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## **RESEARCH ARTICLE - BEES**

# Diversity and Temporal Variation in the Orchid Bee Community (Hymenoptera: Apidae) of a Remnant of a Neotropical Seasonal Semi-deciduous Forest

S MATEUS, ACR ANDRADE-SILVA, CA GARÓFALO

Universidade de São Paulo, FFCLRP-USP, Ribeirão Preto-SP, Brazil

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#### **Corresponding author**

Sidnei Mateus Departamento de Biologia Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto-USP Av. Bandeirantes, 3900 – Monte Alegre 14040-901,Ribeirão Preto-SP, Brazil E-Mail: sidneim@ffcIrp.usp.br

# Abstract

Multiple biotic and abiotic variables control the composition, diversity, and temporal fluctuations of insect communities. In particular, the assembly of bee communities is strongly influenced by climatic factors as well as variation in food resource availability, which in turn also fluctuates in response to the same factors. The goal of the present study was to investigate the species composition, the species richness and the abundance of the euglossine bees in a remnant of the seasonal semi-deciduous forest of northeastern São Paulo state, and evaluate the temporal variation in those parameters over time. From January to December 1990, males of euglossine bees were sampled using three aromatic baits. We collected 643 males belonging to 12 species and three genera. Euglossa imperialis Cockerell was the most abundant species, followed by Eulaema nigrita Lepeletier and Euglossa melanotricha Moure. The composition and species richness showed temporal variation throughout the year, with the highest values being observed during the rainiest months. Such variations occurred by the presence of males from six species which were sampled only during the rainiest months. Of the five most abundant species, Eq. imperialis and Eq. melanotricha were the only species sampled throughout the year. Only El. nigrita and Eq. melanotricha, had the monthly abundance of males correlated with precipitation. Based on the assumption that male euglossine capture rates reflect actual abundance at the sampled habitats, we believe that Eq. imperialis, usually represented by few male in other studies, has a population very well established in the habitat here studied.

## Introduction

The tribe Euglossini consists of approximately 230 described species (Nemésio & Rasmussen, 2011). In the last years, the use of odorous baits to attract males has allowed detailed investigations of the ecological characteristics of these bees, the effects of habitat fragmentation on the diversity of their communities and aspects of their geographic distribution (Tonhasca et al., 2002; Brosi, 2009).

Euglossine bees have been studied in a variety of ecosystems, including the Amazonian forest (e.g., Powell & Powell, 1987; Abrahamczyk et al., 2011), Central American forests (Brosi, 2009), the Cerrado (Brazilian savannas) in central Brazil (Faria & Silveira, 2011; Silveira et al., 2015), and Caatinga (dry scrub forests) in northeast Brazil (Andrade-Silva et al., 2012). Studies have also been conducted in the

Atlantic Forest (Aguiar & Gaglianone, 2012; Rocha-Filho & Garófalo, 2013), both in areas covered by Rain Forests and in areas covered by Semi-deciduous Forests, the two major vegetation types that comprise such biome (Morellato & Haddad, 2000). The Atlantic Rain Forest covers mostly low to medium elevations (<= 1000 m.a.s.l.) of the eastern slopes of the mountain chain that runs along the coastline from southern to northeastern Brazil, while the Atlantic Semi-deciduous Forest extends across the plateau (usually > 600 m.a.s.l.) in the center and southeastern interior of the country (Morellato & Haddad, 2000).

Despite being a key area for conservation, the Atlantic Forest biome remains severely threatened due to its proximity to urban centers and areas of agricultural monoculture such as coffee, orange, sugar cane, and eucalyptus plantations (Morellato & Haddad, 2000). The seasonal semi-deciduous



forests of this biome are one of its most vulnerable and impacted ecosystems, especially in regions where the terrain facilitates the establishment of mechanized agriculture. These forests include distinct enclaves of habitat associated with specific environmental conditions, such as those found in the northeastern extreme of São Paulo state (Durigan et al., 2002). This region is characterized by a relatively high proportion of plant species that are either rare or have a restricted distribution, and has the highest priority for conservation due to its ecological and sociological characteristics, and the degree of habitat fragmentation, being one of the state's most devastated areas over the past 30 years (Kronka et al., 1998).

Given the importance of the conservation of the biodiversity of isolated fragments of habitat, the investigation of a specific group, such as euglossine bees, may contribute to the diagnosis of environmental quality, providing important insights for conservation management, as well as contributing to the investigation of distribution patterns. In this context, the present study describes the diversity and composition of the euglossine bee community in a remnant of the seasonal semideciduous forest of northeastern São Paulo state, and evaluates the temporal variation in these parameters over time.

#### **Material and Methods**

#### Study area

The study was conducted in the Furnas do Bom Jesus State Park (20°14'55" S, 47°28'48" W), which is located in the municipality of Pedregulho, São Paulo, Brazil.

The Park has an area of 2,069.06 ha with altitudes ranging from 750 to 1063 m above sea level. The territory of the Park is mostly covered with shrubs and small trees ("capoeiras") and seasonal semi-deciduous forests that occupy the scarp of caves in the valley of the Córrego do Pedregulho. In the highest regions, there are areas with different savanna physiognomies: "campo sujo", "campo cerrado" and "cerrado" *sensu stricto*. The original forest cover has not significantly changed, and represents the typical semideciduous vegetation found in the region, now distributed in discontinuous patches (Branco et al., 1991).

The local climate is mesothermal, with warm summers (temperatures of 18–32°C) and relatively dry winters (3–13°C), the driest months being June, July, and August, with monthly precipitation of less than 100 mm.

#### Male sampling

Cineole (C), eugenol (E) and vanillin (V) were used as male attractants because these chemicals are considered to be the most effective for attracting males of most euglossine species (Dressler, 1982). These chemicals were applied to small balls of filter paper, which were tied to tree branches 1.5 meters above the ground and 10 m apart, and approximately 50 meters from the edge of the forest. From January to December 1990, once a month, between 10 a.m. and 1 p.m., on sunny days, male euglossine bees were captured with an entomological net as they arrived at the chemical baits. The baits were replenished once an hour due to the volatility of the scents. Local temperature was measured every 30 minutes during the sampling period. Bees were identified in the laboratory and deposited in the entomological collection of the Chemical Ecology and Animal Behavior Laboratory, Biology Department (FFCLRP-USP), Ribeirão Preto, São Paulo, Brazil. Data on precipitation were obtained from Chapadão farm, located 3 km from the study area (20°15'75" S, 47°27'58" W) (Fig 1).



**Fig 1.** Mean temperature (line) of the periods of sampling of males and total precipitation (columns) recorded each month from January to December 1990, in the region of Furnas do Bom Jesus State Park, Pedregulho, São Paulo, Brazil.

#### Data analyses

The adequacy of the samples for the evaluation of species richness was assessed by plotting cumulative species curves, and analyzing the data using the richness estimators (Chao 1, Jackknife 1, Bootstrap, and ICE) available in the EstimateS 8.2 program (Colwell, 2006). Diversity was estimated by the Shannon-Wiener index (Krebs, 1999). While this index may be the most appropriate for comparing diversity within and among species assemblages (Magnussen & Boyle, 1995), Magurran (2004) has identified some limitations, such as the difficulty of comparing values and the assumption that all species are represented in the sample. To mediate these problems, the exponential values– exp (H<sup>2</sup>) – were used, allowing us to estimate effective number of species (Hill, 1973). Equitability and dominance were quantified following Pielou Index and Berger-Parker Index, respectively (Magurran, 2004).

The potential effect of abiotic variable precipitation on the number of males collected monthly and number of species sampled monthly was evaluated using Pearson correlation coefficient (r), based on the total precipitation recorded each month.

Differences in the number of individuals attracted by different types of baits were analyzed using the KruskalWallis test followed by multiple Mann-Whitney comparisons with the Bonferroni correction (Sokal & Rohlf, 1995). The statistical significance of differences in the attractiveness of each bait was calculated using a G test for independent samples (Zar, 1996). The analyses were performed by using the statistical package SigmaStat 3.1 for Windows (Point Richmond, CA, USA, 2004).

#### Results

A total of 643 males belonging to 12 species representing three genera were collected: (1) *Eufriesea* (= *Ef*.) with two species - *Ef. auriceps* and *Ef. violacea*; (2) *Eulaema* (= *El*.) with one species - *El. nigrita*; and (3) *Euglossa* (= *Eg.*), represented by nine species - *Eg. imperialis, Eg. melanotricha, Eg. fimbriata, Eg. leucotricha, Eg. pleosticta, Eg. truncata, Eg. securigera, Eg. annectans* and *Eg. cordata*. Species accumulation curve reached the asymptote well before the end of the study (Fig 2) suggesting that an increase in sampling effort would probably not result in the addition of any meaningful number of new species, as confirmed by the species richness estimators.

The most abundant species were *Eg. imperialis* (44.5% of the males collected), *El. nigrita* (20.0%), *Eg. melanotricha* (12.1%), *Eg. fimbriata* (7.0%), and *Eg. pleosticta* (7.0%). The remaining species (n = 7) accounted for 9.4% of the total males collected (Table 1). The diversity and equitability indices of the

sampled community were H' = 1.70 and J' = 0.88, respectively. *Euglossa imperialis*, the most abundant species, reached a 0.44 dominance according to its Berger-Parker index (d). Both the monthly species richness and abundance of males were significantly correlated with monthly precipitation values (r = 0.86 and r = 0.68; p < 0.05 for both, respectively). In addition, species richness and abundance of males were also correlated with each other (r = 0.65; p < 0.05).



**Fig 2.** Cumulative species curve and richness estimators for euglossine bees captured in the Furnas do Bom Jesus State Park, Pedregulho, São Paulo, Brazil, from January to December 1990. The continuous lines represent the mean value, and the dotted lines the lower and upper limits of the standard deviation.

Species	Number of males captured												
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total (%)
Ef. auriceps Friese	3	2	2									1	8 (1.0)
Ef. violacea Blanchard	6									1	4	7	18 (3.0)
Eg. annectans Dressler		1									1	3	5 (0.8)
Eg. carolina Nemésio	3		1									1	5 (0.8)
<i>Eg. leucotricha</i> Rebêlo & Moure	7												7 (1.1)
Eg. fimbriata Moure	4	9	14	10		2		2	2	1		4	48 (7.0)
Eg. imperialis Moure	19	25	64	49	36	9	5	22	21	13	7	16	286(44.5)
Eg. melanotricha Moure	11	6	12	5	2	3	1	5	10	9	6	8	78 (12.1)
Eg. pleosticta Dressler	11	3	10	9	2		1	1	4	2			43 (7.0)
Eg. securigera Dressler	2		3							1	1		7 (1.1)
<i>Eg. truncata</i> Rebêlo & Moure	2		2					1	3	2			10 (1.6)
El. nigrita Lepeletier	17	19	36	13	5	1		2	5	10	8	12	128 (20.0)
Total of males	85	65	144	86	45	15	7	33	45	39	27	52	643
Number of species	10	7	9	5	4	4	3	6	6	8	6	8	12

 Table 1. Number of male euglossine bees attracted to chemical baits in the Furnas do Bom Jesus State Park, Pedregulho, São

 Paulo, Brazil, from January to December 1990. Ef. (=Eufriesea), El. (=Eulaema), Eg. (=Euglossa).

Of the five most abundant species, Eg. imperialis and Eg. melanotricha were the only species sampled throughout the year, with peaks of abundance in March. Males of El. nigrita did not occur in July and males of Eg. fimbriata and Eg. pleosticta did not visit the baits in May, June, November and June, November and December, respectively. Of these species, males of El. nigrita and Eg. fimbriata were more abundant in March and the males of Eg. pleosticta, in January. The remaining seven species had their males sampling scattered by the months from January to March and from September to December. Except for Ef. violacea and Eg. annectans, that had the greatest number of males sampled in October, November and December, males of Ef. auriceps, Eg. cordata, Eg. leucotricha, Eg. securigera and Eg. truncata were recorded in greater numbers in the first three months of the year (Table 1). Only two species, El. nigrita and Eg. melanotricha, had the monthly abundance of males significantly correlated with precipitation (r = 0.81 and r = 0.76, p < 0.05 for both, respectively).

Significant differences in attractiveness between the odor baits were observed (Kruskal-Wallis: H = 23.49; p <0.05). Furthermore, the attraction pattern between the species was different (G = 351.60; df = 22; p < 0.05). The number of aromatic compounds that the individual species were attracted ranged from one (Eg. imperialis, Eg. fimbriata, Eg. cordata, *Eg. leucotricha* and *Ef. auriceps*) to three (*Eg. pleosticta*).

Cineole was the most attractive compound, having attracted males from 11 out of 12 sampled species and around of 85% of the collected males. Vanillin and eugenol were more effective than cineole only on attracting males of Ef. auriceps and Ef. violaceae and males of Eg. truncata, respectively (Fig 3).



□Cineole ■Vanillin □Eugenol

Fig 3. Frequency of euglossine males captured at each scent bait (cineole, eugenol and vanillin) in the Furnas do Bom Jesus State Park, Pedregulho, São Paulo, Brazil, from January to December 1990.

#### Discussion

Different phytophysiognomies, forest fragment size (Brosi, 2009), differences in sampling effort (Silveira et al., 2011), number and aromatic compound used (Rocha-Filho & Garófalo, 2013), and efficiency of sampling method (Nemésio & Morato, 2004) are some aspects that can affect the results on species richness and abundance in euglossine bee communities. In the present study, the stabilization of the cumulative species curve, reinforced by the richness estimators, indicates that the sampling effort (12 months) was adequate for the full inventory of the euglossine species present in the study area (12 species), considering the sampling procedure used. In other studies made in areas also covered by semi-deciduous forests the richness ranged from seven to 14 species (Giangarelli et al., 2015). This variation must be interpreted cautiously because, in addition to the aspects mentioned above, the results may also be affected by other factors such as patch characteristics, isolation from other fragments and landscape context. The finding of a few species represented by a large number of males - they are the dominants in the community - whereas most of other species are relatively rare, an attribute of the communities, as observed in this study, has also been reported by other authors working with other communities (Aguiar & Gaglianone, 2008; Rocha-Filho & Garófalo, 2013).

The species composition of the community here studied is similar to those reported by other authors working in areas also covered by seasonal semi-deciduous forests (e.g., Rebêlo & Garófalo, 1991; Silveira et al. 2011; Knoll & Penatti, 2012; Giangarelli et al., 2015). Nonetheless, even in areas with the same type of vegetation and great similarity in the structure of the communities some differences can be observed. These differences occur mainly due to the presence or absence of species usually represented by few males and by changes in the relative frequencies of each species in each community, as emphasized by Knoll and Penatti (2012). An example is the data reported by Viotti et al. (2013) showing Eg. leucotricha, usually represented by few males, as dominant species in a community of high-altitude rocky fields.

A similar and surprising example is the occurrence of Eg. imperialis as dominant species in the community here studied, a result unobserved in other studies in environments with the same phytophysionomy. This species has a geographic distribution from Central America to Brazil reaching São Paulo state (Rebêlo & Moure 1995). Analyzing a set of relevant morphological features, Rebêlo and Moure (1995) compared males sampled in this study with males from populations of the Amazon basin and Atlantic Forest. According to the authors, no populations differed from each other, *i.e.*, no significant difference between males can be found. Although without any evidence, the occurrence of Eg. imperialis in the regions west of Minas Gerais and north of São Paulo led Rebêlo and Moure (1995) to suggest the connection of these populations with the population of the Amazon basin through the central Brazil. In this context, it is interesting to note the observations by Moura and Schlindwein (2009) reporting that species of the Atlantic Rainforest like Eg. imperialis, Eg. truncata and El. cingulata occur in the gallery forest of the São Francisco river under the semi-arid climate of the caatinga region. In a study on the diversity of the euglossine bees in forest and woody savanna remnants within the Brazilian savanna domain, Silveira et al. (2015) reported the occurrence of Eg. imperialis in seasonal semideciduous forests, woody savanna and in a gallery forest where the highest abundance of this species happened. According to those authors, the presence of typical species of the Amazon and Atlantic Forest in the sampled remnants reinforces the hypothesis that both gallery and seasonal semideciduous forest acted as bio-corridors, especially for those species that depend on higher humidity, as emphasized by Moura and Schlindwein (2009). The importance of gallery forests as mesic corridors, that open the way to the colonization of the savanna landscape by forest-dependent organisms with ranges centered in the neighboring Amazon and Atlantic Forest was suggested by Sick (1966) and Willis (1992). Moreover, the occurrence of Aglae caerulea, a cleptoparasite of Eulaema nests, in the gallery forest of the Vale do Véu de Noiva in the Parque Nacional da Chapada dos Guimaraes was interpreted by Anjos-Silva et al. (2006) as the "gateway" of that species to Mato Grosso state. Recently, the role of gallery forests as important dispersal alternatives for several species, including A. caerulea, dwelling the Amazon and the Atlantic Forest was highlighted by Silva et al. (2013).

Regardless of the abundance, some recent records show the presence of *Eg. imperialis* in savanna areas (Nemésio & Faria, 2004, Alvarenga et al. 2007, Silva & De Marco, 2014; Silveira et al., 2015). However, the close association of this species to forest habitats (Rebêlo & Moure, 1995) can be confirmed by the increased abundance of it in communities occurring in areas with that phytophysiognomy, as reported by Silveira et al. (2015) and also observed in this study.

Multiple biotic and abiotic variables control the diversity, composition, and temporal fluctuations of insect communities. In particular, the assembly of bee pollinator communities is strongly influenced by climatic factors as well as variation in food resource availability, which in turn also fluctuates in response to climatic variables (Ramirez et al., 2015). As reported by some authors (Rebêlo & Garófalo, 1991; Silveira et al., 2011; Andrade-Silva et al., 2012; Viotti et al., 2013; Rocha-Filho & Garófalo, 2013) and observed in the present study, species richness showed temporal variation throughout the year, with the highest values being observed during the rainiest months. The variation in species composition and species richness here reported occurred by the presence of males from six species which were sampled only during the rainiest months.

Individual analysis of the five most abundant species showed the highest frequencies of male collection of *Eg. melanotricha* and *El. nigrita* occurring during the rainy months, following the pattern most commonly reported to the orchid bees (e.g., Silveira et al., 2011; Andrade-Silva et al., 2012; Rocha-Filho & Garófalo, 2013), while this tendency was not observed for the males of *Eg. imperialis, Eg. fimbriata* and *Eg. pleosticta*. In addition to these patterns of temporal variation, males of *Ef. violaceae, Ef. auriceps, Eg. annectans, Eg. carolina, Eg. leucotricha* and *Eg. securigera* were active only in the rainiest months. Of these six species, only *Ef. auriceps* and *Ef. violacea* have one generation per year, with the immature undergoing a prepupal diapause during the coldest and driest months, and adults with activities for a relatively short period during the hottest and rainiest months (Roubik & Ackerman 1987; Rocha-Filho & Garófalo, 2013).

Although the abundances of males of *Eg. imperialis, Eg. pleosticta* and *Eg. fimbriata* have not been correlated with the values of precipitation, the abundances of *Eg. melanotricha* and *El. nigrita* and the total abundance of sampled individuals were positively related to that climatic parameter. As the seasonal fluctuations in the abundance of adult bees are primarily associated with the food supply (Rêbelo & Garófalo, 1991), the tendency for a positive correlation between precipitation and abundance of males can best be explained with an increase in flowering of hosts plants and consequently in food resource availability, as suggested by Thiele (2005). And, with nectar and pollen sources available, the bees could take advantage of the high level of food for the nesting activities or to coincide adult emergences for that period (Rebêlo & Garófalo, 1991).

The efficiency of cineole, eugenol and vanillin in attractiveness of euglossine males, as observed in this study, have been shown in dozens of inventories carried out in several neotropical regions (Rebêlo & Garófalo, 1991 Silveira et al., 2011; Rocha-Filho & Garófalo 2013). In a study to investigate phenological patterns and seasonal and geographic variations in the preference for fragrances of euglossine males, in two coastal areas of Atlantic Forest in Ubatuba, SP, Rocha-Filho and Garófalo (2013) utilized 14 aromatic baits, three of them, cineole, eugenol and vanillin, in the first year and the other 11 in the second year. During the first year 18 species were sampled and 987 males were collected while in the second year 488 males belonging to ten species were attracted. These results confirm the efficiency of those three compounds in the attraction of euglossine males.

Although the species composition of the community here studied was similar to those occurring in areas also covered by seasonal semi-deciduous forests, the presence of *Eg. imperialis* as the most abundant species was a main factor differentiating the communities. Thus, based on the assumption that male euglossine capture rates reflect actual abundance at the sampled habitats (Otero & Sandino, 2003), we believe that *Eg. imperialis* has a population very well established in the habitat here studied.

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