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Is the capture success of orchid bees (Hymenoptera, Apoidea) influenced by different baited trap designs? A case study from southern Brazil

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ABSTRACT

Orchid bees are increasingly applied on Neotropical biomonitoring and bioindication studies due to the relative easiness of sampling and identification when compared to other bee groups. A considerable number of orchid bee community studies have been adopting baited traps as a sampling method, especially for replication purposes. However, the trap attributes are variable, and hitherto no evaluation of different designs was carried out. Here, five attributes of baited traps were tested: trap volume, number of entrance holes, presence of landing platform, kind of landing platform, and fixation content. We use Mann-Whitney tests to access differences in richness and abundance capture rates for each trap design. We found that volume, number of entrance holes, and fixation content do not influence orchid bees capture. However, the design without landing platforms had a significantly higher capture rate for richness when compared with sanded landing platforms. On the other hand, analyzing the kind of landing platform, we detected a significantly higher richness and abundance for the trap with landing platforms glued with sand. Despite the fact that the effects of different designs tested here were very punctual, we consider that results from samples taken with different baited trap designs are comparable. Some adjustments on trap design can be done according to the particularities of future studies.

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Introduction

Orchid bees (Euglossina sensu Roig-Alsina and Michener, 1993; Melo and Gonçalves, 2005) are long-tongued and mostly metallic colored bees, particularly known for males which collect aromatic compounds from different plant families in order to attract females to courtship (Dresssler, 1982; Roubik and Hanson, 2004). There are about 200 species of Euglossina (Moure et al., 2007; Ramírez et al., 2010), and the group is widely distributed in the Neotropical region, occurring from southern United States to northern Argentina (Michener, 2007). Orchid bees have a better known taxonomy and are also easily sampled when compared to most bee groups, being a strong candidate to conservation, monitoring and bioindicator programs (Hedström et al., 2006).

In the late 1960s, Dodson and colleagues (Dodson and Hills, 1966; Dodson et al., 1969) discovered the use of commercially available aromatic compounds to attract male orchid bees, easing the capture of attracted male with hand nets. A further development was made by Campos et al. (1989) after considering previous trapping reports such as Lopez (1963), who proposed the utilization of baited traps, a passive sampling method in which the aromatic compound is placed into a plastic bottle trap with lateral openings acting as entrance holes. By joining active sampling with hand-netting, these traps are currently used for sampling orchid bees. Still, other kinds of traps, such as Van Someren and McPhail traps, are marginally used in the literature (see Brosi, 2009 for an example).

Some studies suggest that baited trapping would be less effective than hand-netting (Justino and Augusto, 2010; Nemésio and Morato, 2004, 2006; Storck-Tonon et al., 2009) in spite of the notion that they are not directly comparable (*e.g.* Morato, 1998) or complementary (Aguiar and Gaglianone, 2011). Recently, Nemésio and Vasconcelos (2014) retrieved data from five comparative studies showing that hand-netting collected more bee species than baited trap, and that the bee composition is different between the methods. These authors claimed that the sole use of baited traps should be avoided due to sampling bias and incorrect assessments about the orchid bee fauna. However, a benefit of the baited trap, when faced with hand-netting, is the possibility of a higher number of replications, which maximizes the sampling effort (Knoll and Penatti, 2012) without demanding a large number of well-trained active collectors. This

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feature is desirable for comparative sampling design studies (Nemésio and Vasconcelos, 2014) and also for encompassing large scale habitat heterogeneity (Ambruster, 1993).

The baited trap design is variable among papers, and usually the studies do not bring accurate details of the baited trap (but see Ramalho et al., 2009). For instance, the trap volume varies from 500 mL (Gonçalves et al., 2014; Storck-Tonon et al., 2009) to 2L commercial bottles (Bezerra and Martins, 2001; Freitas, 2009; Justino and Augusto, 2010; Ramalho et al., 2009). Also, the number of entrance holes on the traps varies from two (Bezerra and Martins, 2001; Justino and Augusto, 2010; Silva et al., 2009), to three (Freitas, 2009; Ramalho et al., 2009) or four (Matozzo et al., 2011). Faria et al. (in press) indicate that most orchid bee researchers (88%) implement a kind of landing platform, normally made out of the top of a plastic bottle, that could both facilitate the bees entering into the bottle and prevent bee escaping. Still, some researchers developed a landing platform with higher friction, sanding it with a sandpaper or gluing sand (Freitas, 2009; Justino and Augusto, 2010).

Nemésio (2012) declared that the lack of standardization relative to sampling procedures hampers the comparative studies on the biogeography and ecology of orchid bees. Despite the numerous baited trap designs adopted by different researchers, no effect of alternative designs on sampling rates has been reported so far, since methodological studies are only focused on the baited trapping versus hand-netting dichotomy (see Nemésio and Vasconcelos, 2014). Therefore, the goal of this study is to analyze if different trap designs influence the capture success, defined here as abundance and richness rates of bees by trap unit. Thus, it will be possible to answer whether different baited traps are equally effective and whether the available Euglossina studies applying this sampling method are directly comparable.

Material and methods

The study was conducted in Parque Estadual de São Camilo (PRPA), located in western Paraná state, in the Palotina municipality (UTM -24.312998, -53.917491). PRPA is a 385.34 ha conservation unit, located under a Submontane Seasonal Semideciduous Forest, Atlantic Forest Biome (IAP, 2006). The area is surrounded by alternate soybean and corn crops, being one of the few forest fragments under conservation in western Paraná.

Sampling was carried out from 8:30 a.m. to 2:30 p.m., in two sampling phases, from November to December 2012 and from November 2013 to January 2014. The attribute tests described below were performed at each sampling phase, summing up twelve sampling days. A total of 560 bottle traps, 72 hours of sampling, and 3,360 hours of trapping were carried out. The sampling season was chosen according to the annual peak of euglossine bees in western Paraná, which happens in this period, according to Gonçalves et al. (2014). The traps were installed in two transects with 600 m each, separated by 2 km of contiguous forest. Both transects were at the border of the forest, subject to the same edge effects. Each transect was divided in 30 points, 20 m apart, and the exact location of trap installation as well as the trap design were determined through randomization, performed by Sample function with R program (R Development Core Team, 2014).

To enable comparisons between different traps, one trap design was defined as model, based on traps previously utilized by our lab (Gonçalves et al., 2014), and variations were made from it. The trap model is manufactured from a commercial plastic bottle of 500 mL, with two entrance holes with landing platforms, being the landing platform sanded with sandpaper, with 70% ethanol inside the bottle (Fig. 1). Only one variable was tested at a time, and only the 1,8-cineol scent was applied, as this scent has been the most effective one in western Paraná (Gonçalves et al., 2014). The scent was provided at the top of the bottle, on three cotton swabs attached to the bottle cap
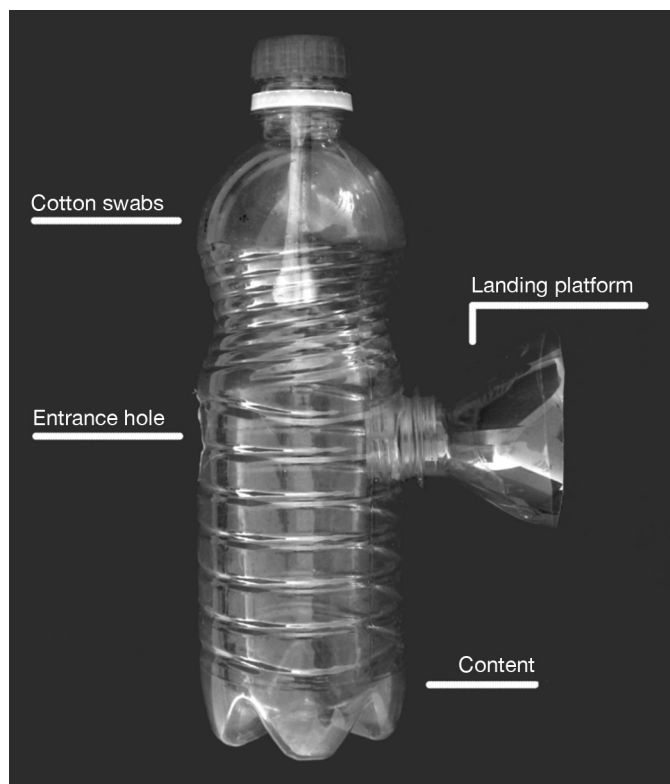

Figure 1. Baited trap made with 500 mL bottle; left landing platform not shown to indicate position of the entrance hole.

with a nail. The bees were fixed with ethanol, pinned, databased, and are deposited in the entomological collection of Setor Palotina, Universidade Federal do Paraná. The species were identified by comparison with previously identified specimens deposited in this institution.

The following attributes were tested: 1) Trap volume (500 mL or 2 L) – 40 traps were prepared according to the model described above, one half made with 500 mL bottles, and another half with 2 L bottles; 2) Trap fixation content (water, ethanol or empty bottle) – among 60 traps, one third had 70 mL of ethanol at the bottom, one third had water, and one third was empty; 3) Entrance holes (two or three) – 20 traps with two opposites holes, and 20 traps with three regularly spaced holes for bees entrance; 4) Landing platform (present or absent) – among 40 traps, 20 were prepared with a 3 cm landing platform, and 5) Landing platform type (sanded, with glued sand, or without any treatment) – 60 traps were prepared according to the model, one third received no treatment to improve the friction, one third was sanded with sandpaper, and the remainder received a mixture of commercial white glue and sand. Each described test was carried out twice, one at each sampling phase. Finally, a sixth test comparing traps without landing platform and traps with landing platforms with glued sand was made; this test was carried out twice in January 2014, the only test performed within a single sampling phase.

For each analysis, we pooled the data from the sampling phases for each attribute test variable and used the raw data. Table 1 presents a summary for the mean rate of abundance and richness by trap. We also analyzed the six attributes at genus level in order to access the influences on different size and behavior of orchid bee genera. The Shapiro-Wilk normality test was carried out, and as our data does not fit the normality curve we adopted the Mann-Whitney test, always comparing two variables at a time, including the cases with three variables. Both tests were carried out with R (R Development Core Team, 2014).

Results

A sum of 939 specimens distributed among nine species and four genera was sampled in PRPA. The most abundant species was *Eufriesea violacea* (Blanchard, 1840) with 528 sampled specimens, followed by *Euglossa fimbriata* Moure, 1968 with 218 specimens; *Euglossa annectans* Dressler, 1982 with 94; *Eulaema nigrita* Lepeletier, 1841 with 40; *Euglossa cordata* (Linnaeus, 1758) with 38; *Euglossa pleosticta* Dressler, 1982 with 18, and *Euglossa* aff. *melanotricha* Moure, 1967, *Eufriesea* aff. *auriceps* (Friese, 1899), and *Exaerete smaragdina* (Guérin, 1844) with only one sampled individual each.

Table 1 presents the Mann-Whitney test for all variables tested here. For volume, trap content and entrance holes, tests do not show any significant difference for richness and abundance (p > 0.05). However, significant results were found for presence or absence as well as kind of landing platform. When the presence of landing platform was tested, the trap without platform was more effective for richness (p < 0.05). About the alternative treatments of landing platform, glued sand platform traps were more effective than sandpapered platform traps for richness, and more effective than non-treated platforms for richness and abundance (p < 0.05), but no significant difference was found when comparing traps with sandpapered platform and non-treated platforms. Besides, the comparison between traps without landing platform and traps with glued sand platforms did not show significant results, despite the higher capture rate of traps with no landing platform. For the genus level analysis, no significant results were found.

Discussion

Our study area was preliminarily sampled by Gonçalves et al. (2014), a study in which seven orchid bee species were recorded, and the additional species sampled here were *Eufriesea* aff. *auriceps* and *Euglossa* aff. *melanotricha*, with one specimen each. The richness and abundance of orchid bees in South Atlantic forests, like the PRPA semideciduous forest, are smaller than those from the North Atlantic and Amazonian forests, what had already been shown by the negative correlation of orchid bees and the Neotropical latitudinal gradient (Sydney et al., 2010). Another known pattern is the lower richness in inland Atlantic forests than that of coastal forests in the same latitude (Giangarelli et al., 2014; Mattozo et al., 2011). One of the remarkable differences between assemblages from southern and northern portions of orchid bee distribution is the lack of a higher diversity of large bees. Only *Eufriesea violacea* and *Eulaema nigrita* are common on southern forests and both are not among the larger species of each genera, especially *E. violacea*. Nemésio and Vasconcelos (2014) pointed to the common observation that larger bees tended to be more frequent in traps (but see Aguiar and Gaglianone, 2011 and Justino and Augusto, 2010), contrasting with small species (genus *Euglossa*), so this bias must be considered when examining our results. In the present study the medium sized *Eufriesea violacea* and two small species of *Euglossa* were more abundant than the larger *Eulaema nigrita*; however, it is not possible to determine if this result reflects the community structure or a sampling bias, since we did not design the present study to compare baited traps with hand-netting. Even if representing a lower diversity, our assemblage included four orchid bee genera, validating our results as representative of the entire group.

Trap volume. Most researches use traps made out of bottles between 1.5 and 3 L (Bezerra and Martins, 2001; Freitas, 2009; Justino and Augusto, 2010; Ramalho et al., 2009), and only 15% use 500 mL bottles (as Gonçalves et al., 2014; Storck-Tonon et al., 2009). The smaller model built with a 500mL bottle is recommended for easiness to handle and transport traps; however, it can be a poor alternative in areas of higher abundance of orchid bees, due to low storage capacity. We found no significant difference among trap volumes, an expected result after the low abundance of orchid bees in the sampled area, although different results can be obtained in areas with higher abundance rates.

Trap fixation content. There is no study, to our knowledge, that explicits the use of a fixation substance inside traps, although some researchers have mentioned the use of ethanol or water (Faria et al., in press). It was frequently observed that bees are rapidly fixed inside traps containing ethanol, which besides being a fair way to promote bee fixation, can also prevent occasional escaping, a fact frequently reported for small bees (Nemésio and Vasconcelos, 2014). According to our test, this attribute did not influence the capture of bees, and by extension it does not diminish escaping. The present study was conducted at the border of a forest fragment, in a very sunny location, and many bees were already dead in empty traps. Nevertheless, in colder or humid areas it is plausible that lower abundance and richness can be recorded in water-filled or empty traps due to the possibility of bees escaping.

Entrance holes. The entrance hole is crucial in the process of trapping, and authors suggest that the hole diameter influences the composition of assemblage of baited traps, given that largest species of

Table 1.
Comparison of different baited trap designs for capturing orchid bees. P values relative to Mann-Whitney test.

Attribute	Variables	Abundance rate	p value	Richness rate	p value
Trap volume	500 mL bottle	1.28	0.31	0.83	0.22
	2 L bottle	0.7		0.43	
Trap content	Water (w)	0.73	w × e = 0.42	0.68	w × e = 0.36
	Ethanol (e)	0.73	e × n = 0.97	0.55	e × n = 0.78
	No treatment (n)	0.85	n × w = 0.45	0.48	n × w = 0.22
Entrance holes	Two	2.2	0.21	0.95	0.07
	Three	1.45		0.68	
Landing platform	Presence	4.05	0.15	1.25	0.01
	Absence	4.86		1.85	
Landing platform	Sanded with sandpaper (s)	1.35	s × g = 0.09	0.65	s × g = 0.01
	Glued with sand (g)	1.55	g × n = 0.0001	1.08	g × n = 0.0003
	No treatment (n)	0.55	n × s = 0.11	0.48	n × s = 0.29
Landing platform	Absence	1.98	0.11	1.33	0.44
	Glued with sand	1.32		1.05	

Eufriesea, Eulaema and *Exaerete* are more representative than smaller species, mostly *Euglossa* (Justino and Augusto, 2010; Nemésio, 2012; Nemésio and Morato, 2004, 2006; Nemésio and Vasconcelos, 2014). The main question is that having entrance holes big enough to allow the largest bees to enter also facilitates the escape of the smaller ones (Morato, 1998; Nemésio and Morato, 2004, 2006). Likewise, one could expect that more holes increase the sampling rate of large species but also the escape of small species. Researches commonly use traps with two (Bezerra and Martins, 2001; Justino and Augusto, 2010; Silva et al., 2009) or three holes (*e.g.* Nemésio and Morato, 2006; Ramalho et al., 2009). Mattozo et al. (2011) used a four-entrance trap, recommending orientation of the holes in different directions as a desirable characteristic. In spite of these preferences, the number of entrances did not influence orchid bee capture according to our results. Additionally, we tested the abundance rates in the genus level, but no significant results were found.

Landing platform. The original trap designed by Campos et al. (1989) does not have landing platforms, as well as the first traps used (Lopez, 1963). Nowadays, landing platforms are used in most studies and authors claim that they can not only help the entrance of bees, but also avoid or diminish the possibility of escapes (Nemésio and Morato, 2006). Following this, Andrade-Silva et al. (2012) used short inverted funnels inside the holes to avoid escaping, given that the inside diameter is smaller than the outside diameter of the entrance. Nevertheless, traps with landing platforms sampled a significant lower richness than traps without a landing platform in the present study. Interestingly, one species, *E. pleosticta*, was only sampled in traps without landing platforms, in spite of the previous records of the sampling of this species with landing platforms (*e.g.* Ramalho et al., 2009). Some bees exhibit a patrolling behavior around the trap to check the entrances before landing and entering. Orchid bee species can take a longer time before entering into baited traps, a behavior noticed for *Eulaema* by Nemésio (2012) and Nemésio and Vasconcelos (2014) and for *Euglossa* by Justino and Augusto (2010), so landing platforms can retard the entrance with protracted patrolling behavior. Most researchers built a platform with glued sand (Faria et al., in press), which is the most efficient landing platform friction approach tested here. About the comparison between traps without landing platforms (which are preferable to those with landing platforms) and glued sand platforms (the most effective of those employing landing platforms), the first sampled 20 more individuals than the second, but no statistically significant difference between them was found. Thus, the landing platform seems to not be an essential feature of baited traps.

Baited traps are recognized by most authors as a poorer option than hand-netting for overall diversity and composition assessment, but this method should not be discarded a priori, since it is recommended for answering questions that need robust sampling design in terms of replication. In this line, our results point that the alternative baited trap designs do not have much influence on the richness and abundance parameters (except for the landing platform), thus making most studies directly comparable. Also, we found that richness was more sensitive than abundance to presence and alternative of landing platforms. We recommend that trap volume should be selected taking into account the predicted area abundance; the use of ethanol on the bottom of traps should be employed to promote a fast and fair fixation of bees, and landing platforms are unnecessary. Surely, these tests should be replicated in other landscapes for validating the prediction of our interpretation.

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Conflicts of interest

The authors declare no conflicts of interest.

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