte, J. & Olesen, J.M. (2003). Invariant properties in coevolutionary networks of plant-animal interactions. Ecol. Lett., 6: 69-81.

Kaiser-Bunbury, C.N., Muff, S., Memmott, J., Muller, C.B. & Caflisch, A. (2010). The robustness of pollination networks to the loss of species and interactions: a quantitative approach incorporating pollinator behaviour. Ecol. Lett., 13: 442-452.

Koschnitzke, C. & Sazima, M. (1997). Biologia floral de cinco espécies de *Passiflora* L. (Passifloraceae) em mata semidecídua. Rev. Bras. Bot., 20: 19-126.

Magurran, A.E. (1988). Ecological diversity and its measurement. Princeton University Press. Princeton.

Memmott, J., Craze, P.G., Waser, N.M. & Price, M.V. (2007). Global warming and the disruption of plant-pollinator interactions. Ecol. Lett., 10: 710-717.

Memmott, J., Waser, N.M. & Price, M.V. (2004). Tolerance of pollination networks to species extinctions. Proc. Biol. Sci., 271: 2605-2611.

Montoya, J.M., Pimm, S.L. & Sole, R.V. (2006). Ecological networks and their fragility. Nature, 442: 259-264.

Morellato, PL.P.C.,Talora, D.T., Takahasi, A., Bencke, C.C., Romera E.C. & Zipparro, V.B. (2000). Phenology of Atlantic Rain Forest Trees: A Comparative Study. Biotropica, 32: 811-823.

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. Ann. Bot.-London, 103: 1471-1480.

Pocock, M.J.O., Evans, D.M. & Memmott, J. (2012). The ro-

bustness and restoration of a network of ecological networks. Science, 335: 973-977.

Ratchcke, B. & Lacey, E.P. (1985). Phenological patterns of terrestrial plants. Annu. Rev. Ecol. Syst., 16: 179-214.

Richards, A.J. (1986). Plant breeding systems. Allen & Unwin, London.

Rizzini, C. T. (1979). Tratado de Fitogeografia do Brasil - aspectos sociológicos e florísticos. v.2. Editora HUCITEC / EDUSP. São Paulo, SP.

Santos, G.M.M., Aguiar, C.M.L., Genini, J., Martins, C.F., Zanella, F.C.V, Mello, M.A.R. (2012). Invasive Africanized honeybees change the structure of native pollination networks in Brazil. Biol. Invasions, 14: 2369-2378. doi 10.1007/s10530-012-0235-8.

Sazima, M. & Sazima, I. (1978). Bat pollination of the Passion flower, *Passiflora mucronata*, in Southeastern Brazil. Biotropica, 10: 100-109.

Silva, G.C. & Nascimento, M.T. (2001). Fitossociologia de um remanescente de mata sobre tabuleiro no Norte do Estado do Rio de Janeiro (Mata do Carvão). Rev. Bras. Bot., 24, 51-62.

Souza, V.C. & Lorenzi, H. (2005). Botânica sistemática: Guia ilustrado para identificação das famílias de Angiospermas da flora brasileira, baseado em APG II. Plantarum, Nova Odessa.

Staniczenko, P.P.A., Lewis, O.T., Jones, N.S. & Reed-Tsochas, F. (2010). Structural dynamics and robustness of food webs. Ecol. Lett., 13: 891-899.

Tylianakis, J.M., Didham, R.K., Bascompte, J. & Wardle, D.A. (2008). Global change and species interactions in terrestrial ecosystems. Ecol. Lett., 11: 1351-1363

Varassin, I.G. & Silva, A.G. (1999). A melitofilia em *Passiflora alata* Dryander (Passifloraceae), em Vegetação de Restinga. Rodriguésia, 50(76/77): 5-17.

Varassin, I. G., Trigo, J. R. & Sazima, M. (2001).The role of nectar production, flower pigments and odour in the pollination of four species of *Passiflora* (Passifloraceae) in southeastern Brazil. Bot. J. Linn. Soc., 136: 139-152.

Villela, D.M., Nascimento, M.T., Aragão, L.E.O.C., Da Gama, D.M. (2006). Effect of selective logging on forest structure and nutrient cycling in a seasonally dry Brazilian Atlantic forest. J. Biogeogr., 33: 506-516.

Vitta, F.A. (1997). *Passiflora loefgrenii* (Passifloraceae), a New Species in Subgenus *Passiflora* from the Brazilian Atlantic Rain forest. Novon, 7: 210-212.

Vogel, S. (1983). Ecophysiology of zoophilic pollination. In Lage, O.L., Nobel, P.S., Osmond, C.B. & Ziegler, H. (Eds.). Physiological Plant Ecology. III. Encyclopedia of Plant Physiology. (pp. 559-624) Heidelburg: Springer-Verlag.

Waddington, K.D. (1983). Foraging behavior of pollinators. Pollination Biol., 9: 213-223.

Yamamoto, M., Silva, C.I., Augusto, S.C., Barbosa, A.A.A.& Oliveira, P.E. (2012). The role of bee diversity in pollination and fruit set of yellow passion fruit (*Passiflora edulis* forma *flavicarpa*, Passifloraceae) crop in Central Brazil. Apidologie, 43: 515-526, doi: 10.1007/s13592-012-0120-6