Pollination of *Peltogyne chrysopis*: an endemic tree of the Atlantic Forest

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ABSTRACT

*Peltogyne chrysopis* is an arboreal legume endemic to the Atlantic Forest and known only from the state of Bahia, Brazil. Focal observations were made of anthesis, pollen availability, stigma receptivity, nectar production, and the presence of osmophores and UV-reflective pigments for the species. Floral visitors were also observed and classified based on the timing and frequency of their visits and their foraging behavior. The breeding system was inferred from the pollen-ovule ratio and pollen tube growth after pollination treatments. *Peltogyne chrysopis* was found to be melittophilous, with anthesis occurring from 02h00min to 05h00min, and protogynous and xenogamous, with flower scent emission and pollen release before sunrise. *Xenochlora nigrofemorata* was the main pollinator, as it effectively collected and transferred pollen grains. Nectar production appears to be a secondary resource to ensure the attraction of a diversity of floral visitors and potential pollinators in the absence of effective pollinators. The results of the present study contribute to understanding the pollination mechanisms of *Peltogyne*, a genus that has been neglected with regard to its reproductive mechanism, and documents, for the first time, the role of the bee genus *Xenochlora* in plant pollination.

Keywords: anthesis, compatibility system, floral rewards, Leguminosae, melittophily, pollinator efficiency, *Xenochlora nigrofemorata*

Introduction

Studies on reproductive biology (e.g., floral biology, breeding system, pollination) have been widely recognized as important tools for identifying the factors associated with the reproductive success of plants and the subsequent maintenance of populations, especially when dealing with endemic, rare or endangered species (Schemske et al. 1994; Rodríguez-Pérez 2005; Castro et al. 2008). However, many aspects of reproductive biology that influence the distribution and maintenance of tree species in fragmented tropical Atlantic forests remain poorly investigated.

According to Girão et al. (2007), forest fragmentation may be promoting shifts in the relative abundances of the reproductive traits of trees (e.g., hermaphroditic and xenogamic species predominate in forest fragments and in forest interiors over self-compatible species). The Atlantic Forest phytogeographical domain has long been recognized as a biodiversity hotspot, although its forests have suffered massive fragmentation and serious environmental disturbances and currently occupy less than 8% of their original area of distribution (Galindo-Leal & Câmara 2005). Habitat destruction and fragmentation have been the main factors reducing the viabilities of plant populations and driving biodiversity losses and extinctions.
is a tree species of the subfamily Faboideae (Leguminosae) that grows 20 to 25 m tall and has bi-foliolate leaves and small white flowers gathered in terminal panicles (Barney 1994). It is endemic to the Atlantic Forest (Lima & Cordula 2015) where it occurs in low population densities (Thomas et al. 2009), with the few records available for it in herbaria collections being from Bahia State in northeastern Brazil (Fig. 1; data obtained from CRIA – Centro de Referência em Informação Ambiental, http://splink.cria.org.br/).

The present study was carried out from June to August 2016 (comprising four field expeditions for 10 days, totaling 105 hours of focal observation), with a population of *P. chrysopis* distributed along the anthropogenically impacted margins of the Jatimane River in the municipality of Nilo Peçanha, Bahia State. The vegetation, classified as dense ombrophilous forest or dense alluvial forest, is characterized by the presence of phanerophytes and large numbers of palm trees, lianas, and epiphytes (Veloso et al. 1991). The site has an elevation of 111 m and an Af-type climate, with intense rainfall throughout the year and no dry season. Mean monthly precipitation varies between 126 and 189 mm, while mean monthly temperature varies from 22.7 °C (July) to 26.7 °C (February) (Alvares et al. 2013). A voucher specimen (I.M. Souza 322) was deposited in the herbarium at the Universidade Estadual de Feira de Santana.

**Floral biology**

The timing, sequence, and duration of anthesis were observed constantly in 35 pre-anthesis flower buds from 00:00 to 17h00min (on one individual) for three days during the course of a two-week period. The restricted number of focal individuals reflects the difficulty of accompanying flowering in such an arboreal species due to its crown heights and the absence of blooming in by other individuals in the small focal population (*n = 3*). Stigma receptivity was evaluated by exposing the stigmas of 30 flowers to 3 different pollinator groups, diurnal and nocturnal foragers often exhibit morpho-physiological constraints. For example, enlarged ocelli and compound eyes are common characters for nocturnal or dim-light bees and have been associated with neurophysiological traits that enhance light sensitivity (Tierney et al. 2012). Few studies have focused on the role of nocturnal or dim-light pollinators (e.g., Hopkins et al. 2000; Franco & Gimenes 2011; Braun et al. 2012; Krug et al. 2015; Cordeiro et al. 2016; Oliveira et al. 2016), while temporal niche partitioning between them and diurnal pollinators has been even less explored (Smith et al. 2017).

The present work focused on *Peltogyne chrysopis* Barney, a legume species endemic to the Atlantic Forest, and for which there are currently only records from the coastal ecosystems of Bahia State in northeastern Brazil. The species appears to be restricted to rainforest forest fragments (Lima & Cordula 2015), where it occurs primarily in riparian habitats. According to the IUCN Red List Categories and Criteria, *P. chrysopis* is currently considered "vulnerable" based on its limited extent of occurrence and few collection records.

There have been no published studies concerning the breeding systems or pollinators of species of *Peltogyne*, even though they are widely distributed in the Neotropics (Silva 1976), and especially in tropical moist forest phytogeographical domains of Brazil (Amazonian = 16 species; Atlantic Forest = 7 species out of 24 for Brazil) (Lima & Cordula 2015). The genus includes several taxa of ecological, phytochemical, and/or economic importance (Silva 1976).

We describe the floral biology of *P. chrysopis* and some aspects of its breeding system, with emphasis on the temporal availability of floral resources, plant-animal interactions during the flowering cycle, and pollination efficiency. To better understand the floral mechanisms underlying pollinator interactions, the following questions were addressed: (1) What is the pollination syndrome exhibited by *P. chrysopis*? (2) What are the floral resources offered and do they vary throughout the flowering cycle? (3) What are the floral visitors and potential pollinators? (4) Is *P. chrysopis* exclusively dependent on pollen vectors given its compatibility system? The present study was designed to contribute knowledge on the reproductive mechanisms of *P. chrysopis*, a legume species endemic to the Atlantic Forest, and generate useful information about its current state of conservation.

**Materials and methods**

**Study species and site**

*Peltogyne chrysopis* is a tree species of the subfamily Detarioideae (Leguminosae) that grows 20 to 25 m tall and has bi-foliolate leaves and small white flowers gathered in terminal panicles (Barney 1994). It is endemic to the Atlantic Forest (Lima & Cordula 2015) where it occurs in low population densities (Thomas et al. 2009), with the few records available for it in herbaria collections being from Bahia State in northeastern Brazil (Fig. 1; data obtained from CRIA – Centro de Referência em Informação Ambiental, http://splink.cria.org.br/).

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hydrogen peroxide (at one-hour intervals from 03h00min to 08h00min) to detect peroxidase activity (Dafni et al. 2005; Dey et al. 2016).

Pollen availability (i.e., the number of anthers open with pollen) was assessed by observing 30 non-bagged floral buds during their entire floral cycle. Bagged flowers and pre-anthesis buds (n = 30) were fixed in FAA (50%) at different time intervals throughout anthesis (03h00min, 04h00min, 05h00min, 06h00min, 12h00min, 15h00min) to estimate pollen viability with 2% acetic carmine solution (Nadia et al. 2013; Costa & Machado 2017). Nectar production was assessed by monitoring: (i) the total volume of nectar accumulated in the flowers (n = 14) for 10 hours; and (ii) the volume of nectar secreted by a second group of flowers (n = 4) at pre-defined time intervals (06h00min, 08h00min, 10h00min, 12h00min, 14h00min, and 15h00min). These flowers were all bagged at pre-anthesis to avoid floral visitor intervention. Nectar volumes were measured using a graduated micro-syringe (0–25 µL, Hamilton, Reno, Nevada, USA). Non-parametric Wilcoxon test was used to determine if the nectar volumes secreted by the flowers varied during the day.

Breeding system inference using in vivo pollen germination techniques

We inferred the breeding system of *P. chrysopis* by estimating the pollen/ovule ratio (P/O), as proposed by Cruden (1977). This ratio was obtained by counting all pollen grains from closed anthers of five flowers (under a microscope) and the number of ovules on the same flowers (under a stereomicroscope).

Pollination experiments were performed to test the hypothesis that *P. chrysopis* is self-incompatible and dependent on biotic vectors for pollen transfer. We analyzed the flowers of an individual tree (the only one in bloom) by performing the following treatments: (1) autonomous self-pollination (n = 10), which tested spontaneous pollen transference in isolated flowers; (2) manual self-pollination (n = 12), in which pollen grains from the anthers of one
flower were manually transferred to the stigmas of the same flower; (3) manual cross-pollination, or geitonogamy (n = 12), in which pollen grains from one flower were transferred to the stigma of a second emasculated flower; and (4) natural pollination (control group) (n = 12), in which flowers were marked and left under natural conditions to evaluate natural pollen transfer. All of the pollination treatments, except natural pollination, were performed using pre-anthesis flower buds that were previously isolated in voile bags. After treatments 2 and 3, the flowers were again bagged to avoid floral visitor intervention, and approximately 10 hours after all of the treatments the flowers were fixed in 50% FAA.

Pistils were rinsed in distilled water, transferred to 70 % ethanol, and rinsed in 10N NaOH at 60 °C for 10 minutes and subsequently transferred to distilled water overnight. The pistils were then cleared in 2 % sodium hypochlorite for 1 h, rinsed in distilled water, and subsequently stained with 0.2 % aniline blue (modified from Tangmitscharoen & Owens 1997). Each pistil was sliced in half and then placed on a microscope slide to be squashed under a coverslip.

**Figure 2.** Floral biology of *Peltogyne chrysopis* Barneby. **A–C.** Anthesis development, with white arrows indicating stigmatic receptivity (**A.** 03:00 h; **B.** 04:00 h; **C.** 05:00 h); and red arrows indicating anther dehiscence (**B.** Closed anthers; **C.** Opened anthers; Scale bar: 2 mm); **D.** *Xenochlora nigrofemorata* collecting pollen on a flower (white arrow indicates the position of the stigma; red arrow indicates empty anthers); **E.** Pollen tubes growing in the style after visitation by *Xenochlora nigrofemorata*; **F.** Pollen tubes growing on the stigmatic surface after manual cross-pollination treatments (geitonogamy); **G.** Nectar droplets at the base of the ovary, indicated by a green arrow; **H.** Distribution of UV pigments in the flower, indicated by the yellow color; **I.** Distribution of osmophores in the flower, indicated by reddish dots.
The presence of pollen tubes in the stigmatic tissue and/or in the style was then evaluated using epi-fluorescence microscopy.

**Floral visitors and pollinators**

Focal observations were made constantly from 05h00min to 17h00min for five days, for a total of 60 h of observations (distributed over two weeks), with the time and frequency of visits, and foraging behavior being recorded. The insects were captured with an entomological net, mounted, dried, deposited in the Museu de Zoologia da Universidade Estadual de Feira de Santana – MZUEFS, and identified by experts on their respective groups. The activities of the floral visitors were filmed and photographed to aid in describing their behaviors.

The floral visitors were classified as potential pollinators, occasional pollinators, or thieves based on their body size, visitation time, frequency and duration of visits, and foraging behavior on the flowers. Potential pollinators combined: (i) visitation during periods of stigmatic receptivity and pollen availability; (ii) high frequency of visits; and (iii) body size large enough to carry pollen grains and to come into contact with the stigmas of the flowers during visits. Occasional pollinators were those visitors with body sizes and behaviors suitable for pollen transfer, but with low visitation frequencies and durations. Thieves were floral visitors that collected pollen and/or nectar without contacting the anthers and/or stigma (Inouye 1980).

The efficiency of potential pollinators was tested by bagging the flowers soon after their visits. The pistils were fixed in 50 % FAA 10 hours after bagging the flowers, and the presence of pollen tubes in the stigmatic tissues and/or style were evaluated by preparing pistils for epi-fluorescence microscopy analysis, as previously described.

**Results**

**Floral biology and pollination syndrome**

The flowers of *Peltogyne chrysopis* are arranged in terminal panicles, subtended by a leaf, with a mean length of 15.6 ± 3.3 cm, ca. 123 ± 37.65 flowers per inflorescence, and ca. 500 flowers per branch. The flowers are hermaphroditic, slightly zygomorphic, and with four greenish sepals (6–8 × 5–7 mm) and five white petals (6.5–9.5 × 2–4.1 mm). The androecium is composed of 10 stamens with rimose yellow anthers (2–2.5 × 1–1.5 mm); the ovary is pubescent, superous and stipitate (4 × 3 mm) with seven ovules; the style is elongated (ca. 6 mm long) in which the most internal whorl of five stamens is shorter than the pistil, while the anthers in the most external whorl are the same length as the pistil (Fig. 2). Nectariferous tissue is found at the base of the ovary surrounding the stipe (Fig. 2G).

The species has melittophilous flowers; anthesis begins near 02h00min with the slow distension of the sepal and petals; by approximately 05h00min the flowers are completely open with a receptive stigma and dehiscent anthers. The pistil becomes projected out of the floral bud in the first hours of anthesis (ca. 03h00min), with a receptive stigma that suggests protogyny (Fig. 2A). The flowers have osmophores on the margins of sepal and petals that release a slight sweet scent at the end of anthesis (Fig. 2I). UV pigments are present in the sepals, petals, and at the base of the filaments (Fig. 2H). The number of flowers opening per day varied from 5 to 43 during the study period; they lasted ca. 24 h, with the calyx and corolla persisting as they withered.

Anther dehiscence begins at approximately 04h20min and lasts 40–60 minutes (Figs. 2C, 3) with pollen viability varying from 99.75 ± 0.5 % (03h00min) to 97.75 ± 1.89 % (15h00min). Nectar is secreted at the base of ovary (as droplets) beginning at the end of anthesis and is produced throughout the day in volumes varying from 1.2 to 3.3 µL (Fig. 3); the secreted volumes did not vary significantly among different times of the day (Tab. 1). The total volume of nectar accumulated for a single day was ca. 1.6 ± 0.41 µL per flower. Nectar concentration was not precisely assessed due to methodological problems.

**Table 1.** Comparison of nectar volume (µL) secreted by flowers of *Peltogyne chrysopis* Barneby at different times during the flowering cycle. Wilcoxon test results are indicated below the diagonal while P-values are above.

<table>
<thead>
<tr>
<th>Time</th>
<th>06h:00min</th>
<th>08h:00min</th>
<th>09h:00min</th>
<th>10h:00min</th>
<th>12h:00min</th>
<th>15h:00min</th>
</tr>
</thead>
<tbody>
<tr>
<td>06h:00min</td>
<td>0.538</td>
<td>0.068</td>
<td>0.655</td>
<td>0.144</td>
<td>0.068</td>
<td></td>
</tr>
<tr>
<td>08h:00min</td>
<td>0.55</td>
<td>0.144</td>
<td>0.854</td>
<td>0.068</td>
<td>0.144</td>
<td></td>
</tr>
<tr>
<td>09h:00min</td>
<td>1.83</td>
<td>1.46</td>
<td>0.068</td>
<td>0.068</td>
<td>0.715</td>
<td></td>
</tr>
<tr>
<td>10h:00min</td>
<td>0.45</td>
<td>0.18</td>
<td>1.83</td>
<td>0.461</td>
<td>0.068</td>
<td></td>
</tr>
<tr>
<td>12h:00min</td>
<td>1.46</td>
<td>1.83</td>
<td>1.83</td>
<td>0.74</td>
<td>0.066</td>
<td></td>
</tr>
<tr>
<td>15h:00min</td>
<td>1.83</td>
<td>1.46</td>
<td>0.36</td>
<td>1.83</td>
<td>1.84</td>
<td></td>
</tr>
</tbody>
</table>

**Breeding system**

The pollen/ovule ratio for *Peltogyne chrysopis* was 5191.43 ± 1389.91, indicating it is a xenogamous species. The results of the breeding system experiments indicated that the species is self-compatible, as high percentages of stigmas were found with germinating pollen grains after geitonogamy and manual self-pollination treatments (Tab. 2). The species did not exhibit evidences of sporophytic or gametophytic self-incompatibility mechanisms (Fig. 2E-F) and depends on biotic vectors for pollen transfer.
Table 2. Results of the treatments used to investigate the reproductive system of *Peltogyne chrysopis* in rainforest vegetation along the margins of the Jetimane River, Nilo Peçanha, Bahia State, Brazil.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Flowers/Pollen tube germination (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autonomous self-pollination</td>
<td>10/0 (0)</td>
</tr>
<tr>
<td>Manual self-pollination</td>
<td>12/6 (50)</td>
</tr>
<tr>
<td>Manual cross-pollination (geitonogamy)</td>
<td>12/8 (66.6)</td>
</tr>
<tr>
<td>Natural conditions (control)</td>
<td>12/12 (100)</td>
</tr>
<tr>
<td>Efficiency of <em>Xenochlora nigrofemorata</em></td>
<td>10/10 (100)</td>
</tr>
</tbody>
</table>

Flower visitors and pollinators

We recorded visits by one beetle (Coleoptera), two dipterans (Diptera), and four different bee species (Hymenoptera) to *P. chrysopis* flowers. The beetle had a small body and simply walked on the petals and sepals while feeding on nectar (Fig. 2G); the dipterans landed on the anthers and fed on residual pollen grains (Fig. 3I). These floral visitors did not touch the stigma nor damaged the flowers and were therefore classified as thieves.

Among the bee species, *Xenochlora nigrofemorata* (Halictidae) was considered a potential pollinator. These bees visited all of the flowers as soon as anthesis ended (between 05h20min and 06h20min) with higher frequencies (one to four visits per flower); they visited the flowers searching for pollen and almost depleting the anthers. The relatively large body size of this species (in relation to the flower) and its foraging behavior (embracing a set of anthers, buzzing them, and then collecting pollen) enabled pollen transfer since the bees touched the stigma during their visits (Figs. 2D, 3H). The pollination efficiency of *X. nigrofemorata* was confirmed by epi-fluorescence microscopy analyses, with the presence of germinating pollen grains on the stigmatic surfaces and in the styles of all the pistils they visited (Fig. 2E, Tab. 2).

The bee *Centris* sp. (Apidae) was classified as an occasional pollinator since it was observed at the beginning of the morning (ca. 05h20min), but was not a frequent visitor to individual flowers (one visit per flower) and visited few flowers (n = 2). Its pollination efficiency could not be assessed due to the few recorded visits. *Trigonisca* sp. was classified as pollen thieving. This small bee visited the flowers at the beginning of the morning (ca. 06h20min), landing on each anther and searching for residual pollen grains, but they did not carry great amounts of pollen nor touch the stigma. *Trigona hyalinata* visited the flowers during the entire day (first records ca. 08h00min), landing on the petals or embracing the pistil and positioning its head toward the base of the ovary to feed on nectar (Fig. 3J-M). During visits by this species, the anthers were almost completely empty, and thus this species was classified as nectar thief.

Discussion

*Peltogyne* is a poorly-studied legume genus with respect to its mating system, with information available for basically only its fruiting phenology (Rocha *et al*. 2006). There is also information in the literature concerning its taxonomy (e.g., Silva *et al*. 1976), phytochemistry (e.g., Almeida *et al*. 1974; Gutiérrez-Macías *et al*. 2016), herbivory ecology (e.g., Nascimento & Proctor 1996; 1997), and population ecology (e.g., Nascimento & Proctor 2001). Thus, the present study is the first to investigate some of the aspects of the breeding
system of a species of *Peltogyne*. Our results indicate that *P. chrysopis*, an endemic species of the Atlantic Forest, possesses melittophilous and protogynic flowers that are mainly xenogamous and self-compatible. In addition, the present study is the first to document in detail pollination mediated by the poorly known bees of the genus *Xenochlora*.

Individuals of *Peltogyne chrysopis* initiate anthesis during dim-light, with the flowers becoming completely opened at the beginning of the morning (ca. 05h00min), yet during dim-light. The flowers release a slight sweet scent that attracts *Xenochlora nigrofemorata*, the first floral visitors and a poorly documented bee species belonging to a small, poorly known and rarely collected bee genus (Engel et al. 1997; Engel 2000; Santos & Melo 2013). Despite anthesis occurring during in the dark, the set of floral traits of *P. chrysopis* (e.g., small and slightly zygomorphic flowers, presence of osmophores and UV-reflective pigments, and pollen as the main floral resource) fit traditional melittophily (Faegri & Pijl 1979).

Pollination is a key process for sexual reproduction of most flowering plants (Aguilar et al. 2006), and bee-pollination has been shown to be prevalent in many different ecosystems. Although mechanisms related to pollination and the reproductive success of tree species have been poorly investigated (especially in the Brazilian Atlantic Forest), empirical evidence indicates that bee-pollination is prevalent, even in the canopies of trees growing in humid habitats (Dulmen 2001; Fidalgo & Kleinert 2009; Vieira et al. 2010; Braun et al. 2012; Franceschinelli et al. 2015), as confirmed by the present study with legitimate visits of *X. nigrofemorata*.

*Xenochlora* is a Neotropical bee genus of the tribe Augochlorini with only four species recorded from tropical moist forests (Michener 2007). The genus is closely related to *Megalopta* (Tierney et al. 2012; Gonçalves 2016), which is widely recognized for the nocturnal or crepuscular activity (Wolda & Roubik 1986; Hopkins et al. 2000; Franco & Gimenes 2011; Carvalho et al. 2012), but *Xenochlora* differs basically by having smaller ocelli and stiff, black setae on the hindlegs (Engel 2000).

*Xenochlora* has been recognized as a diurnal genus (Engel et al. 1997; Tierney et al. 2012), in contrast with its closest relative *Megalopta*. However, this recognition is based only on D. Roubik’s collection (1991), who mentions that the holotype of the genus was collected using “flowers and baits” (Engel et al. 1997). Morphological attributes (e.g., smaller ocelli) have also been used to support the hypothesis of diurnal behavior for the genus (Michener 2007; Wcislo & Tierney 2009). Nevertheless, not all dim-light foraging bees have enlarged ocelli and compound eyes, especially facultative dim-light foragers (Wcislo & Tierney 2009). Despite the lack of confirmed ethological data in the literature, it is not possible to rule out facultative dim-light activity for species of *Xenochlora* (Tierney et al. 2012). In the present study we recorded visits of *X. nigrofemorata* only at the beginning of the morning (i.e., 05h20min), during the transition from dim-light to sunrise. In addition, visits recorded some time later were associated with cloudy days.

*Xenochlora nigrofemorata* is considered the main pollinator of *P. chrysopis* due to: (i) bees visiting flowers when the stigma was receptive and pollen grains viable; (ii) bee body size and foraging behavior favoring contact with the stigmatic surface during visits; and, (iii) bee pollination efficiency (i.e., pollen transference from the anthers to the stigma), as confirmed by the presence of pollen tubes on the stigmatic surface and in the styles of visited flowers. All the information available in the literature thus far for the genus consists of taxonomic treatments (Engel et al. 1997; Engel 2000; Santos & Melo 2013) and phylogenetic analyses of the tribe Augochloroni (Tierney et al. 2012; Gonçalves 2016), and reports on nesting biology and social behavior for two species (Tierney et al. 2008). Thus, the present study is the first to report on the association between flowers and a species of the genus *Xenochlora*, with detailed information on pollination mediated by *X. nigrofemorata* — pollen transference via floral sonication behavior (i.e., buzzing the anthers) as indicated by epifluorescence microscopy, which contradicts that assumed by Cardinal et al. (2018) for the genus.

In addition to *X. nigrofemorata*, another bee species also buzzed the anthers of *P. chrysopis* to collect pollen from their flowers: individuals of *Centris* sp. made fast and punctual visits soon after anthesis ended. Their body size, foraging behavior, and timing and numbers of visits (few visits when the stigma was receptive and pollen was viable) classifies them as occasional pollinators. The behavior of buzzing anthers to collect pollen has been widely associated with pollination (i.e., buzz-pollination with explosive pollen release) of several flowering plants (e.g., Harder & Barclay 1994; Dupont & Olesen 2006; Fidalgo & Kleinert 2009; Franco et al. 2011; Souza et al. 2012).

Buzz-pollination may have evolved as a feature that favored successful fertilization and the control of pollen removal by pollen thieves or less efficient pollinators (Luca & Vallejo-Marín 2013). Empirical evidence indicates that the bee behavior of embracing anthers and then contracting flight muscles at high frequencies (King 1993) is not performed only on flowers with poricidal anthers, but is more closely associated with the actual abilities of the bees distributed among seven hymenopteran families to perform buzzing (Proença 1992; Freitas & Sazima 2003; Fidalgo & Kleinert 2009; Luca & Vallejo-Marín 2013; Cardinal et al. 2018), as seen here with *X. nigrofemorata* and *Centris* sp. during their visits to *P. chrysopis* flowers.

The other two bee species that visited flowers of *P. chrysopis* did not collect pollen by buzzing the anthers. The small species *Trigonisca* sp. visited flowers soon after anthesis ended, but did not touch the stigmatic surface during their visits. *Trigona hyalinata* visited the flowers throughout the day while feeding on nectar, but as they
appeared after visits by efficient pollinators (i.e., *X. nigrofemorata*) and the anthers were virtually empty, they did not carry pollen grains on their bodies. Both of these bee species (*Trigonisca* sp. and *T. hyalinata*) were therefore classified as nectar thieves. Nevertheless, it is important to highlight the potential for pollen transfer by *T. hyalinata* (in the absence of main pollinators) in light of appropriate aspects of its morphology and behavior. Besides, a species of *Trigona* do mediate effective pollination in a population of a congeneric legume species (*P. pauciflora*) in northern Bahia State (IM Souza unpubl. res.).

Our observations suggest temporal segregation of floral visitors according to floral resource availability, with anther dehiscence before sunrise attracting the pollinators *X. nigrofemorata* and *Centris* sp., followed by the secretion of highly viscous nectar droplets soon after the end of anthesis, and persisting throughout the day, attracting *T. hyalinata*. This temporal segregation is an important feature commonly associated with reduced overlap among the temporal niches of bee species (Willmer & Corbet 1981; Biesmeijer & Richter 1999; Barônio & Torezan-Silingardi 2017). This partitioning of floral visitation reduces competition for floral resources and might optimize pollination and, consequently, reproductive success of *P. chrysopis*, since the species is exclusively dependent on biotic vectors for pollen transference.

Assuming that xenogamy (which improves gene flow and genetic variation among populations) is the main reproductive strategy of *P. chrysopis*, self-compatibility (e.g., geitonogamy) may still provide advantages of reproductive assurance (O’Brien 1994; Buza et al. 2000; Navarro & Guitián 2002). Self-compatibility can also be associated with reproductive flexibility for colonizing harsh habitats (Navarro & Guitián 2002) or disturbed areas – such as the Atlantic Forest fragments where *P. chrysopis* is found.

The flowering of *P. chrysopis* appears to be asynchronous (only one individual bloomed during the study period) and occurs in the coldest months of the year, since the study region experiences relatively low temperatures in June, July, and August (23.3, 22.7, and 23.3 °C respectively) (Alvares et al. 2013). The supposed asynchronous flowering of *P. chrysopis*, combined with its self-compatibility, may assure its reproductive success.

The information gathered here concerning the floral biology and pollination strategies of this endemic Atlantic Forest legume species (*P. chrysopis*) contributes to the knowledge of the reproductive biology of the genus *Peltogyne* as a whole and the role of *X. nigrofemorata*, which belongs to a poorly documented genus, in the pollination of plant species. Future challenges will involve broadening research efforts to cover other related species and synthesizing that information in a phylogenetic context to help elucidate evolution within the subfamily Detarioideae (LPWG 2017).

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