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Influence of experience on homing ability of foragers of *Melipona mandacaia* Smith (Hymenoptera: Apidae: Meliponini)

F Rodrigues[1,2], MF Ribeiro[3]

1 - Universidade Federal do Vale do São Francisco, Petrolina, PE, Brazil
2 - Universidade Federal Rural do Semiárido (UFERSA), Mossoró, RN, Brazil
3 - Empresa Brasileira de Pesquisa Agropecuária (Embrapa), Petrolina, PE, Brazil

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Corresponding author
Francimária Rodrigues
Universidade Federal Rural do Semiárido, UFERSA
Departamento de Ciências Animais, DCAn
Prédio do DCAn II, Lado Oeste
Av. Francisco Mota, 572
Bairro Costa e Silva, 59.625-900
Mossoró, RN, Brazil
E-Mail: francimaria.rodrigues@ufersa.edu.br

Abstract

The distance a bee can fly to collect food is quite relevant, among other aspects, for successful pollination. However, studies on this aspect concerning stingless bees usually do not take into consideration their homing ability. The objectives of this study were to verify the maximum distance that foragers of *Melipona mandacaia* Smith can fly, and whether experience is relevant for their homing ability in a Caatinga region of Northeast Brazil. Five colonies were used to collect foragers. These were marked and released starting from 100 m from their nests and at every 100 m up to a maximum distance on which there would be no bee returning to the nest. To evaluate the influence of experience, after being marked, another group of bees was put back into colonies, collected again after eight days and released in five distances only (500, 1,000, 1,500, 2,000 and 2,500 m). In both experiments, as the distance increased, the returning success of the bees decreased significantly. In fact, there was a significant negative correlation between their returning success and the distances they were released. The maximum distance a translocate bee returned to its hive was 2,700 m. The percentage of success was very high for bees released at 500 and 1,000 m (100% and 77%, respectively), suggesting this is the common flight range for the species. In most cases, average percentage of success was significantly higher for experienced bees than for other bees reinforcing the idea that experience is quite relevant for homing ability.

Introduction

Stingless bees fly in order to collect the necessary food resources (pollen and nectar) and nest-building materials (resin, mud, etc.), and they need to travel certain distances for that (Roubik, 1989). In fact, bees are faced constantly with the task of navigating back to their nests from remote food sources and this is called 'homing ability'. Honey bees are the most studied bees concerning these aspects. They evolved several methods for doing this, such as compass-direct 'vector' flights, use of learned land marks, and cognitive maps based on spatial memory and on two dimensional snapshots of the surroundings (Anderson, 1977; Cartwright & Collet, 1981, 1983; Gould, 1986; Capaldi & Dyer, 1999; Menzel et al., 2005; Menzel & Giurfa, 2006; Reynolds et al., 2007; Menzel et al., 2012; Cheeseman et al., 2014). Nevertheless, for stingless bees, up to the moment, no studies concerning homing ability have been performed. Investigations have been restricted to foraging activity, recruitment, flight range and maximum flight distances.

The distances traveled by foragers depend on several factors, as density and seasonality of food source, as well as the bee species (Dornhaus et al., 2006), physiology and body size (Araujo et al., 2004; Greenleaf et al., 2007). Moreover, other aspects, isolated or together, may also affect their flight, as internal colony conditions and climatic factors (Hilário et al., 2000).

Flight range have been the object of some studies in a few stingless bee species. In *Trigona corvina* Cockerell, *Partamona* aff. *cupira* (Smith), *Tetragonisca angustula* (Latreille) and *Nannotrigona testaceicornis* (Lepeletier), the maximum distances reached by bees varied from 623 to 853 m (van Nieuwstadt & Ruano Iraheta, 1996). Among bees of the *Melipona* Illiger genus, the maximum distances were

estimated in 2,000 m for *Melipona bicolor* Lepeletier and *Melipona scutellaris* Latreille (Araújo et al., 2004), and 2,100 m for *Melipona mandacaia* Smith (Kuhn-Neto et al., 2009).

The method generally used for such estimations is the training of bees up to a food source. Another method is the capture and recapture of bees, which also allows obtaining information on the maximum flight distance that bees are able to travel to forage (Roubik & Aluja, 1983). There are no studies on the maximum distance a *M. mandacaia* forager can fly using this specific methodology. Moreover, except by the study of Kuhn-Neto et al. (2009), there is no other information concerning the maximum flight distance of this species, specially taking into consideration the experience of bees, which is probably an important factor for homing ability, as demonstrated for honeybees.

M. mandacaia is a very important species for the meliponiculture of Petrolina, Pernambuco state, and Juazeiro, Bahia state, in the Northeastern region of Brazil, being mainly used for honey production (Ribeiro et al., 2012). However, it is relatively little studied, especially concerning its potential for pollination services. In this way, the present study was carried out with the objectives of verifying the maximum distances foragers can fly, their homing ability and the influence of experience on this process.

Material and methods

The experiments were performed at Embrapa Semiárido (09°4'17.53"S 40°19' 10.24" W) in an area of 2,100 ha, at 42 km from the city of Petrolina (Pernambuco state), a semiarid region in Northeast Brazil. The vegetation is typical of hiperxerophile "Caatinga" (Zanella, 2000), a type of savanna. The plants are used to low precipitation, that is restrict to a few months of the year, intense hours of sun and high temperatures, and many of them loose their leaves during the drought period.

M. mandacaia, popularly known as 'mandaçaia', occurs naturally in this Bioma and it is distributed along the São Francisco River, in the states of Bahia, Ceará, Paraíba, Pernambuco e Piauí (Batalha-Filho et al., 2011).

Maintenance of colonies

Five colonies of *M. mandacaia* were used in the experiments. The colonies were installed in hives kept at the Entomology sector of Embrapa Semiárido, in a room maintained at ambient temperature (around 27 °C). Bees had free access to the external environment through a plastic tube in the wall. Supplementary food (*Apis mellifera* Linnaeus honey) was provided in average every eight days, according to a usual beekeeping practice. In the days of the experiments, the colonies did not receive any food.

Two experiments were performed from August 2011 to June 2012, which are described below, totaling 11 consecutive months. Taking into account the possible effects of climate during the experimental period, the release of bees (as explained bellow) occurred in similar conditions of weather and daytime. Thus, bees were released between 8 a.m. to 10 a.m., with average temperature of 27 °C and relative humidity of 65%. In days considered unfavorable (i.e., cloudy, windy, rainy, or with much different conditions concerning temperature and humidity) the release did not happen.

Experiment 1: Homing ability of mixed foragers' group (experienced and inexperienced bees)

In order to be sure only foragers would be used in the experiment, bees were collected at the nest entrance with an insect aspirator when they were arriving from the field. Afterwards, they were placed in acrylic cages (20 x 20 x 20 cm) containing food (*A. mellifera* honey), being one cage for each colony. These bees were then marked on the thorax with plastic nontoxic paint (one color for each investigated distance). Soon after, they were put into wooden boxes and were kept there up to the following day, when they were released.

Initially, because we could not differentiate experienced from inexperienced bees, mixed foragers groups were used. Therefore, mixed foragers groups of 25 bees from each colony were released at distance intervals of 100 m from their nest, up to the distance where no bee returned, i.e. from 100 m, 200 m and so on, up to 2,800 m. The distances from the releasing points were measured with a GPS in a straight line in relation to the nests' entrances (Fig 1). For registering the bees that returned to their nests, small wooden boxes were placed where the original hives were. Those observation boxes had a transparent glass cover, which allowed the observer to check the number and color of bees that returned. The original hives were kept closed throughout the day in order to avoid that other foragers (not marked) returned to the observation boxes. Thus, marked bees were used only once, and for each evaluated distance, new marked bees were used.

The percentage of success was calculated considering the number of bees released, and the number of bees that returned to their nests.

Experiment 2: Homing ability of experienced foragers

During the previous experiment, it was observed that sometimes the bees did not arrive. However, with repetitions of the same distances, other bees were able to return to their nests. Therefore, in order to test the hypothesis that more experienced bees were more capable of recognizing the areas where they were released, and so could find the route back to their nests more easily, another experiment was carried out. The collection and marking of bees was done according to the same methodology already described. Nevertheless, after being marked, the bees were put back into their own colonies. After eight days, these bees were collected again and placed into wooden boxes with

Fig 1. Geographical location of the distances where the *Melipona mandacaia* bees were released. Font: Laboratory of Geoprocessing, Embrapa Semiárido.

food. They were kept there until the next day, when they were released. In this way, these bees had in common eight days of flight experience. However, because we could not collect all marked bees, since some could have died, the number of bees released was defined according to the number of bees that we were able to collect on that day. The registration of returning bees was performed in the same way, but only five distances were tested (500 m; 1,000 m; 1,500 m; 2,000 m; 2,500 m) due to the lower availability of the bees.

At the end of the experiment there were two groups of bees that were compared: a first group, where the experience was not considered (and therefore, could include bees with and/or without experience, i.e., a "mixed group"), and a second group, where only bees with experience were tested ("experienced group").

Statistical analysis

In order to verify if the colonies were statistically different, the Kruskal-Wallis test was applied. To assess the differences found for the returning success between the two groups of bees ("mixed group" and "experienced group"), a Chi-square was applied. The relation between the returning success of the bees and the distance they traveled was tested using a linear regression (Zar, 2010).

Results

Experiment 1: Homing ability of mixed foragers group (experienced and inexperienced bees)

This experiment used 3,225 individuals for releasing, being 25 bees from each colony in each distance. However, some colonies did not reach the same maximum distance (2,700 m), but smaller ones. Thus, for each evaluated distance, in average 125 bees were released, but from 2,400 m, a smaller number of bees could be released (75-100 bees).

Nevertheless, colonies did not show differences among themselves (Kruskal-Wallis, P= 0.463; n= 27 distances) and for this reason the data related to the returning success of the bees were analyzed together.

The number of bees that returned to their nests decreased gradually with increasing distance. As mentioned above, they reached a maximum distance of 2,700 m (Fig 2).

In general, bees had low success in coming back to their nests when released up to the distance of 2,300 m, 2,500 m and 2,700 m (4% of success, for these distances). It was clear that with increasing distance the percentage of success decreased, and there was a strong correlation between these two factors (Fig 2). Indeed, there was a highly significant negative correlation between the returning success of the bees and the distance from where they were released (Spearman ranking correlation, rho= -0.937, P= 0.000, N= 27 distances; Fig 2).

Experiment 2: Homing ability of experienced foragers

In this experiment, 113 individuals were released, being 12-35 bees in each distance, according to the availability of bees, as mentioned above.

The returning success of the bees reached 100% when they were released up to 500 m (Fig 2). Even at the distance of 1,000 m the percentage of success was quite high (77%), reinforcing the idea that experience must be important for their homing ability. This percentage was twice as big as the one found for bees of the previous experiment (Fig 2).

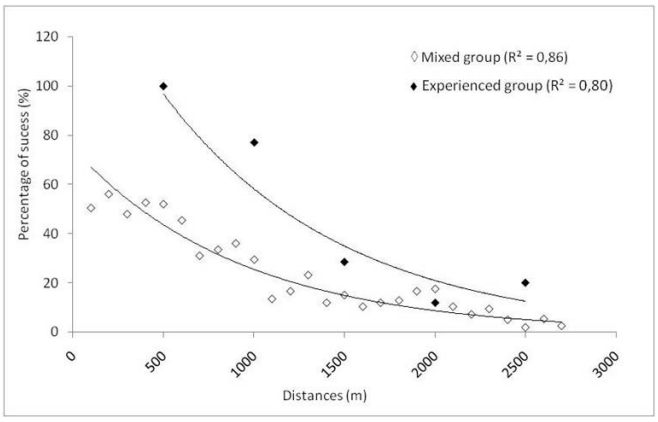

Fig 2. Percentages of success of *Melipona mandacaia* bees returning to their nests in relation to the distance from where they were released for the "Experienced group" and "Mixed group".

When the two groups of bees ("mixed group" and "experienced group") were compared, most the results were higher when experienced bees were used (Table 1). The only exception was for 2,000 m. Thus, both groups of bees were very different in returning success (P values were highly significant at the level of 0.001, Table 1). In the same way, as the distance increased, the returning success decreased significantly, showing a negative correlation (rho= -0.949, P= 0.014, N= 5 distances; Fig 2).

Table 1. Comparison between the number of bees released and returning success of the bees (as well the percentage of success) for the two groups of *Melipona mandacaia* bees ("mixed group" and "experienced group"), and statistical analysis ($\chi c2$ and P values, Chi-square test), for the different distances.

Distances (m)	Number of bees released		Number of returned bees		Percentage of success (%)		Xc^2 (P values)
	Mg	Eg	Mg	Eg	Mg	Eg	
500	125	12	65	12	52.0	100.0	15.2 (P<<0.001)
1,000	125	13	37	10	29.6	77.0	21.1 (P<<0.001)
1,500	125	35	19	10	15.2	28.6	4.1 (P<<0.001)
2,000	125	33	22	4	17.7	12.1	1.0 (P<<0.001)
2,500	100	20	2	4	2.0	20.0	14.7 (P<<0.001)

Mg: "Mixed group"; Eg: "Experienced group".

Discussion

Roubik and Aluja (1983) performed studies using a similar method applied in the present study and found that *Melipona fasciata* Latreille bees returned to their nests when released at the distance of 2,100 m, and *Cephalotrigona capitata* (Smith) returned from 1,500 m. These authors observed that there was a relation between the head size and the distance the bees could fly. Moreover, they also made an estimative through regression tests, and verified that the maximum distances would be 2,400 m and 1,700 m respectively.

Kuhn-Neto et al. (2009) studied the flight of *M. mandacaia* through the training of foragers to the food source. They verified that the maximum distance reached was 2,100 m for larger bees, and 1,560 m for the smaller ones. This confirms what was found by Nieuwstadt and Iraheta (1996) when studying the relationship between the size of some bees (*Trigona corvina*, *Partamona* aff. *cupira*, *Tetragonisca angustula* and *Nannotrigona testaceicornis*) and their foraging range. The authors emphasized that the maximum distances obtained in the experiment of capture and recapture increased ca. 300 m in comparison to the experiment that used the artificial feeder.

In the present study, with another methodology, *M. mandacaia* bees traveled larger distances (2,700 m) than in the study of Khun-Neto et al. (2009), i.e., from 2,100 to 1,560 m. We did not analyzed the size of the bees. However, it is possible that the discrepancy between the results found by us and by Khun-Neto et al. (2009) were due to the different methods used. On the other hand, both methods present limitations. The feeder method could underestimate the flight range since a feeder would not be as attractive for the bee as a flower. In addition, the method of releasing bees could fail when bees do not know the location where they are released (Niewstadt & Ihareta, 1996).

Another fact to be considered is that *M. mandacaia* is a stingless bee endemic from the "Caatinga" region (Zanella, 2000; Batalha-Filho et al., 2011) and this Bioma is characterized by a low density of natural sources and a prolonged drought (Drumond et al., 2000). Thus, plant physiognomy and biology could force the bees to fly longer distances to feed. Although both localities of the experiments (from Khun-Neto et al, 2009, and our study) are in "Caatinga" areas, it is possible that in our case the region presented harder conditions (as the extreme drought of the last years).

Capaldi and Dyer (1999), studying the homing ability of *A. mellifera*, concluded that several factors influence the performance of bees, as for example their learning in relation to nest location and place where they were released, as well as whether they are experienced in foraging. In fact, the data found in the present study with *M. mandacaia* suggest that flight experience is indeed important to bees for homing ability.

Sánchez et al. (2007) studied foraging experience in *Scaptotrigona mexicana* (Guérin) and found that more experienced bees tend to change to other food sources more easily than less experienced bees. When analyzing the effect of experience on the distance reached by *S. mexicana*, they observed that indeed the experience and not the distance of the feeders was the most relevant factor that affected the choice for the food source. These authors concluded that foraging experience could be an advantage for the colony since it allows the exploitation of new food sources, as contributes for diminishing the competition among foragers.

The experience is usually neglected in flight distances studies of stingless bees. The homing ability of an experienced bee in relation to the ability of a naive (or less experienced)

one is remarkable in our results. In fact, the present study is the first one to investigate this factor. In addition, as it was observed, the returning success increased about 30% when more experienced bees were released at 500 m, according to Table 1.

In our experiments, we found that the returning success of the bees was higher for "experienced bees" than for the "mixed group" in all distances, except for 2,000 m (Table 1). For this distance, the result was opposite, and the reason could be that, by chance, the bees released in that distance had a longer experience in the "mixed group" than in the other group. This question remains to be clarified. However, it was remarkable that in all other tested distances the number of succeeded bees was significantly higher for the "experienced group".

The mechanisms by which bees find their way back to their nests were not investigated in this study. However, it is possible that stingless bees use the same learning tools as honeybees, such as landmarks and spatial memory, as previously mentioned. In bumblebees, it was observed that bees that were released presented a 'circling' behavior: flying on circles over the release site (Goulson & Stout, 2001). The same was registered in our experiments and probably was used for initial orientation of the bees.

Conclusions

Although the maximum flight distance reached for *M. mandacaia* was 2,700 m, it was outstanding that all bees released at 500 m were able to return to their nests, and even at 1,000 m the large majority returned, suggesting that these distances are part of the common flight range of foragers. As mentioned by Nogueira-Neto (1997), studies on flight distances of stingless bees are relevant since they provide information on flight range of bees in relation to availability of food sources and possibilities for productivity. Thus, the results presented in this study may be useful in crop pollination programs since the distance of hives in relation to cultivated areas may influence pollination efficiency, and consequently, productivity of crops. Moreover, adequate bee pasture should be included in the common flight range of bees in order to guarantee their production.

Finally, our results demonstrated for the first time that experience can limit or improve the homing ability of stingless bees, and this aspect should be considered in future investigations.

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