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The influence of nectar production and floral visitors on the female reproductive success of *Inga* (Fabaceae): a field experiment

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Floral morphology, nectar secretion strategies and the contribution of pollinators to the reproductive success of plants provide important clues regarding the levels of generalization or specialization in pollination systems. Anthesis throughout the day and night allows flowers to be visited by diurnal and nocturnal pollinators, promoting generalization or specialization. We studied three species in the diverse tropical genus *Inga* to: (1) quantify the response of flowers to successive nectar extractions and (2) determine the contribution of diurnal and nocturnal floral visitors to female reproductive success. *Inga* flowers could be clearly distinguished mainly on the basis of the staminal tube diameter and the quantities of filaments and pollen grains. Successive nectar removals led to a decrease of 60% in the total nectar secretion in *I. vera* and to increases of 20% in *I. ingoides* and 10% in *I. striata*. Despite these differences, the studied *Inga* spp. exhibited similar patterns of visitation rates and shared diurnal and nocturnal pollinators. Nocturnal pollinators contributed ten times more than diurnal pollinators to the female reproductive success of *Inga*. Floral morphology, nectar secretion patterns and pollination ecology data suggest an evolutionary trend towards specialization for nocturnal pollinators in *Inga* spp. with crepuscular or nocturnal flowers. *Botanical Journal of the Linnean Society*, 2015, **177**, 230–245.

ADDITIONAL KEYWORDS: Atlantic forest – bats – fruit set – hummingbirds – pollination systems – seed set – hawkmoths.

INTRODUCTION

Although generalist pollination systems are globally widespread (e.g. Johnson & Steiner, 2000; Olesen & Jordano, 2002; Fenster *et al*., 2004) and represent successful evolutionary strategies in some plant groups (Torres & Galetto, 2002), they are poorly understood in biodiverse tropical forests. Generalist pollination systems can evolve from specialized syndromes (Tripp & Manos, 2008), but the idea that interactions between plants and biotic pollinators are prone to diversification and specialization in tropical ecosystems is widespread in pollination biology (Ollerton, 1996; Olesen & Jordano, 2002; Fenster *et al*., 2004; Alcantara & Lohmann, 2010). It is possible that generalized or mixed pollination systems found in tropical forests represent incomplete evolutionary pathways. Studies of the contribution of pollinator groups to fruit set in generalized systems may reveal the occurrence of a single or a few groups of effective pollinators (EPs), leading to a better understanding of the evolutionary mechanisms of pollination systems (Fleming *et al*., 2001; Fenster *et al*., 2004).

The quantity and distribution of floral resources exert strong influences on the foraging behaviour of pollinators and provide important clues regarding the

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evolution of pollination systems. Increases in nectar availability favour pollinator attraction, promoting high numbers of floral visits (Hodges, 1995; Klinkhamer, De-Jong & Linnebank, 2001; Longo & Fischer, 2006) and increases in pollen flow between flowers and individuals (Fisogni *et al*., 2011). Successive removals may change nectar production by the flowers, leading to increases, decreases, interruptions of secretion or alterations in sugar content during anthesis (Koptur, 1983; Galetto, Bernardello & Juliani, 1994; Galetto, Bernardello & Rivera, 1997; Freitas & Sazima, 2001; Castellanos, Wilson & Thomson, 2002; Galetto & Bernardello, 2004; Musicante & Galetto, 2008; Nepi & Stpiczýnska, 2008; Heil, 2011; Bobrowiec & Oliveira, 2012). Many plant species in tropical and subtropical ecosystems secrete more nectar in terms of volume and sugar content following successive removals, which may affect flower visitation rate, pollen flow and reproduction (e.g. Koptur, 1983; Galetto & Bernardello, 2004; Longo & Fischer, 2006; Ornelas & Lara, 2009; Bobrowiec & Oliveira, 2012).

Although pollen limitation of fruit set in plants occurs when flowers do not receive adequate pollinator visits, excessive visitation to individual flowers might also reduce fruit set because of the increased receipt of incompatible pollen or removal of compatible pollen grains from the stigmatic surface (Pyke, 1984). Thus, an equilibrium might exist between floral resource production and the frequency of visits by EPs to optimize the reproductive success of plants. Despite their specialized floral attributes, many species of plant are visited by several species of pollinator (Koptur, 1983; Ollerton, 1996; Ollerton *et al*., 2009; Cruz-Neto *et al*., 2011; Amorim, Galetto & Sazima, 2013). The genus *Inga* Mill. comprises *c*. 300 species with diurnal, crepuscular or nocturnal brush-type flowers, which are mainly visited by hummingbirds, bats and hawkmoths (Koptur, 1983; Pennington, 1997; Cruz-Neto *et al*., 2011; Amorim *et al*., 2013; Barros, Webber & Machado, 2013).

The aim of this study was to investigate the levels of generalization and specialization of the pollination systems of *Inga* in relation to the expectations from floral trait syndromes. Specifically, we aimed to understand how the relationship between nectar secretion and flower visitation rates by the different pollinators affects the reproductive success of *Inga*. We tested the following hypotheses: (1) *Inga* flowers secrete a larger amount of nectar when they are subject to more nectar extractions; (2) naturally pollinated flowers produce reduced numbers of fruits and seeds relative to those that are hand pollinated, suggesting pollen limitation; and (3) if evolutionary processes guide the specialization of pollination systems, flowers visited by nocturnal pollinators will present both larger fruit set and seed set than flowers exclusively visited by diurnal animals.

MATERIAL AND METHODS

DATA

Study site and species

The study was carried out in the forest remnant Mata de Coimbra (Coimbra Forest), located on a private property in the state of Alagoas, north-eastern Brazil (9°00′S, 35°52′W). Coimbra Forest is the largest fragment of this system, and is also one of the largest forests in north-eastern Brazil, at *c*. 3500 ha (Girão *et al*., 2007; Lopes *et al*., 2009). The area is located in the Borborema Plateau and can be classified as moist tropical forest under the Holdridge system. With annual precipitation ranging from 1700 to 2500 mm, the study site exhibits a seasonal climate with a dry season from October to February and a rainy season from April to September (climate data for the period 1922–2001, Usina Serra Grande). The richest plant families in the study area are Fabaceae (30 species, 14 of which belong to Mimosoideae and eight to *Inga*), followed by Sapotaceae (13 species), Lauraceae (11 species) and Sapindaceae (eight species) (Girão *et al*., 2007).

The species *Inga vera* subsp. *affinis* (DC.) T.D. Penn. occurs in Central and South America, from Mexico to Argentina. *Inga striata* Benth. occurs only in South America and is found in Guiana, on the Brazilian coast, in the Amazon, Bolivia, Peru, Ecuador and Colombia. *Inga ingoides* (Rich.) Willd. occurs in the Antilles and South America, from Bolivia to the state of Minas Gerais in Brazil. The three studied species are trees up to 20 m in height, and occur preferentially in forest edges, secondary forests, periodically flooded areas and along water courses (*sensu* Pennington, 1997). We selected these three species because of their high abundance in the study fragment.

Floral biology and anthesis

We measured the length of the calyx, length and diameter of the corolla, length of the staminal tube, length of the filaments, number of stamens and length of the styles of 30 flowers for each species. To estimate the number of flowers per inflorescence, we counted the number of flower buds in 30 inflorescences per species. A maximum of five flowers per individual was used to record morphometric data. We monitored anthesis in 50 pre-anthesis flower buds per species. We recorded the time of rupture of the corolla, total opening of flowers, flower withering and the viability and time of receptivity of male and female reproductive verticils.

The total number of polyads per anther was counted for 50 anthers, and the number of pollen

grains per polyad was counted from 30 polyads. In both cases, we used 30 flowers per species for counting. The total number of pollen grains per flower was counted using a methodology adapted from Koptur (1984) in ten flowers per species, and the pollen viability was checked following the cytoplasmic staining technique with acetic carmine (Radford *et al*., 1974). The stigmatic receptivity was tested in the field with potassium permanganate ($KMnO_4$, 0.25%; Robinsohn, 1924). We counted the number of ovules per flower based on the longitudinal sections of the ovaries. The counting and estimation of the number of pollen grains and ovules on pre-anthesis flower buds and flowers were carried out at the Laboratório de Biologia Floral e Reprodutiva (POLINIZAR) at the Universidade Federal de Pernambuco, Brazil.

Field experiments

Nectar secretion

To describe the nectar secretion pattern throughout the lifespan of a flower and the effects of successive removals on nectar production, we followed the procedure outlined by Galetto & Bernardello (2005). We isolated, with semi-permeable paper bags, 180 pre-anthesis flower buds (60 per species) to test the hypothesis that *Inga* flowers submitted to a larger number of extractions secrete more nectar. We separated six groups with ten flowers each for the three species. At 20:00 h, we extracted the nectar and measured the nectar volume and sugar concentration for each flower of the first group. At 22:00 h, we extracted the nectar and measured the nectar volume and sugar concentration for the first time in the second group and for the second time in the first group of flowers. Thus, every 2 h, a new group of ten flowers with accumulated quantities of nectar was added. These measurements were carried out until 06:00 h after the beginning of anthesis. The maximum number of extractions for the first group of flowers was six. These times of extraction were based on the attributes of floral anthesis, such as stigmatic receptivity, anther dehiscence, nectar availability, the beginning of flower withering and the interruption of nectar secretion. Nectar was extracted during the flowering peak of each *Inga* species; we considered the flowering peak to be a flowering intensity (the proportion of flowers open), at the population level, equal to or greater than 75% (for details, see Cruz-Neto *et al*., 2011).

Microsyringes (Microliter® 10 and 25 μL) were used for volume measurements, and a pocket refractometer (Atago® 0–50%) was used for measurements of sugar content in nectar. We used the values of the volume and concentration to estimate sugar content in the nectar (in milligrams) following Bolten *et al*. (1979) and Galetto & Bernardello (2005). Although we cannot eliminate the possibility that nectar removal might have damaged nectariferous tissues, and thus may have influenced the measured nectar secretion patterns, a single person completed all of the extractions to minimize this effect across species and to allow comparisons.

Breeding system

We analysed the breeding system of *Inga* by monitoring fruit set rates in the four treatments performed to test: (1) autogamy (bagged, no hand pollination); (2) self-compatibility (bagged, self-hand pollination); (3) outcross fruit set (bagged, outcross hand pollination); and (4) natural pollination (unbagged) following standard protocols used in floral biology (Dafni, Kevan & Husband, 2005). For the autogamy experiment, we marked between 16 and 24 flowers distributed among five individuals for each species. For the self-compatibility and outcross fruit set experiments, we performed hand pollinations using six flowers per tree, also distributed among five individuals per species. In the case of the outcross hand pollinations, all individuals contributed with pollen to the crosses. We did not perform crosses between species. For the natural pollination, we marked at least 1400 flowers per species, which were isolated or grouped in inflorescences. A maximum of four flowers per inflorescence was used in this experiment, and eight individuals of *I. vera*, five of *I. striata* and seven of *I. ingoides* were sampled. Trees of the same species were separated by at least 500 m to avoid close relationships between them.

Floral visitors

We observed the frequency of visits to flowers during anthesis, recorded the group of floral visitor (i.e. bees, wasps, birds, moths and bats) and counted the number of visits received by the flowers at 30-min intervals every hour. In total, ten flowers of each *Inga* sp. were observed during the diurnal and nocturnal anthesis periods. These observations were carried out for two days and two nights, for a total of 42 h of monitoring for each *Inga* sp. During the nocturnal observations, we monitored the flowers against the sky light, which is clearer than the light beneath the forest canopy. In addition, these nocturnal observations were carried out on full moon nights to facilitate the monitoring of floral visitors.

The roles of the floral visitors were estimated on the basis of the relationship between the number of visits in which the pollinator touched the reproductive structures of the flowers (anthers and stigma) and the time of visits. We classified floral visitors into three categories: larcenists (LA), which did not touch the reproductive verticils or damage the floral

structures; occasional pollinators (OPs), which touched the reproductive verticils in up to 60% of the visits; and EPs, which touched active reproductive verticils in > 60% of the visits.

Using an insect net, we collected diurnal and nocturnal insects whilst they visited the flowers. For the nocturnal insects, we also used light traps as a complementary sampling method (for details, see Cruz-Neto *et al*., 2011). Observations of nocturnal floral visitors and the use of light traps took place on different nights. All moths were identified using identification guides (e.g. D'Abrera, 1986; Kitching & Cadiou, 2000). For the remaining insects, we consulted the entomological collection of the Laboratório de Biologia Floral e Reprodutiva (POLINIZAR) at the Universidade Federal de Pernambuco, where all specimens were deposited.

Selective exposure of flowers

Semi-permeable paper bags were used to isolate *c*. 2000 pre-anthesis flower buds per *Inga* sp. We selectively maintained *c*. 1000 bagged flowers from the beginning of anthesis to avoid visits by diurnal pollinators and then exposed them to nocturnal visitors from 18:00 to 05:00 h; we then bagged the flowers. We exposed the remaining flowers to diurnal pollinators from the beginning of anthesis, from 12:00 to 18:00 h, and during the morning after anthesis, from 05:00 to 12:00 h. We bagged the flowers submitted to diurnal pollinators from 18:00 to 05:00 h to avoid visits by nocturnal pollinators. For these experiments, we used eight individuals of *I. vera* (125 flowers per individual), five of *I. striata* (200 flowers per individual) and seven of *I. ingoides* (143 flowers per individual). We collected all fruits formed by each of the three *Inga* spp. in the experiments and counted the numbers of seeds in them.

Analyses

Differences in each floral trait between the studied *Inga* individuals were analysed using one-way analysis of variance (ANOVA). We investigated the differences in the floral and nectar features among *Inga* species using principal component analysis (PCA). The sample size used to study the morphological and nectar features in the studied *Inga* ranged from ten to 30 flowers depending on the floral attribute. To standardize our sampling for PCA, we used ten flowers per species. For those cases in which we sampled 30 flowers, we randomly selected ten flowers for PCA.

For the nectar secretion pattern and nectar strategy experiments, we compared the volume, concentration and milligrams of sugar per flower among the six sets of flowers per species (see the section on Nectar secretion above for details). Comparisons of the first measurements of these nectar features among sets were conducted to identify the nectar secretion pattern of *Inga*. To identify the nectar secretion strategy, we compared the total amount of nectar (volume and milligrams of sugar) secreted by these sets of flowers submitted to different numbers of removals. Data for the nectar secretion patterns of the three studied *Inga* species were square root transformed, whereas the data for the nectar strategies of *I. striata* and *I. ingoides* were log transformed (Sokal & Rohlf, 1981). We analysed the nectar secretion patterns of the three studied *Inga* spp. and the nectar strategy data of *I. striata* and *I. ingoides* using one-way ANOVA followed by a Tukey test. The data on the nectar strategy of *I. vera* were analysed with the Kruskal–Wallis test followed by a Dunn test.

To determine whether diurnal pollinators were more frequent than nocturnal pollinators, we compared the total number of visits received by the same set of flowers during the day and night using a *t*-test for dependent samples (Sokal & Rohlf, 1981). We grouped the visitation frequency data into 12 2-h intervals. These intervals represent the total lifespan of the flowers for the three studied *Inga* spp. We checked the differences between these intervals using a one-way ANOVA followed by a Tukey test (Sokal & Rohlf, 1981). All of these data on visitation frequency were square root transformed prior to the statistical analyses.

To investigate the limitation in the fruit set rate of *Inga*, we compared the frequency of fruit set per flower between manual cross-pollination and natural pollination experiments in each species. We also compared the frequency of fruit set per flower among pollinations by diurnal, nocturnal and both groups of floral visitors. Generalized linear models (GLMs) with binomial data structure were used for these comparisons of fruit set. We selected a nested design controlled for the variance in instances in which many flowers were sampled per tree. The Wald chi-squared (χ^2) values were reported for all of these comparisons. Finally, to compare the numbers of seeds per fruit in the selective exposure experiments, we used a Kruskal–Wallis test followed by a Dunn test (Sokal & Rohlf, 1981). All tests of nectar secretion, visitation frequency and fruit set were carried out in Statistica 8.0 (StatSoft Inc., 2007). The PCA tests were carried out in InfoStat/F v. 2013 (Di Rienzo *et al*., 2013).

RESULTS

Floral biology and anthesis

The inflorescences of the three *Inga* spp. are axillary racemose, with an average number of 14 ± 2 flowers for *I. vera*, 12 ± 5 for *I. striata* and 9 ± 5 for *I. ingoides*. We found up to six inflorescences per axil in these species. We recorded up to four open flowers per

Figure 1. Flowers of *Inga* at the Coimbra remnant, AL, Brazil. A, Flower of *Inga vera*. B, Inflorescence of *Inga striata*. C, Flower of *Inga ingoides*. D, A bat, *Glossophaga soricina*, visiting a flower of *I. ingoides*. White lines under the letters A, B and C represent 1 cm. Arrows in B and C indicate the style of *I. striata* and *I. ingoides*, respectively. Photographs: A, B, O. Cruz-Neto; C, D, A. V. Lopes.

inflorescence during the flowering peak for *I. vera* and *I. striata* and three flowers per inflorescence for *I. ingoides*.

The flowers of the three species are actinomorphic, hermaphroditic and pentamerous, and can be classified as brush type (Fig. 1). The androecium is polystemonous, with connate stamens at the base forming a narrow staminal tube. The anthers of the three species are dorsifixed and exhibit longitudinal dehiscence. The gynoecium is composed of a single stigma, a long style and a unicarpellate, unilocular and pluriovulate ovary (Table 1).

Despite the similar morphology, *Inga* spp. could be clearly separated on the basis of their floral traits. *Inga vera* exhibited significantly greater corolla diameter ($F_{2,87} = 78.3$, $P < 0.01$), stamen number ($F_{2,87} = 27$, $P < 0.01$), staminal tube diameter ($F_{2,87} = 64.53$, $P < 0.01$), number of polyads per anther ($F_{2,87} = 511.92$, $P < 0.01$) and number of pollen grains per flower ($F_{2,87} = 99.8$, $P < 0.01$) than *I. striata* and *I. ingoides*. The lengths of the style and staminal tube were significantly higher in *I. ingoides* relative to the other studied species (style length: $F_{2,87} = 320.32$, $P < 0.01$; staminal tube: $F_{2,87} = 157.9$, $P < 0.01$). Calyx length ($F_{2,87} = 1.8$, $P = 0.16$), number of ovules per flower ($F_{2,87} = 21.4$, $P = 0.2$) and pollen viability ($F_{2,87} = 88.8$, $P = 0.17$) did not vary among the studied *Inga* species (Table 1). The first two components of the PCA explained 65% of the variation in these morphological floral traits, with *I. striata* and *I. ingoides* being more similar to each other than to *I. vera* (Fig. 2).

Flower opening began at approximately 12:00 h (*I. vera*) or 13:30 h (*I. striata* and *I. ingoides*) and lasted until 17:00 h (*I. ingoides*) or 17:30 h (*I. striata* and *I. vera*), when the flowers were completely open and exhibited white reproductive verticils. The withering of flowers began at 05:00 h on the following day and lasted until 09:30 h for *I. vera*, from 06:00 to 11:00 h for *I. striata*, and from 06:00 to 09:30 h for *I. ingoides*. Stigmas of the three species were receptive from the beginning of anthesis until withering. The anthers began to release polyads early in the night (approximately 17:30 h), when it was possible to extract nectar from the flowers. The flowers of the three species were functional (i.e. capable of receiving and releasing pollen through pollinators) with nectar and polyads available simultaneously for 10 h in *I. vera*, for 11–12 h in *I. striata* and for 13 h in *I. ingoides*.

Table 1. Morphometric and quantitative characteristics of flowers and pollen viability (PV) of *Inga vera*, *I. striata* and *I. ingoides* (Fabaceae-Mimosoideae) at the Coimbra remnant, Usina Serra Grande, AL, Brazil

		Inga vera	*Inga striata*	*Inga ingoides*	ANOVA	
Floral character	Attribute		(mean ± SD)		*F*	*P*
Calyx	Length (cm)*	0.8 ± 0.1[a]	0.9 ± 0.12[a]	0.8 ± 0.03[a]	1.80	0.17
Corolla	Length (cm)*	1.4 ± 0.1[a]	1.0 ± 0.055[b]	1.4 ± 0.2[a]	49.53	0.01
	Diameter (cm)*	0.6 ± 0.05[a]	0.4 ± 0.056[c]	0.5 ± 0.1[b]	78.30	0.01
Style	Length (cm)*	4.5 ± 0.2[c]	7.1 ± 0.5[b]	7.3 ± 0.5[a]	320.32	0.01
Stamens	Number*	54.9 ± 10.6[a]	46.0 ± 3.7[c]	52.4 ± 3.1[b]	27.00	0.01
	Length (cm)*	3.7 ± 0.5[b]	5.2 ± 0.2[a]	5.4 ± 0.2[a]	229.70	0.01
Staminal tube	Length (cm)*	1.2 ± 0.1[b]	1.2 ± 0.1[b]	1.47 ± 0.08[a]	157.90	0.01
	Diameter (cm)*	0.4 ± 0.06[a]	0.2 ± 0.05[c]	0.33 ± 0.06[b]	64.53	0.01
Ovules/ovary	Number*	15 ± 1[a]	18 ± 0[a]	18 ± 0[a]	21.40	0.20
Pollen	Polyads/anther†	21.1 ± 2.2[a]	15.6 ± 0.76[b]	8.9 ± 1.0[c]	511.92	0.01
	Pollen grains/polyad‡	19.6 ± 3.2[a]	17.3 ± 1.9[b]	18.8 ± 2.3[a]	5.88	0.01
	Pollen grains/flower§	21560 ± 501.1[a]	12507.8 ± 65.4[b]	8937.2 ± 36.4[c]	99.80	0.01
	PV (%)‡	94[a]	100[a]	100[a]	88.80	0.17

*N = 30 flowers.
†N = 50 anthers.
‡N = 30 polyads.
§N = 10 flowers.
Values in the same line followed by distinct letters were statistically different at $P < 0.05$.

Dynamics of nectar secretion and removal effects

Nectar secretion began when the corolla started to open, at approximately 13:00 h, but in amounts of < 1 µL during the first 4 h of anthesis. The three species secreted *c*. 45–55 µL of nectar per flower at a concentration of 19%, which is equivalent to *c*. 8 mg of solutes per flower (Table 2). We found a single period of active secretion from 18:00 to 00:00 h for *I. vera*, which intensified from 22:00 to 00:00 h (Fig. 3A, B). Following the secretion period, we identified active nectar resorption from 00:00 h to 06:00 h (Fig. 4B), in which flowers of *I. vera* exhibited a reduction from 9.6 ± 1.2 mg of sugar at 00:00 h to 7.2 ± 1.1 mg of sugar at 06:00 h ($F_{5,54} = 86.4$, $P < 0.01$). The other two species exhibited continuous secretion without active resorption (Figs 3C–F, 4D, F). The nectar volume increased until 22:00 h (*I. striata*: $F_{5,54} = 74.53$, $P < 0.001$; *I. ingoides*: $F_{5,54} = 74.93$, $P < 0.01$), whereas the sugar concentration did not change significantly during the nectar secretion period (*I. striata*: $F_{5,54} = 41.18$, $P = 0.06$; *I. ingoides*: $F_{5,54} = 58.31$, $P = 0.06$). The number of milligrams of sugar per flower increased until 22:00 h and remained constant until the end of the nectar secretion period (*I. striata*: $F_{5,54} = 38.41$, $P < 0.01$; *I. ingoides*: $F_{5,54} = 27.6$, $P < 0.001$).

The studied *Inga* spp. showed different responses to the extractions of floral nectar (Table 2). Flowers of *I. vera* submitted to more than four extractions

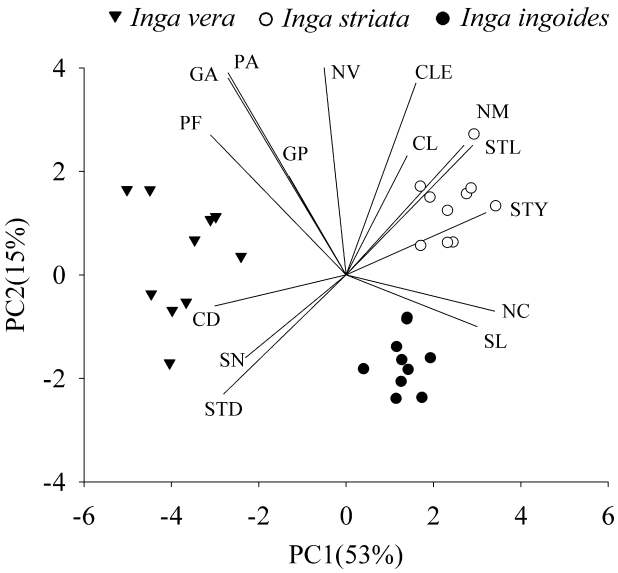

Figure 2. Results of the principal component analysis (PCA) related to flower and nectar features of *Inga* at the Coimbra remnant, AL, Brazil. The PCA was based on 15 variables related to floral morphology and nectar features of *I. vera*, *I. striata* and *I. ingoides*. CD, corolla diameter; CL, calyx length; CLE, corolla length; GA, grains/anther; GP, grains/polyad; NC, nectar concentration; NM, milligrams of sugar in the nectar; NV, nectar volume; PA, polyads/anther; PF, pollen grains/flower; SL, stamen length; SN, stamen number; STD, staminal tube diameter; STL, staminal tube length; STY, style length.

Table 2. Effects of successive nectar extractions on nectar production [volume (μL) and sugar content (mg/flower)] in flowers of *Inga vera*, *I. striata* and *I. ingoides* (Fabaceae-Mimosoideae) at the Coimbra remnant, AL, Brazil

		Time of extraction (h)													
		20:00		22:00		00:00		02:00		04:00		06:00		Total	
		V (μL)	mg/flower	*V* (μL)	mg/flower	*V* (μL)	mg/flower	*V* (μL)	mg/flower	*V* (μL)	mg/flower	*V* (μL)	mg/flower	*V* (μL)*	mg/flower*
Species	Sets	Mean ± SD													
I. vera	1	16 ± 3.5	2 ± 0.4	4.6 ± 3.3	0.3 ± 0.2	3.3 ± 0.6	0.2 ± 0.1	2.9 ± 0.6	0.2 ± 0.1	2.5 ± 0.4	0.13 ± 0.1	1.65 ± 0.8	0.08 ± 0.1	30.95 ± 6.3^{b}	2.9 ± 0.5^{c}
	2			20.6 ± 1.4	2.53 ± 0.2	2.06 ± 0.3	0.15 ± 0.02	2 ± 0.5	0.16 ± 0.05	2.3 ± 0.4	0.16 ± 0.03	1.9 ± 0.7	0.13 ± 0.04	28.5 ± 2.1^{b}	3.1 ± 0.2^{c}
	3					46.06 ± 9	9.57 ± 1.9	2.14 ± 0.3	0.17 ± 0.03	2.34 ± 0.4	0.14 ± 0.04	1.7 ± 0.6	0.07 ± 0.02	52.2 ± 8.7^{a}	10 ± 1.9^{a}
	4							52.26 ± 4.9	8.51 ± 0.6	2.1 ± 0.47	0.13 ± 0.03	1.54 ± 0.4	0.08 ± 0.02	55.9 ± 1.1^{a}	8.7 ± 0.6^{a}
	5									54.89 ± 3.7	7.24 ± 0.6	1.6 ± 0.6	0.59 ± 0.04	56.5 ± 4.1^{a}	7.34 ± 0.6^{b}
	6											54.04 ± 9.8	7.19 ± 1.5	54.04 ± 9.9^{a}	7.2 ± 1.5^{b}
I. striata	1	25.6 ± 3.8	5.8 ± 0.8	6.6 ± 0.9	1.1 ± 0.2	7.2 ± 0.9	1.2 ± 0.17	7.61 ± 0.8	1.3 ± 0.2	7.35 ± 1.4	1.3 ± 0.3	3.3 ± 0.8	0.64 ± 0.2	57.6 ± 3.3^{a}	11.3 ± 0.7$^{a/b}$
	2			37.3 ± 3.8	8.4 ± 0.8	5.2 ± 0.7	0.9 ± 0.1	5 ± 0.7	0.9 ± 0.1	4.4 ± 0.4	0.89 ± 0.1	3.03 ± 0.6	0.6 ± 0.1	54.9 ± 4.6$^{a/b}$	11.7 ± 0.97^{a}
	3					39.43 ± 4.1	9.2 ± 0.9	4.63 ± 0.9	1.1 ± 0.2	3.65 ± 0.7	0.76 ± 0.1	2.9 ± 0.6	0.9 ± 0.2	50.6 ± 4.4$^{b/c/d}$	11.9 ± 1.05^{a}
	4							45.03 ± 2.5	10.13 ± 0.7	2.14 ± 0.5	0.42 ± 0.1	2.1 ± 0.4	0.4 ± 0.07	49.2 ± 2.3$^{c/d}$	10.9 ± 0.7$^{b/c}$
	5									46.01 ± 2.3	9.4 ± 0.6	1.6 ± 0.6	0.32 ± 0.1	47.7 ± 2.4^{d}	9.7 ± 0.65^{c}
	6											53.9 ± 4	10.3 ± 0.9	53.9 ± 4$^{a/b/c}$	10.3 ± 0.9^{c}
I. ingoides	1	30.5 ± 0.8	4.8 ± 0.3	5.5 ± 0.8	0.9 ± 0.2	6.2 ± 0.7	1.3 ± 0.2	7.7 ± 0.4	1.65 ± 0.1	8.5 ± 0.4	1.6 ± 0.1	4.36 ± 0.6	0.78 ± 0.1	62.7 ± 2.8^{a}	11.03 ± 0.8^{b}
	2			38.4 ± 1.0	7.3 ± 0.4	5.7 ± 0.4	1.1 ± 0.1	6.5 ± 0.4	1.4 ± 0.1	7.68 ± 0.4	1.4 ± 0.1	4.8 ± 0.5	0.8 ± 0.1	63.1 ± 0.8^{a}	12.08 ± 0.3^{a}
	3					41.3 ± 0.5	8.3 ± 0.1	5.9 ± 0.4	1.3 ± 0.1	6.4 ± 0.4	1.2 ± 0.1	3.89 ± 0.31	0.67 ± 0.07	57.5 ± 1^{b}	11.4 ± 0.13^{b}
	4							40.5 ± 13.3	8.3 ± 2.7	5.12 ± 1.7	0.93 ± 0.29	3.9 ± 1.3	0.67 ± 0.21	49.5 ± 16.1^{d}	9.9 ± 3.3^{c}
	5									46.71 ± 0.5	8.73 ± 0.2	5.57 ± 0.48	1.03 ± 0.09	52.3 ± 0.6^{c}	9.8 ± 0.26^{c}
	6											45.22 ± 0.5	8.39 ± 0.27	45.2 ± 0.6^{e}	8.4 ± 0.3^{d}

*Volumes (μL) and milligrams of sugar in the nectar per flower (mg/flower) in the same column followed by different letters, for each species, were statistically significantly different. *I. vera*: V ($H_{2,30}$ = 18.49; P = 0.0001), mg/flower ($H_{2,30}$ = 20.19; P < 0.001); *I. striata*: V ($F_{5,54}$ = 10.9; P < 0.001), mg/flower ($F_{5,54}$ = 7.89; P < 0.001); *I. ingoides*: V ($F_{5,54}$ = 217.52; P < 0.001), mg/flower ($F_{5,54}$ = 86.27; P < 0.001).

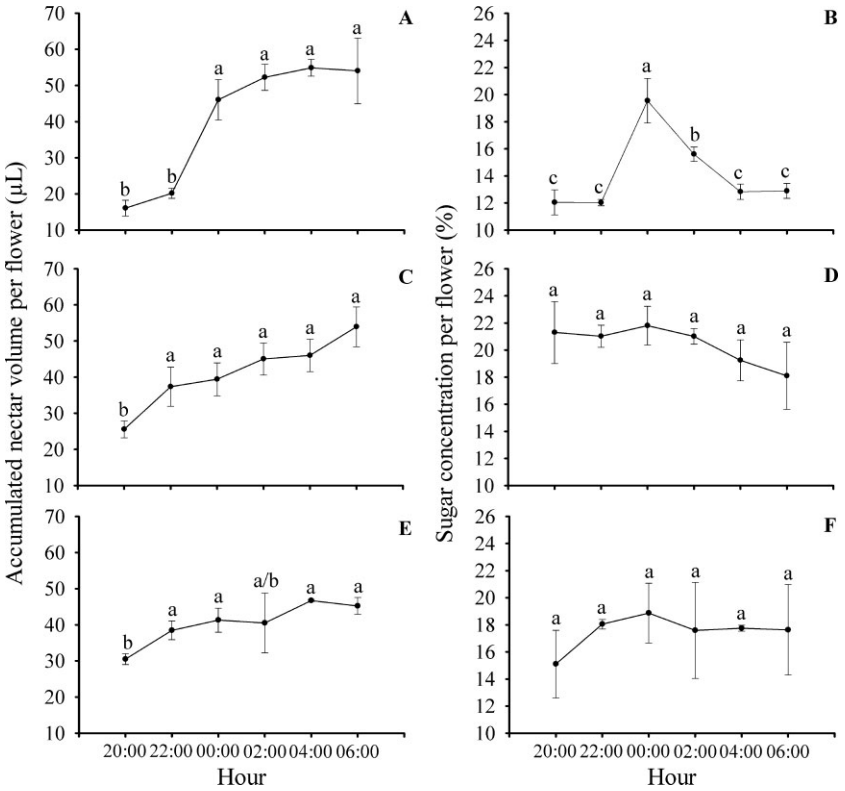

Figure 3. Volume (µL) and nectar sugar concentration (%) in flowers of *Inga vera* (A, B), *I. striata* (C, D) and *I. ingoides* (E, F) at the Coimbra remnant, AL, Brazil. Different letters for each set of nectar extraction indicate significant differences (A: $F_{5,54} = 89.3$, $P < 0.001$; B: $F_{5,54} = 89.0$, $P < 0.001$; C: $F_{5,54} = 74.5$, $P < 0.001$; D: $F_{5,54} = 41.1$, $P = 0.07$; E: $F_{5,54} = 74.9$, $P < 0.01$; F: $F_{5,54} = 58.3$, $P = 0.07$). Circles represent the mean values; vertical bars represent the standard deviation.

secreted a smaller volume of nectar per flower ($H_{2,30} = 18.49$, $P < 0.0001$) and milligrams of sugar per flower ($H_{2,30} = 20.19$, $P < 0.001$), with a reduction of approximately 60% in the production, reinforcing the nectar resorption for this species. The effect of successive extractions in *I. striata* contributed to a small increase, of less than 10%, in nectar secretion volume ($F_{5,54} = 10.9$, $P < 0.001$) and milligrams of sugar per flower ($F_{5,54} = 7.89$, $P < 0.001$). The increase in the number of extractions in *I. ingoides* led to an increase of *c.* 17 µL ($F_{5,54} = 217.52$, $P < 0.001$) or approximately 4 mg of sugars ($F_{5,54} = 86.27$, $P < 0.001$), values that corresponded to approximately 28% of the storage capacity of the staminal tube.

Pollen and breeding system

Pollen grains are grouped into polyads, with a variable number of pollen grains for the three species (Table 1). The viability of these grains was 94% for *I. vera* and 100% for the other species. The number of pollen grains per flower varied from 8937 for *I. ingoides* to 21 560 for *I. vera*.

The three species are self-incompatible, as no fruit was set after the experiments involving manual and spontaneous self-pollination (Table 3). The potential fruit set seems to be unfulfilled for the studied species because we observed an increase in fruit production of up to 10.4 times ($\chi^2_{(2)} = 11.5$; $P = 0.003$) for *I. vera*, 5.78 times ($\chi^2_{(2)} = 172.3$; $P < 0.01$) for *I. striata* and 6.58 times ($\chi^2_{(2)} = 10.3$; $P = 0.002$) for *I. ingoides* in the treatment of hand cross-pollination relative to natural pollination.

Diurnal visitors

We recorded 16 species of diurnal visitor (48% of total): bees ($N = 6$), wasps ($N = 2$), hummingbirds ($N = 7$) and another bird species of the family Emberizidae that was observed only on *I. vera* (Table 4). One bee species (*Trigona fuscipennis*) and one bird species (*Coereba flaveola*) were considered to be nectar robbers, because they visited flowers during the afternoon when the anthers were closed and did not touch the reproductive structures.

The most important OPs for the three *Inga* spp., as indicated by their behaviour, high frequency and time of visit, were the hummingbirds *Amazilia fimbriata* and *Phaetornis ruber*. Hummingbirds, when hovering, introduced their beaks into the staminal tubes of the

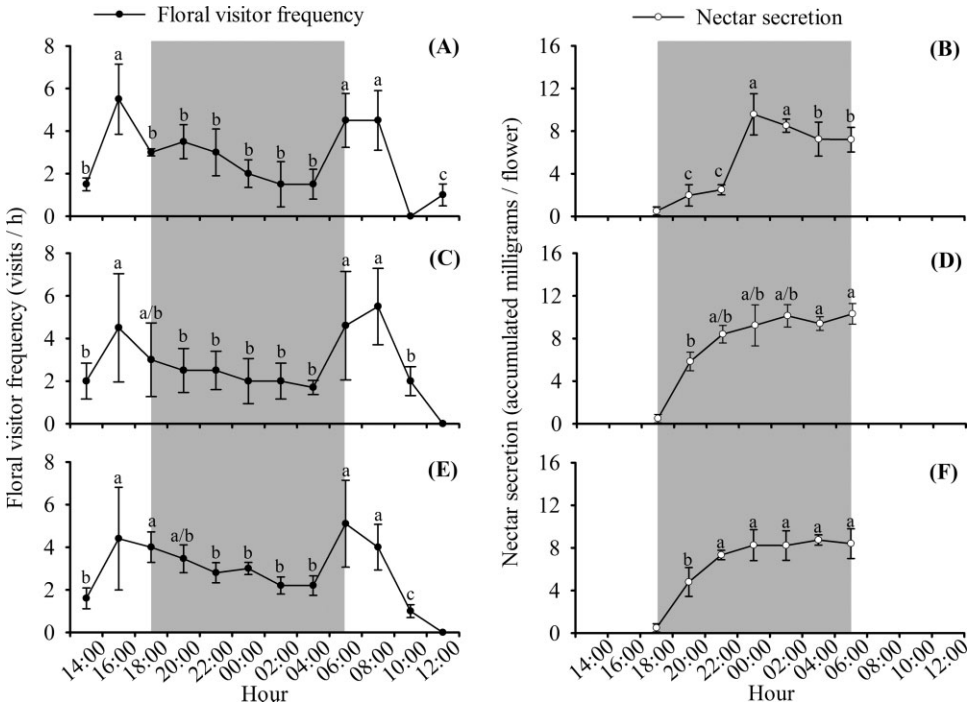

Figure 4. Relationship between nectar secretion (milligrams of sugar/flower) and flower visitor frequency (number of visits/h) in flowers of *Inga vera* (A, B), *I. striata* (C, D) and *I. ingoides* (E, F) at the Coimbra remnant, AL, Brazil The grey bar represents the nocturnal period; circles represent the mean values; vertical bars represent the standard deviations.

Table 3. Breeding systems of *Inga vera*, *I. striata* and *I. ingoides* (Fabaceae-Mimosoideae) at the Coimbra remnant, AL, Brazil [success (fruit set/flower number)]. Natural fruit set (control), spontaneous self-pollination, self-hand pollination and outcross hand pollination experiments were performed to check the breeding system of *Inga*

	I. vera	*I. striata*	*I. ingoides*
Control (natural fruit set)	1.9% (30/1561)^{b*}	1.1% (17/1493)^{b†}	1.5% (22/1447)^{b‡}
Spontaneous self-pollination	0% (0/100)	0% (0/100)	0% (0/100)
Self-hand pollination	0% (0/30)	0% (0/30)	0% (0/30)
Outcross hand pollination	20% (6/30)^{a}	6.6% (2/30)^{a}	10% (3/30)^{a}

Values in the same column followed by different letters were significantly different: $^{*}\chi^2_{(2)} = 11.5$, $P = 0.003$; $\dagger\chi^2_{(2)} = 172.3$, $P < 0.01$; $\ddagger\chi^2_{(2)} = 10.3$, $P = 0.002$.

flowers to collect nectar and touched the anthers and stigmas in a behaviour similar to that of nocturnal pollinators. Moreover, we recorded visits of hummingbirds until 17:50 h, the time at which *Inga* flowers received visits from hawkmoths, such as *Aellopos ceculus*, and when polyads were already available.

Nocturnal visitors

We recorded 17 species (52% of total) of nocturnal visitors: noctuids ($N = 4$), hawkmoths ($N = 12$) and bats ($N = 1$) (Table 4). We considered the nocturnal visitors as EPs, as they visited *Inga* flowers when anthers were dehiscent and the stigma was receptive. Moreover, there was contact between the bodies of these animals and the reproductive structures of the flowers in all visits. The most frequent nocturnal pollinators in the studied *Inga* spp. were hawkmoths, mainly *Aellopos ceculus*, *Manduca hannibal* and *Neogene dynaeus*, which visited flowers of the three *Inga* spp., and *Xylophanes loelia*, which visited flowers of *I. striata* and *I. ingoides*.

We collected five specimens of hawkmoths of five different species with insect nets when they visited *Inga* flowers. We captured, with a light trap, specimens of seven other species, which had polyads of *Inga* attached to their bodies (see Cruz-Neto *et al.*, 2011; Table 4). Hawkmoths visited *Inga* flowers for a longer

Table 4. Diurnal and nocturnal floral visitors of *Inga vera*, *I. striata* and *I. ingoides* and their effectiveness and behaviour in the Coimbra remnant, AL, Brazil

Diurnal (D) and nocturnal (N) floral visitors	*I. vera*	*I. striata*	*I. ingoides*	Effectiveness*	Behaviour†
Bees (D)					
Hymenoptera – Apidae					
Apis mellifera	X	X	X	30%	OP
Centris aenea		X		30%	OP
Centris sponsa	X			20%	OP
Trigona spinipes	X	X	X	10%	OP
Trigona fuscipennis	X	X	X	0%	LA
Xylocopa suspecta	X		X	30%	OP
Wasps (D)					
Hymenoptera – Vespidae					
Synoeca cyanea	X	X	X	40%	OP
Pachodynerus sp.	X	X	X	20%	OP
Birds (D)					
Trochilidae					
Amazilia fimbriata	X	X	X	60%	OP
Amazilia versicolor	X	X	X	60%	OP
Chlorostilbon aureoventris	X	X	X	60%	OP
Eupetomena macroura		X	X	60%	OP
Glaucis hirsuta		X	X	60%	OP
Melanotrochilus fuscus		X	X	60%	OP
Phaetornis ruber	X	X	X	60%	OP
Emberezidae					
Coereba flaveola	X			0%	LA
Hawkmoths (N)					
Lepidoptera – Sphingidae					
Aellopos ceculus‡	X	X	X	90%	EP
Callionima parce			X	100%	EP
Cocytius antaeus	X		X	100%	EP
Erinnyis lassauxii‡	X			100%	EP
Eumorpha anchemolus‡		X		100%	EP
Manduca florestan		X		100%	EP
Manduca hannibal‡	X	X	X	100%	EP
Neogene dynaeus‡	X	X	X	100%	EP
Pachygonidia caliginosa		X		100%	EP
Protambulyx astygonius		X		100%	EP
Xylophanes anubus		X		100%	EP
Xylophanes loelia		X	X	100%	EP
Moths (N)					
Lepidoptera – Noctuiidae					
Ascalapha odorata	X			100%	EP
Sp2		X		100%	EP
Sp3		X		100%	EP
Ophisma tropicalis	X		X	100%	EP
Bats (N)					
Glossophaginae					
Glossophaga soricina	X	X	X	100%	EP

*Effectiveness: percentage of visits in which the floral visitor touched the reproductive structures (anthers and stigma).
†Behaviour: larcenist (LA), never touches floral reproductive structures or damages the flowers; occasional pollinator (OP), touches the floral reproductive structures on 1–60% of visits; effective pollinator (EP), touches the floral reproductive structures on > 61% of visits.
‡Sphingid species captured with a butterfly net whilst visiting flowers of *Inga*.

Table 5. Fruit set per flower (Fr/Fl) and seed set per fruit (Sd/Fr) ratios of flowers visited only by diurnal (Diurnal), nocturnal (Nocturnal) or both guilds of pollinator (natural pollination – Control) and female reproductive success indices in *Inga vera*, *I. striata* and *I. ingoides* at the Coimbra remnant, AL, Brazil

	Fr/Fl [Success (*N* fruits/*N* flowers)]		
	Diurnal	Nocturnal	Control
I. vera	0.3% (3/1000)	1.4% (14/1000)	1.9% (30/1561)*
I. striata	0.2% (2/1000)	1% (10/1000)	1.1% (17/1493)†
I. ingoides	0.2% (2/987)	1.36% (13/959)	1.5% (22/1447)‡
	Sd/Fr (mean ± SD)		
I. vera	3.3 ± 1.57[b]	8.2 ± 3.12[a]	8.8 ± 2.11[a]§
I. striata	6.5 ± 3.53[a]	10.8 ± 2.44[a]	8.8 ± 2.48[a]¶
I. ingoides	5.0 ± 2.83[a]	8.4 ± 2.93[a]	7.1 ± 1.54[a]**
	Female reproductive success index (Fr/Fl × Sd/Fr)		
I. vera	0.01	0.12	0.17
I. striata	0.01	0.10	0.10
I. ingoides	0.01	0.12	0.11

*$\chi^2_{(3)} = 532.12$, $P < 0.001$.
†$\chi^2_{(3)} = 49.81$, $P < 0.001$.
‡$\chi^2_{(3)} = 6.16$, $P = 0.007$.
For the comparisons of seed set per fruit, values in the same row followed by different letters were significantly different: §$H_{2,47} = 7.9843$, $P = 0.0185$; ¶$H_{2,29} = 4.6$, $P = 0.1$; **$H_{2,37} = 3{,}2$, $P = 0.2$.

time than other nocturnal pollinators, from 17:00 to 04:00 h. Bats visited flowers from 18:30 to 03:00 h. Hawkmoths were observed visiting the flowers on all nights of observation, whereas bats were absent on some nights. Noctuids started their visits to the three *Inga* spp. at *c*. 19:00 h and finished at *c*. 23:00 h for *I. ingoides* and *I. vera* and 00:30 h for *I. striata*.

The visitation frequency was reduced in the nocturnal period relative to the diurnal period from a mean ± SD of 19.7 ± 4.5 to 13.3 ± 2.8 in *I. vera* ($t = 0.48$; $P = 0.03$), 21.5 ± 3.6 to 13.8 ± 3.2 in *I. striata* ($t = 3.6$; $P < 0.01$) and 16.8 ± 2.2 to 14.2 ± 1.9 in *I. ingoides* ($t = 2.41$; $P < 0.01$). We found a similar pattern of a low frequency of visits per flower per hour (mean ± SD: 2.3 ± 1.2 in *I. vera*, 2.25 ± 0.52 in *I. striata* and 2.91 ± 0.81 in *I. ingoides*) during the night, when the flowers were receptive and actively secreting nectar (Fig. 4B, D, F). The highest frequency of visits, approximately four visits per hour, was registered at the transition periods between the nocturnal and diurnal flower visitors (*I. vera*: $F_{11,108} = 12.99$, $P < 0.01$; *I. striata*: $F_{11,108} = 13.52$, $P < 0.01$; *I. ingoides*: $F_{11,108} = 10.18$, $P < 0.01$), when the flowers did not secrete nectar.

Selective flower exposure to nocturnal and diurnal pollinators

Nocturnal visitors contributed more efficiently than diurnal visitors to the production of fruits in the studied *Inga* spp. (*I. vera*: $\chi^2_{(3)} = 532.12$, $P < 0.001$; *I. striata*: $\chi^2_{(3)} = 49.81$, $P < 0.001$; *I. ingoides*: $\chi^2_{(3)} = 6.16$, $P = 0.007$). The fruit set per flower was four times higher in *I. vera* and between six and seven times higher in *I. striata* and *I. ingoides* in the nocturnal relative to the diurnal period. In addition, the fruit set rates attributed to the activity of nocturnal pollinators were close to the values observed for natural pollinations in the three *Inga* spp. (Table 5). This same pattern was observed for the indices of female reproductive success. There was no difference in the seed set per fruit between nocturnal and diurnal pollinators in *I. striata* or *I. ingoides*. However, in the case of *I. vera*, we found a higher average number of seeds in fruits from flowers exposed to nocturnal pollinators ($H = 7.9843$, d.f. = 2, $P = 0.0185$), reaching more than twice the number of seeds observed in fruits from flowers exposed to diurnal visitors. Female reproductive success was more than ten times greater in nocturnal than diurnal pollinators (Table 5).

DISCUSSION

Detailed information on pollination interactions involving trees of tropical regions is rare in the literature, despite its importance to the knowledge of pollination ecology and evolution. In this article, we describe the occurrence of two clear pollination strategies in three *Inga* spp. that are visited by similar spectra of pollinators and exhibit similar gross floral

morphology. *Inga vera* differs from the other species in having the widest corolla and staminal tube, the highest number of stamens and the largest production of pollen grains per flower. Despite the studied *Inga* spp. having a similar nectar secretion period, a possible resorption of sugars was detected only in *I. vera*. These differences seem to translate into higher reproductive efficiency (Table 5), possibly through improved quality of pollen or through reduced resource limitation of fruit set.

Pollination syndrome of *Inga*

Brush-type flowers with narrow staminal tubes (2.0–4.0 mm) and crepuscular anthesis are characteristics that classify the studied species in the sphingophilous pollination syndrome (*sensu* Faegri & Pijl, 1979). Indeed, hawkmoths were the most frequent group of nocturnal pollinators in the present study, and have also been noted as the main pollinators of other *Inga* spp. (Koptur, 1983). Although the studied *Inga* spp. are sphingophilous, we found species of bats, hummingbirds, bees, wasps and lepidopterans frequently visiting their flowers.

Large numbers of flowers and inflorescences per tree characterize *Inga* spp. as mass-flowering species (*sensu* Gentry, 1974). Some diurnal visitors, such as hummingbirds and bees, react positively to large amounts of floral resources (e.g. Hodges, 1995; Klinkhamer *et al*., 2001; Longo & Fischer, 2006; Fisogni *et al*., 2011; Justino, Maruyama & Oliveira, 2012) and may be intensely attracted to *Inga* flowers, explaining their high visitation rate.

Inga flowers could be distinguished mainly on the basis of stamen quantity and length, staminal tube diameter and length, style length, quantity of pollen grains and nectar strategy, which are probably functionally related to the effectiveness of pollination. *Inga striata* and *I. ingoides* appear to have more similar floral forms to each other in comparison with *I. vera*. Although there is no published phylogenetic study on the three investigated *Inga* spp., *I. vera* and *I. ingoides* appear to be close relatives (Dexter, Pennington & Cunningham, 2010), whereas *I. vera* and *I. striata* do not seem to be close relatives (Richardson *et al*., 2001). Thus, it is possible that the more similar floral form and nectar strategy found in *I. striata* and *I. ingoides* represents convergent evolution, or that these species exhibit an ancestral state, whereas *I. vera* shows a derived morphology and nectar strategy.

Secretion patterns and removal effects of nectar

Large amounts (26–55 μL) of dilute (12–20% sugar) nectar with a slightly sweet scent are characteristic of sphingophilous flowers (*sensu* Faegri & Pijl, 1979). Nectar may be secreted in regular rhythms throughout the lifespan of flowers (Heil, 2011) and has also been associated with the major activity of the pollinators, affecting pollination and thus pollen flow (e.g. Koptur, 1983; Hodges, 1995; Klinkhamer *et al*., 2001; Musicante & Galetto, 2008; Agostini, Sazima & Galetto, 2011). Previous studies on *Inga sessilis* (Vell.) Mart. found that nectar secretion is increased during the activity of nocturnal pollinators (Amorim *et al*., 2013). We also observed significant increases in nectar volume and milligrams of sugar during the first hours of the night for *I. striata* and *I. ingoides*, the period of highest activity of nocturnal pollinators of *Inga*, reinforcing the relationship between nectar secretion and the characteristics of nocturnal visitors.

Nectar secretion can be extremely costly, and adjustments of nectar production rates during the lifespan of the flowers are therefore expected to be frequent in many plant species (Pyke, 1991; Ornelas & Lara, 2009; Heil, 2011). We found that nectar sugar content remains constant throughout anthesis in *I. striata* and *I. ingoides*, but is reduced in *I. vera*. These differences in sugar secretion/resorption probably imply divergence among *Inga* spp. in sugar sensing mechanisms, which allow for the separate regulation of nectar volume and concentration (Nepi & Stpiczýnska, 2008; Heil, 2011).

Successive removals may cause nectar resorption in *I. vera* or enhance nectar secretion in *I. striata* and *I. ingoides*. The strategy of nectar resorption may indicate optimization of resource use by the plant or enhancement of pollen quality delivery through a reduction in reward levels in frequently visited flowers, which would increase the movement of pollinators between different flowers and individuals (Pyke, 1991; Nepi & Stpiczýnska, 2008; Fisogni *et al*., 2011). Flowers in which nectar production is inhibited by an increase in the number of extractions use smaller amounts of energy to attract floral visitors, and therefore may allocate a larger amount of resources to seed production and maturation (Pyke, 1991; Ornelas & Lara, 2009). Conversely, flowers in which nectar secretion is stimulated by an increase in the number of extractions may supply the requirements of pollinators during high-activity periods of visiting and save nectar in periods of low frequency of visitation (Koptur, 1983).

Pollen and breeding system

The number of pollen grains per flower was higher in *I. vera* than in the other studied species. Changes in the quantity of pollen grains per flower among closely related species may be related to the flower morphology, activity of pollinators and/or sexual system

(Cruden, 2000). Because the studied *Inga* spp. are similarly pollinated and exhibit the same sexual system, it is possible that changes in floral morphology, such as a wider corolla and staminal tube and higher number of stamens in *I. vera*, are related to this variation in pollen grain number.

Similar to many tropical trees, *Inga* spp. produce relatively few mature fruits despite their mass flowering strategy (e.g. Koptur, 1983; Amorim *et al*., 2013; Barros *et al*., 2013). Fruit set ranged from 1.1 to 1.9% for the studied *Inga* spp. Low fruit/flower ratios have been mainly attributed to poor pollination success, maternal resource limitation, the selective maturation of fruits and a self-incompatible breeding system (Arroyo, 1976; Stephenson, 1981; Gibbs & Sassaki, 1998; Torres *et al*., 2002). All of these factors may have acted in combination to determine fruit set in *Inga* (Barros *et al*., 2013).

Greater limitations in fruit set of natural relative to manual cross experiments suggest a deficit in pollination, in which none of the pollinators effectively fulfils the potential fruit set of the three studied species. Some bats and hummingbirds are thought to show territorial behaviour (Gribel & Hay, 1993; Justino *et al*., 2012), which may result in pollen flow between flowers of the same individual. Hawkmoths may also contribute to inviable pollen flow because they can visit at least seven flowers in sequence on the same tree. An intense flow of incompatible pollen grains is strongly associated with high rates of abortion of flowers and fruits in Fabaceae (Arroyo, 1976; Koptur, 1984; Huth & Pellmyr, 2000), and may reduce fruit set in self-incompatible species, such as *Inga edulis* Mart. and *I. stipularis* DC. (Barros *et al*., 2013), *I. vera*, *I. striata* and *I. ingoides* (present study).

Despite the possible excess of incompatible pollen flow promoted by the territorial behaviour of nocturnal pollinators in the studied *Inga* spp., bats and hawkmoths are able to fly long distances during their foraging routes (Gribel & Hay, 1993; Elmore, Miller & Villella, 2006; Amorim *et al*., 2013). Cross-pollination between distant individuals and populations of these species may ensure fruit and seed production in *Inga* (Koptur, 1984; Amorim *et al*., 2013; our data). This ability to fly long distances during foraging may be of importance to the maintenance and conservation of species mainly in a fragmented habitat such as the Brazilian Atlantic forest (Lopes *et al*., 2009).

The roles of nocturnal and diurnal pollinators

The quality (efficiencies or efficacies) and quantity (visitation frequency) of pollination services are strongly related to the reproductive success of plants and the evolutionary pathways of flowers and pollinators (Stebbins, 1970; Waser *et al*., 1996; Thomson & Wilson, 2008). Although fruit set was low (< 2%), nocturnal pollinators contributed ten times more than diurnal pollinators to the female reproductive success, suggesting a higher pollination efficiency of bats and hawkmoths, despite their lower overall visitation rates.

We stress that the dehiscence of the anthers may also be an important component for the determination of the contributions of nocturnal and diurnal pollinators to the pollination of *Inga*. According to our field observations, diurnal pollinators visited *Inga* flowers at the beginning of anthesis, when the stigma was receptive but the anthers were still not dehiscent, or at the end of anthesis, when the flowers were becoming senescent and contained little pollen or nectar. Conversely, nocturnal pollinators visited *Inga* flowers when the stigmatic surface was receptive, polyads were available and nectar was secreted in large amounts. Therefore, diurnal pollinators may have a reduced contribution to the pollen flow and fruit set in crepuscular *Inga* spp.

Some plant species can selectively abort fruits with less vigorous seeds depending on the quality of pollen provided by the pollinators (e.g. Huth & Pellmyr, 2000; Torres & Galetto, 2002; Torres *et al*., 2002; Mena Alí & Rocha, 2005; Zhang *et al*., 2011) and stigma age (Young & Gravitz, 2002). In the case of *I. vera*, the number of seeds per fruit was reduced to 50% with diurnal relative to nocturnal pollinations. The reduced number of seeds in diurnal pollinations may be explained by the poor pollen quality because hummingbirds may exhibit territorial behaviour, promoting pollen flow among related individuals.

Pollination system in *Inga*: conclusions

We have described the occurrence of generalist pollination systems for *Inga* in this study. However, we emphasize that these species seem to be specializing on nocturnal pollinators based on the nocturnal floral anthesis and nectar secretion. Because many *Inga* spp. exhibit diurnal or 24-h pollination strategies (Pennington, 1997), the specialization of flowers to nocturnal visitors may represent a derived state. In addition, asymmetric evolution involving hawkmoths, which exhibit polyphagous behaviour, and plants exhibiting a high level of specialization has been recorded recently (Martins & Johnson, 2013) and may be considered for the studied *Inga* in the Atlantic forest.

We found differences in the floral forms and nectar secretion strategies of the studied *Inga* spp. *Inga ingoides* and *I. striata* seem to have a strategy intended to obtain more visits by pollinators, whereas

I. vera restricts nectar secretion for the first visits to flowers. Despite these differences, the species exhibited similar visitation rates and timing of visits and shared many species of pollinator. It is possible that selective processes during evolution may have driven *I. vera*, *I. striata* and *I. ingoides* to develop different floral forms and nectar secretion strategies. However, in the present and recently fragmented environment, the selective pressures in terms of pollination are similar for all three species. Moreover, the *Inga* spp. may exhibit genetic constraints that restrict changes in their characteristics at the present time, and they may not have had time to evolutionarily change their floral form or nectar secretion to affect the behaviour of pollinators.

With regard to conservation strategies, species of the common tropical genus *Inga* may interact with many diurnal and nocturnal pollinator species. According to our data, three species of *Inga* interact with 33 species of pollinator, providing nectar or pollen for them. In addition, at our study site, the *Inga* spp. interact with 85% of the hawkmoth community (Cruz-Neto *et al*., 2011). We therefore recommend *Inga* as a genus with key species for the conservation of the Atlantic forest, the fourth most threatened biodiversity hotspot worldwide (Mittermeier *et al*., 2005), because of its importance to the maintenance of pollinator communities.

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