

ECOLOGY, BEHAVIOR AND BIONOMICS

Eufriesea violacea (Blanchard) (Hymenoptera: Apidae): an Orchid Bee Apparently Sensitive to Size Reduction in Forest Patches

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Eufriesea violacea (Blanchard) (Hymenoptera: Apidae): uma Abelha das Orquídeas Aparentemente Sensível à Redução no Tamanho dos Fragmentos Florestais

RESUMO - *Eufriesea violacea* (Blanchard) é uma espécie de Euglossini bastante sazonal, de ocorrência mais frequente no Sul e Sudeste do Brasil. Vários estudos revelaram grandes variações na abundância de machos de *E. violacea* em remanescentes de Mata Atlântica dessas duas regiões brasileiras. Neste artigo, relatamos variações na abundância de machos de *E. violacea* de vários fragmentos florestais de tamanhos distintos (variando de 10 a 580 ha), e sugerimos que a espécie seja provavelmente sensível à redução no tamanho da área do fragmento florestal. As amostragens foram realizadas em nove remanescentes florestais de Mata Atlântica, localizados no Norte do Paraná, Sul do Brasil. Machos euglossíneos foram coletados com rede entomológica durante suas visitas às iscas-odores, entre 10:00h e 13:00h, de outubro a dezembro de 2001 e 2006. O total de 360 machos de *E. violacea* foi coletado nos fragmentos estudados. O número de abelhas atraídas às iscas em cada área variou de zero a 261 indivíduos. O tamanho do fragmento foi proporcionalmente relacionado ($r = 0,993$) à taxa de visitação de machos de *E. violacea* nas diferentes áreas, sendo que o maior número médio de machos (43,5) por amostragem foi observado para abelhas do fragmento florestal maior. Embora hipóteses alternativas para o declínio na abundância ou ausência de *E. violacea* em fragmentos florestais menores não devam ser descartadas, esses resultados indicam que populações de *E. violacea* necessitam de áreas florestais maiores para sua sobrevivência.

PALAVRAS-CHAVE: Floresta Atlântica, abelha euglossina, Euglossini, abundância de abelhas, neotrópico

ABSTRACT - *Eufriesea violacea* (Blanchard) is a very seasonal euglossine species, more frequently found in the southern and southeastern regions of Brazil. A number of studies have revealed large variations in the abundance of males of this species present in Atlantic Forest remnants throughout both regions. In this paper, we report variations in the abundance of *E. violacea* males sampled in several forest patches of different sizes (ranging from 10 to 580 ha), and we propose that this species is possibly sensitive to the reduction in size of forest remnants. Surveys were carried out in nine forest remnants of Atlantic rainforest located in northern Paraná State, southern Brazil. Male euglossine bees were collected with an entomological net when visiting scent-baits, between 10:00 am and 1:00 pm, from October to December of 2001 and 2006. A total of 360 *E. violacea* males were captured in the nine forest fragments studied. The number of bees attracted to scent baits in each forest patch varied from zero to 261. A very high association ($r = 0.993$) was detected between the forest patch size and the visitation rate of *E. violacea* males at different sites, with the highest mean number of males visiting baits/sampling (43.5) being observed for bees from the largest forest remnant. Although alternative hypothesis should not be discharged for the decline in the abundance or absence of *E. violacea* in small forest patches, our results indicate that populations of this euglossine species need larger forest areas for existing.

KEY WORDS: Brazilian Atlantic rainforest, euglossine bee, Euglossini, bee abundance, Neotropics

Orchid bees (Apidae: Euglossini) are shy, fast-flying bees, rarely collected at flowers (Dressler 1982, Nemésio & Silveira 2007a), and are considered elusive pollinators throughout the American tropics (Cameron 2004), where

they are visitors of a large number of plant families including Orchidaceae (Dressler 1982, Roubik & Hanson 2004). The group is primarily native from Mexico to northern Argentina (Dressler 1982, Roubik & Hanson 2004), but occurrence of

euglossine bees have also been reported to USA (Minckley & Reyes 1996, Pemberton & Wheeler 2006).

There is evidence indicating that forest fragments can undergo a decline in the number of males in some euglossine species (Powell & Powell 1987, Morato 1994, Sofia & Suzuki 2004). Recently, Parra-H & Nates-Parra (2007) suggested that it is possible to determine habitat quality using data obtained from orchid bee array.

Eufriesea violacea (Blanchard) is a robust, medium-size bee (ca. 16 mm long), with a metallic integument, predominantly green with violet parts in males and violet colour in females. Considered an endemic species to the Atlantic Forest (Nemésio & Silveira 2007b), the occurrence of *E. violacea* has been documented mainly to the southern and southeastern regions of Brazil (Peruquetti & Campos 1997, Rebêlo & Garófalo 1991, Sofia & Suzuki 2004, Wittmann *et al* 1989). While females of *E. violacea* exhibit solitary nesting behaviour and nest in natural and man-made cavities, frequently constructing nests in aggregations (Peruquetti & Campos 1997), males are usually attracted to scent-baits of eucalyptol, vanillin and others chemicals during the wet-warm season (Wittmann *et al* 1989, Sofia & Suzuki 2004, Uehara & Garófalo 2006). This species has one generation per year (Wittmann *et al* 1989), with males usually emerging in September and females after October (Peruquetti & Campos 1997). Like other *Eufriesea* species, *E. violacea* spend most of the annual cycle as diapausing prepupae (Dressler 1982, Kimsey 1982, Uehara-Prado & Garófalo 2006).

Although several authors have usually considered species of *Eufriesea* as rare (e.g., Kimsey 1982, Nemésio & Silveira 2004, Nemésio 2005), a number of studies carried out in Atlantic Forest remnants in Brazil have revealed surprisingly high frequencies of males of *E. violacea* (Wittmann *et al* 1989, Sofia *et al* 2004), representing above 50% of the euglossine samples in these studies. This fact attracted the attention of Nemésio & Silveira (2007b), who pointed out the variation in abundance of this species in different studies (Wittmann *et al* 1989, Rebêlo & Garófalo 1997, Nemésio 2004, Sofia *et al* 2004). Comparing the euglossine fauna from three forest remnants in southern Brazil, Sofia & Suzuki (2004) detected that a reduction in fragment size negatively affected the frequency of *E. violacea* males, suggesting that this species could be a possible bioindicator of habitat quality.

According to Roubik (2001), population studies of bees allow for a graphic understanding of trends or possible decline in pollinator abundance, and perhaps more fundamentally, the kinds of abundance variations that can be expected. In this paper, we report the variation in the abundance of *E. violacea* males sampled in nine forest patches of different sizes and suggest that this species is sensitive to reduction of forest remnant size.

Material and Methods

Study sites. This study was conducted in nine forest fragments located in northern Paraná State, in southern Brazil (Fig 1). The dominant vegetation in the region is the semi-deciduous forest, a tropical seasonal forest that represents an ecosystem of the Atlantic Forest (Morellato & Haddad 2000). The local climate is classified as Cfa, humid subtropical with

warm summer, with a mean annual temperature of 21°C. The mean annual rainfall is 1,600 mm (Mendonça 2000), where rain is distributed in all the seasons, with a decline in rainfall in the winter (June, July and August).

Of the nine sites studied, five constitute areas on privately owned ranches, showing different sizes, as follows: two large forest fragments (> 100 ha), named sites A and B; one medium-size fragment (86 ha), site C; and two small forest fragments (10-15 ha), corresponding to sites D and E (Table 1). These fragments are areas where a minimum of 20% of the land must be preserved as wooded areas on private properties, established by a Brazilian Federal law. The vegetation of these fragments has been selectively logged for many decades.

Two forest fragments constitute governmental reserves: Parque Estadual Mata dos Godoy - GF (site F) and Parque Municipal Arthur Thomas (site G). GF is a governmental reserve that includes an area of 580 ha of very well preserved native vegetation. It has a wooded area that is more or less connected with other native forest fragments situated outside the protected area of the park, constituting a forest fragment with a total area of around 2800 ha (Sofia *et al* 2005). Parque Municipal Arthur Thomas consists of 66 ha and is situated within the urban area of Londrina, a city of around 500,000 inhabitants. The last two forest patches are also located inside an urban area: one of them corresponding to 13.4 ha is situated at the campus of the Universidade Estadual de Londrina (site H) and a forest patch of around 18 ha surrounding an urban stream (site I) (Fig 1 and Table 1). All forest fragments exhibit signs of past and present anthropogenic disturbance and most part of them are surrounded by crop and pasture areas.

Methods and analysis. Male euglossine bees were collected with an entomological net after they were attracted to scent-baits of eugenol, eucalyptol and vanillin, between 10:00 am and 1:00 pm (2h of sampling/site), from November to December of 2001 and from October to December of 2006, which is the period of higher activity of *E. violacea* in the northern part of Paraná State (Sofia & Suzuki 2004, Sofia *et al* 2004).

To make the census more effective and minimize sampling error caused by weather, only clear and warm days were chosen for samplings (Powell & Powell 1987). The baits were placed in the shade, hung on strings tied to vegetation in the border of the fragment. Chemicals were replenished on the baits every half hour, in order to maintain a high and homogeneous attractiveness (Uehara-Prado & Garófalo 2006). Only one site per forest fragment was established for samplings. After being collected at the baits, the easily identifiable *E. violacea* males were marked with a dot of paint (Posca: Mitsubishi Pencil Co., Ltd.) on the thorax and released. The procedure of marking the bees was employed to avoid counting the same bee more than once. Only a few individuals of *E. violacea* were collected and voucher specimens were deposited at the Zoology Museum of the Universidade Estadual de Londrina (MZUEL).

Visitation rate (mean number of bees/sampling) was used to compare possible variation in *E. violacea* abundance in different forest patches. The statistical analyses included the chi-square test (one-sample) and the Kruskal-Wallis test (which was based on the median values of number of males attracted to chemical baits) (Siegel & Castellan

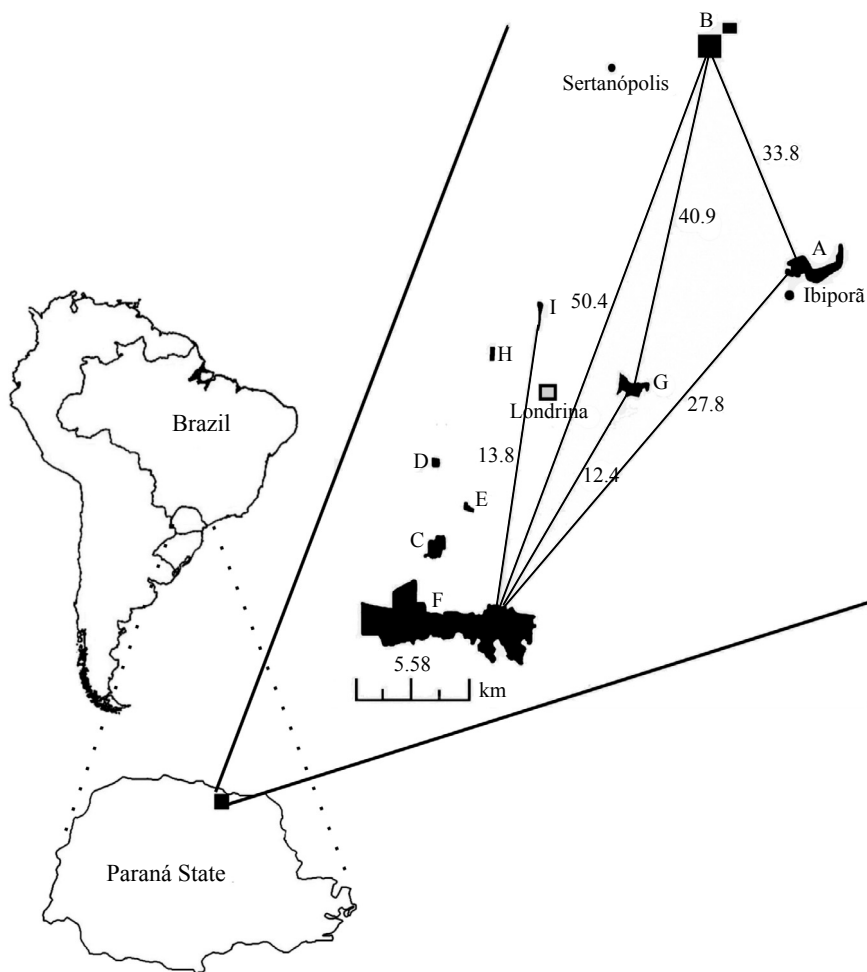


Fig 1 Map of the study area showing the nine forest fragments sampled for abundance of *E. violacea* males. Only forest fragments A and B are out of scale. The numbers in the figure correspond to the distance in kilometers between forest fragments. Distances between forest fragments: A-B (33.8 km), A-F (27.8 km) B-F (50.4 km) and B-G (40.9 km).

1988). Also, the association between mean number of bees captured per sampling and fragment size data was examined for significance using a correlation coefficient test. All values were considered significantly different if $P < 0.05$.

Results and Discussion

A total of 360 *E. violacea* males were captured in the nine forest fragments studied. The number of bees attracted to scent baits in each forest patch varied from zero (sites D, H and I) to 261 at site F, which showed the highest mean number of males visiting baits/sampling (43.5) amongst all forest fragments (Table 1). Despite variation in the number of samplings per area (ranging from $n = 3$ to $n = 6$), there was an obvious wide variation between some forest fragments in the number of individuals collected ($\chi^2 = 202.144$, $P < 0.0001$; $H = 31.380$, $P < 0.001$, $df = 8$). The three larger forest fragments (A, B and F) showed some variation in the number of bees attracted to scent baits among the different samplings (Fig 2). However, a high correlation ($r = 0.993$) was detected between forest patch size and visitation rate of *E. violacea*

males surveyed at baits at different sites (Fig 3).

Euglossine bees have been extensively recognized as strong fliers, especially the larger euglossine species (Janzen 1971, Dressler 1982). As a medium-sized and robust bee *E. violacea* is potentially able to fly long distances. However, in a fragmented landscape, the matrix has strong influence on fragmented populations, such as precluding the movement of some species (Laurence *et al* 2002). On the other hand, the movement of euglossine bees between forest fragments, crossing open areas, has been reported by different authors (Raw 1989, Murren 2002, Tonhasca *et al* 2003).

In the present study, it is possible that the geographic proximity of site F (the largest and best preserved site surveyed) favours small forest patches located in the vicinity, such as sites C and E, located respectively 1.3 km and 4.2 km from the site F fragment. Thus, site F could be functionally acting as a "mainland" and a source of visitors to small fragments located in close proximity (Nol *et al* 2005). According to Castro & Fernandes (2004), although large forest fragments may be a potential source of colonizers and/or immigrants, these events should be much more frequent among fragments due to the much shorter

Table 1 Characteristics of forest patches (size in hectares, location, type of reserve and vegetation), number of samplings per site and total of *E. violacea* males sampled in each study site and visitation rate (number of bees/sampling) from November to December/2001 and from October to December/2006.

Forest fragment (site)	Size (ha) (type)	City (geographic coordinate)	Reserve	Type of vegetation	Number of samplings	Total of males (visitation rate)
A	170 (large)	Ibiporã (23°16' S; 51°03' W)	Private	Primary	5	42 (8.4)
B	180* (large)	Sertanópolis (22°59'S; 50°59'W)	Private	Secondary	3	35 (11.7)
C	86 (medium)	Londrina (23°25'S; 51°14'W)	Private	Secondary	3	10 (3.3)
D	14.6 (small)	Londrina (23°22'S; 51°14'W)	Private	Secondary	3	0
E	10.2 (small)	Londrina (23°23'S; 51°13')	Private	Secondary	5	8 (1.6)
F	580 (+ 2200) (large)	Londrina (23°27' S; 51°15' W)	Governmental	Primary	6	261 (43.5)
G	66 (medium)	Londrina (23°15' S; 51°15'W)	Governmental	Secondary	4	8 (2.0)
H	13.4 (small)	Londrina (23°19'S; 51°12'W)	Urban	Secondary	4	0
I	18.5 (small)	Londrina (23°19'S; 51°10'W)	Urban	Secondary	4	0

*Fragment size = the sum of two smaller fragments, showing 66.7 and 117.3 ha, sited apart 230 m and considered as a single one.

distances that separate them from each other. Assuming that *E. violacea* from a large forest fragment (site F) are moving among small fragments in the vicinity, it means that the well-preserved conditions of this forest patch, compared to all other eight fragments surveyed, are not preventing *E.*

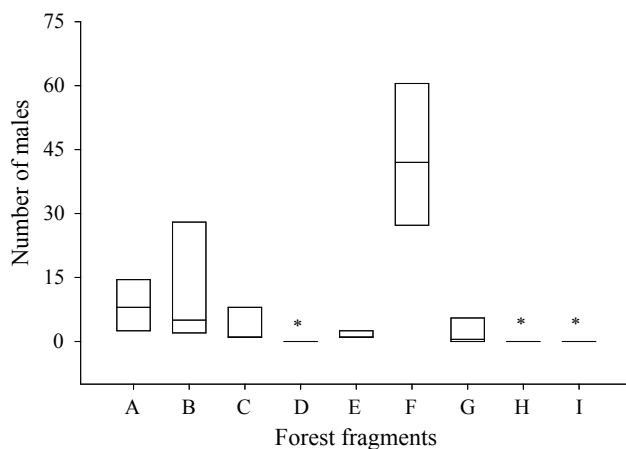


Fig 2 Variation in number of *E. violacea* males collected in different samplings, carried out in nine forest fragments studied (named A to I). Horizontal line inside the vertical box represents the median and the upper and lower limits of the box represent the percentiles (75% and 25%, respectively). Number of samplings per area varied from three to six. * = statistically different from fragment F ($P < 0.05$).

violacea males to exploit other forest patches. It has been suggested that the density of male euglossine bees may be higher at sites with greater resource availability, but the local concentration of resources does not prevent bees to access nearby areas (Tonhasca *et al* 2002a). Also, Murren (2002) attributed the reproductive success of island populations of *Catasetum viridiflavum* Hooker (Asparagales, Orchidaceae), a euglossine bee-pollinated orchid species, to mainland populations of *Eulaema cingulata* (Fabricius) (Hymenoptera, Apidae), which were found to fly across water to reach bait traps available on the island. According to this author, males

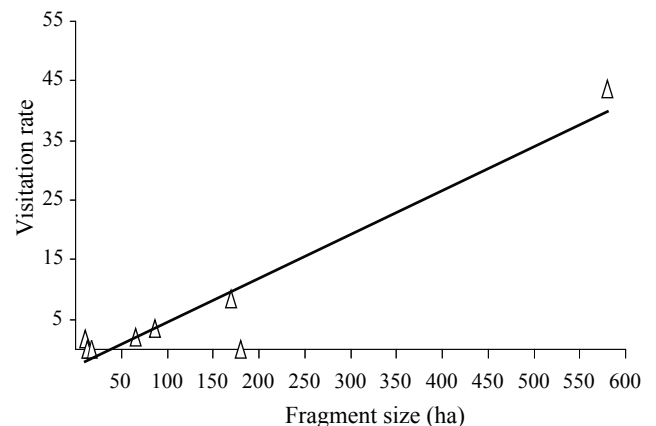


Fig 3 The interaction between visitation rate (mean number of bees/sampling) of *E. violacea* males and forest fragment size.

of *E. cingulata* are probably not island residents, but rather transient visitors from larger continuous forest areas.

Assessing the genetic structure of *E. violacea* males in three forest remnants of the Atlantic Forest in the study region of the current work, Sofia *et al* (2005) reported the occurrence of a single panmictic population among three forest fragments studied, attributing this finding to reduced history of fragmentation in the region (around 80 years) and to the potential ability of males to move among nearby forest patches. However, to better understand the foraging behaviour of *E. violacea* males, concerning movement between forest patches, future studies using marking and recapture methods must be considered (Raw 1989, Murren 2002, Tonhasca *et al* 2003).

In opposition to the above, the short distance between sites D and F was not sufficient to explain the absence of *E. violacea* at site D, which is also located close (around 2.5 km away) to the large fragment, site F (Fig 1). However, in a fragment containing a small population of inhabiting bees or occasionally visited by bees from the “neighbourhood”, the limited number of samplings per site could explain the failure in surveying bees at baits, but other fragmentation effects on local fauna cannot be ignored in this case.

Also, the high association detected between forest fragment size and visitation rate of euglossine males suggests that it is a potential area-sensitive species. Area-sensitive species by definition are species that prefer to breed in large tracts of forest; however, species may still be considered area-sensitive if their probability of occurrence increases with habitat patch size (Fraser & Stutchbury 2004). Even though population dynamics in unprotected habitats are less predictable or more compromised by exotic organisms (Roubik 2001), the abundance pattern observed for *E. violacea* in the present study makes this euglossine bee a possible area-sensitive species, corroborating the idea that euglossine bees are possible bioindicator of quality of environmental conditions (Peruquetti *et al* 1999, Tonhasca *et al* 2002b, Parra & Nates-Parra 2007).

While forest fragmentation is associated with population decline of euglossine bees (Powell & Powell 1987, Morato 1994, Sofia & Suzuki 2004), a higher euglossine bee abundance in 10 ha and 100 ha fragments compared to a continuous forest is reported in the literature, with a significant reduction in euglossine bees visiting baits occurring only in very small forest patches (1 ha) (Becker *et al* 1991). In a very long-term study of orchid-bees carried out in a protected tropical moist forest in Panama, Roubik (2001) demonstrated that even though populations of bees varied considerably between years, no decline in euglossine bees in the study area occurred over 20 years. They concluded that long-term studies substantially clarified results taken on a small temporal scale.

An alternative explanation for the absence or low number of males of *E. violacea* visiting baits at some forest patches may be the availability of alternative scent sources in these areas, such as a high concentration of resources (displayed as “hot spots”) (Armbruster 1993), reducing the chance of bees being attracted to baits. As males do not collect fragrances everyday, but do feed frequently, it seems logical to suppose nectar distribution as a main, but not sole, causal factor of capturing frequencies (Otero & Sandino 2003).

Our results indicate that populations of *E. violacea* need larger forest areas for existing. Therefore, the absence of *E. violacea* in some forest patches could indicate a high degree of disturbance, demonstrating the need for their better management. It is worth noting that in northern Paraná State these forest patches are among the last remains of the Brazilian Atlantic rainforest and as such probable reservoirs of local biodiversity.

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