SHORT COMMUNICATION

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High precision during food recruitment of experienced (reactivated) foragers in the stingless bee *Scaptotrigona mexicana* (Apidae, Meliponini)

Abstract Several studies have examined the existence of recruitment communication mechanisms in stingless bees. However, the spatial accuracy of location-specific recruitment has not been examined. Moreover, the location-specific recruitment of reactivated foragers, i.e., foragers that have previously experienced the same food source at a different location and time, has not been explicitly examined. However, such foragers may also play a significant role in colony foraging, particularly in small colonies. Here we report that reactivated *Scaptotrigona mexicana* foragers can recruit with high precision to a specific food location. The recruitment precision of reactivated foragers was evaluated by placing control feeders to the left and the right of the training feeder (direction-precision tests) and between the nest and the training feeder and beyond it (distance-precision tests). Reactivated foragers arrived at the correct location with high precision: 98.44% arrived at the training feeder in the direction trials (five-feeder fan-shaped array, accuracy of at least ±6° of azimuth at 50 m from the nest), and 88.62% arrived at the training feeder in the distance trials (five-feeder linear array, accuracy of at least ±5 m or ±10% at 50 m from the nest). Thus, *S. mexicana* reactivated foragers can find the indicated food source at a specific distance and direction with high precision, higher than that shown by honeybees, *Apis mellifera*, which do not communicate food location at such close distances to the nest.

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Introduction

Scouts of stingless bee species have various ways to guide nestmates to a food source. These ways, called communication mechanisms, range from simple alarming (random searching) to the indication of a food source spatial location (directed searching). They can be classified into two categories: (1) nest-based communication mechanisms, which include alarm, sounds, excitatory movements, and trophallactic (responses to the odor and taste of the food) behaviors (Esch et al. 1965; Nieh 1998; Nieh and Roubik 1998); and (2) field-based communication mechanisms, such as piloting, scent trail, food marking, local enhancement, and local inhibition (Kerr et al. 1981; Kerr 1994; Aguilar and Sommeijer 2001; Slaa 2003; Slaa et al. 2003).

These communication mechanisms may facilitate precise location communication. Nonetheless, the accuracy of location communication has not been studied in stingless bees. Schmidt et al. (2003) reported that the stingless bee *Scaptotrigona* aff. *depilis* could distinguish between paired feeders such that 97.5–100% of newcomers arrived at the odor-marked feeder, even when the feeders were only separated by 1.7 m. This shows that stingless bee foragers and, in particular, olfactory communication, may allow foragers to pinpoint a particular spatial location. However, accuracy can only be measured with multiple feeders placed simultaneously in fan-shaped arrays to determine directional accuracy and in linear arrays to determine distance accuracy (von Frisch 1967; Towne and Gould 1988). Because there are no other options, accuracy cannot be measured when foragers can only make a choice between two feeders.

Precise recruitment also requires that the recruiter has a good ability to learn the spatio-temporal characteristics of the food source. When a bee visits a food source, she is said to be experienced because she has started learning the resource's characteristics. She can then use this information as a search image to inform her nestmates of similar food sources once the initial food source is exhausted at a particular time and location. Such reactivated foraging may play an important role in the foraging

strategy of stingless bees (Biesmeijer et al. 1998). When a food source is below certain profitability threshold, the bee returns to its colony, waiting for that food source to be profitable again. This kind of bee is therefore called an unemployed experienced forager (Biesmeijer and de Vries 2001). No work has explicitly evaluated the recruitment precision of reactivated foragers in stingless bees, which could be important in understanding overall meliponine foraging strategies.

In this paper we chose to study *Scaptotrigona mexicana* (Apidae: Meliponini), a stingless bee inhabiting southeast Mexico, where it shares resources with at least other 30 species of stingless bees (Ayala 1999). We wished to answer the following question: how precisely can experienced foragers recruit newcomers and reactivated foragers to a specific distance and direction?

Materials and methods

Study site

A shrimp farm in Puerto Madero, Chiapas (direction experiments, April and May 2002) and a soy farm (direction and distance precision experiments, June–November 2003) in Tapachula Chiapas were chosen as the locations for the experiments, which were performed between 08:00 and 14:00 hours. *Scaptotrigona mexicana* colonies other than ours were not found at the chosen sites. We used four colonies of *S. mexicana* consisting of 1,500–2,500 individuals housed in wooden boxes.

The feeders (control and training) were made of a plastic plate supporting a 225-cm² piece of yellow foam. A spherical piece of cottonwool was placed on the foam square and constantly soaked in saturated table-sugar solution during experiments. A 1.0-m-high tripod supported these components.

Bee training

Communication evidence experiments

We trained the bees by placing the training feeder close to the nest entrance and placing 1 ml of 2.5 M sucrose solution in it. Once the bees started to feed, the training feeder was gradually moved to the desired location. All the bees that landed on this feeder were trapped, except one, which was marked on its thorax with water-based paint. Once the training feeder was at the experimental distance (50 m) and direction (either SW, SE, NW, or NE) a control feeder was placed in the opposite direction but at the same distance. For the next 30 min, all the bees landing on both feeders were trapped and counted, except the marked one. In the next trial, the directions of the feeders were reversed to check for site bias effects. After ten trials, the directions were changed by 90°, and ten more trials were run. At the end of each trial, the trapped bees were marked and released.

Accuracy experiments

The bees were trained as previously outlined, but this time at 5 m from the nest. At this location, the bees were fed on 2.5 M sucrose solution ad libitum. This procedure induced mass-recruitment. Because of the large number of bees feeding during mass-recruitment (100–200 individuals) and the defensive nature of *S. mexicana*, we were unable to trap and mark all the bees without eliciting any alarm behavior. To ensure that all the bees from the colony became experienced, we let them feed for about 1 h. Thus we could be sure that most of the bees were experienced. Next, the concentration was lowered to 0.5 M until only a few bees remained feeding. In this way 3–5 bees were trained to the desired location (50 m SE or NW). The piece of foam was then changed for a clean, different-colored one and the feeder was moved 10 m to the left or to the right. This was done in order to prevent the recruits from choosing on the basis of their own memory (color and direction), rather than on the basis of the parameters communicated by the recruiter bee.

Next, four control feeders, identical in color and shape to the training feeder, were placed according to the experiment (direction or distance), but instead of food they were offered water. This was done in order to prevent the trained bees from consistently choosing these feeders. After 20–30 min, the water was changed for food identical to that in the training feeder. Two behaviors were then tested: (1) trained bees choosing control feeders and (2) trained bees using us as cues. If, for 10–15 min, the trained bees were observed not to change their decision to visit the control feeders due to our presence (standing near a control feeder) or by themselves, the test was allowed to start and the food concentration was increased to 2.5 M to elicit recruitment. All the trained bees were then marked and trapped, except for one that was left free. If this bee was observed not to recruit, then it was trapped and another trapped bee was let free. This procedure was repeated until one bee was observed to recruit. During a period of 30 min, all reactivated foragers (bees that had previously fed at a feeder and were thus marked) were captured in inverted glass tubes with cotton stoppers. All the trapped bees were released after a trial was completed.

Statistical analysis

The hypothesis was that the reactivated foragers would not have a preference for any of the five feeders if there was no communication. In the case of directional communication we expected the number of bees to be higher in the training than in the control feeder. We applied the χ^2 test in both cases.

Results

Evidence of communication

Foragers were recruited to the training feeder in numbers significantly higher ($\chi^2=256$, 1 df, $P<0.001$) than in the control feeder in all 20 trials (five trials per direction, two colonies, see Fig. 1), regardless of the direction. There was no significant effect of the training direction ($\chi^2=6.36$, 3 df, $P<0.05$), and thus no evidence of site bias.

Distance and directional accuracy

In the distance and directional accuracy experiments, we only trained bees to the NW and the SE because preliminary experiments had showed that the effect of direction on the behavior of the bees was not significant. We found a significant difference between the number of reactivated bees visiting the control and training feeders in both direction and distance experiments ($\chi^2=120.25$, 1 df, $P<0.001$, direction trials; $\chi^2=73.4$, 1 df, $P<0.001$, distance trials, see Fig. 2). Reactivated foragers arrived at the correct location with high precision: 98.44% arrived at the training feeder in the direction trials (azimuthal accuracy of at least ±6° at 50 m from the nest), and 88.62% arrived at the training feeder in the distance trials (accuracy of at least ±5 m or ±10% at 50 m from the nest).
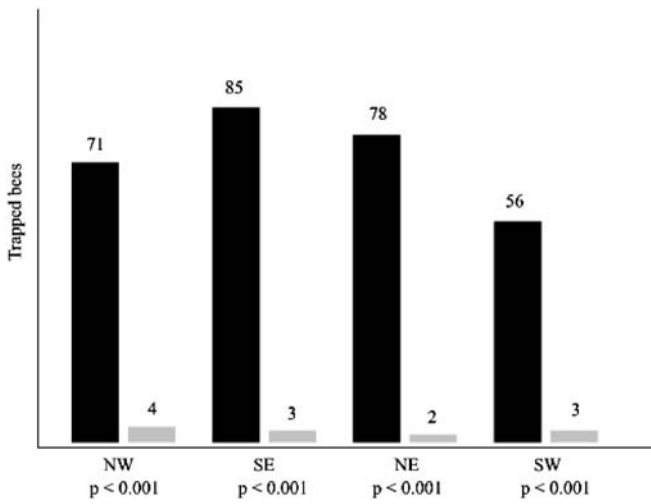
Fig. 1 Number of trapped bees in the control and training feeders in each of the four directions tested (all feeders at 50 m from the nest) in the directional communication tests. *Black bar* = training feeder, *grey bar* = control feeder placed in the opposite direction

Discussion

Scaptotrigona mexicana directional communication and recruitment precision

Despite of the presence of an identical control feeder in the opposite direction, there were always more bees visiting the training feeder [290 bees (96%) in the training feeders; 12 bees (4%) in the control feeders over 20 trials], resulting in a statistically significant difference. Other species of *Scaptotrigona*, e.g., *S. postica*, *S. bipunctata*, and *S.* aff. *depilis*, also recruit nestmates to a specific food

location. Therefore, it seems that all the species within this genus may have similar abilities to communicate specific food location (Lindauer and Kerr 1960; Kerr et al. 1981; Schmidt et al. 2003). Furthermore, our results show that *S. mexicana* foragers can communicate distance and direction with a high degree of precision, with 126 and 2 reactivated foragers, respectively, trapped at the training and control feeders in the direction trials (at least 98.44% azimuthal accuracy at $\pm6°$ and 50 m from the nest) and 109 and 14 reactivated bees trapped in the training and control feeders, respectively, in the distance trials (88.6% precision of ±5 m or $\pm10\%$ at 50 m from the nest). No reactivated foragers ever landed at the ±10 m feeders in any of the direction or distance trials (Fig. 2). Distance accuracy was approximately 10% less than directional accuracy at ±5 m, perhaps due to the fact that foragers always flew over the control feeders located between the training feeder and the colony when going from the nest to the food source. However, further experiments are necessary to test this hypothesis. In honeybees, Towne and Gould (1988) found that the distance scatter is similar in magnitude to the directional scatter. The angular scatter is $\pm7°$ of azimuth at 700 m and $\pm30°$ at 100 m. At distances ranging from 0 m to approximately 100 m (in *Apis mellifera*; von Frisch 1967), honeybees use a round dance that communicates food profitability but does not communicate food location to nestmates in distances close to the nest (Dyer 2002). Unlike Towne and Gould (1988), we did not observe a gradual trend in which foragers were scattered in their arrival locations over multiple feeders. Thus the accuracy that we have measured may be an underestimate. A denser array of feeders (perhaps at 0.5 m intervals) may reveal that reactivated foragers exhibit an even higher degree of accuracy. Such accuracy may derive

Fig. 2 Trapped bees on each feeder in the precision tests. Direction trials to (**a**) Northwest and (**b**) Southeast. Distance trials to (**c**) Northwest and (**d**) Southeast. Five trials per direction. *10 m* and *5 m* refer to the distance of the control feeders relative to the training feeder
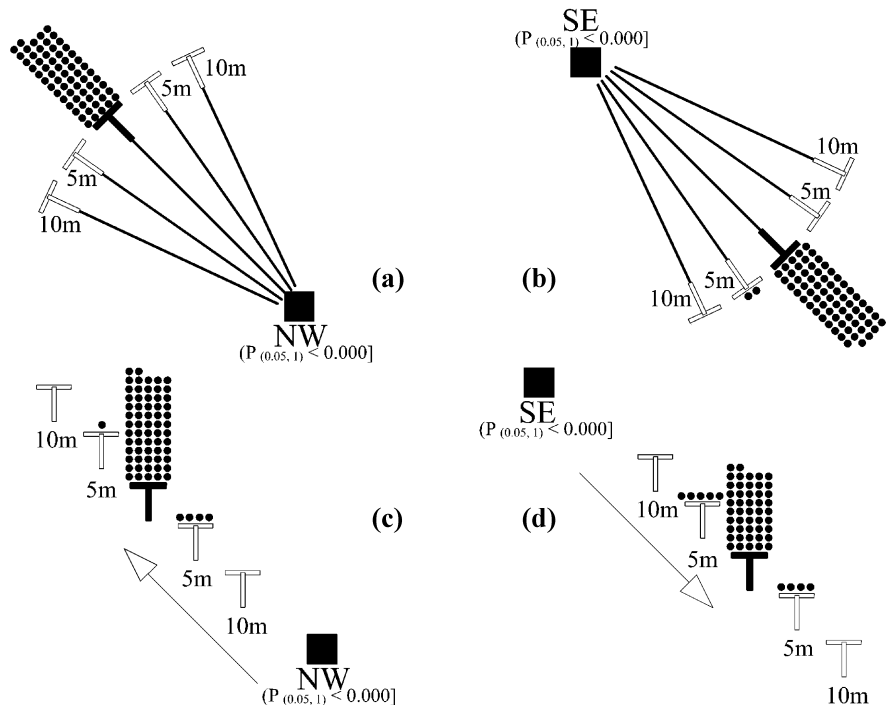
from the olfactory communication mechanism reported in the genus *Scaptotrigona*. Schmidt et al. (2003) and Kerr et al. (1981) showed that *Scaptotrigona* foragers of other species odor-mark feeders, and this may result in nestmates arriving very precisely at the correct distance and direction if *S. mexicana* foragers also odor-mark.

Reactivation of foragers

In previous work with *S. mexicana*, we noted that the bees (even the trained ones) often fed on the control feeders, and our data (unpublished) were very similar to those of Towne and Gould (1988). However, when the color of the piece of foam was changed in the training and color feeders, as in our study, the reactivated foragers showed a definite preference towards the training feeder. This means that unemployed experienced bees may visit the previous site where they fed as a first option when they are stimulated by scouts. As a second option, the bees may follow the scouts' indications to get a similar new food source at a different site.

It is unclear to what degree the reactivated foragers received information inside the nest from recruiters in order to go out and search for the food source. It is possible that reactivated foragers searching for the food source came across odor marks deposited by recruiting foragers without first contacting the recruiting foragers inside the nest. In future studies, we plan to examine the role of intra- and extranidal information in the reactivation of foragers.

Possible communication mechanisms of *S. mexicana*

Previous studies have revealed that the main recruiting mechanism for food collecting used by *Scaptotrigona postica* is pheromone marking (Lindauer and Kerr 1960; Kerr et al. 1981). This mechanism allows a very high efficiency for food localization. Because of the high precision observed in our trials, we suspect this mechanism is used by *S. mexicana* foragers. We have observed departing foragers landing on the feeder and nearby substrates, and rubbing their mouthparts against this substrate, a behavior which is thought to deposit odor marks, and which has been observed in several different meliponine species (Lindauer and Kerr 1960, Schmidt et al. 2003). In fact, Villa and Weiss (1990) demonstrated that *Trigona* (*Scaptotrigona*) *mexicana* foragers were able to mark the feeding site, thus improving the "right" choice of recruits. However, the experimental set up in that study consisted of only two feeders and therefore did not measure the orientation accuracy.

It is also possible that *S. mexicana* recruits use local enhancement as another field-based mechanism (Slaa et al. 2003). We observed foragers landing with less delay onto feeders when other foragers were on the feeder than when no other foragers were on it. On two occasions,

mass-recruitment behavior was observed with almost all the bees landing onto the training feeder and very few choosing the control. Nonetheless, other experiments are needed in order to verify and quantify the relative importance of local enhancement and odor-marking in correct newcomer orientation to a food source in *S. mexicana*.

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