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Pollen Analysis of the Post-Emergence Residue of *Euglossa* Bees (Apidae: Euglossini) Nesting in an Urban Fragment

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Abstract

Euglossini bees are considered pollinators of a wide variety of plants in the Neotropical region, but little is known about their floral preferences. In this study, we identified the botanical species used as pollen and nectar sources by three *Euglossa* species, *Euglossa cordata* (Linnaeus), *E. townsendi* Cockerell, and *E. securigera* Dressler using pollen residue found in brood cells from trap nests installed in an urban fragment in São Luís, Maranhão, Brazil. In 14 analyzed *E. cordata* nests, 23 pollen types were observed, in seven *E. townsendi* nests, ten pollen types were observed, and in one nest of *E. securigera*, six morphotypes were identified. *Solanum* (Solanaceae), *Zanthoxylum* (Rutaceae), *Mimosa pudica* L. (Fabaceae), and *Chamaecrista* (Fabaceae) pollen types were common to all three bee species. A principal component analysis showed 83.04% variability on the first two axes, demonstrating substantial similarity among the samples. *Solanum*, *Mimosa pudica*, and *Zanthoxylum* were the principal components in the ranking. Larger diversity values (mean = 0.80) in some samples indicated that the species gathered resources in a heterogeneous manner; this is consistent with the findings of other studies of *Euglossa*. In general, the bees exhibited overlapping niches with regard to the most abundant pollen grains in the nests, but the females showed individual plasticity when gathering floral resources.

Introduction

The Euglossini tribe (Hymenoptera: Apidae) is distributed only in the Neotropical region and it is divided into five genera: *Euglossa* Latreille, *Eulaema* Lepeletier, and *Eufriesea* Cockerell consist of pollen-gathering species, whereas *Aglae* Lepeletier and Serville as well as *Exaerete* Hoffmannsegg are cleptoparasites (Michener, 2007). The *Euglossa* genus has the largest number of species, with 129 species already described (Nemésio & Rasmussen, 2011), which are distributed from Mexico to northern Argentina and in the Caribbean Islands (Cameron, 2004; Michener, 2007).

In the Corbiculate Apidae, Euglossini bees are the only species that are not, strictly speaking, social. Certain *Euglossa* species are solitary or can form community nests with various females born in the nest (Garófalo et al., 1998; Michener, 2007; Augusto & Garófalo, 2011). Initially, when a *Euglossa*

female finds an appropriate place to build her nest, she forms the first cell made with resin, where she stores the larval food. Next, she lays her egg and closes the cell, then begins to build new brood cells (Garófalo et al., 1998; Ramírez et al., 2002).

Nests of *Euglossa* can be aerial or constructed in different types of cavities found in natural substrates; additionally, they can also be hosted by artificial man-made structures (Augusto & Garófalo, 2004; Cameron, 2004). The use of trap nests has enabled researchers to become acquainted with the different species of bees nesting in such cavities in different localities, as well as providing information on species biology (Garófalo et al., 1993). This includes the food resources utilized by adult bees and larvae (Arriaga & Hernández, 1998; Silva et al., 2016).

Euglossini bees are pollinators of a wide variety of plants and can fly long distances in tropical forests (Janzen, 1971). To date, the resources that these bees use for food are not well known; most previous studies are based on



direct observations of flowers (Ramírez, 2002; Rocha-Filho et al., 2012). Some studies have identified the floral resources through pollen analysis of larval food; e.g. *Euglossa atrovirena* Dressler (Arriaga & Hernández, 1998), *E. annectans* Dressler (Cortopassi-Laurino et al., 2009), *E. viridissima* Friese, *E. dilemma* Bembé & Eltz (Villanueva-Gutierrez et al., 2013), *E. nigropilosa* Moure (Otero et al., 2014), and *E. townsendi* Cockerell (Silva et al., 2016).

In spite of this, there is a need for more research into the different biomes in which these bees are found. *Euglossa cordata* (Linnaeus), *E. securigera* Dressler, 1982, and *E. townsendi* are widely distributed in the Neotropics. In Brazil, they occur in the humid Amazon forest and in vast areas in the northeast and the central-south (Rêbello et al., 2003). In the state of Maranhão, these species are widely distributed throughout the various phytogeographic regions (Rebêlo & Silva, 1999). Maranhão is undergoing rapid deforestation, and is located in a region of transition between the Amazon forest, the Cerrado of the Central Plains, and the semi-arid Caatinga in the northeast (Rêbello & Silva, 1999). Therefore, it is necessary to conduct further research to understand the biodiversity and ecological relationships of these flora and fauna.

To this end, the aim of the present study was to determine the floral resources utilized by *E. cordata*, *E. townsendi*, and *E. securigera* via the analysis of post-emergence pollen residue collected from trap nests in an urban fragment.

Material and Methods

Study area

The study was conducted at the 120.95-ha Integral Protection Conservation Unit in the Sítio do Rangedor State Park, located in the municipality of São Luís, Maranhão, Brazil (decree no. 23303/2007). The park serves as a climate control and water capture unit for the municipality (SEMA, 2017).

The vegetation in Rangedor State Park is a remnant fragment of the Amazon Forest. Estimates indicate that there are more than 120 botanical species in the region, distributed in 52 plant families, with *Syagrus oleracea* (Mart.) Becc. (Arecaceae), *Attalea speciosa* Mart ex. Spreng. (Arecaceae), *Pithecellobium* sp. (Fabaceae), and *Himatanthus drasticus* (Mart.) Plumel (Apocynaceae) constituting approximately 45% of the tree population (SEMA, 2017).

The city of São Luís is located on the northern coast of Maranhão. It has a humid tropical climate, with average temperatures ranging from 26 to 28 °C. Annual rainfall reaches 2000 mm³, with the period of most intense rainfall from January to June, and the dry season from July to December (Silva et al., 2008).

Nest sampling

Trap nests were installed in the study area for one year (April 2010 to April 2011). The nest diameters were 8, 10, 12, 14, and 16 mm. They consisted of two pieces of wood joined together with adhesive tape (3 cm high x 3 cm wide x 16 cm

long), with a 10 cm deep longitudinal slit. In all, 500 nests were used, with 100 of each diameter. They were organized into 100 clusters protected by PET bottles, with one nest of each diameter in the cluster.

The blocks were placed in two sampling units, selected according to the physiognomic characteristics of the vegetation. Area I (2°9'54"S, 44°15'51"W) was selected for its open vegetation, mainly composed by shrubs and herbaceous plants, and greater exposure to the sun. Area II (2°29'48"S, 44°16'07"W) was chosen for its naturally regenerating vegetation and shaded environment; this area corresponds to dry land forest, which represents 50% of the plant cover of the park (SEMA, 2017). In each area, we installed 50 blocks on the branches of trees, hanging at a height of 1.5 m from the ground.

Every two weeks, the trap nests were inspected. Those in which bees had established were taken to the laboratory until the individuals emerged. They were then sacrificed in chambers with ethyl acetate, pinned, and labeled. The *Euglossa* genus, identified by a specialist, was common in the trap nests in both Area I and Area II. The species were found to belong to the subgenus *Euglossa* (*Euglossa*) and to be highly similar morphologically. Identification was made possible through the males that were born in the nests, since they were the only ones with identification keys.

Pollen samples

Each nest occupied by bees was considered a sample (*E. cordata*: Ec.01–Ec.14, *E. townsendi*: Et. 01–Et.07, and *E. securigera*: Es.01). After all the individuals had emerged, the residual pollen grains and feces found in the brood cells were obtained so that the floral resources utilized by the bees. The pollen material was prepared using the acetolysis method described by Erdtman (1960).

Under the optical microscope, pollen types were identified and 500 pollen grains were counted per sample. In this manner, we were able to determine the frequency of pollen type occurrence. When possible, we identified pollen grains at the species level, and we also used the pollen type criterion for genus or family, in which pollen grains that present a set of very similar morphological characteristics comprise a group of plants that share the same characteristics (Barth, 1989). Pollen slides from the Palynothecca of the Federal University of Maranhão and pollen catalogues were used to identify the botanical affinity of the pollen types (e.g. Roubik & Moreno, 1991; Silva et al., 2014; Lorente et al., 2017).

Data analysis

To verify the similarity of pollen types among samples, multivariate analysis was conducted using principal components analysis (PCA). First, a matrix with the absolute values for each pollen type present in the samples was transformed into natural logarithms. Next, ranking was conducted using a covariance matrix and employing the PAST program. Variability among samples was expressed using the first two axes of the PCA.

Table 2. Frequency of occurrence of pollen types in *Euglossa townsendi* and *E. securigera* nests.

Samples	<i>Euglossa townsendi</i>							Mean	<i>E. securigera</i>
	Et.01	Et.02	Et.03	Et.04	Et.05	Et.06	Et.07		
Date	Jun/10	Jul/10	Aug/10	Mar/11	Mar/11	Apr/11	Apr/11		Feb/11
Diam. (mm) / Site	14 / II	14 / II	16 / II	14 / II	10 / II	12 / II	12 / II		16 / I
Number of cells	4	8	6	8	9	9	9		3
Pollen types	Frequency of pollen types (%)								
Areaceae									
<i>Copernicia prunifera</i>				7.4				1.06	
Fabaceae									
<i>Chamaecrista</i>					1.0			0.14	0.60
<i>Mimosa pudica</i>	56.4	30.8	1.8	89.0	2.2	40.2		31.49	4.40
<i>Schrankia leptocarpa</i>	0.8								
Malpighiaceae									
<i>Byrsonima</i>				0.4				0.06	11.40
Malvaceae									
<i>Hibiscus</i>							0.2	0.03	
Melastomataceae									
<i>Micinia</i>									3.80
Rutaceae									
<i>Zanthoxylum</i>	38.6	4.0	13.4	3.2	13.2	5.6	5.8	11.97	23.80
Sapindaceae									
<i>Cupania diphylla</i>							0.4	0.06	
Solanaceae									
<i>Solanum</i>	4.2	65.2	84.8		83.4	54.2	93.6	55.06	56.00
Not identified									
NI3					0.2			0.03	

Twenty-three pollen types occurred in the nests of *E. cordata*, belonging to 10 families and 20 genera; two were not identified (Table 1). For *E. townsendi*, 10 pollen types were identified, distributed among seven families and nine genera; one was not identified. *E. securigera*, with just one nest evaluated, exhibited six pollen types belonging to five families and six genera (Table 2).

The most frequent types of pollen grains for *E. cordata* were *Solanum* (50.63%) (Fig 1A), *Mimosa pudica* L. (20.91%) (Fig 1B), *Zanthoxylum* (15.69%) (Fig 1C), *Eugenia* (3.59%) (Fig 1D), *Chamaecrista* (3.41%), *Delonix* (1.89%), and *Dalechampia* (1.29%) (Fig 1E); the remaining 16 pollen types

constituted less than 1% of grains counted (Table 1). For *E. townsendi*, *Solanum* (55.06%), *Mimosa pudica* (31.49%), *Zanthoxylum* (11.97%), and *Copernicia prunifera* (Mill.) H.E. Moore (1.06%) were the most frequent pollen types; the remaining six pollen types represented less than 1%. For *E. securigera*, the most important pollen types were *Solanum* (56%), *Zanthoxylum* (23.80%), *Byrsonima* (11.40%), *Mimosa pudica* (4.40%), and *Micinia* (3.80%) (Table 2).

The pollen grains in common for all three bee species were *Chamaecrista*, *M. pudica*, *Zanthoxylum*, and *Solanum*. Sixteen pollen types were exclusive to *E. cordata*, three to *E. townsendi*, and only one to *E. securigera* (Table 3).

Table 3. Exclusive pollen types (*) shared (+) by *Euglossa cordata*, *E. townsendi* and *E. securigera* nesting in trap nests in the Sítio do Rangedor State Park, Maranhão, Brazil.

	<i>E. cordata</i>	<i>E. townsendi</i>	<i>E. securigera</i>
<i>E. cordata</i>	* <i>Mauritia</i> . <i>Wedelia pilosa</i> . <i>Bignonia</i> . <i>Dalechampia</i> . <i>Cassia</i> . <i>Centrosema brasiliense</i> . <i>Copaifera</i> . <i>Delonix</i> . <i>Dioclea</i> . <i>Mimosa</i> tipo 2. <i>Eugenia</i> . <i>Myrcia fallax</i> . <i>Manettia</i> . <i>Lantana</i> . Not identified (NI 1. NI 2)	+ <i>Copernicia prunifera</i> . <i>Chamaecrista</i> . <i>Mimosa pudica</i> . <i>Schankia leptocarpa</i> . <i>Zanthoxylum</i> . <i>Solanum</i>	+ <i>Chamaecrista</i> . <i>Mimosa pudica</i> . <i>Zanthoxylum</i> . <i>Solanum</i>
<i>E. townsendi</i>		* <i>Hibiscus</i> . <i>Cupania diphylla</i> . Not identified (NI 3)	+ <i>Chamaecrista</i> . <i>Mimosa pudica</i> . <i>Byrsonima</i> . <i>Zanthoxylum</i> . <i>Solanum</i>
<i>E. securigera</i>			* <i>Micinia</i>



Fig 1. Photomicrographs of certain pollen types observed in the nests of *Euglossa* females. (A) *Solanum*, (B) *Mimosa pudica*, (C) *Zanthoxylum*, (D) *Eugenia*, (E) *Dalechampia* and (F) *Centrosema brasilianum* (scale bars = 10 μ m).

The pollen type samples were ordered using PCA according to the similarity of their occurrence. Figure 2 shows that the variability covered 83.04% on the first two PCA axes, which demonstrates high similarity among the samples. *Solanum*, *M. pudica*, and *Zanthoxylum* were the principal components used for ranking the samples. The aggregate of

types, on the left side of the PCA, indicates species with few grains or those encountered in just a few samples.

The Ec.10 sample had the highest richness of pollen types (10). In contrast, samples Ec.01, Et.02, Ec.06, Et.03, and Et.06 had the lowest number of pollen types (3 each). On average, there were 4.95 types per nest [standard deviation (SD): ± 1.78].

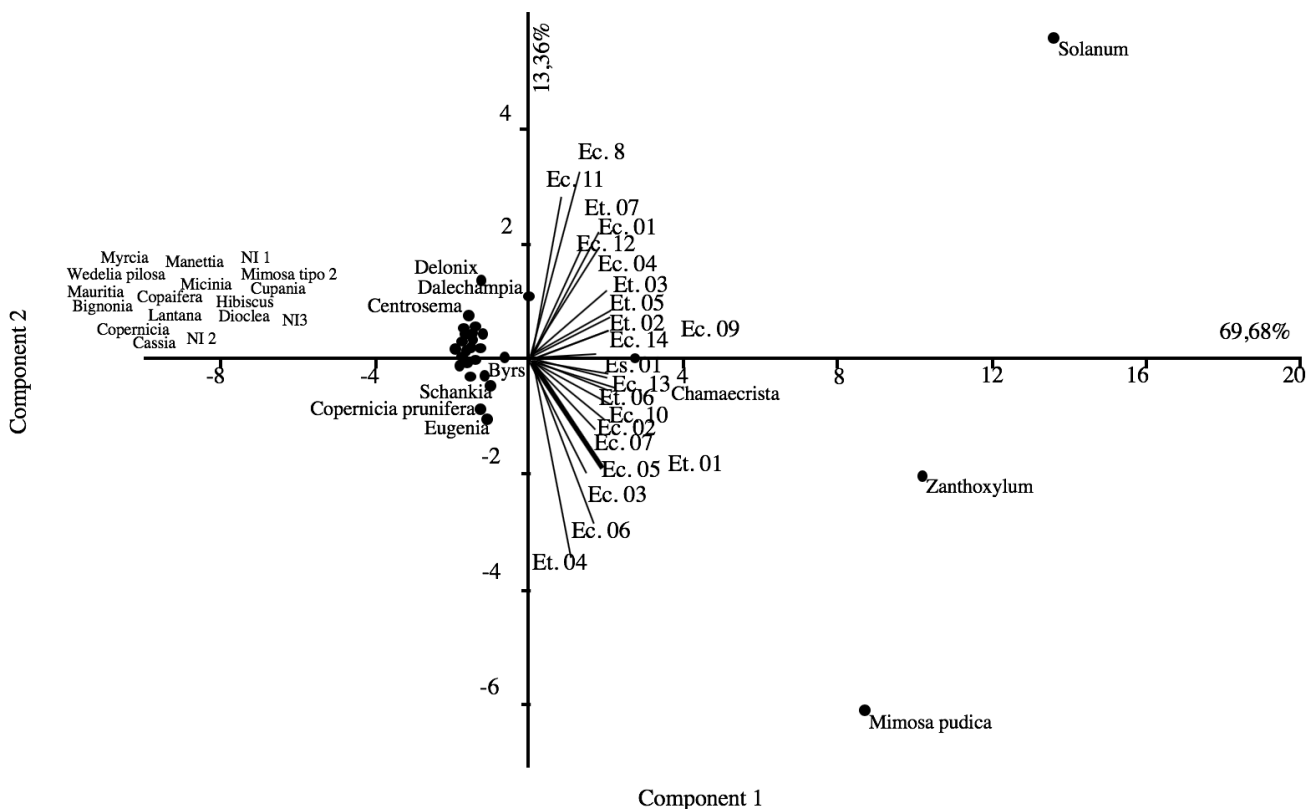


Fig 2. Ranking of principal components analysis (PCA) for nests of *Euglossa cordata* (Ec.01 to Ec.14), *Euglossa townsendi* (Et.01 to Et.07) and *Euglossa securigera* (Es.01). (● = pollen type).

As for the Shannon diversity index, sample Ec.10 presented the highest value ($H' = 1.371$) and Ec.08 had the lowest ($H' = 0.135$) (0.80 ± 0.34) (Fig 3). The mean equitability index was $0.52 (\pm 0.18)$; sample Et.06 had the highest value ($J' = 0.782$) and Ec.08 had the lowest value ($J' = 0.084$) (Fig 3).

Discussion

The predominance of nests obtained from *E. cordata* was due to the fact that the species is one of the most frequent species in the ecosystems of the northern region of the State of Maranhão (Rêbello et al., 2003) and in several other regions, while *E. townsendi* and *E. securigera* are less frequent in surveys (e.g. Neves & Viana, 1997; Mendes et al., 2008; Ramalho et al., 2009). In spite of the difference in the number of nests obtained, the floral resources identified in this analysis contribute to the knowledge of the plants used by the bee species studied, particularly for *E. securigera*, as the food resource usage of which has not been previously assessed.

The *Euglossa* species are considered generalists when foraging for food resources. Nevertheless, they show preferences for certain plant families. Overall, regardless of the area of the park in which they nested, *E. cordata*, *E. townsendi*, and *E. securigera* exhibited important source sharing. This result was similar to those of a previous study on *E. viridissima* and *E. dilemma*, which demonstrated overlapping niches (Villanueva-Gutierrez et al., 2013).

In this study, 23, 10, and 6 pollen types were observed in the nests of *E. cordata*, *E. townsendi*, and *E. securigera*,

respectively, which was similar to that observed by Boff and Alves-dos-Santos (2018) in nests of *E. cordata* (20 pollen types), but less than that observed in nests of *E. atrovoneta* (74) (Arriaga & Hernández, 1998), *E. annectans* (74) (Cortopassi-Laurino et al., 2009), *E. viridissima*, and *E. dilemma* (45) (Villanueva-Gutierrez et al., 2013). However, a smaller number of nests was assessed in the Sítio do Rangedor State Park, especially for *E. securigera*, for which only a single nest was obtained for the analysis. Furthermore, the pollen analyzed in our study was the residue present in the brood cells. Even so, the floral preferences were quite similar to those found in previous studies.

Even for *E. townsendi*, which had 10 pollen types identified in seven nests, we observed the lowest degree of exploited species richness compared to a previous study of the same bee species (Silva et al., 2016), in which 21 pollen types were found in six nests. Because the Sítio do Rangedor State Park consists of a forest fragment with reduced plant diversity (SEMA, 2017), the number of botanical species exploited by *E. townsendi* in the locality may have been limited. However, both studies showed that this bee species has botanical preferences and, in the state of São Paulo, the greatest preference was for *Miconia chamissois* Naudin (Melastomataceae), followed to a lesser extent by *Solanum palinacanthum* Dunal (Solanaceae) (Silva et al., 2016).

Plant species of Solanaceae (*Solanum*), Fabaceae (*Chamaecrista* and *Cassia*), and Melastomaceae (*Miconia*) have flowers with poricidal anthers; in order to obtain pollen from these flowers, bees must powerfully vibrate their wings

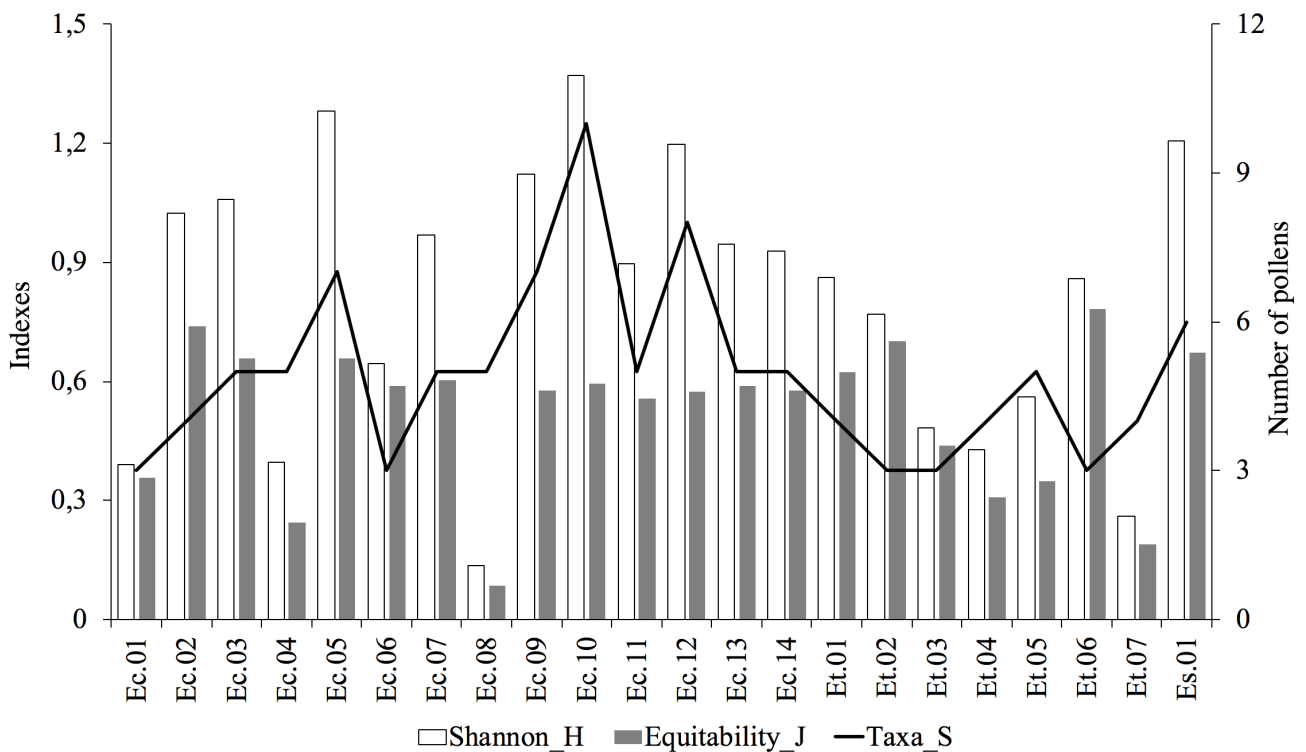


Fig 3. Richness (S), Diversity Index (H') and Equitability (J') of pollen grains found in *Euglossa cordata* (Ec.01 – Ec.14) and *Euglossa townsendi* (Et.01 to Et.07) nests.

in a behavior known as “buzz pollination” (Buchmann, 1985; Nunes-Silva et al., 2010). Furthermore, other polleniferous species without poricidal anthers are important for *Euglossa* (Arriaga & Hernandez, 1998), such as *Eugenia* (Myrtaceae) and *M. pudica* (Fabaceae), which exhibited high frequencies in some samples. As for the latter, *M. pudica* is a ruderal species with flowering throughout the year but greater abundance in months with higher rainfall, and is considered crucial for the survival of several bee species (Marques et al., 2011).

If Euglossini females do have preferences regarding pollen gathering, their search for nectar seems to be less specific (López-Urbe et al., 2008). Plants with nectar are frequently cited as being visited by Euglossini females and males (Janzen, 1971; Ramírez et al., 2002; Rocha-Filho et al., 2012), and some foraging by females was recorded in this study: e.g. *C. prunifera* (Arecaceae), *Wedelia pilosa* Baker (Asteraceae), *Bignonia* (Bignoniaceae), *Centrosema brasiliense* (L.) Benth., *Copaifera*, *Delonix* and *Dioclea* (Fabaceae), *Hibiscus* (Malvaceae), *Manettia* (Rubiaceae), *Zanthoxylum* (Rutaceae), *Cupania diphylla* Vahl (Sapindaceae), and *Lantana* (Verbenaceae).

The *Dalechampia* (Euphorbiaceae) pollen type, which occurred in six samples of *E. cordata*, may be singled out as the main source of floral resin for nest building (Armbruster, 1993; Opedal et al., 2016). Plants of this botanical genus are frequently pollinated by Euglossini bees, which has been well documented in the literature (Armbruster & Webster, 1981; Sazima et al., 1985; Opedal et al., 2016).

The pollen of *Byrsonima*, which was shared between *E. townsendi* and *E. securigera* (with a relative frequency of 11.40% for the latter), is not commonly gathered by these bees. The Malpighiaceae family is frequently visited by ‘oil-gathering bees’ because of the use of floral oil to feed the larvae (Rêgo & Albuquerque, 2006); however, they are also visited for pollen gathering, as recorded among *Eulaema* (Ramírez et al., 2002). Thus, nothing prevents *Euglossa* females from also visiting these plants in search of this resource. The only nest of *E. securigera* occurred in Area I, and based on our observations, the locality contains many *Byrsonima* shrubs. On the other hand, *E. townsendi* also collected pollen from this plant, which is significant because it only nested in Area II, where plants of this genus were unusual; this indicates that the bees were not limited to the immediate vicinity of their nesting area for foraging.

According to Janzen (1971), plants foraged by Euglossini bees provide high quality rewards as they flower over long periods of time. Thus, these bees habitually and repeatedly visit the same species when it is flowering. An example of a species foraged over a long period of time is *Zanthoxylum* (Rutaceae), which was very important for bee species in this study, possibly as a source of both pollen and nectar. Additionally, the pollen of Rutaceae (*Citrus*) was observed over nine months in *E. atrovirens* samples, although with low pollen frequency (Arriaga & Hernández, 1998). On the other hand, shorter flowering species can also be visited by these bees, as shown by the rare occurrence of these pollen types in the samples.

Notably, the pollen types of *Solanum*, *M. pudica*, and *Zanthoxylum* were observed in most months during which the nests were established. They were of greater or lesser importance depending on the sample employed as the major pollen in the PCA ranking. Even samples of nests obtained in the same month, i.e. both *E. cordata* and *E. townsendi*, varied in terms of importance of the principal pollen type. According to Arriaga and Hernández (1998), an interesting behavior of these bees is that they can forage the same plants at the moment of nidification, but the way the resource is exploited can change; furthermore, individual preferences may differ among females, indicating a plasticity at the individual level. This is consistent with the findings of Cortopassi-Laurino et al. (2009), where the contents of brood cells were not homogeneous, and the polylectic behavior of *Euglossa* was observed over a matter of days.

The higher diversity values in some samples show that the bees gathered resources in a heterogeneous manner, with up to ten pollen types in nest Ec.10. The mean diversity value of 0.80 was similar to that found for *E. atrovirens* (Arriaga & Hernández, 1998), which was 0.83. As for the uniformity of the samples, Et.06 had the greatest index, which shows that the female of that nest foraged resources in a more homogeneous manner (*Solanum* 54.2%, *M. pudica* 40.2%, and *Zanthoxylum* 5.6%). On the other hand, sample Ec.08, with the least uniformity (0.084), indicates that the female gathered her resources in a heterogeneous manner, while having the greatest preference for *Solanum* (97.8%). This variation in the uniformity and diversity of pollen grains encountered in the nests can also be explained by the individual-level plasticity of female foraging.

The Euglossini bees have considerable ability to fly several distances in search of food resources (Janzen, 1971). Thus, we consider that, although the bees founded their nests in different areas (open vegetation vs. shaded areas), this does not preclude species from foraging for their resources throughout the region of the Park and surrounding area and sharing their preferred resources. The *Euglossa* bees that nest and forage in the Rangedor State Park contribute to the pollination of the plant species present in the area, and by their capacity for displacement, can mediate gene flow with individuals located in other urban fragments, which is of prime importance for the conservation of vegetation.

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