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Foraging behavior of the small-sized stingless bee *Plebeia* aff. *flavocincta*[1]

Comportamento de forrageio da abelha sem ferrão de pequeno porte *Plebeia* aff. *flavocincta*

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ABSTRACT - The aim of the study was to investigate the flight range and foraging behavior of the stingless bee *Plebeia* aff. *flavocincta*, as well as testing its attraction to different sugar syrup concentrations within different distances to the colony. Foraging pattern along the day, preference of bees for sugar concentration in nectar and their relationship with distance from the colony were assessed by the means of manipulating those parameters with artificial flowers containing different sugar syrup concentrations and displayed at varying distances. The bees *P.* aff. *flavocincta* foraged from 6 a. m. to 5:30 p. m., with a foraging peak from 9 a. m. to 11:30 a. m. and showed preference for higher syrup concentrations (60% and 70%) though have foraged indiscriminately in all tested sugar concentrations and varying distances. We have concluded that due to its small body size, this species needs higher temperatures to forage when compared to large-body species, and shows preference for more concentrated sugar sources, but also explores the food source with lower sugar concentrations within their flight range. Such strategy may help to understand how a small-sized and short-flight bee such as *P.* aff. *flavocincta* achieved a vast geographic distribution in the country and is well succeeded in colonizing highly anthropized areas where natural sources of nectar are usually scarce within short distances.

Key words: Artificial feeding. Flight range. Nectar concentration. Meliponini. Body size.

RESUMO - O objetivo desse trabalho foi investigar o raio e comportamento de voo no forrageamento da abelha *Plebeia* aff. *flavocincta*, e testar a sua atração para diferentes concentrações de xarope de açúcar em diferentes distâncias da colônia. O padrão de forrageio ao longo do dia, a preferência das abelhas pela concentração em açúcar do néctar e sua relação com a distância da colônia foram investigados por meio de manipulações desses parâmetros com flores artificiais contendo diferentes concentrações de xarope e colocadas a distâncias variadas das colmeias. As abelhas *P.* aff. *flavocincta* forragearam das 6 h às 17 h 30, com pico de forrageio das 9 h às 11 h 30 e apresentaram preferência por maiores concentrações de xarope (60% e 70%), embora tenham forrageado indiscriminadamente em todas as concentrações e distâncias variáveis. Conclui-se que devido ao seu porte reduzido, essa espécie necessita de temperaturas mais altas que espécies de porte maior para forragear plenamente e apresenta preferência por fontes mais concentradas de açúcar, no entanto não abre mão de explorar outras fontes com menores concentrações existentes dentro do seu raio de voo. Essa estratégia pode auxiliar a compreender como uma abelha de porte reduzido e raio de voo curto como *P.* aff. *flavocincta* consegue apresentar uma distribuição geográfica tão ampla e colonizar áreas altamente antropizadas onde fontes naturais de néctar são geralmente escassas a curtas distâncias.

Palavras-chave: Alimentação artificial. Concentração de néctar. Meliponíneo. Raio de voo. Tamanho corporal.

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INTRODUCTION

In social bees, foraging is a costly work consisting in the dispersion of worker bees to explore the nest surroundings seeking for food sources and bringing resources to the colony (DORNHAUS *et al*., 2006; JARAU *et al*., 2000). Studies investigating bee foraging behavior are essential for understanding the relationship of these pollinators with the plant species they visit (MARZINZIG *et al*., 2018; SIQUEIRA *et al*., 2018). Decisions taken by the individuals during food searching tend to maximize the energetic rewards with minimal additional costs for obtaining the resource, in order the losses are not greater than the gains (MACARTHUR; PIANKA, 1966).

The behavioral characteristics for food searching vary in the diverse bee species, being influenced by biotic and abiotic factors such as the body size of the individual, weather, and resources explored (OLIVEIRA *et al*., 2012; OYEN; DILLON, 2018). Although some bees are crepuscular species with activities under low temperature and luminosity (SIQUEIRA *et al*., 2018; SOUZA; NASCIMENTO, 2018), most species show greater foraging activity in the time of the day when the temperature is higher and there is more luminosity, as well as low humidity and wind speed (POLATTO; CHAUD-NETTO; ALVES-JUNIOR, 2014). External aspects are of great influence on the foraging behavior of bees, especially for small-sized species, which due to their reduced body size present difficulty for maintaining optimal foraging temperature when outside their nests in cool environments (CAMPOS; GOIS; CARNEIRO, 2010; SILVA *et al*., 2013; SILVA; RAMALHO; ROSA, 2011).

In stingless bees (Meliponini) body size varies considerably within the many species. In Brazil, there is a great taxonomic diversity of these bees, with species from only 2.6 mm as for *Plebeia minima* to species sized up to 14 mm as *Melipona grandis* (OLIVEIRA *et al*., 2013). The species from the genus *Plebeia* stand out for being among the smallest individuals, with many of them not reaching 3.5 mm in length (MICHENER, 2007).

Studies on the foraging behavior of stingless bees are still reduced and most of them aimed towards the species with greater body sizes (LINCHTENBERG; IMPERATRIZ-FONSECA; NIEH, 2010), thus it has still been limited the knowledge with respect to the foraging activity in small-sized bees, such as *Plebeia* spp., both related to the foraging distance as well to ecological conditions that influences them. However, these small-sized meliponines are largely spread in the American continent, occurring from Mexico down to the central region of Argentina both in natural and anthropized areas. Considering the reduced body size, these bees might present a short flight range indicating the need of foraging strategies that make possible for them to efficiently explore the resources available in areas much smaller than those areas usually explored by larger bees (ZURBUCHEN *et al*., 2010).

Therefore, the present work aimed to study the flight range and flight behavior of the small-sized stingless bee *Plebeia* aff. *flavocinta*, as well as to test the attractiveness of sugar syrups with different sugar concentrations within different distances from the nest. We intend to provide information for the future development of techniques for the management and natural or artificial feeding for this bee species.

MATERIAL AND METHODS

Experimental area

The study was carried out at the Meliponary of the Bee Unit of the Department of Animal Sciences, Center of Agricultural Science of the Federal University of Ceará (UFC), Fortaleza, Brazil (3°44'33.70" S e 38°34'45.46" O) from July to December, 2017. The climate of the region, according to the Köeppen classification, is Aw', rainy tropical, with annual average precipitation of 1350 mm concentrated from January to April, average temperature of 26.5 °C and relative air humidity of 80%.

Four colonies of *Plebeia* aff. *flavocincta* were used for data collection. These colonies were randomly chosen among 16 colonies present in the Meliponary.

Artificial flower

The bees *Plebeia* aff. *flavocincta* were trained to collect an energy-rich syrup resource 1:1 (sugar : water) containing attractive vanilla essence and offered in artificial flowers. These flowers were made of four Eppendorf® tubes settled in radial conformation around a 10 mL plastic storage tube container, with a yellow synthetic wool pad for attracting the bees attention (Figure 1). The sugar syrup resource was stored inside the Eppendorf tubes with lids previously punched with 2 mm holes in order to allow the entry of small bees, but not larger flower visitors.

Sugar syrup preparation

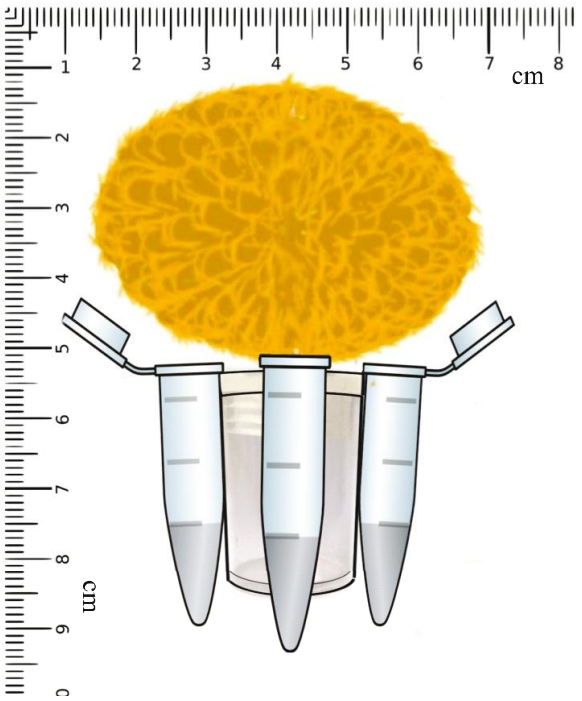
The syrups used in the tests were of different sugar concentrations (0%, 30%, 40%, 50%, 60% and 70%) prepared in the laboratory of the UFC Bee Unit calculating the amount of water and sugar for obtaining the concentration at the desired proportion. The necessary volume of water for obtaining the proportion of syrup was measured in graduated beakers, and the sugar was weighed in a digital scale with precision of four digits (GEHAKA BK3000). Afterwards, the volume of water

was transferred to a beaker on hot plate and heated up to the boiling point and, right after, adding the sugar and stirring continuously until the total dissolution. After the syrup cooled down, the concentration was checked with the aid of a manual refractometer (SIMOKIT 0-90%) and, if necessary, corrections were made. The sugar syrup was then stored in glass jars before adding the juice of half of a lime because the citric acid preserves the sugar syrup for longer time. The jars were tagged with the information of syrup concentrations and stored in a refrigerator to be used in the artificial flowers during the experiments.

Experiment of *Plebeia* aff. *flavocincta* visits to flowers throughout the day

To learn about the foraging pattern of *Plebeia* aff. *flavocincta* throughout the day, five artificial flowers containing sugar and water syrup solution 1:1 were placed in a shaded area at 30 meters from the meliponary. The flowers were previously filled with 1.5 ml of sugar syrup on the day before the observations were taken and stored in a refrigerator, before taking them to the field at 5:30 a.m. in the following morning. The observations were made for seven consecutive days, counting the number of bees visiting the artificial flowers during the day at every interval of half an hour, from 6 a.m. to 5 p.m. When necessary, the artificial flowers were refilled with sugar syrup. During the observations, notes were taken of the number of bees *P.* aff. *flavocincta* collecting syrup in each artificial flower. Data were grouped in intervals of 2.5 hours for statistical analyses.

Figure 1 - Schematic drawing of artificial flowers used in the experiments with small-sized bee *Plebeia* aff. *flavocincta*, made of a 10 mL plastic storage tube container, four Eppendorf® tubes settled in radial conformation, and a yellow synthetic wool pad simulating an inflorescence



Experiment of syrup concentrations

To learn about the preference of bees for sugar concentration in nectar, it was performed the experiment with different concentrations of sugar in syrup (30%, 40%, 50%, 60%, and 70%) in addition to the null treatment (0%) where the flowers were supplied only with water. Therefore, artificial flowers were previously prepared and tagged according to the sugar concentration in the syrup they contained, and three flower replicates were prepared for each sugar concentration. The flowers were then taken to the field at 5:30 a.m. and arranged in a shaded area at a distance of 30 meters from the meliponary. The observations were performed in accordance with the preceding experiment. During the observations, notes were taken about the number of the bees *P.* aff. *flavocincta* that were surrounding, exploring, or collecting syrup at each artificial flower. The results of observations made at every half an hour were grouped in intervals of 2.5 hours for statistical analyses (6 a. m. - 8:30 a. m.; 9 a. m. - 11:30 a. m.; 12 p. m. - 2:30 p. m.; 3 p. m. - 5:30 p. m.).

Tests of sugar concentration and foraging distance

In order to learn if differences in sugar concentration would influence the distance *P.* aff, *flavocincta* workers would fly for collecting food, four experiments manipulating sugar syrup concentrations and hive distances were performed, as follows:

Experiment A - A syrup with 30% sugar concentration was prepared and split in four portions where each of them was treated with a distinct food coloring dye (two drops/10 ml of syrup). Then, each colored sugar syrup was assigned to one distance from the artificial flowers to the hives, as follow; blue – 0 m, red – 15 m, green – 30 m, yellow – 45 m. During the days of the experiment, three artificial flowers were filled with each of the colored syrups and arranged in their respective distances from the meliponary. The observations were made at the entrance of four hives, for ten alternate days. The bees were counted for 10 minutes, at every half hour, starting at 6 a. m. and finishing at 5:30 p. m. every day. Since the abdominal exoskeleton cuticle of *P.* aff, *flavocincta* workers is transparent, the distance in which the bee had gone for collecting food was easily deducted by the color their abdomens acquired when consuming

the syrup (Figures 2 and 3A). Previous tests counting the number of bees in artificial flowers arranged at the same distance from the hives and containing the colored syrups used in the present experiment, besides a fifth one not artificially colored, have shown that the syrup color does not interfere with the foraging preference of these bees.

Experiment B - The same as experiment A, except that all sugar syrups contained a sugar concentration of 60% (Figure 3B);

Experiment C - Similar to the prior experiments, however syrups had increasing sugar concentrations (30%, 40%, 50%, and 60%) with the extending distance from the hives (Figure 3C);

Experiment D - Similar to experiment C, different by the fact that sugar concentrations decreased with the extending distance from the hives (Figure 3D).

During the experiment, the meteorological conditions measured in the area were maximum and minimum temperature of 29.1 °C and 26.5 °C respectively, and the average temperature of 28.3 °C. The relative air humidity varied between 61% and 85%, and an average of

Figure 2 - *Plebeia* aff. *flavocincta* workers with colored abdomens due to foraging in artificial flowers containing dyed sugar syrup allowed to deduct the distance they were foraging from their colonies. f: **A)** green; **B)** blue; **C)** yellow; **D)** red

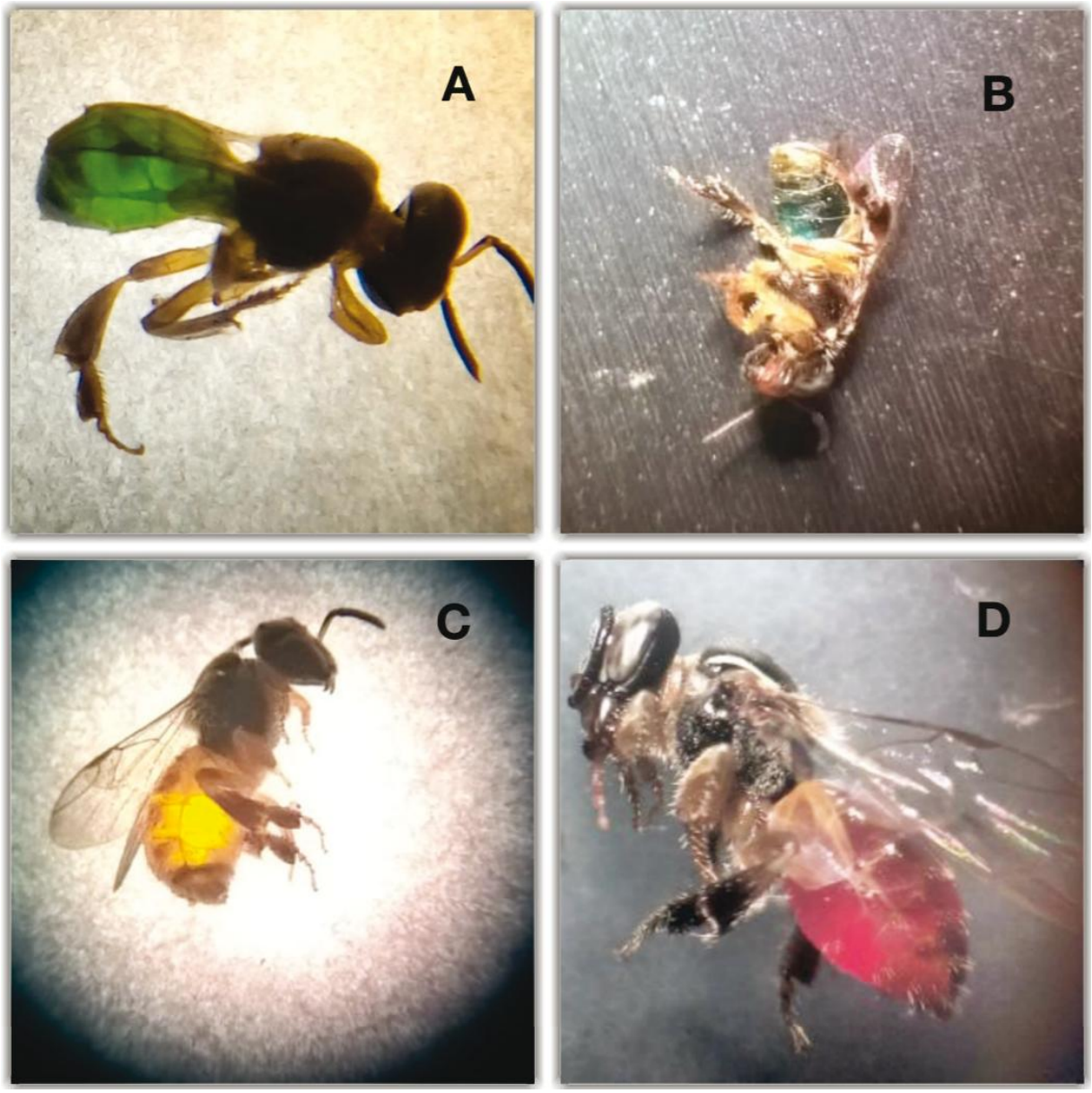
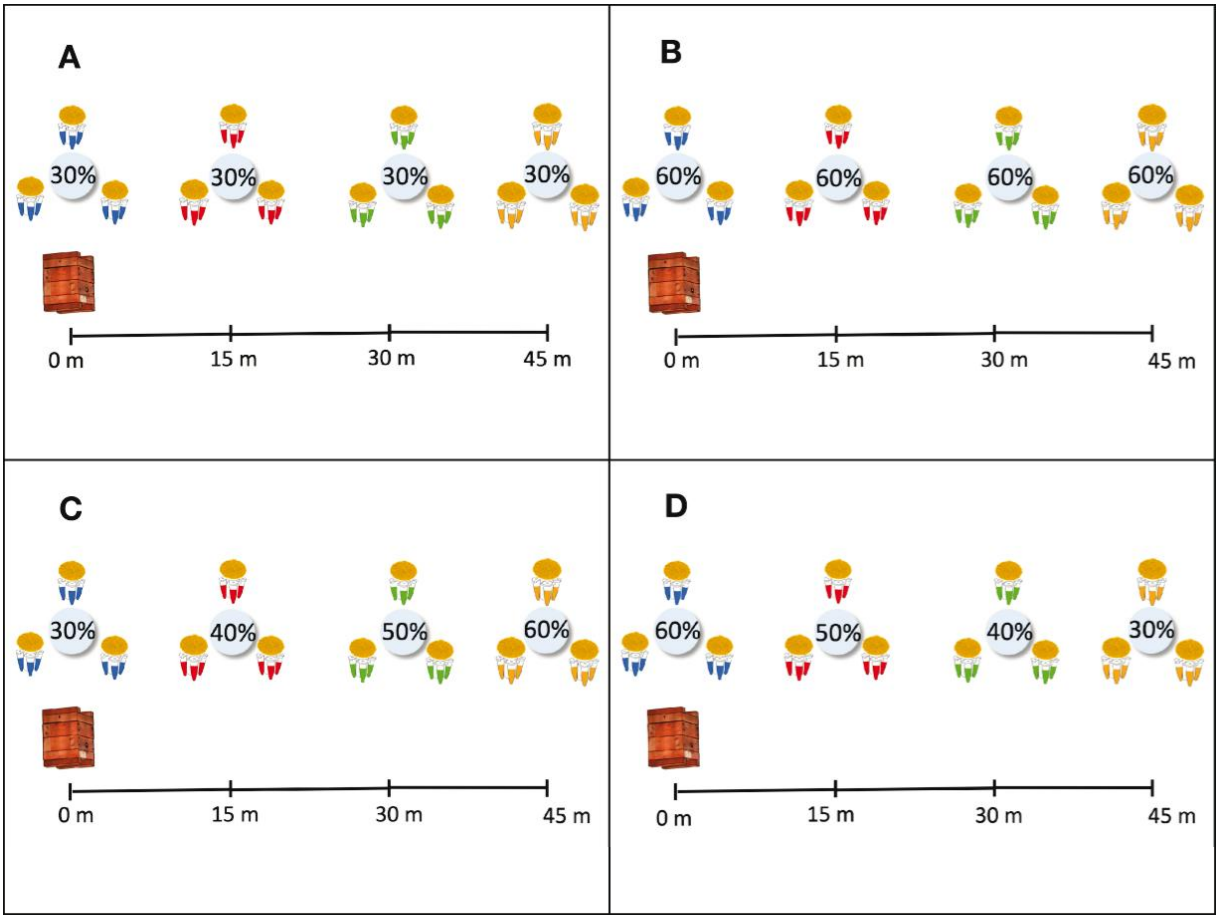


Figure 3 - Schematic drawing of the experiment of foraging distances from the colonies using four sugar syrup concentrations in the artificial flowers. **A)** Concentration of 30% in all distances; **B)** Concentration of 60% in all distances; **C)** Concentrations of 30%, 40%, 50%, 60% at the distances of 0 m, 15 m, 30 m and 45 m respectively; **D)** Concentrations of 60%, 50%, 40% and 30% at the distances of 0 m, 15 m and 45 m respectively



70.4%. The wind speed varied between 2.3 $m.s^{-1}$ and 4.3 $m.s^{-1}$ and an average of 3.2 $m.s^{-1}$.

All data analyses were carried out using the R Statistical Software. The tests of bee visitation to artificial flowers, sugar syrup concentration, and variation of sugar syrup concentration in four different distances were performed using Kruskal Wallis nonparametric test, followed by Dunn's test, with P significant when < 0.05%.

RESULTS AND DISCUSSION

Distribution of *Plebeia* aff. *flavocincta* visits throughout the day

Plebeia aff. *flavocincta* workers foraged the artificial flowers containing 50% sugar syrup and placed 30 m from the hives throughout the day, with mean numbers ranging from 2.28 to 9.23 individuals at any time (Table 1). However, they were less frequent in the interval between 6 a. m. and 8:30 a. m. differing significantly ($p<0.05$) from the numbers of visits at other times during the day (Table 1). There were no significant differences in the number of bees foraging among other times of the day (Table 1).

Results showed the presence of few bees visiting the artificial flowers during the first hours of the day, but the number of visits quickly increased at 9 a. m. and foraging frequency remained high most part of the day, decreasing only by the end of observations after 3 p. m. (Table 1). Usually bees do not maintain high foraging frequency after midday, even when climatic conditions are adequate for flight, because the resources they seek on flowers becomes scarce as the day progresses (POLATTO; CHAUD-NETO; ALVES-JUNIOR 2014;

Table 1 - Instantaneous mean number of *Plebeia* aff. *flavocincta* workers visiting artificial flowers with syrup at 50% concentration of sugar, in four intervals of time during the day. Data collected during the dry period of the year 2017

Intervals (Times)	N	Mean number of bees per artificial flower (± s. e. m.)
6 a. m. - 8:30 a. m.	35	2.28 ± 0.58 b
9 a. m. - 11:30 a. m.	35	9.23 ± 1.04 a
12 p. m. - 3:30 p. m.	35	8.63 ± 1.40 a
3 p. m. - 5:30 p. m.	35	6.65 ± 0.91 a

Mean values followed by the same letters on the column do not differ at $p<0.05$

VILLANUEVA-GUTIERREZ; ROUBIK; POTER-BOLLAND, 2015). However, the constant presence of *P.* aff. *flavocincta* workers in the artificial flowers during the entire day can be explained by the fact that the resources had on no occasion become scarce because we refilled the artificial flowers with sugar syrup whenever necessary.

The considerable increase of bees in the artificial flowers starting from 9 a. m. demonstrates that *P.* aff. *flavocincta* bees had remained longer inside their nests during the first hours in the morning, differently from large-sized bees such as *Xylocopa* spp. and *Apis mellifera* that, under similar climatic conditions, begin to forage as early as 5 a. m. (SILVA *et al*., 2015; SOUZA; NASCIMENTO, 2018). Bees do not leave their colonies to forage before the optimal environmental conditions; of these, temperature stands out as one of the main factors related to foraging activity (HILÁRIO *et al*., 2001).

Body temperature in bees varies according to the activity they perform, and major heat losses take place during foraging activity in cold climate (TAN *et al*., 2012). Thus, many bee species prefer to forage during the times of day with higher temperatures, as well as in low relative air humidity (HEMALATHA *et al*., 2018; POLATTO; CHAUD-NETO; ALVES-JUNIOR, 2014). Small-sized bee species, such as *P.* aff. *flavocincta* used in the present study, are more likely to be affected by changes of climatic conditions, as owing to the small body size they lose heat more quickly than larger bees, presenting, therefore, proportionally higher energy consumption in order to maintain the optimal body temperature for flight (OYEN; DILLON, 2018). Hence, taking into consideration that the distance to the artificial flowers as well as sugar syrup quantity and concentration were kept constant during all day, it was noticed that *P.* aff. *flavocincta* had preferred to forage in higher numbers only after the temperature reached means above 28 °C, starting from 9 a. m. Indeed, in the present study, foraging was not recorded with temperatures below 24.6 °C at the observed colonies, which suggests that these bees need higher temperatures to thoroughly forage. However, Hilário *et al*. (2001) observed for another species of the same genus (*Plebeia pugnax)* flight activity starting with temperatures of 15 °C in São Paulo, Brazil, suggesting that some small-sized bee species can present adaptations to forage at temperatures lower than other species in function of ecological conditions in the regions where those species have evolved. The present study was carried out during the dry period of the year, in which relative air humidity varied from 55 to 81%, not influencing the flight activity of the bee *P.* aff. *flavocincta*.

Experiments of sugar syrup concentrations

In the experiment with constant distance from the artificial flowers to the hives, however, varying syrup concentration from 0 to 70% sugar, the results showed that *P.* aff. *flavocincta* presented significant ($p<0.05$) preference for the more concentrated sugar syrups, certainly for offering higher energetic rewards. Indeed, in three out of four of the studied intervals, the bees had visited in significantly ($p<0.05$) greater numbers the artificial flowers containing syrups with higher sugar concentrations (60% and 70%), indicating that, when possible, the bee *P*. aff. *flavocincta* visit flowers with higher energetic compensation.

Bees, in general, forage in nectar concentrations ranging from 35 to 65% sugar (ROUBIK *et al*., 1995), and different bee species can present preference for nectars in different concentrations (BASARI; RAMLI; KHAIRI, 2018). Body size is pointed out as a characteristc also related to the bee preference for different sugar concentrations in nectar, therefore, large-sized bees present preference for nectar with higher sugar concentrations, while small-sized bees reach optimal foraging with lower sugar concentrations (BASARI; RAMLI; KHAIRI, 2018; ROUBIK *et al*., 1995). However, in the present study, *P.* aff. *flavocincta*, even though being a small-sized species (3 mm), has preferred higher sugar syrup concentrations. In addition, syrup viscosity increases as sugar concentration also increases, and Nicolson *et al*. (2013) remark that nectar viscosity is a more important factor than concentration when choosing food sources since low viscosity enables

bees to drink more quickly reducing imbibing time, in this way, allowing for transporting greater volumes to the nest.

As already discussed in the previous experiment of visits throughout the day, here *P.* aff. *flavocincta* workers also presented a low number of visits to the artificial flowers at the first hours of the day, regardless of the tested sugar concentrations, not differing significantly ($p>0.05$) among treatments. Increase in foraging frequency has only been observed starting from the second interval (9 a. m. - 11:30 a. m.) for the higher concentrations (50%, 60%, and 70%) tested, remaining constant until the end of observations (Table 2). This pattern is similar to the one that has been obtained in the previous experiment with the constant concentration of 50%, reinforcing the temperature outside the nest as a more determining factor for foraging activity in this bee species than any potential reward.

Experiment of foraging distance

The arrangement with increasing sugar concentration (30%, 40%, 50%, 60%) in syrup was the only one showing significant difference ($p<0.05$) among the tested distances, in which the number of bees foraging at 45 m from the hives differed in relation to that at 0 m (Table 3). The other arrangements did not differ ($p>0.05$) among the tested distances. Jarau *et al*. (2000) observed that *Melipona scutellaris* bees tend to first search for food within an approximate distance of 30 m from the colony and only after that explore more distant, behavior not observed significantly for *Plebeia* aff. *flavocincta*. Similar foraging performance presented at the different distances studied when sugar syrup concentrations were kept the same, both for 30 and 60%, suggest that *P.* aff. *flavocincta* workers are capable of foraging up to 45 m from their colonies, and this distance is not an obstacle for exploring food resources existing within this flight radius. This is reinforced by the fact that the number of foragers significantly increased with rising sugar concentrations in the syrup, even at greater distances up to 45 m from the nest. In addition, studies suggest that greater distances are covered in case the energetic costs obtained by the food resource is worth the consumption of energy during the flight (ABOU-SHAARA, 2014; FREIRE; PIGOZZO, 2014).

Table 2 - Instantaneous mean number of *Plebeia* aff. *flavocincta* workers visiting artificial flowers containing syrup in different sugar concentrations in four intervals of time during the day. Data collected during the dry period of the year 2017

Sugar concentrations (%)	N	Mean number of visitation of bees per artificial flower (± e. p. m.)			
		(6 a. m.-8:30 a. m.)	(9 a. m.-11:30 a. m.)	(12 p. m.-2:30 p. m.)	(3 p. m.-5:30 p. m.)
0	24	0.12 ± 0.07 Aa	0.83 ± 0.24 Ac	0.79 ± 0.25 Ac	0.29 ± 0.18 Ac
30	24	0.29 ± 0.09 Aa	0.29 ± 0.09 Aa	1.00 ± 0.35 Ac	0.96 ± 0.43 Abc
40	24	0.25 ± 0.11 Aa	1.46 ± 0.41 Abc	0.87 ± 0.19 Ac	0.54 ± 0.23 Abc
50	24	0.12 ± 0.07 Ba	1.4 ± 0.53 ABbc	2.08 ± 0.68 Abc	2.08 ± 0.44 ABa
60	24	0.12 ± 0.07 Ba	6.5 ± 1.97 Aab	9.62 ± 2.48 Aab	6.5 ± 1.62 Aa
70	24	0.08 ± 0.06 Ba	12.58 ± 2.86 Aa	20.71 ± 4.34 Aa	9.96 ± 2.13 Aa

Means values followed by the same lowercase letters on the column and uppercase letters on the line do not differ at $p > 0.005$

Tabela 3 - Mean number (M) and medians (Md) of *Plebeia* aff. *flavocincta* workers entering the hive at intervals of 10 minutes after have been foraging in artificial flowers containing syrups of different sugar concentrations and arranged at varying distances from their hives. Data collected during the dry period of the year 2017

Distance (m)	N	Syrup concentration in function of distance (%)							
		30, 30, 30, 30		60, 60, 60, 60		30, 40, 50, 60		60, 50, 40, 30	
		M	Md	M	Md	M	Md	M	Md
0	40	10.15 ± 1.96	4.5 a*	23.25 ± 6.72	6.5 a	9.32 ± 3.26	0 b	5.37 ± 1.63	0 a
15	40	12.70 ± 3.87	1 a	50.87 ± 10.53	16.5 a	16.02 ± 3.47	5 ab	11.92 ± 5.01	1 a
30	40	16.62 ± 5.08	0.5 a	32.67 ± 7.04	4 a	31.10 ± 6.86	8 ab	12.45 ± 3.15	3 a
45	40	15.02 ± 4.19	3 a	23.42 ± 7.44	4 a	28.65 ± 6.46	11 a	6.80 ± 2.33	1 a

*In the context of nonparametric analysis, median values were taken into consideration for the data comparison; Medians followed by same letters on the column do not differ at $p>0.05$

However, even though the quality of the resources available as well as the body size of the bee species are factors that influence the foraging distance of a colony (DORNHAUS *et al*., 2006), it was noticed that the treatments of decreasing concentrations in relation to the distance from hives did not differ ($p>0.05$) among themselves, which shows that the bees were foraging evenly in the area regardless the syrup concentrations and their distances from the hives. Once again, our data reinforces that within the tested 45 m flight radius, these bees had explored evenly the nectar resources available regardless their concentrations, which suggests that their successful foraging can be related to an extremely generalist behavior, fairly exploring the available resources within flight range. Indeed, these bees are easily found in a variety of flower species, as well varied sugar sources such as sodas, ice cream, candies, etc. made available by humans in anthropized areas (SAMEJIMA *et al*., 2004). Such strategy may help to understand how a small-sized and short-flight bee such as *P*. aff. *flavocincta* achieved a vast geographic distribution in the country and is well succeeded in colonizing highly anthropized areas where natural sources of nectar are usually scarce within short distances. Finally, our study provide beekeepers with relevant information on specificities of weather, foraging distance and sugar concentration in nectar that they should consider when feeding *P*. aff. *flavocincta* colonies.

CONCLUSIONS

1. *Pebleia* aff. *flavocincta* has a foraging flight range of at least 45 m from the colony since it easily foraged on artificial flowers placed up to that distance. However, it is necessary to investigate longer distances to determine the maximum flight range of this species;
2. *Pebleia* aff. *flavocincta* forages plentifully at 28 °C and higher temperatures and nectars with a wide range of sugar concentration, preferring those of higher sugar concentrations when available in the field.

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