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ARTÍCULO DE INVESTIGACIÓN / ORIGINAL RESEARCH PAPER

ORCHID BEES (APIDAE: EUGLOSSINI) IN A FOREST FRAGMENT IN THE ECOTONE CERRADO-AMAZONIAN FOREST, BRAZIL

Abejas de orquídeas (Apidae: Euglossini) en un fragmento de bosque en el ecotono Cerrado-Selva Amazónica, Brasil

José Max Barbosa de OLIVEIRA-JUNIOR^{1,2,3}, Sara Miranda ALMEIDA^{1,2}, Lucirene RODRIGUES², Ailton Jacinto SILVÉRIO JÚNIOR²,
Evandson José dos ANJOS-SILVA⁴.

¹ Programa de Pós-Graduação em Zoologia, Universidade Federal do Pará. Rua Augusto Correia, n.º 1, Guamá, 66075-110. Belém, Brazil.

² Programa de Pós-Graduação em Ecologia e Conservação, Universidade do Estado de Mato Grosso. Avenida Prof. Dr. Renato Figueiro Varella, s/n, 78690-000. Nova Xavantina, Brazil.

³ Instituto de Ciências e Tecnologia das Águas, Universidade Federal do Oeste do Pará, Avenida Mendonça Furtado, n.º 2946, Fátima, 68040-470, Santarém, Pará, Brazil.

⁴ Departamento de Biologia, Universidade do Estado de Mato Grosso. Avenida Tancredo Neves, s/n, Cavallhada, 78200-000. Cáceres, Brazil.

For correspondence. maxbio@hotmail.com

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ABSTRACT

This paper presents information about the species richness and abundance of orchid bees in the transitional area between the Cerrado and the Amazon Forest in the Xingu river basin. The males were collected in a forest fragment in the municipality of Ribeirão Cascalheira, northeast Mato Grosso state, Brazil, between 1 and 5 November 2011. Benzyl benzoate, 1,8 cineole, eugenol and vanillin were used as baits, to attract the bees using insect nets and methyl salicylate was used only in the bottle traps. A total of 168 males belonged to four of five Euglossini genera distributed among 16 species were recorded. The greatest species richness was registered in the genus *Euglossa*, however *Eulaema nigrita*, was the most abundant Euglossine species in the community. The species richness varied between the edge and the first point within the fragment. The males were more active between 10:00 and 11:00 h, and the 1,8 cineole was the most attractive substance. The fragment studied contained representative species richness of orchid bees in a region of widespread environmental heterogeneity, which is still largely unknown as regards its pollinators and deserves attention for the compilation of new inventories.

Keywords: attraction efficiency, baits, Brazil, fragmentation, pollinators.

RESUMEN

En este trabajo se presenta información sobre la riqueza de especies y abundancia de Euglossini en la zona de transición entre el Cerrado y la selva amazónica, en la cuenca del río Xingu. Las muestras se recogieron en un fragmento de bosque en el municipio de Ribeirão Cascalheira, nordeste de Mato Grosso, Brasil, entre el 1 y 5 de noviembre de 2011. Las sustancias puras de benzoato de bencilo, 1,8 cineol, eugenol y la vainillina fueron utilizadas como cebos para atraer los machos usando red de insectos aunque el salicilato de metilo se utilizó en trampas pasivas. Un total de 168 machos pertenecientes a cuatro géneros fueron registrados, distribuidos en 16 especies. La mayor riqueza de especies se registró en el género *Euglossa*, aunque *Eulaema nigrita* fue la especie más abundante en la comunidad. La riqueza de especies varió entre el borde y el primer punto dentro del fragmento. Los machos de

abejas de orquídeas eran más activos entre 10:00-11:00 h, y el 1,8 cineol fue la sustancia pura más atractiva. El fragmento estudiado contenía un alto grado de riqueza de especies de abejas de orquídeas en una región de heterogeneidad ambiental extensa, que sigue siendo en gran parte desconocido con relación a su fauna de insectos polinizadores y merece la atención para la elaboración de nuevos inventarios.

Palabras clave: cebos, Brasil, eficiencia de atracción, fragmentación, polinizadores.

INTRODUCTION

Orchid bees comprise the tribe Euglossini (Hymenoptera: Apidae) and are distributed exclusively in the Neotropical region, with greatest biological diversity in the warm and humid equatorial zones (Dressler, 1982). These corbiculate bees have also been reported in the Cerrado (Carvalho and Bego, 1996; Alvarenga *et al.*, 2007), Pantanal (Anjos-Silva, 2006a), Caatinga (Aguilar, 1990; Neves and Viana, 1999), gallery forests (Neves and Viana, 1999; Anjos-Silva, 2006b; Anjos-Silva *et al.*, 2006) and even in high altitude regions, such as the Andes (Ramírez, 2005; Ramírez, 2006; Parra and Nates-Parra, 2012).

For Brazil there are more than 200 known species of Euglossines distributed among five genera: *Aglæ* Lepeletier and Serville, 1825 (= *Ag.*), *Eulaema* Lepeletier, 1841 (= *El.*), *Eufriesea* Cockerell, 1908 (= *Ef.*), *Euglossa* Latreille, 1802 (= *Ag.*) and *Exaerete* Hoffmannsegg, 1817 (= *Ex.*) (Ramírez *et al.*, 2002; Anjos-Silva, 2007; Anjos-Silva, 2008; Anjos-Silva, 2010). Euglossines are capable of flying long distances (≈ 23 km) in continuous tropical forests (Janzen, 1971), are highly vagile and visit the flowers of more than 30 plant families (Lloyd and Barret, 1996), including more than 2000 species of orchids (Ramírez *et al.*, 2002; Cameron, 2004).

In Brazil, studies on communities Euglossini are more frequent in the Central Amazon region (Powell and Powell, 1987; Becker *et al.*, 1991; Morato *et al.*, 1992) and northeast of the São Paulo state (Rebêlo and Garófalo, 1991). These studies are restricted in the Cerrado, and still scarcer in the Cerrado-Amazon Forest ecotone (Mesquita-Neto *et al.*, 2012). The larger geographical gap in the diversity of Euglossini for these regions is certainly related to the few samplings (Nemésio and Silveira, 2007), or only basic information such as the geographical distribution of species (Silveira and Campos, 1995). The Euglossines collect aromatic compounds in plants and one hypothesis is that substances such as terpenes and sesquiterpenes, for example, are used as sexual pheromones (Williams and Whitten, 1983). Such substances, analogous to those recorded in orchid flowers, have been produced synthetically and their use has enabled significant advances in our understanding of this peculiar insect fauna, especially because certain pure substances was tested to attract the orchid bee males, which has made this a widespread technique allowing for the compilation of inventories in various neotropical regions (Williams and Whitten, 1983).

Prior to this discovery, orchid bees sampling relied mostly on capturing bees foraging in flowers (see Sakagami *et al.*,

1967). Through the use of baits to attract orchid bees, the daily and seasonal patterns can now be observed and understood as similar to those observed in the actual plants visited, making it possible to obtain precious information about the patterns of species diversity, population density, seasonal and geographic variation, activity times, as well providing useful data as regards the male longevity, age structure and specificity of bee attraction to the chemicals used in the Neotropics (Ackerman, 1983; Oliveira and Campos, 1995; Rebêlo and Garófalo, 1997; Peruquetti *et al.*, 1999; Bezerra and Martins, 2001).

The number of males visiting the pure substances reflects emerging patterns and is correlated to the males' searching for natural resources (nectar and pollen) and, consequently, may indicate the abundance of males in a given geographic region (Ackerman, 1983; Roubik and Hanson, 2004). The significant biological diversity, the variation in male abundance and the ecological importance of orchid bees in several neotropical ecosystems suggest that these bees could be considered bioindicators of the quality of the pollination environmental services in natural, semi-natural, conserved and protected areas (Tonhasca Jr. *et al.*, 2002; Souza *et al.*, 2005).

The Euglossines are therefore suitable subjects for studies into the direct and indirect effects of forest fragmentation on the structure and dynamic of biological communities throughout the neotropic ecozone (Becker *et al.*, 1991; Brosi *et al.*, 2008; Brosi, 2009; Andrade-Silva *et al.*, 2012). In this context, this paper aimed to verify the species richness and abundance of Euglossini in the transitional forest between the Cerrado and the Amazon Forest, presenting preliminary data for the municipality of Ribeirão Cascalheira, in the Xingu river basin, eastern Mato Grosso state, Brazil. We hope that the study area has a high species richness of Euglossini compared with other studies due to the wide availability of microhabitats that region.

MATERIALS AND METHODS

Study area

This study was performed in a forest fragment at the Destino Farm (1.535m²) (12°52'12.5" S and 52°05'8.5" W) in the municipality of Ribeirão Cascalheira, northeast Mato Grosso state, Central Brazil (Fig. 1). The fragment is isolated from other fragments by a distance greater than 30 m and is surrounded by a matrix of low biomass and grazing. This region represents the transitional area between the Cerrado

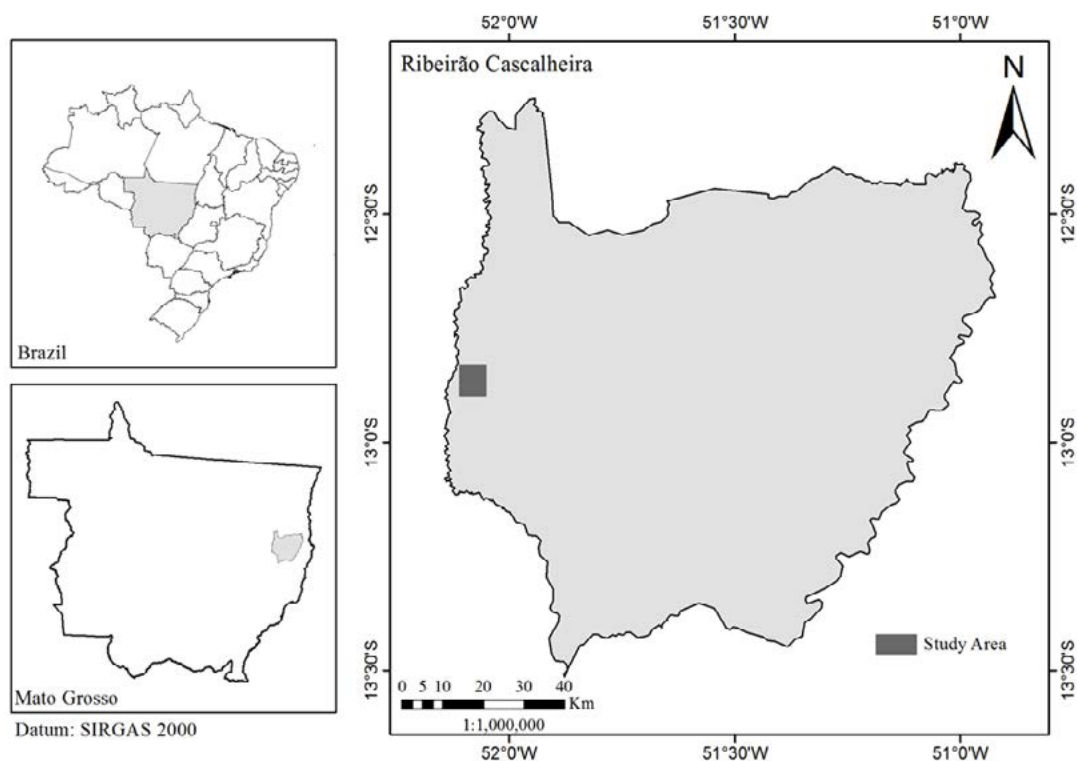


Figure 1. Map of Mato Grosso, with inset of the municipality of Ribeirão Cascalheira, Brazil. The study area was a forest fragment (in dark grey) located in the Destino Farm.

and the Amazon Forest (Ratter *et al.*, 1978), characterised by pre-Amazonian forest formations, as well as savannahs and forests typical of the Brazilian Cerrado (Marimon *et al.*, 2006). The region climate is humid tropical with a well-defined dry season, precipitation of less than 100 mm in the driest month, and mean temperature above 18°C in the coldest month. Mean annual precipitation of 1900 mm, and there two well-defined seasons, with the peak of the dry season occurring between June and September, and intense rains between December and March (Peel *et al.*, 2007).

DATA COLLECTION

Sampling of Euglossini and methodological procedures

For sampling the males, the baits benzyl benzoate, 1.8 cineole, eugenol and vanillin were used with the aid of insect nets, while methyl salicylate was used in bottle traps (modified Campos *et al.*, 1989). Further information about collection techniques for sampling orchid bees can be found in Lopez (1963), Sakagami *et al.* (1967), Bennett (1972), Folsom (1985) and Campos *et al.* (1989).

Specimens were collected from 1 to 5 November 2011 seven 80 m transects with baits were established to collection, 150 m apart, starting at the edge and moving into the forest

fragment. In the active collection (insect nets) the transects ($n = 4$) were defined alternately with the transects of the bottle traps ($n = 3$). In each of the transects three sequences of four pure substance were arranged, consisting of a cotton pad attached to the branches of the trees transversal to the main line, totalling four substances per transect.

For each of the seven transects a sequence of the pure substances was arranged as follows: the first sequence was established at the edge of the fragment, the second 40 m from the edge, and the third 80 m from the edge. In each transect, each of the substances were placed 5 m apart and in the following order: benzyl benzoate, 1.8 cineole, eugenol and vanillin (the last diluted in alcohol) (Fig. 2). Due to the different evaporation rates, a 1 ml refill was added to each pure substance every hour during the sampling (Anjos-Silva, 2007).

Four collectors (one per transect), equipped with insect nets simultaneously inspected the scent baits in each transect of the fragment at 20-minute intervals, between 08:00 and 16:00. The bottle traps soaked with methyl salicylate bait was distributed in similar way of insect nets collections, resulting in a sampling effort of eight hours per day per collector during five consecutive days.

The bottle traps were set between 07:00 and 08:00 and inspected daily, after 16:00, when the captured specimens

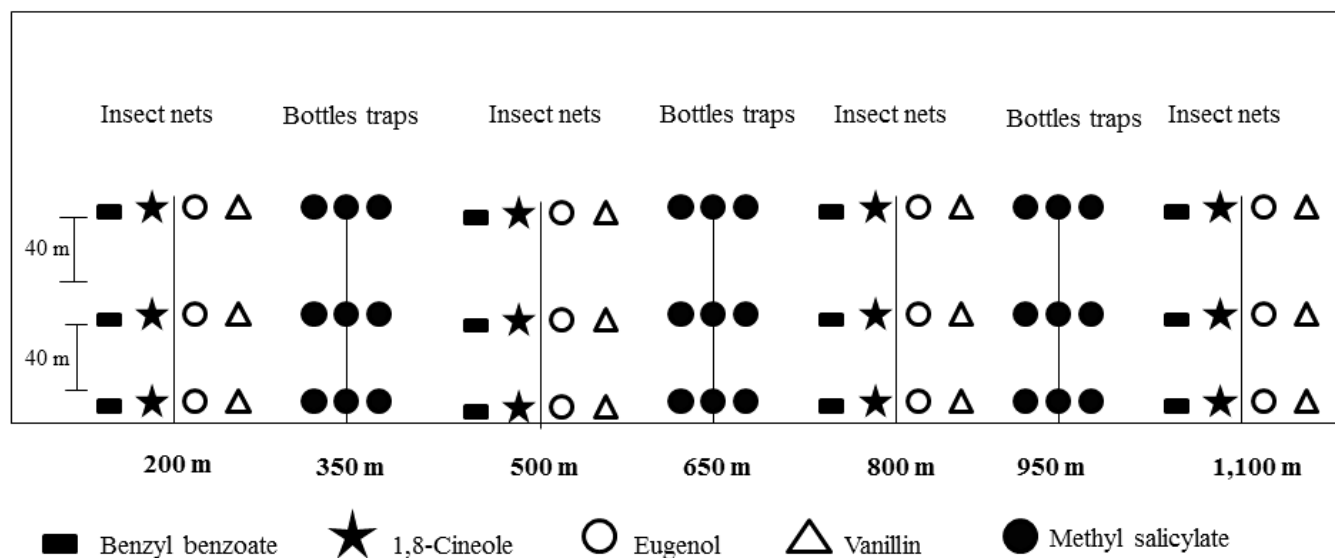


Figure 2. Sampling design used in the forest fragment showing the transections, collection methods and the respective scented baits used to attract male Euglossini (Apidae) bees in a forest fragment in the Cerrado-Amazonian Forest transitional area in Ribeirão Cascalheira, Mato Grosso, Brazil.

were collected during four consecutive days and preserved in 70 % alcohol solution and then identified (collection permit IBAMA/SISBIO #12778-1). Voucher specimens were deposited in the collection of the Laboratory for Neotropical Bees and Wasps (LABEVE), Universidade do Estado de Mato Grosso (UNEMAT), *Campus Cáceres*, Mato Grosso, Brazil.

DATA ANALYSIS

Absolute and relative frequency calculations were made for the Euglossini bees collected attracted to the pure substances using insect nets and bottle traps methods. A Student's *t*-test (Zar, 1999) was conducted to verify if the bees' visiting frequency differed in relation to the type of attractive substance. Histograms of abundance were plotted to illustrate the number of individuals related to the time interval and the preference for chemicals. Statistical analyses were performed using routines on the statistical platform R (R Development Core Team, 2011).

The species richness was estimated using a nonparametric first-order Jackknife estimator with 100 randomizations (Colwell and Coddington, 1994), with continuous control of the sampling effort. The species richness procedure was calculated using EstimateS software (Statistical Estimation of Species Richness and Shared Species from Samples) version 7.5.0 (Colwell, 2005).

The abundance data were expressed as logarithms to illustrate the rank species abundance. The 1-way ANOVA was used to verify the existence of any difference in the species richness between the edge (P1) and the interior of the forest fragment, 40 m in (P2) and 80 m in (P3) from

the edge, and also to compare the males' visiting frequency to the three most attractive chemicals. To perform the ANOVA, the data were expressed as logarithms and after verifying any differences between the groups compared by the 1-way ANOVA, a Tukey's test was performed to detect such difference. The level of significance considered for the analyses was 5 %. The ANOVA and *t* Test analyses were performed once the premises of data homogeneity and normality of variance were analysed (Zar, 1999).

RESULTS

The orchid bee community

A total of 168 male orchid bees were collected during the sampling, distributed among the genera *Eulaema* (four species), *Eufriesea* (one specie), *Euglossa* (ten species) and *Exaerete* (one specie), and belonging to 16 species. The genus with the greatest male abundance was *Eulaema* (106 ♂♂; 63 %), while the least abundant was *Exaerete* (7 ♂♂; 4 %). The most abundant species was represented by *El. nigrita* (40 %), *El. cingulata* (19 %) and *Ef. surinamensis* (11 %).

In the bottle traps only eight individuals (4.76%) belonging to five species were recorded, with *Ef. surinamensis* the most representative. A total of 160 males (95.24 %) belonging to 16 species were collected using insect nets, with *El. nigrita* and *El. cingulata* being the most abundant species (41.88 % and 20 %, respectively) in the community (Table 1).

There has been a disparity in the species abundance of orchid bees, with several species represented by many individuals and other species represented by few individuals in the community (Fig. 3).

Table 1. Absolute abundance (N) and relative abundance (%) of males of orchid bee species (Apidae: Euglossini) recorded in a forest fragment in the Cerrado-Amazon Rainforest transitional area in Ribeirão Cascalheira, northeast Mato Grosso, Central Brazil, attracted to the pure substances 1.8 cineole (CI.), eugenol (EU.), vanillin (VA) benzyl benzoate (BB) (insect nets) and methyl salicylate (MS) (bottle traps) in the period from 1 to 5 November, 2011.

Taxon	Insect nets		Bottle traps		Pure substances (baits)						
	N	%	N	%	Total	%	CI	EU	VA	BB	MS
<i>Eulaema</i> Lepeletier, 1841											
<i>Eulaema bombiformis</i> (Packard, 1869)	3	1.87	1	12.5	4	2.38	3	0	0	0	1
<i>Eulaema cingulata</i> (Fabricius, 1804)	32	20	-	-	32	19.06	1	28	3	0	0
<i>Eulaema mocsaryi</i> (Friese, 1898)	1	0.62	1	12.5	2	1.2	0	0	1	0	1
<i>Eulaema nigrita</i> Lepeletier, 1841	67	41.88	1	12.5	68	40.48	61	3	3	0	1
<i>Eufriesea</i> Cockerell, 1908											
<i>Eufriesea surinamensis</i> (L., 1758)	15	9.38	4	50	19	11.3	1	0	14	0	4
<i>Euglossa</i> Latreille, 1802											
<i>Euglossa bidentata</i> Dressler, 1982	3	1.87	-	-	3	1.78	1	1	1	0	0
<i>Euglossa bursigera</i> Moure, 1970	1	0.62	-	-	1	0.59	0	1	0	0	0
<i>Euglossa chalybeata</i> Friese, 1925	3	1.87	1	12.5	4	2.38	1	1	1	0	1
<i>Euglossa cordata</i> Linnaeus, 1758	2	1.26	-	-	2	1.2	0	1	1	0	0
<i>Euglossa</i> gr. <i>cordata</i>	4	2.5	-	-	4	2.38	4	0	0	0	0
<i>Euglossa ignita</i> Smith, 1874	1	0.62	-	-	1	0.59	0	0	0	1	0
<i>Euglossa imperialis</i> Cockerell, 1922	1	0.62	-	-	1	0.59	1	0	0	0	0
<i>Euglossa pleosticta</i> Dressler, 1982	15	9.38	-	-	15	8.93	11	3	1	0	0
<i>Euglossa</i> gr. <i>purpurea</i>	2	1.26	-	-	2	1.2	2	0	0	0	0
<i>Euglossa securigera</i> Dressler, 1982	3	1.87	-	-	3	1.78	3	0	0	0	0
<i>Exaerete</i> Hoffmannsegg, 1817											
<i>Exaerete smaragdina</i> (Guérin-Ménéville, 1845)	7	4.37	-	-	7	4.16	2	2	3	0	0
Total	160	100%	8	100%	168	100	91	40	28	1	8

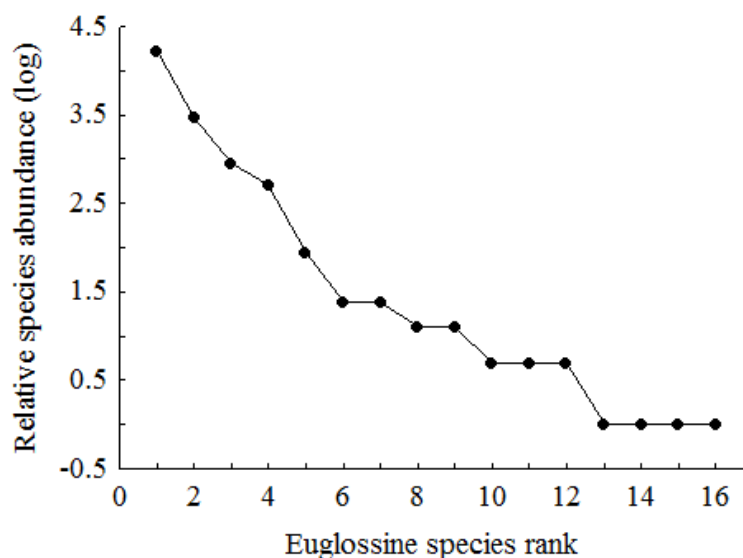


Figure 3. Relative species abundance (in logarithmic scale) of orchid bees (Hymenoptera: Euglossini) attracted to the scented baits of methyl salicylate, benzyl benzoate, 1.8 cineole, eugenol and vanillin in the period 1 to 5 November 2011, in a forest fragment in the Cerrado-Amazonian Forest transitional area in Ribeirão Cascalheira, Mato Grosso, Brazil.

The species richness and the males' abundance were lower at the edge of the fragment (P1), where 48 males belonging to six species were collected. At the mid-point of the fragment (P2) 71 males were captured, distributed among 16 species. At the innermost point of the forest fragment (P3) 49 males were recorded belonging to 11 species. Such differences in the abundance of males could be explained by chance ($F_{(2, 31)} = 0.666$; $p = 0.52$). However, when we observed the Euglossine species richness in the study area, we found a difference between the points at the edge and the interior of the forest fragment ($F_{(2, 50)} = 4.012$; $p = 0.024$) (Fig. 4).

The Tukey's test demonstrated that the difference is found between the edge point (P1) and the mid-point (P2), since P2 showed 11 more species than P1, and chance was not

sufficient to explain such a difference ($p = 0.018$; $g.l = 50$). However, no difference was observed between P1 and P3 ($p = 0.387$, $g.l = 50$), nor between P2 and P3 ($p = 0.286$; $g.l = 50$).

Activity times of male orchid bees

The most intense activity of the orchid bee males was observed in the matutinal period, especially between 10:00 and 11:00 (Fig. 5a). The 1.8 cineole attracted 91 males (54.16 %) belonging to 15 species (78.95 %), followed by the eugenol, with 40 males (23.81 %) and belonging to eight species (4.76 %) and the vanillin, which attracted 28 males (16.66 %) from ten species (52.63 %). The 1.8 cineole was, therefore, more attractive than the eugenol, vanillin and the benzyl benzoate (Fig. 5b) in the Xingu basin, seeing as the 1.8

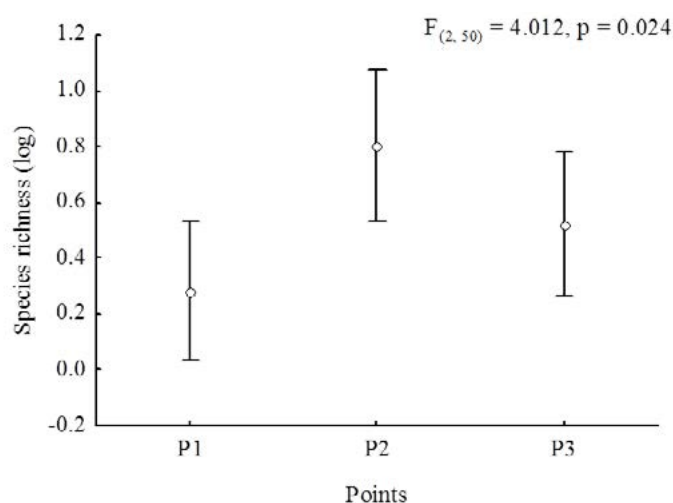


Figure 4. Comparison between species richness of orchid bees (Apidae: Euglossini) sampled at the points situated at the edge of the fragment (P1), and 40 m (P2) and 80 m (P3) from the edge of a forest fragment in the Cerrado-Amazonian Forest transitional area in Ribeirão Cascalheira, Mato Grosso, Brazil.

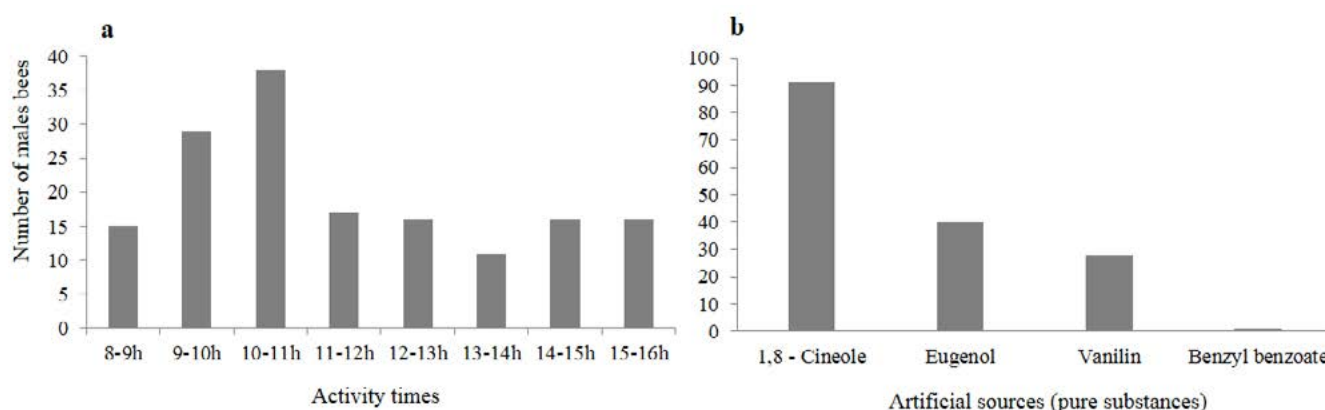


Figure 5. Activity times of male orchid bees (Apidae: Euglossini) attracted to the scented baits of benzyl benzoate, 1.8 cineole, eugenol and vanillin in the period 1 to 5 November 2011, in a forest fragment in the Cerrado-Amazonian Forest transitional area in Ribeirão Cascalheira, Mato Grosso, Brazil.

cineole attracted on average 2.28 more individuals than the other baits put together. In this case, the difference cannot be explained by chance ($t = 3.453$; $g.l = 32$; $p = 0.001$).

Among the most abundant species captured with insect nets, the *El. nigrita* males showed a preference for the 1.8 cineole ($n = 67$; 91.04 %), whereas *El. cingulata* preferred the eugenol ($n = 32$; 87.5 %), and the *Ef. surinamensis* were most attracted to the vanillin ($n = 15$; 93.33 %). In other way, only one *Eg. ignita* male was captured, attracted to the benzyl benzoate.

When comparing the frequency of the male visits to the three most attractive pure substances (1.8 cineole, eugenol and vanillin), a significant difference was also found between the males' preferences ($F_{(2,30)} = 5.49$; $p = 0.009$) (Fig. 6). By means of the Tukey's test it was possible to affirm that the 1.8 cineole attracted 2.47 times more males than the eugenol ($p < 0.001$) and 1.94 times more than the vanillin ($p = 0.04$), which differences cannot be explained by chance. However, when comparing vanillin and eugenol, the differences were not found to be significant ($p = 0.848$).

DISCUSSION

The distribution of the orchid bee males among the species was similar to the results obtained in other studies (Ricklefs *et al.*, 1969; Ackerman, 1983; Ramalho *et al.*, 2009), in line with the tropical patterns, with numerous individuals distributed among few species, and numerous species with little representation in the community.

The higher species richness of the genus *Euglossa* follows the patterns already observed in other inventories (Brito and Rêgo, 2001; Anjos-Silva, 2008; Ramalho *et al.*, 2009; Anjos-Silva, 2011), which is represented by species that prefer humid environments, but that occurs in regions with Amazon-like climate and vegetation (Anjos-Silva, 2011).

The genus *Eufriesea* was the third most abundant, but less diverse, represented by 19 *Ef. surinamensis* males. All the species of this genus are highly seasonal and begin their foraging activities early in the wet season and remaining active during the rainy season (Ackerman, 1983; Cameron, 2004), but one exception exists for *Ef. Pulchra* observed in August, during the dry season (Anjos-Silva, 2010). The presence of just one species of this genus in the fragment studied must be related to the start of the rainy season in this region of Mato Grosso.

Of all the species recorded, the most abundant was *El. nigrita*, a common species in the Pantanal wetlands (Anjos-Silva, 2006b) and in the Cerrado (Nemésio and Faria Jr., 2004), typical of open and relatively dry landscapes and in the transitional zone between the Cerrado and the Amazon Rainforest (Rebêlo and Silva, 1999). It is a bioindicator of environmental impacts as it is abundant in disturbed areas (Carvalho Filho, 2010), probably due to their physiological plasticity, which makes these bees resistant to conditions of environmental stress (Silva *et al.*, 2009).

El. cingulata is a polytypical species (according to Engel, 2014) and was the second most abundant species in the

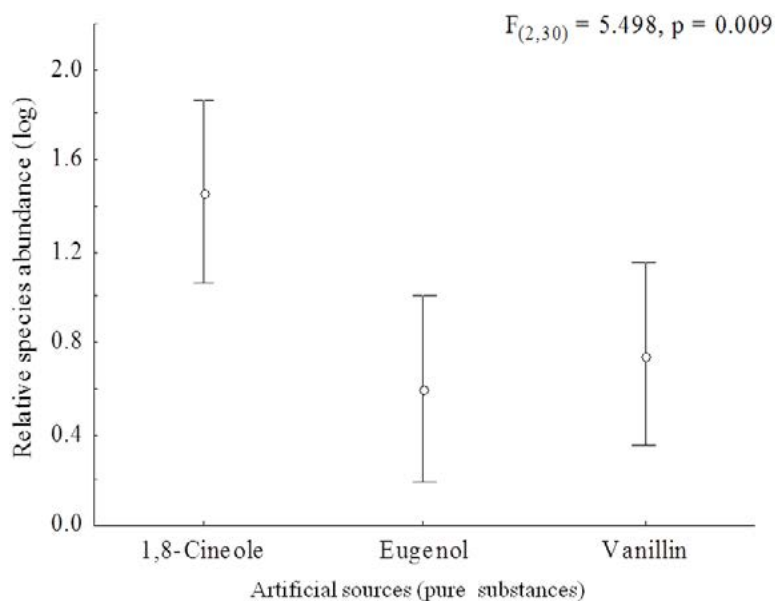


Figure 6. Comparison between the number of male orchid bees (Apidae: Euglossini) attracted to the three most attractive scented baits (1.8 cineole, eugenol and vanillin) in the period 1 to 5 November 2011, in a forest fragment in the Cerrado-Amazonian Forest transitional area in Ribeirão Cascalheira, Mato Grosso, Brazil.

community, which has a wide geographic distribution, reported from the North of Mexico to Bolivia and the South of Brazil (Ramírez *et al.*, 2002; Anjos-Silva, 2007).

The Euglossine species richness registered in the northeast of Mato Grosso is considerable and stands out in relation to other Brazilian states. In a seasonal forest in the state of Rio de Janeiro, 4069 males were collected belonging to 13 species of the four genera recorded here (Aguar and Gaglianone, 2008). Peruquetti *et al.*, (1999) collected 308 males belonged to 15 species and 893 males belonged to ten species in remnant areas of Atlantic forest of Minas Gerais, respectively. At the end, they produced a list of 57 recorded species in the Atlantic forest, based on the literature (Peruquetti *et al.*, 1999).

In Maranhão, where orchid bee species richness is considered higher, seven different environments were samples, registering 44 species (Rebêlo and Silva, 1999). In fragments of firm ground in the Amazon 290 males from 16 Euglossini species were collected, but by using McPhail traps (Becker *et al.*, 1991). In the first systematic inventory compiled in Mato Grosso state, 264 males from 35 Euglossini species were captured, of which 13 species were found in the Pantanal (Anjos-Silva, 2006b), 17 species in the Cerradão and 25 in gallery forest areas in the Chapada dos Guimarães National Park (Anjos-Silva, 2006a). These works demonstrate the scarcity of data for Mato Grosso, which possesses a great variety of phyto-physiognomies distributed in the Cerrado, Amazon and Pantanal regions, including the transitional zone between the Cerrado and the Amazon Rainforest.

The five Euglossini genera already reported in the state of Mato Grosso (Anjos-Silva and Rebêlo, 2006) were all recorded in this study with the exception of *Aglae* (Anjos-Silva *et al.*, 2006), which has not yet been recorded in the Xingu river basin (for additional information's, see Silva *et al.*, 2013).

The fact that no significant differences were found in the abundance between the edge and the interior of the forest fragment might be related to the high vagility of the orchid bee males, capable of travelling long distances (Janzen, 1971; Kroodsmas, 1975). However, we can note that the species richness registered at the edge point (P1) significantly differed to one of the inner points (P2). This result may be related to the fact that the edge of the fragment contains fewer resources for foraging bees in search of fragrances, suggesting less activity of males at the edge of forest fragments.

In this regard, Morato (1994) observed greater abundance of these bees further inside the forest (58.7 %) and less abundance at the edge of the fragment (27 %) and in pastures (14.2 %). Brosi *et al.*, (2008), meanwhile, reported that Euglossini bees are severely affected by forest fragmentation, with reduced abundance at the edge of forests near pasture (15 %), and with no male catalogued outside the forest fragment.

There are several possible factors behind the species richness and abundance of local Euglossini bees, to the extent that ecological theory suggests that the abundance of food resources would benefit large populations and that diverse food sources would favour greater richness of consumer species (Silveira *et al.*, 2002). Variations in the species richness of Euglossini between different neotropical regions may be attributed to the differences in collection methods, the types of traps used, the types of pure compounds used (Ramírez *et al.*, 2002), as well as the sampling effort and, above all, the geographic region and time of the year and whether an inventory is compiled.

The results presented here for the northeast of Mato Grosso are preliminary, but provide indications of the considerable species richness of orchid bees in the Cerrado-Amazon Rainforest transitional zone, bearing in mind that in this study the estimated species richness is relatively high, despite only one single forest fragment being sampled. New inventories made over longer sampling periods are required at other sites and at different times of the year in order to discover this exuberant native bee fauna.

The Euglossines observed in this study displayed the highest level of activity between 10:00 and 11:00, with the increase in temperature and lower humidity interfering in foraging activities, which is in line with existing literature (Dodson *et al.*, 1969; Braga, 1976; Oliveira, 1999; Brito and Rêgo, 2001; Santos and Sofia, 2002). Fragrance collection by the males is unquestionably more intense during the morning (Dressler, 1982), and one of the possible biotic factors that influences the males' activity is the production of odorous compounds by the plants (Oliveira, 1999).

The low foraging activity of *Eulaema* and *Euglossa* males in the afternoon might be a response to the normal abundance patterns of sources of fragrances (Armbruster and Berg, 1994). For the pollinators to visit the flowers at times of the day when the temperature is lower, the flower must offer a reasonable quantity of resources, such as nectar, for the bee to be able to regulate its body temperature (Heinrich and Raven, 1972).

In our study the active collection (insect net) was more efficient, attracting 95.45 % of the males compared to the passive collection (PET bottle traps). In our study area, the baits in the bottle traps attracted specimens of the *Eufriesea*, *Eulaema* and *Exaerete*, however *Ef. surinamensis* was the most abundant species (50 %) in the community using this method. In the studies conducted in the northeast of Brazil, this type of trap failed to sample any specimen of the *Eufriesea* genus, as observed in Bahia (Neves and Viana, 1997; Viana *et al.*, 2002) and Pernambuco (Bezerra and Martins, 2001).

Viana *et al.* (2002) catalogued five Euglossine species belonged to three genera in the restinga (salt marsh) vegetation in Bahia state using insect net and also capturing individuals on flowers by the sweeping method. Ramalho *et*

al. (2009) captured 4094 individuals belonging to 17 species from three genera (*Euglossa*, *Eulaema* and *Exaerete*) over the course of one year in five fragments of Atlantic Forest, using bottle traps.

The most appropriate method for capturing orchid bees can vary according to the logistics of the work and the collection site, and although one technique or another may be the most commonly employed and recommended for bee surveying. The best results in species numbers registration are obtained when multiple methods are used with this purpose (Pinheiro-Machado and Silveira, 2006). Therefore, the associated use of different methodologies becomes complementary for bee sampling and allows the results to cover a greater number of bee specimens from different species, thus producing a sample of greater significance and amplitude (Smith-Pardo and Gonzales, 2007).

As regards the preferred baits, we observed that cineole, eugenol and vanillin were the most attractive to males, in agreement with Brito and Rêgo (2001), who found that the most attractive essences were cineole (44.32 %), eugenol (19.49 %), methyl salicylate (19.0 %) and vanillin (16.92 %), with the exception of the order of vanillin and methyl salicylate.

Cineole, vanillin and methyl salicylate have proven to be efficient chemicals in attracting orchid bee specimens for capture, where the cineole is generally the most efficient substance in terms of abundance of males attracted, followed by the vanillin and the methyl salicylate (Neves and Viana, 1999), corroborating with the results achieved in this study, since of the four baits used cineole attracted significantly more bees.

The *El. nigrita*, *Ef. surinamensis* and *El. cingulata* species have been frequently sampled in studies to assess bait preferences for Euglossines in different regions of Brazil (Peruquetti *et al.*, 1999; Santos and Sofia, 2002; Anjos-Silva *et al.*, 2006; Anjos-Silva, 2010).

It is important to highlight that the bees' preference for a certain type of chemicals should be evaluated with great care, since there are several intrinsic and extrinsic factors that may influence this choice. For example, the wind or sunlight may affect the level of volatility of the fragrance at collection sites. Cineole, for instance, has a low molecular weight and is therefore a very volatile substance, which can be dispersed more easily and attract individuals from afar (Silva and Rebêlo, 2002).

Carvalho *et al.* (2006) stress that the differences reported under artificial conditions may result from competitive interactions for the exploration of floral resources, as several plant species may be visited by Euglossini species. A greater quantity of visits occurs, especially when the same source of odours is explored by the males.

Due to the lack of studies that evaluate the foraging sites of orchid bees in Amazon forest fragments, it cannot be affirmed that the bees tend to forage at the centre of

the fragments, although orchid bees can travel across great distances (Janzen, 1971; Williams and Dodson, 1972). The need arises, therefore, to conduct new studies in different sized fragments with the aim of achieving solid scientific grounds to confirm this hypothesis.

CONCLUSION

We have demonstrated that the fragment studied in the Xingu Basin presented a considerable species richness of orchid bees in a region of ample spatial heterogeneity, resulting in the need for further studies in this transitional region, given the lack of data.

We observed that the most efficient technic for sampling orchid bees in the Xingu Basin was the insect net. However, the use of multiple methods is also important, allowing the collection of a greater number of bees of different species and thus providing a faunal sample of greater significance and amplitude in the Neotropics.

In relation to the orchid bee males' activity times, the reduced foraging of these bees in the afternoon may be due to the males' response to the patterns of supply of the floral fragrances in the environments studied.

We found that cineole was the most attractive chemical, supporting the results of other studies that have evaluated bait preferences among Euglossines. However, the use of different kind of chemicals is important in faunal surveys, allowing for a more complete sampling and thus better understanding of the orchid bees in the Neotropical region.

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