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Long-Term Male Aggregations of *Euglossa melanotricha* Moure (Hymenoptera: Apidae) on Fern Fronds *Serpocaulon triseriale* (Pteridophyta: Polypodiaceae)

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Introduction

Male solitary bees do not generally pass the night in maternal nests like females, but tend to aggregate in other locations, forming communal dormitories (Alvesdos-Santos *et al* 2009). These dormitories are locations at which a number of individuals of the same or from different species gather to rest for a few hours, generally close to one another (Adams 1999).

Three types of communal dormitories have been identified in insects (Endringer *et al* 2004): facultative (formed during migrations), diapause (in response to seasonal variation) and circadian (nocturnal roosting of groups of male bees). In the latter case, the same sites may be used continuously over many years by successive generations of insects (Mallet & Gilbert 1995). In bees, this type of behavior has only been observed in males,

Abstract

A communal dormitory of male orchid bees, *Euglossa melanotricha* Moure, was monitored over a one-year period, when they passed the night in the fronds of a *Serpocaulon triseriale* (Polypodiaceae) fern. The bees used the same fronds continuously, moving to neighboring fronds as senescence set in. As many as 49 males were observed together on any one night, clinging to the midribs on the abaxial surface of up to five fern blades with their mandibles. A number of males returned to the same site to pass the night continuously over a number of months, and were observed making physical contact with one another without provoking agonistic behavior. Males of *E. melanotricha* appeared to prefer sleeping at sites close to nests and potential sources of odoriferous essences, such as orchids (Orchidaceae).

although the selection of sites is an unknown process.

The benefits of aggregation may include anti-predator defense (Treisman 1975, Hoogland & Sherman 1976, Azevedo & Faria Jr 2007), thermoregulation (Yom–Tov et al 1976), more efficient access to females (Wagner 1993) and the synchronous rearing or education of offspring (Emlen & Demong 1975). However, males may also simply congregate as a consequence of environmental restrictions, such as the scarcity of appropriate roosts, rather than behavioral or social factors (Endringer et al 2004). Evans & Linsley (1960) suggested that the gregarious behavior may have a protective function, but when males begin to congregate (preliminary concentration), they are more exposed to certain types of predators (Mallet & Gilbert 1995, Oliveira & Castro 2002, Endringer et al 2004).

A number of studies have focused on aggregations in Apoidea (Linsley 1962, Miyanaga & Maeta 1998,

Stehmann & Semir 2001, Alves-dos-Santos *et al* 2009), although most have involved relatively short-term and qualitative observations. The paucity of long-term studies, that have monitored dormitories over a number of years (Alcock 1998), or experimental approaches (Wcislo 2003) is probably due primarily to the difficulties of locating males in the wild and determining their preferred nocturnal roosts. In addition, males rarely use sites for more than a few days. In fact, most observations refer to the use of sites for only a single night. In the present study, aggregations of male orchid bees of the species *Euglossa melanotricha* Moure were observed continuously using fern fronds *Serpocaulon triseriale* as communal dormitories over an extended period.

Material and Methods

The present study was conducted in an urban residential area of 0.5 ha located in the municipality of Campo Formoso in the Chapada Diamantina region in the state of Bahia, Brazil (10°30′17.71″S,40°19′10.99″W). Local altitude ranges from 400 m to 800 masl. The region's climate is hot and semi-arid (BSh) (Köppen 1948), with annual precipitation of 302 mm to 1,935 mm, and a marked rainy season from March to August (Bahia 2010).

The orchid bees of the genus *Euglossa* are essentially Neotropical in distribution, with a total of 122 recognized species (Nemésio 2009). They are found from Paraguay to Mexico, but are absent from Chile and most of the West Indies, except for a single species in Jamaica, *Euglossa jamaicensis* (Silveira *et al* 2002, Moure *et al* 2007). *Euglossa melanotricha* is found in southern, central, and eastern Brazil (Moure *et al* 2007), typically in open vegetation, such as the savanna and semi-arid habitats of the Cerrado and Caatinga biomes, respectively. The species appears to be most abundant in Cerrado habitats (Nemésio & Faria Jr 2004, Alvarenga et al. 2007).

Serpocaulon triseriale, formerly known as Polypodium triseriale, is very common throughout most of Brazil (Labiak & Hirai 2010). It is frequently used as a decorative plant, and is tolerant to wide variations in humidity and temperature. Observations of the number of males present at the communal dormitory were initiated in January 2002, and continued until December 2003, when we finalized our observations. From October 2002 and onwards, daily records were kept of the time at which males began to occupy the site at the end of each afternoon, and when they left in the morning. Observations were conducted in two sessions, one between 05:00 am and 10:00 am (07:15 am \pm 01:30, n = 342), and the other between 4:30 pm and 8:50 pm (6:25 pm \pm 02:08, n = 331). During these observations, over a total of 600h, the temperature and relative humidity of the air were recorded using a thermo-hygrometer with an accuracy of 0.1. In January 2002, all males in the aggregation (n = 23) were marked with permanent opaque white ink on top of the torax, to determine which individuals returned to the site, and their frequency of visitation.

The bee species was identified by Dr. André Nemésio of the Laboratório de Ecologia e Sistemática de Abelhas, Depto de Zoologia, Univ Federal de Minas Gerais (UFMG), MG, Brazil, based on ten specimens, which were deposited in his collection. The plant species was identified by MSc Maria Lenise Silva Guedes, curator of the Herbário Alexandre Leal Costa, Univ Federal da Bahia, BA, Brazil, where the voucher specimen is deposited. Descriptive statistics (means and standard deviations) were provided by Microsoft Excel 2007, and Pearson's correlation coefficient was calculated in BioEstat 5.0.

Results

The aggregation of *E. melanotricha* males was first observed on January 11th, 2002, when 23 males were observed on two blades of a *S. triseriale* fern. All males abandoned the site in the following morning (07:00 am), but 30 specimens returned in the afternoon (4:00 pm).

Fifty-six cultivated orchids belonging to eight species were observed in the study area, which would flower at different times over the course of the year, although with some overlap in the flowering period (Fig 1).

Between October 2002 and October 2003, up to 49 males of *E. melanotricha* were observed occupying primarily five fern blades of *S. triseriale*. During this period, the mean number of males observed together in the dormitory varied was 9.2 ± 12.2 (range = 1-49, n = 342).

The first male landed on the *S. triseriale* frond between 4:30 pm and 08:50 pm (mean = 6:25 pm \pm 02:08 dp, n = 342), and other males would continue to arrive for around the next hour. The first bee clings with its mandibles to the midrib of the basal region of the blade (Fig 2). The hind

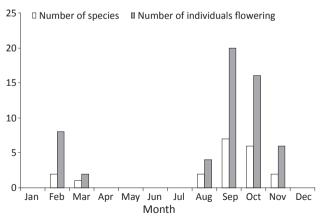


Fig 1 Orchid species observed at the study site in Campo Formoso, Brazil, and the number of individuals flowering each month between October 2002 and October 2003.



Fig 2 Aggregation of *Euglossa melanotricha* males on a *Serpocaulon triseriale* frond. Males preferred to rest at the midrib of the blades and were closely grouped.

legs of the male were free, and were rubbed continuously over the surface of its body, as if spreading some kind of substance over the body. The second male to arrive took up a similar position a little below the first male, the third just below the second, and so on.

This behavior would continue until the blade was fully occupied, with the last male located a little below the medial region of the blade. Males arriving later would begin to occupy a neighboring blade in the same manner. Males tended to be grouped very closely together, and would sometime interact, but were never aggressive towards one another. On a number of occasions, recently-arrived males were observed touching the antennae or other part of the body of a resting male when settling into position. The same fern fronds were used consecutively for up to 14 days, until their natural senescence impeded their use. At this point, the *E. melanotricha* males would begin occupying a new blade, generally beside the one previously occupied (Fig 2).

The mean number of males occupying the dormitory per night was higher during the dry season, between September and February (Z = 5.68, P < 0.0001, n = 395) (Fig 3). Despite this variation, no correlation was found between the number of males in the aggregation and either ambient temperature (r = 0.006, P = 0.90, n = 342) or humidity (r = 0.03 P = 0.65; n = 342). On rainy days, most of the males remained in the aggregation. Data on the activity of E. melanotricha males in forested areas within the same region as the study site indicate that they are active throughout the year, but are more abundant during the dry season, in particular in November and December (ACR Andrade-Silva, pers. comm.).

On sunny days, males dispersed from the dormitory at around 7:00 am and returned a little later (8:00 pm). On cloudy days, they left later, at around 10:00 am, and landed earlier, starting at 3:00 pm. Few males did not abandon the dormitory. Observations of the marked

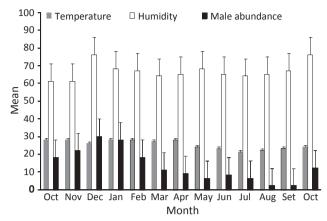


Fig 3 Abundance of *Euglossa melanotricha* males congregating on fronds of *Serpocaulon triseriale* between October 2002 and October 2003.

bees confirmed that while individuals did return to the dormitory to pass the night, there was no strict site fidelity. Of the 23 marked males, only five (21.7%) returned to the dormitory on seven consecutive nights, and only two (8.7%) were still using the site on the 17th day of observation.

Discussion

The use of the same roosts over long periods of time by different males may be related to the presence of many natural nests (N = 14) and the relative abundance of orchids, particularly Catasetum, Notylia and Ocyndium (Fig 1). These orchids are important sources of odoriferous essences to males, which visited them during the flowering period. We observed that more *E*. melanotricha bees are born in the warmer months and, as a consequence, there were more males in the roost. Alternatively, site fidelity would allow males to spend less time searching for an appropriate roost each night, allowing them to invest more energy the following day in the search for the odoriferous essences used to attract females. In addition, the presence of natural nests, associated with the certainty of the emergence of virgin females, and adequate overnight shelter would be extra incentives for the males to stay in this area.

There are few records in the Apoidea of males using the same dormitory over long periods (Alves-dos-Santos et al 2009). In fact, studies of males of Augochlorella neglectula (Cockerell) in Panama (Wcislo 2003) and Idiomelissodes duplocincta (Cockerell) in Arizona (Alcock 1998) recorded no sleeping site fidelity. However, the data indicate that this behavior may be related to the high mobility of males, which travel relatively long distances searching for virgin females or for areas with high concentrations of flowers towards which these females move on emerging from their nests, as suggested for

the aggregation of males in *Centris fuscata* (Lepeletier) (Azevedo & Faria Jr. 2007).

Females of *E. melanotricha* (Moure) usually construct their nests in ground holes, cavities in buildings or mounds (Sakagami *et al* 1967, Augusto & Garófalo 2007). Although the nesting behavior of females is well-known, the behavior of the males is rarely described. According to the classification of Alcock (1998), they congregate in dense aggregations, maintain contact with one another, and use the same dormitories continuously over periods of a number of months. The lack of agonistic interactions indicates that the species is characterized by some level of sociality, as recorded in other species of the superfamily Apoidea (Alcock 1998, Azevedo & Faria Jr 2007).

One interpretation of the continued use of the dormitory site is that males of *E. melanotricha* mark the *S. triseriale* fronds with pheromones in order to facilitate the task of locating the site in the following afternoon. In this case, the male that "decides" to return to the dormitory earlier will be able to find the site relatively easily, and then signal to the following males. The first male presumably releases a signal odor, which indicates its location to other males, allowing these individuals to follow the trail and aggregate at the site. Males that arrive earlier will be able to occupy the internal portion of the blade, where they will be better protected from nocturnal predators.

Most of the hypotheses used to explain aggregating behavior in males of Apoidea favor anti-predator strategies and thermoregulation (Miy Anaga & Maeta 1998, Alves-dos-Santos 1999, Stehmann & Semir 2001, Oliveira & Castro 2002, Alves-dos-Santos et al 2009). The present study indicates that the local abundance of natural nests, the eclosion of virgin females throughout the year, a safe shelter to spend the night and the availability of floral essences may have favored the persistence of males of *E*. *melanotricha* in the area for such a relatively long period of time. However, further studies are needed to investigate the frequency of aggregations of *E. melanotricha* males in urban areas, and whether the prolonged use of a specific substrate is related to the availability of locations with concentrations of orchids or is a common behavior in the species. It would also be important to verify whether male marking may affect their behavior, and whether marked males abandon the dormitory due to the loss of their characteristic odor, impeding their recognition by the other members of the "group".

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