

Short Communication

Bait traps remain attractive to euglossine bees even after two weeks: a report from Brazilian Atlantic forest


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ABSTRACT

Bait traps are effective and commonly used method in the studies of orchid bees. Important questions in the context of this method, including those related to bait dispersion, how long baits remain attractive, the distance from which males are supposed to be attracted to lures and so on, are still open subjects. Data on the attractiveness of bait traps that have remained in the field during two weeks in a large Atlantic forest preserve are presented. Four main results emerge from the data: (i) the abundance of specimens collected per day decreased in all the attractants as the traps were left on the field; (ii) despite this decrease, the absolute number of individuals collected after eight and fifteen days is remarkably, mostly in eugenol and vanillin baits; (iii) the vast majority of species, 22 of 25, was already collected on the first sample day; (iv) a consistent variation in the relative abundance of individuals collected in each scent as collections were made. We urge that bait traps should not be left in the field beyond what is strictly necessary since there is a real possibility of collecting a significant number of individuals as these traps remain available.

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Orchid bees (Hymenoptera, Apidae, Euglossina) have been regarded as an effective model for studying several ecological questions (e.g. [Nemésio and Silveira, 2006](#)). One of the main reasons behind this practice is the possibility of attracting euglossine males to synthetic compounds that mimic the resources they gather from natural sources ([Dodson et al., 1969](#)). It seems crystal clear there is an overall methodology for collecting orchid bees, but a simple analysis of papers presenting local surveys of euglossine bees reveals that the way attractants are used is quite variable from one study to another, regarding, for instance, the number of used scents, the selected scents, the distance baits are placed from the ground and apart each other, if baits are offered in traps or hung for direct capture by insect nets and so on ([Nemésio, 2012; Faria et al., 2015](#)).

Bait trapping euglossine bees is itself a rather controversial issue. Active sampling (i.e. sampling euglossine bees through insect nets) is regarded to collect significantly more bee species than passive sampling (through bait traps) and leads to significantly different species composition ([Nemésio and Vasconcelos, 2014](#)). On the other hand, it is undeniable that this latter method increases per capita sampling effort, particularly when large areas or many sites are surveyed simultaneously ([Mattozo et al., 2011](#)) and/or

when robust sampling designs are required in terms of replication ([Sydney and Gonçalves, 2015](#)).

In studies where bait traps are employed for attracting males, for instance, these traps are sometimes offered to bees during a given period of a single day (e.g. [Bezerra and Martins, 2001; Ramalho et al., 2009](#)), or traps are left in the field overnight (e.g. [Botsch et al., 2017; Coswosk et al., 2018](#)) and even longer (e.g. 3–4 days; [Storck-Tonon et al., 2009; Knoll and Penatti, 2012](#)). Replenishing of chemicals on the baits is also a common concern in euglossine assessments (see e.g. [Sofia and Suzuki, 2004; Nemésio and Silveira, 2006; Silveira et al., 2011](#)). This practice is regarded to be a way of trying to ensure that compounds with different volatility profiles could be evenly available to males (e.g. [Uehara-Prado and Garófalo, 2006](#)), since alcohol-based scents would disperse faster than oil-based scents (see [Nemésio, 2012](#)). [Nemésio \(2012\)](#) presented and extensively discussed some methodological concerns in ecological studies with orchid bees, including those related to bait dispersion, distance from which males are supposed to be attracted to lures and so on. The main conclusion one can draw from the vast controversy presented by [Nemésio \(2012\)](#) is that we do not entirely understand how baits work. In particular, one key aspect has to do with the duration of the attractiveness of these baits to males or how long bait traps should be offered to bees for sufficient sample, although a suggestion that this period may be as long as multiple weeks was given by [Roubik \(1993\)](#). However, two relevant

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questions should be stressed: (i) Roubik (1993) presented information only on the attractiveness of cineole, while we present data on seven compounds; and, moreover, (ii) Roubik (1993) reported the use of 8–15 mL of cineole per trap, while we just utilized cotton swabs soaked with a given aromatic compound (see methods below).

This short communication aims at presenting some general data on the attractiveness of bait traps that were left in the field during two weeks in a large Atlantic forest preserve.

Fieldwork was carried out at Reserva Natural Vale (RNV), a large remnant of Brazilian Atlantic forest, encompassing ca. 22,700 ha in the municipalities of Linhares, Jaguaré and Sooretama (19°06'–19°18'S and 39°45'–40°19'W), northern Espírito Santo state, Brazil (Lopes and Mello-Silva, 2014). Reserva Natural Vale, together with Reserva Biológica Sooretama (ca. 24,000 ha), are by far recognized (Amorim, 1984) as the last Tabuleiro forest massif in northern Espírito Santo (reviewed by Silva, 2014). Among the four natural vegetation types found in the region, Mata Alta (tall forest), which presents a thick canopy layer reaching up to 40 m, occupies ca. 70% of the total area of the Reserva Natural Vale (Peixoto et al., 2008). Baits were installed in this physiognomy.

A total of 175 bait traps (35 traps for each of the following seven attractants: benzyl acetate, β -ionone, eucalyptol, eugenol, methyl salicylate, methyl trans-cinnamate and vanillin) was offered to bees along an edge-interior transect within the fragment. Complete description of bait traps and experimental design may be found elsewhere (Coswosk et al., 2018). It is important to highlight that attractants were not replenished after the traps were placed in the field. Traps were installed in early December 2012, and samples were carried out one (sample 1), eight (sample 2) and fifteen (sample 3) days after their installation. In this way, sample 2 was carried out seven days after sample 1 and sample 3 was carried out seven days after sample 2 (fourteen days after sample 1). Bees lured were gently removed from traps, transferred to 70% ethanol, and pinned. Males were identified with help of taxonomic keys (Rebêlo and Moure, 1996; Faria and Melo, 2007, 2012; Nemésio, 2009) and by comparison with specimens previously identified. Taxonomy follows Moure et al. (2012). All the individuals are deposited in the “Coleção Zoológica Norte Capixaba” (CZNC), Centro Universitário Norte do Espírito Santo, Universidade Federal do Espírito Santo, São Mateus, Brazil.

A total of 3114 males of 25 species was collected at the three moments (sample 1: 683; sample 2: 1648; and sample 3: 783 specimens) (Table 1). Four main results are highlighted: (i) the abundance of specimens collected per day (sample 1: the absolute number of individuals collected after the first day; sample 2 and 3: the number of individuals divided by the seven days between samples 1 and 2 and samples 2 and 3) decreased in all the attractants as the traps were left on the field (Fig. 1A); (ii) despite this decrease, the absolute number of individuals collected after eight and fifteen days is remarkable, mostly in eugenol and vanillin baits (Fig. 1B); (iii) the vast majority of species, 22 of 25, was already collected on the first sample (Fig. 1C); (iv) there is a notable variation in the relative abundance of individuals collected in each scent as the collections were made (Fig. 1D).

Regarding the decrease in attractiveness of the compounds, what could be inferred by the decline in the number of males collected per day, the abrupt drop in eucalyptol attractiveness after the first sample is remarkable. This compound is the most employed by orchid bee researchers, at least in Brazil (Faria et al., 2015), and is regarded to be the most attractive compound with respect to the number of species collected (Rebêlo, 2001). The evidence presented here supports the statement that using eucalyptol baited traps for a long period of time, without replenishment, seems to be ineffective since it is a highly volatile compound. On the other hand, the decrease was much less pronounced in eugenol and vanillin

baits, two other widely used compounds (Faria et al., 2015), that keep attracting a reasonable number of males per day even after two weeks. In the specific case of vanillin, this bait becomes more attractive as alcohol evaporates and the compound crystallizes, so that bait replenishment would not be desirable at first (Coswosk, pers. obs.).

These two baits could then be employed in sampling designs where the availability of bait traps in the field for long periods is useful, for instance in studies where population sizes of focal species that are known to be attracted to these compounds are estimated. Intensive sampling schemes for some rare species could also be settled, improving the chances of collecting them. Some rare orchid bees of seasonal genus *Eufriesea*, for instance, are known to be attracted to eugenol and vanillin baits (e.g. *Eufriesea brasilianorum* (Friese, 1899), see Nemésio, 2009).

These decisions should obviously consider the risk of collecting several specimens of the most abundant species and should be defined in a cost-effective context. After all, we have to keep in mind that the number of bees collected in samples 2 and 3 (2431 specimens) is quite high. When we consider that the decline of populations of forest dependent euglossine species is a real matter (e.g. Nemésio, 2013), with the possibility of becoming even worse (Faleiro et al., 2018), and that data on the conservation status of a large number of species is deficient (see Nemésio, 2009), we really should consider the real need of collecting and sacrificing euglossine males (see Nemésio, 2012).

The results presented here also give support to the statement that most euglossine species are expected to be inventoried in the first sampling days. Some studies suggest that short-term intensive sampling has been demonstrated to be useful in characterizing the local faunas of Euglossina, both in Atlantic areas where a large regional species pool is found (e.g. Nemésio, 2010) and in Cerrado, where euglossine richness is not that high (e.g. Tosta et al., 2017). However, it should be stressed that the results presented here, and also by Nemésio (2010) and Tosta et al. (2017) were obtained during the rainy season. As suggested by Nemésio (2016), rapid inventories should be carried out during the rainy season when the overall community composition, including those in the highly seasonal genus *Eufriesea*, are likely to be sampled. Obviously, an assessment aims at collecting the entire local fauna, so the lack of records for three species in the first day matters. When it is assumed that surveying all species is a virtually impossible task (e.g. Nilsson et al., 2001) and that all sampling methods have inherent and usually unknown sampling biases that favor the detection of some species but not others (Gotelli and Colwell, 2011), the fact that 22 of 25 species were sampled in just one day is outstanding.

The availability of data on euglossine richness and composition from long-term studies is a bottleneck for conducting extensive comparative studies, e.g. on biogeography (e.g. Aguiar et al., 2014) of these bees. As the results of short-term assessments appear increasingly robust, data associated with orchid bee distribution and seasonality will greatly increase and comparative studies on euglossine assemblages will surely thrive.

Finally, even considering the results presented here come from a region where euglossine bees are abundant and speciose, we urge that bait traps should not be left in the field beyond what is strictly necessary, even in places where orchid bees are not that speciose and/or abundant, since there is a real possibility of collecting a significant number of individuals as these traps remain available to males.

Contributors

All authors conceived the research and went to field to perform the experiments. The last author wrote a first version of the

Table 1
Abundance of euglossine species collected in seven different attractants over three consecutive samples (one, eight and fifteen days) at the Reserve Natural Vale, northern Espírito Santo state, Brazil (BA: benzyl acetate; BI: β -ionone; EC: eucalyptol; EG: eugenol; MC: methyl trans-cinnamate; MS: methyl salicylate; VN: vanillin).

Species	Sample 1							Total	Sample 2							Total	Sample 3							Total	Total by species
	BA	BI	EC	EG	MC	MS	VN		BA	BI	EC	EG	MC	MS	VN		BA	BI	EC	EG	MC	MS	VN		
<i>Eulaema cingulata</i> (Fabricius, 1804)	68	42	0	24	1	0	69	204	108	222	0	153	0	23	260	766	6	159	0	184	0	0	188	537	1507
<i>Euglossa cordata</i> (Linnaeus, 1758)	0	14	157	8	45	0	0	224	11	46	4	142	241	0	0	444	0	3	0	80	3	0	0	86	754
<i>Eulaema nigrita</i> Lepeletier, 1841	0	0	45	0	0	0	24	69	0	0	0	0	0	3	207	210	0	0	0	0	0	0	93	93	372
<i>Euglossa securigera</i> Dressler, 1982	0	1	4	10	0	1	0	16	1	0	0	81	11	0	0	93	0	0	0	32	0	0	0	32	141
<i>Euglossa iopocila</i> Dressler, 1982	0	0	3	11	1	0	12	27	0	0	0	17	0	0	10	27	0	0	0	19	0	0	4	23	77
<i>Euglossa ignita</i> Smith, 1874	4	0	6	5	10	6	0	31	13	0	0	5	8	6	0	32	0	0	0	0	0	0	0	0	63
<i>Euglossa fimbriata</i> Moure, 1968	0	4	10	2	1	0	0	17	3	24	0	5	0	0	0	32	0	0	0	2	0	0	0	2	51
<i>Euglossa gaiinii</i> Dressler, 1982	1	22	1	0	0	0	0	24	0	0	0	2	0	0	0	2	0	0	0	0	0	0	0	0	26
<i>Eulaema niveofasciata</i> (Friese, 1899)	0	0	0	0	2	5	0	7	2	0	0	0	0	12	0	14	0	0	0	0	0	0	0	0	21
<i>Euglossa cognata</i> Moure, 1970	0	0	0	1	0	7	0	8	0	0	0	1	0	5	1	7	0	0	0	0	0	0	0	0	15
<i>Euglossa imperialis</i> Cockerell, 1922	0	0	0	1	0	8	0	9	0	0	0	1	0	5	0	6	0	0	0	0	0	0	0	0	15
<i>Eulaema atlantica</i> Nemésio, 2009	2	2	2	0	0	2	1	9	0	4	0	0	0	2	0	6	0	0	0	0	0	0	0	0	15
<i>Exaerete smaragdina</i> (Guérin, 1844)	1	0	3	2	1	0	0	7	1	0	0	0	0	1	0	2	0	0	1	2	0	0	0	3	12
<i>Euglossa adiantola</i> Hinojosa-Díaz, Nemésio & Engel, 2012	0	0	1	2	0	1	0	4	0	0	0	1	0	0	1	2	0	0	0	3	0	0	0	3	9
<i>Euglossa botocuda</i> Faria & Melo, 2012	0	0	5	0	0	4	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
<i>Euglossa marianae</i> Nemésio, 2011	0	0	9	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
<i>Eufriesea dentilabris</i> (Mocsáry, 1897)	0	0	0	0	0	0	2	2	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	4
<i>Euglossa pleosticta</i> Dressler, 1982	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2	0	0	0	2	0	0	0	2	4
<i>Eufriesea surinamensis</i> (Linnaeus, 1758)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2
<i>Exaerete salsai</i> Nemésio, 2011	0	0	0	0	0	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
<i>Exaerete frontalis</i> (Guérin, 1844)	0	0	1	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
<i>Eufriesea atlantica</i> Nemésio, 2008	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Euglossa avicula</i> Dressler, 1982	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1
<i>Euglossa leucotricha</i> Rebêlo & Moure, 1996	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Euglossa milena</i> Bembé, 2007	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Total (scent/sample)	76	85	247	68	62	36	109		139	296	4	410	260	57	482		6	162	1	324	3	0	287		3114
Total (sample)				683								1648								783					

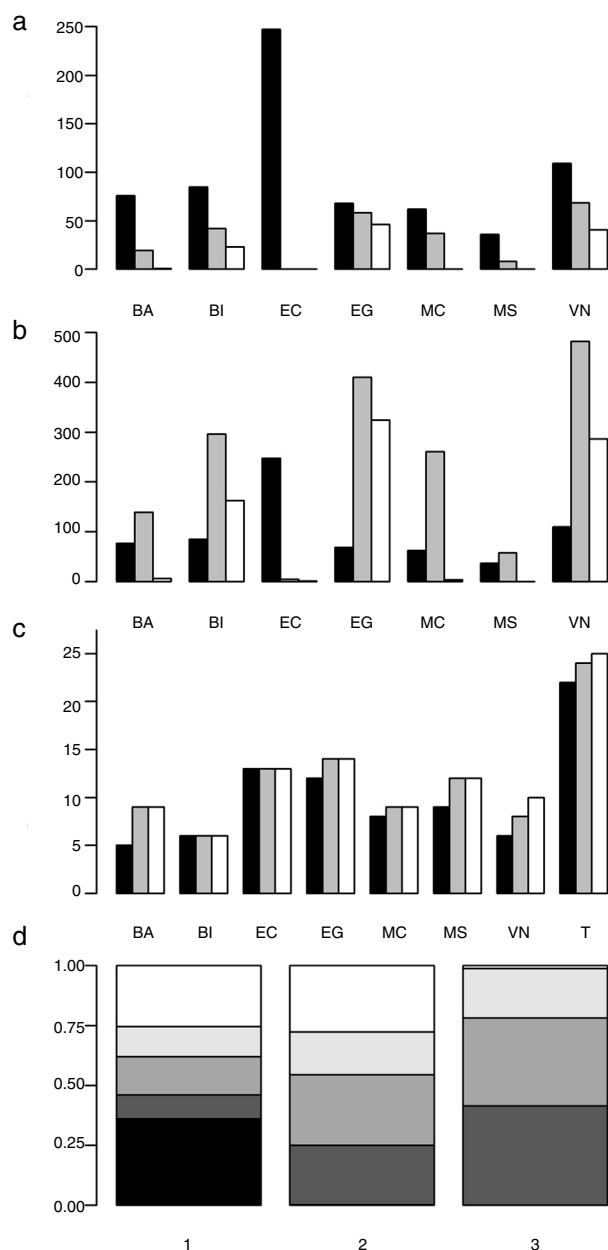


Fig. 1. Variation in the number of individuals and species collected in RNV according to the attractive bait and each performed sample. a: abundance of specimens collected per day in each sample; b: absolute number of specimens collected in each sample; c: accumulated number of species after samples (a–c: sample 1, black; sample 2, gray; sample 3, white); BA: benzyl acetate; BI: β -ionone; EC: eucalyptol; EG: eugenol; MC: methyl trans-cinnamate; MS: methyl salicylate; VN: vanillin; T: total per sample; d: relative abundance of specimens collected in each scent (EC, black; EG, dark gray; VN, gray; BI, light gray; BA+MC+MS: white; numbers refer sequentially to first, second and third collections).

paper and all authors contributed equally reviewing critically the manuscript for important intellectual content. All authors approved the final version of this manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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