

# Do euglossine males (Apidae, Euglossini) leave tropical rainforest to collect fragrances in sugarcane monocultures?

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**ABSTRACT.** Euglossine bees are known to be long-distance pollinators in tropical rainforests. But there is controversy concerning to the flight ranges of these bees between forest fragments. In an isolated fragment of Atlantic Rainforest in Pernambuco, NE Brazil, surrounded by sugarcane monocultures, it was examined if euglossine males leave closed rainforest to collect fragrances. In a straight-line transect leading from forest into a sugarcane plantation, euglossine males were simultaneously captured by scent baits at seven distinct points: inside the forest, forest edge, outside the forest in the sugarcane fields at distances of 10 m, 50 m, 100 m, 250 m and 500 m from the forest edge. A total of 945 euglossine bees of 16 species were recorded. The results demonstrate different relations of the Euglossini species to the closed forest. Males of 11 species did not leave the forest. Such species, together with the plants they are linked to, seem to be the most threatened by habitat fragmentation. Only bees of five species were found at the scent baits in the sugarcane fields. Already the 10 m sampling point outside the forest showed a drastic reduction in species richness, indicating that the forest edge functions as a barrier for most euglossine species.

**KEY WORDS.** Atlantic Rainforest, habitat fragmentation, Northeast Brazil, orchid bees, scent baits.

**RESUMO. Machos de Euglossini deixam a floresta tropical para coletar fragrâncias em uma monocultura de cana-de-açúcar?** Abelhas Euglossini são conhecidas por serem polinizadores de longas distâncias em florestas tropicais. No entanto, existem controvérsias sobre o raio de vôo dessas abelhas entre fragmentos florestais. Em um fragmento isolado de Mata Atlântica, cercado por monoculturas de cana-de-açúcar, em Pernambuco, Nordeste brasileiro, foi examinado se os machos de Euglossini deixam a mata para coletar fragrâncias. Machos de Euglossini foram capturados simultaneamente por iscas odoríferas ao longo de um transecto em sete pontos: interior da mata, borda da mata e no canavial, a distâncias de 10 m, 50 m, 100 m, 250 m e 500 m da borda. Foram registrados 945 indivíduos de Euglossini pertencentes a 16 espécies. Os resultados mostram diferentes relações das espécies de Euglossini com a floresta fechada. Machos de 11 espécies não saíram da mata. Essas espécies, juntamente com suas plantas relacionadas, parecem ser as mais ameaçadas pela fragmentação do habitat. Apenas abelhas de cinco espécies foram encontradas em iscas odoríferas no canavial. Além do mais, o ponto de amostragem de 10 m fora da mata mostrou uma acentuada redução na riqueza de espécies, indicando que a borda da mata é uma barreira para a maioria das espécies de Euglossini.

**PALAVRAS CHAVES.** Fragmentação de habitat, iscas odoríferas, Mata Atlântica, Nordeste brasileiro.

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The Euglossini are neotropical bees, most of which are found in hot and humid equatorial regions (MOURE 1967). As effective pollinators they display relationships with numerous associated plant species, including buzz-pollination flowers, resin flowers, perfume flowers and long-tubed nectar flowers, playing thus, a key role in maintaining diversity in tropical rainforests (DRESSLER 1982, BUCHMANN 1983, ACKERMAN 1985, ROUBIK 1989, SCHLINDWEIN 2000, CAMERON 2004).

One of the outstanding features of euglossine bees is that males collect floral perfumes (volatile lipids) produced in osmophores of more than 700 species of Orchidaceae and other plant families (VOGEL 1962, 1966, 1999, DRESSLER 1982, CAMERON 2004).

Several components of these fragrances that make part of the flowers' bouquet may be used as chemically pure substances to attract euglossine males. This method has been re-

peatedly used to survey Euglossini and to perform studies about abundance, seasonality and population dynamics in various neotropical forest formations (see for example ROUBIK & ACKERMAN 1987, WITTMANN *et al.* 1988, BEZERRA & MARTINS 2001, ROUBIK 2001, 2004, NEVES & VIANA 2003).

The Brazilian Atlantic Rainforest currently covers less than 8% of its original area, encompassing around 91,000 km<sup>2</sup>. In Pernambuco and other states of NE-Brazil, only small fragments of Atlantic Forest, that are usually surrounded by sugarcane plantations, are currently remaining (RANTA *et al.* 1998). Such monocultures do not offer any flower resource or nesting sites required by the bees, therefore isolating the fragments of forest. To guarantee the reproductive success of plants that depend on pollination by euglossine males like those of *Catasetum* Rich. ex Kunth., 1932, *Stanhopea* Frost ex Hook., 1829 and *Gongora* Ruiz & Pavón, 1794 species, flights of these males among forest fragments are necessary (MARTINI *et al.* 2003).

Euglossine bees are known to be long-distance pollinators in closed rainforests where flight ranges of more than 20 km have been demonstrated (JANZEN 1971, KROODSMA 1975, ACKERMAN *et al.* 1982). On the other hand, there is controversial information concerning to the flight ranges of these bees between forest fragments and the relationships between fragment size and abundance and species richness of Euglossini (POWELL & POWELL 1987, BECKER *et al.* 1991, FRANCESCHINELLI *et al.* 2003, TONHASCA *et al.* 2003). To get information on the flight behavior of euglossine males and on the relationship of the different euglossine species to forest habitats we tested attraction to scent baits in the extreme landscape of forest fragments inside a matrix of sugarcane monoculture in Pernambuco and asked the following questions: 1) Do Euglossini males leave the forest to collect fragrances outside the forest?, 2) Are there differences among the euglossine species in relation to their dependence on forest habitats?, and 3) To what distance do the male bees advance into sugarcane fields in search of fragrances?

## MATERIAL AND METHODS

### Study site

The field study was carried out in the forest fragment "Mata de Bujary" (476 ha, altitude 95 m MSL, 07°36.403'S, 34°58.925'W), municipality of Goiana, an area in the "Zona da Mata" region of Pernambuco, where vast sugarcane fields prevail and surround fragments of native Atlantic Rainforest. The studied forest remnant was separated from the next Atlantic Rainforest fragment by a distance of at least two kilometers (Fig. 1).

### Sampling of Euglossini

The following seven sampling points were established along a transect: a) within the forest fragment, b) at the forest edge, c) within the sugarcane plantation 10 m distant from the forest edge, d) 50 m distant, e) 100 m distant, f) 250 m distant, and g) 500 m distant.



Figure 1. Satellite image with the rainforest fragment "Mata de Bujary" (1) surrounded by sugarcane monocultures (3). The smallest distance to the neighboring fragment is 2 km (2). Transect with the sampling points (4).

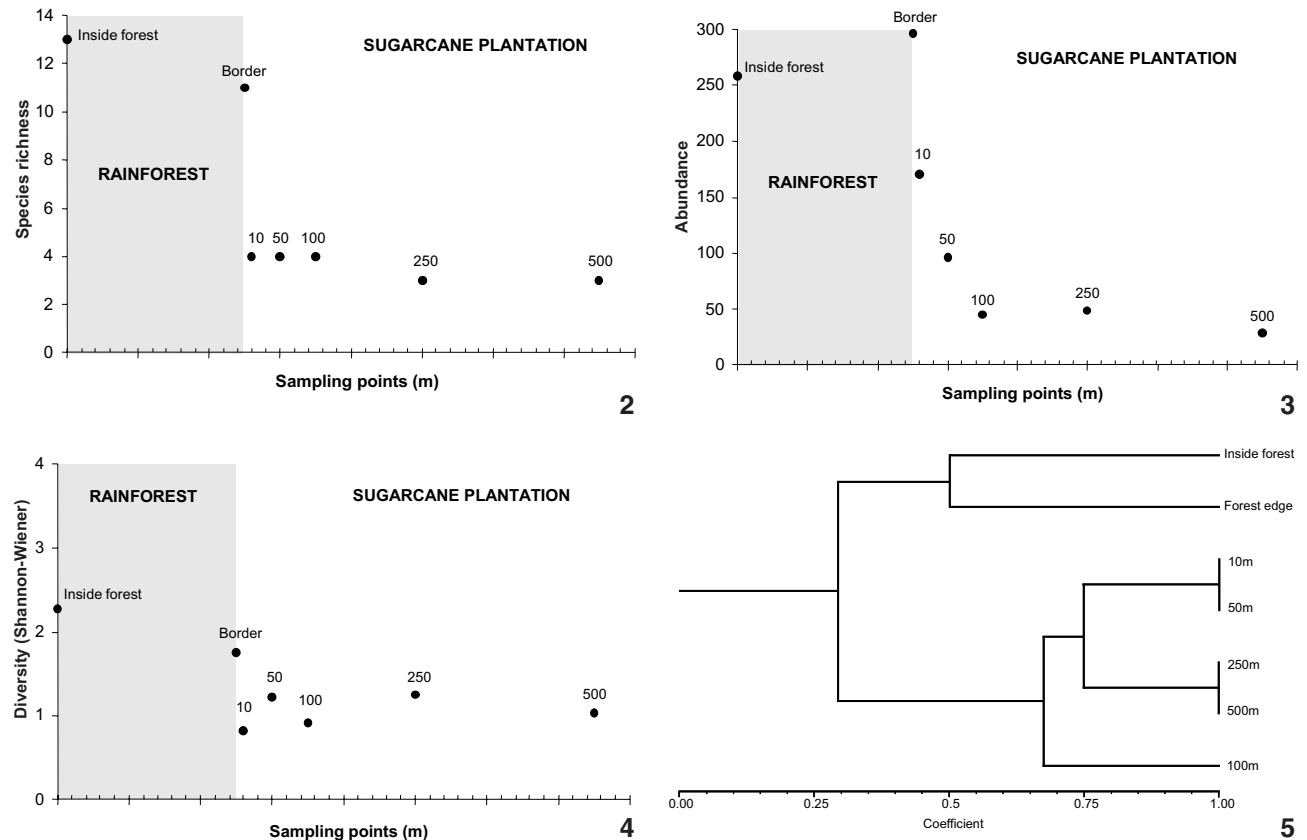
Five samples were collected on the following days: 25/XI/2002, 09/XII/2002, 17/III/2003, 16/IV/2003 and 01/VIII/2003. The euglossine males were attracted by scent baits (pieces of filter paper containing droplets of fragrance solution) between 9:00 h and 12:00 h, at peak bee activity. The traps were posted in trees within the forest, at its edge and in sugarcane plants inside the plantations, allowing a minimum distance of three meters between them. The baits were monitored simultaneously with one person at each point. Five fragrances that proved to be effective in attracting euglossine males at the study site were used at all points (benzyl acetate,  $\beta$ -ionone, skatol, eugenol and methyl salicylate). The bees were caught with entomological nets and stored in glasses containing droplets of ethyl acetate and later identified in the laboratory by comparison with the reference collection of the workgroup. The bees were deposited in the Entomological Collection of the Federal University of Pernambuco, Recife. Collection data were entered into a database program.

### Data Analysis

We calculated the Shannon-Wiener index of diversity ( $H'$ ) to the base  $\log_2$  for each sampling point. The Shannon-Wiener indices of diversity were compared using Hutcheson's t-test (ZAR 1996).

The frequency of species and individual abundance between the sampling points was analyzed through G-test for one sample – adherence (ZAR 1996). This test was performed with the software Systat 8.0 (WILKINSON 1998).

We used Jaccard index (KREBS 1989) through NTSYSpC 2.01t software (ROHLF 2000) to evaluate qualitatively the bees' similarity between each sampling point. From the pairwise similarity we generated a single-linkage dendrogram (VALENTIN 2000), where the sampling points were grouped by UPGMA (Unweighted Pair Group Method with Arithmetic mean) method.



Figures 2-5. Euglossini at seven monitoring points on a transect in "Mata de Bujary", NE, Brazil. The points were on a transect from closed rainforest into sugarcane plantations. The distance between sampling point "forest" and sampling point border was 250 m. (2) Species richness; (3) abundance; (4) diversity (Shannon-Wiener index); (5) Similarity (Jaccard) of Euglossini bees at the different sampling points.

## RESULTS

A total of 945 individuals of Euglossini were recorded: 259 inside the forest, 297 at the edge, 171 at a distance of 10m from the edge, 96 at 50 m, 45 at 100 m, 49 at 250m and 28 at 500 m distance. The bees belonged to 16 species: 4 to *Eulaema* Lepeletier, 1841, 9 to *Euglossa* Latreille, 1802, 2 to *Exaerete* Hoffmannsegg, 1817, and 1 to *Eufriesea* Cockerell, 1908 (Tab. I).

Males of only five euglossine species left the rainforest: *Euglossa* (*Euglossa*) *cordata* Linnaeus, 1758, *E. (Euglossa) violaceifrons* Rebêlo & Moure, 1995, *Eulaema* (*Apeulaema*) *nigrita* Lepeletier, 1841, *E. (Apeulaema) cingulata* Fabricius, 1804 and *E. (Eulaema) flavescens* Friese, 1899. Only *E. cordata*, *E. nigrita* and *E. cingulata* were collected at 250 m and 500 m distances from the forest edge. On the other hand, bees like *Exaerete frontalis* Guérin, 1845, *E. smaragdina* Guérin, 1845, *Eufriesea mussitans* Fabricius, 1787, *Eulaema* (*Eulaema*) *bombiformis* Packard, 1869, *Euglossa* (*Euglossella*) *augaspis* Dressler, 1982, *E. (Glossurella) crassipunctata* Moure, 1968, *E. (Euglossa) gaianii* Dressler, 1982, *E. (Glossura) ignita* Smith, 1874, *E. (Euglossa) iopyrrha*

Dressler, 1982, *E. (Euglossella) perpulchra* Moure & Schindwein, 2002 and *E. (Euglossa) securigera* Dressler, 1982, were not recorded outside the forest (Tab. I).

Species richness ( $G = 15.389$ ,  $g.l = 6$ ,  $p = 0.0209$ ) and abundance ( $G = 534.95$ ,  $g.l = 6$ ,  $p = 0$ ) were significantly different between the sampling sites (Figs 2 and 3). The same was true for diversity (Shannon-Wiener index, Fig. 4). The sampling point inside the rainforest showed the highest species richness (13 species) and diversity ( $H' = 2.27$ ), followed by the point at the edge (11 species and  $H' = 1.76$ ). The total diversity of all points together was  $H' = 2.06$ .

The sampling point inside the forest showed a high similarity to the edge point, whereas the sampling points inside the sugarcane plantation were very similar to each other and dissimilar to the forest and edge points (Fig. 5).

The scent which attracted the highest number of individuals was  $\beta$ -ionone (63.8%), followed by skatol (22.9%) and benzyl-acetate (8%) (Tab. II). Methyl salicylate, eugenol and benzyl-acetate baits showed poor attractivity outside the forest (Tab. II).

Table I. Species of Euglossini with number of males recorded during simultaneous collection with scent baits at seven sampling points on a transect from closed rainforest into sugarcane plantation to 500 m distant from the forest edge.

Species	Sampling points						
	Forest	Edge	10 m	50 m	100 m	250 m	500 m
<i>Eufriesea mussitans</i>	1	0	0	0	0	0	0
<i>Euglossa cordata</i>	121	181	140	64	36	27	20
<i>Euglossa ignita</i>	8	3	0	0	0	0	0
<i>Euglossa iopyrrha</i>	3	0	0	0	0	0	0
<i>Euglossa securigera</i>	0	2	0	0	0	0	0
<i>Euglossa augaspis</i>	2	3	0	0	0	0	0
<i>Euglossa gaianii</i>	4	1	0	0	0	0	0
<i>Euglossa crassipunctata</i>	0	1	0	0	0	0	0
<i>Euglossa violaceifrons</i>	1	6	0	0	1	0	0
<i>Euglossa perpulchra</i>	1	0	0	0	0	0	0
<i>Eulaema cingulata</i>	53	50	5	6	1	3	1
<i>Eulaema bombiformis</i>	8	0	0	0	0	0	0
<i>Eulaema flavescens</i>	6	6	1	1	0	0	0
<i>Eulaema nigrita</i>	47	43	25	25	7	19	7
<i>Exaerete frontalis</i>	4	0	0	0	0	0	0
<i>Exaerete smaragdina</i>	0	1	0	0	0	0	0
Total	259	297	171	96	45	49	28

Table II. Number of individuals of Euglossini collected at the seven sampling points by scent.

Scent	Sampling points						
	Forest	Edge	10 m	50 m	100 m	250 m	500 m
Skatol	60	56	28	30	9	25	8
Methyl salicylate	23	5	0	0	0	0	0
$\beta$ -Ionone	135	185	141	64	36	22	20
Benzyl-acetate	30	42	2	1	0	1	0
Eugenol	11	9	0	1	0	1	0
Total	259	297	171	96	45	49	28

## DISCUSSION

The baiting experiment shows that the different species of Euglossini have different connections to the forest. To males of 11 out of a total of 16 species, the sugarcane plantation seems to be a barrier to their activity range. These species are closely bound to forest habitats and the males seem to avoid flying over open areas. These species should be more affected by Atlantic Rainforest destruction and fragmentation. Nevertheless, this conclusion has to be treated with care, as some of the forest-restricted species were rare.

In the Brazilian Amazon, POWELL & POWELL (1987) showed that the conversion of rainforest to pasture forms barriers to

euglossine males. BECKER *et al.* (1991) suggest that the bees' size is related to the distance between fragments that they can cross. In this context, males of *Eulaema bombiformis* or *E. flavescens* should be more capable to leave the forest than smaller bees. The present results show, however, that the different connections of the species of Euglossine to closed forest are not related to body size. Males of the considerably smaller *Euglossa cordata* occurred in high numbers at the most distant sampling points while no male of *Eulaema bombiformis* was recorded outside the forest.

Males of species that were frequently recorded at the sampling points in the sugarcane plantation, like *Eulaema nigrita*, *E. cingulata* and *Euglossa cordata* show a high flexibility and capacity of adaptation to disturbed areas. Several studies showed

that *Eulaema nigrita* and *Euglossa cordata* occur in urban environments where they were also found building nests (ZUCCHI *et al.* 1969, WITTMANN *et al.* 2000, SILVEIRA *et al.* 2002, DARRAULT *et al.* 2003). Males of these species should be able to link isolated rainforest fragments.

In other studies related to the flight behavior of euglossine males in fragmented habitats *Euglossa cordata*, *Eulaema nigrita* and *E. cingulata* were also sampled outside forests (RAW 1989, WITTMANN *et al.* 2000). In an experiment of marking and recapture, TONHASCA *et al.* (2003) showed that these species cross open areas between fragments of Atlantic Rainforest in the state of Rio de Janeiro. Moreover, *Euglossa cordata* is frequently collected in the region of open *Caatinga* (succulent thorn-shrub savannah) in Northeastern Brazil (NEVES & VIANA 2003).

All studies were performed attracting males with scent baits. Unfortunately, there is only little information on female behavior due to the difficulty of sampling. This would be especially important because the activity range of females is limited by the nest locality. Females make foraging flights and always return to their nest, while males can move by chance in a given region (JANZEN 1971, ACKERMAN *et al.* 1982).

JANZEN (1971) showed that females of *Euplusia surinamensis* (Linnaeus, 1758) returned to their nest when released more than 23 km away. This activity range, however, was determined in an area of continuous rainforest. In regions where forest habitats are restricted to distant fragments, most of the euglossine species seem to stay in the fragments, avoiding open areas. In this condition they consequently may not act as long-distance pollinators.

The reduced or absent flights between forest fragments certainly have a negative effect on the reproductive success of plants that depend on pollination by euglossine bees, especially orchids with perfume flowers. In small forest remnants, the original population of these orchid species is reduced to tiny rest-populations, which may lose their capacity of reproduction. Such a small rest-populations of *Gongora quinquenervis* (Orchidaceae), for instance, showed no fruit set despite of a 100% pollinaria removal rate by *Euglossa cordata* and *E. perpulchra* in a small forest fragment near the present study site (MARTINI *et al.* 2003). Species of *Catasetum* (Orchidaceae) are threatened in the same way, as these dioecious orchids in the Atlantic Rainforest of Pernambuco are pollinated exclusively by males of *Eulaema bombiformis* (CARVALHO & MACHADO 2002), bees that in our study did not leave the closed rainforest.

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