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Original article

Effect of forager-deposited odors on the intra-patch accuracy of recruitment of the stingless bees *Melipona panamica* and *Partamona peckolti* (Apidae, Meliponini)*

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Abstract – We show that the stingless bees *Melipona panamica* and *Partamona peckolti* have a high precision of intra-patch recruitment that is influenced by forager-deposited odor marks in the field. We trained foragers to a 2.5 M sucrose feeder in the center of an array of five identical feeders (20 cm between feeders) at different distances and directions from the nest and measured the distribution of recruit visitations. In the *free-foraging* phase foragers could odor mark a filter paper around the experimental feeder, and in the *odor-removal* phase we substituted it each five minutes by a clean one. Significantly more recruits in both species chose the experimental feeder over the controls in the distance and directional arrays ($P \leqslant 0.034$) and odor removal significantly decreased precision of recruitment in both species ($P \leqslant 0.034$). Scent marks in both species thus play a significant role in orienting recruits to already known profitable food sources.

odor marking / recruitment / stingless bees / intra-patch precision / Meliponini

1. INTRODUCTION

Stingless bees (Tribe Meliponini, Michener, 2000) can recruit nestmates to food sources (Jarau et al., 2000, 2003; Slaa et al., 2003; Biesmeijer and Slaa, 2004; Aguilar et al., 2005), and meliponine communication of food source location by foragers has been a subject of interest since the pioneering work of Lindauer and Kerr (1958, 1960). To recruit, they use several different mechanisms of food communication, ranging from sound pulse production (which may encode the distance and quality of food source: Esch et al., 1965; Esch, 1967; Aguilar and Briceño, 2002; Nieh et al., 2003; Hrncir et al., 2006), scent trail deposition (which indicates the direction from the food source to the nest of some stingless bees, Kerr, 1960; Lindauer and Kerr, 1960; Nieh et al., 2003, 2004; Schmidt et al., 2003; Jarau et al., 2004), point source odor marking of the food source (Aguilar and Sommeijer, 2001; Nieh et al., 2003; Schmidt et al., 2003, 2005; Hrncir et al., 2004; Jarau et al., 2005), pilot flights (Aguilar et al., 2005) to simple alerting (which evidently stimulate foragers to search for food, without providing any location information, Lindauer and Kerr, 1958; Kerr et al., 1963).

However, relatively little is known about the precision of location communication in stingless bees and the influence of the different mechanisms of communication on this precision. To date, no studies have examined the precision of newcomer recruitment with such feeder arrays, although this is the standard protocol in *Apis mellifera* studies (von Frisch, 1967; Towne and Gould, 1988). To test the

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precision of recruitment communication, it is necessary to offer bees more than two feeders to choose from because the distribution of foragers arriving at the feeders is thereby limited to two points and cannot be accurately assessed (von Frisch, 1967; Towne and Gould, 1988). For example, sampling of an error distribution can yield a biased estimate if limited to two points. Sampling of multiple points is desirable, although there are practical considerations that limit the number of feeders in a given array, and thus most investigators have settled on using arrays with a minimum of five feeders (von Frisch, 1967).

In the meliponine bee *Scaptotrigona mexicana*, Sánchez et al. (2004) used five-feeder arrays to measure recruitment precision in reactivated foragers. Reactivated foragers have previously experienced the feeder at a similar location and may therefore be searching for the feeder on their own (based upon a previously acquired search image) or act on new information provided by recruiters (Biesmeijer and De Vries, 2001). Sánchez et al. (2004) found high precision in the feeder choice of reactivated foragers (approximately 98% of foragers chose the correct feeder in a five feeder directional array and approximately 89% made the correct choice in the five feeder distance array) when feeders were placed 50 m from the nest and space 5 m from each other. This high precision of reactivated forager orientation is likely related to forager odor marking of the feeder (Sánchez et al., 2004). Other studies (Aguilar and Sommeijer, 2001; Schmidt et al., 2003, 2005; Hrncir et al., 2004) using paired feeders showed that newcomers of some stingless bees (*M. favosa*, *M. seminigra*, *Nannotrigona testaceicornis* and *S*. aff. *depilis*) can odor mark the food source. In these studies, most workers ($\geqslant$ 63%) preferred to visit a feeder that was already visited by workers of the same species, when two feeders were offered to the bees in a dual choice experiment. However, no studies have yet examined the effect of odor mark removal on recruits in a feeder-array experiment.

We therefore used fan-shaped feeder arrays to study the spatial precision of recruitment in a small patch of feeders and the influence of odor marks on this precision in two stingless bee species, *Melipona panamica* Cockerell, 1919 and *Partamona peckolti* (Friese, 1901). We tested the precision of recruitment in small-scale feeders arrays (20 cm separation among feeders) in order to determine how these species choose among clumped food sources. *Melipona panamica* foragers can recruit nestmates to a good food source at a specific three-dimensional location (Nieh and Roubik, 1995), deposit attractive odor marks on the food source (but not a scent trail), and produce recruitment sounds that may provide location information inside the nest (Nieh, 1998; Nieh and Roubik, 1998). Colonies of *M. panamica* usually contains 500 to 800 adult individuals (Roubik, 1992; Nieh and Roubik, 1995), nests in hollow trees, and occur from northern Colombia to southern Costa Rica (Roubik, unpubl. data).

In contrast, bees in the genus *Partamona* appear to use a different mechanism of communication. The only study concerning food source communication in *Partamona* was performed by Kerr (1969) in the species *P. helleri* (referred as *Trigona* (*Partamona*) *cupira helleri*). He reported that workers from *P. helleri* were able to communicate the direction of the food source (79% of the recruits reached the experimental feeder located 37 m from the nest in a paired-feeder experiment, $N = 67$ recruits). Noting a strong odor enveloping recruiters, he speculated that foragers might release attractive aerial odors in flight to assist recruit orientation, although this hypothesis still needs to be tested. *Partamona peckolti* usually nests upon cavities and crevices located in epiphytes and other substrates, and several entrances may be found clumped. This species inhabits the Pacific coast, from the northwestern forests of Peru and Ecuador into Colombia, Panama and Venezuela, and it was found in great altitudes (2000 m in the Andes, Camargo and Pedro, 2003).

2. METHODS

This study was performed on Barro Colorado Island at the Smithsonian Tropical Research Institute (STRI), Republic de Panama, from September to November 2005. We used one colony of *P.*

peckolti and two colonies of *M. panamica*. The *P. peckolti* colony (P1) was located in its natural nest (in a log crevice) on a balcony outside the bee laboratory (9°9.923'N, 79°50.193'W). The *M. panamica* colonies (M1 and M2) were placed inside observation colonies (description in Nieh and Roubik, 1995) one inside the bee laboratory, and the other on the balcony. Colony M1 corresponds to colony D (approximately 2000 workers) and colony M2 to a new colony G in the sequence (approximately 800–1000 workers) published in Nieh and Sanchez (2005). Colony M1 was connected to the outside with a 1 cm inner diameter, 10 cm long vinyl tube. Observations were made between 0800 and 1600 h and we closed the entrances of *M. panamica* colonies not under study during our experiment to avoid feeder visitation by non-subject colonies. No other colonies of the studied species were present in the study area.

The feeder consisted of a clear glass bottle (40 mL) filled with unscented 2.5 M sucrose solution (Ultra Pure, ICN Biomedicals, cat# 821721) and inverted over a clear grooved circular plastic plate (10 cm diameter, von Frisch, 1967). The plastic plate was placed on top of a 1 m high tripod, allowing us to move the feeder to any desired location. Because of the filter paper used in the experiments, the color showing through the transparent feeder base and bottle was white. In all experiments, we trained bees by placing the feeder next to the nest entrance and then moving it away to progressively greater distances once bees began to visit (method of von Frisch, 1967). All bees visiting the feeder were individually marked on the thorax with different combinations of colored acrylic paints (Binney and Smith, code#54-0125), and thus experienced foragers could be distinguished from recruits (newcomers who had never previously experienced the feeder at any location at any time, Nieh, 2004). Weather data during the experiments was provided by the Terrestrial Environmental Sciences Program of the Smithsonian Tropical Research Institute. Data was taken from the Barro Colorado Lutz Tower weather station (wind speed was recorded each 15 min).

2.1. *Partamona peckolti* odor marking experiment

In this experiment, we tested if *P. peckolti* foragers could deposit odor marks at a good food source, and if these marks were attractive to nestmates. Underneath the plastic grooved plate, but not in contact with the sugar solution, we placed a Whatman filter paper (5.5 m inner diameter, 12 cm outer diameter) that extended a further 3 cm around from the feeder base and thus allowed bees to walk on it and potentially deposit odor marks. In preliminary trials, we placed an additional 2.5 cm circle of filter paper on top of the feeder to determine if bees would land and deposit odor marks, as is observed in several stingless bee species (Nieh, 2004). However, we never observed bees landing on top of the feeder (8 hours observation time) despite substantial recruitment, and thus we used only filter paper under the feeder base during our experiments.

In the initial odor collection phase, we allowed foragers to visit the feeder for 20 minutes, eliciting a large amount of recruitment. After the 20 min, we closed the feeder, removed the putatively odor-marked filter paper, and immediately opened two new feeders (1 m apart) at 25 m west from the nest, but without food. On one of the feeders we placed the used filter paper (experimental feeder) and, on the other, an unused clean filter paper (control feeder). In the subsequent test phase, we captured all experienced foragers that landed on the feeders. In this phase, all foragers visiting the feeders were experienced, and their choice (experimental or control feeder) was noted. We captured foragers with insect aspirators (Nieh et al., 2003) as soon as they landed on the feeder. At the end of each trial, which lasted 10 min, we placed the captured foragers in an empty plastic box provided with food (2.5 M unscented sucrose solution) and covered with a mesh lid for ventilation. We released these bees only at the end of experiments with this species. We reversed the location of experimental and control feeders each five minutes to avoid any site bias. We performed 20 trials in total, 10 trials with each set of feeder locations.

2.2. *Partamona peckolti* intra-patch precision experiments

We trained five foragers of *P. peckolti* to visit a feeder located 45 meters west of the colony. All other bees were captured using insect aspirators and only released after the end of the experiments. After these bees learned the location of the feeder, we placed four additional identical feeders, for a total of five feeders, in a fan-shape array (Sánchez et al., 2004; von Frisch, 1967) separated from each other by 20 centimeters. We placed the feeders on top of a round plastic table (90 cm diameter × 50 cm high). All feeders had a piece of filter paper between the

grooved plate and the glass bottle (as in the previous experiment) that could be marked by trained bees. The trained bees were only allowed to visit the experimental feeder, located at the center of the feeder array. If a trained bee consistently tried to visit the wrong feeder, she was captured and replaced by a new forager in order to avoid possible odor marking of an experimental feeder. The spatial distribution of the feeders depended on the experiment: direction or distance (Fig. 1). We recorded the arrival of recruits and their choice: the experimental or control feeders. Choices were only counted in the absence of other bees on the feeders to avoid visual social enhancement (Slaa et al., 2003) and allowed experienced foragers to visit the feeder during all the time the trials were performed, to avoid the evaporation of odor marks in this experiment as well as in the *M. panamica* experiment (see below).

We divided each experiment (*direction* and *distance*) in two parts: in the first part, the five foragers could mark the filter paper of the experimental feeder at will (*odor-marking phase*). In the second, we removed the experimental filter paper each five minutes and substituted a clean one (*odor-removal phase*) to remove forager-deposited odors. We performed 20 trials in total, ending each trial when we recorded eight recruit choices. Due to variability in recruitment rates, trials lasted on average 20 min, but could vary from 5 to 40 min.

2.3. *Melipona panamica* intra-patch precision experiments

For *M. panamica*, we repeated the same procedure used in the *Partamona* precision experiment, but for this species, we allowed 15 experienced bees to visit the experimental feeder (to increase the rate of recruitment and thus insure an adequate sample size during the *odor-removal* phase). We aimed to perform each phase (*odor-marking* phase and *odor-removal* phase) at two different distances: 25 and 75 m south from the colonies. However, for colony M1 we only were able to perform the experiments at the distance of 25 m, because recruitment was almost absent due to a bloom of natural food sources when we tried to train the foragers to 75 meters. For colony M2, the experiments were performed at the specified distances. We performed four trials, each one consisting of eight recruit choices, for each phase of the experiments. Due to variability in the rate of recruitment, trials lasted on average 20 min, but could vary from 5 to 40 min.

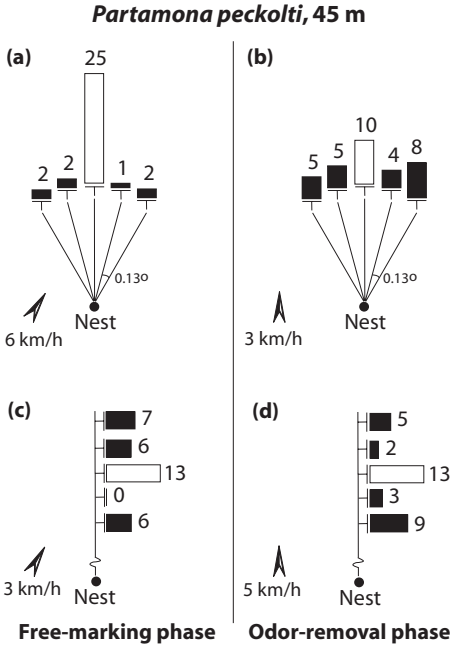

Figure 1. Number of *P. peckolti* recruits in the feeder-array choice experiments. Direction trials in the (a) *odor-marking* and (b) *odor-removal* phases. Distance trials in the (c) *odor-marking* and (d) *odor-removal* phases. Sample sizes given at the tops of bars. White bar: experimental feeder, black bars: control feeders, 45 m refers to the distance from experimental feeder to nest. Feeders were spaced 20 cm from each other (not shown in scale). Arrowheads indicate the average wind direction for each experiment.

2.4. Statistical analysis

In all experiments, we used the χ^2 test to determine if bees preferred the experimental or control feeders in the precision and odor mark attractiveness experiments. For comparisons among different treatments (i.e. comparisons between the precision of recruitment at the *odor-marking* and *odor-removal* phase), we used the heterogeneity G-test. To compare the recruitment precision at different distances from the nest, we used ANOVA. For tests that involved multiple sampling of the same data, we applied a sequential *Bonferroni* correction for multiple analysis as appropriate (Sokal and Rohlf, 1995; Zar, 1999). All angular measurements are reported in degrees (°).

3. RESULTS

3.1. *Partamona peckolti* odor marking experiment

Partamona peckolti foragers usually flew in groups, at a very low height (approximately 0.5 m from ground) and seemed to orient to odors in the air around the experimental feeder. However, no typical odor-marking behavior common to other stingless bees (landing with mandible-rubbing) was observed in the departing foragers. Significantly more bees (54.7%) chose the experimental feeder ($N = 280$) with the previously visited filter paper in the test trials ($\chi^2_1 = 4.5$; $P < 0.034$, $N = 512$). Thus, *P. peckolti* foragers can deposit attractive odor marks on food.

3.2. *Partamona peckolti* intra-patch precision experiments

In the *direction* experiment (0.13° separation between each feeder relative to the nest, total array angle of 0.51° relative to the nest, four trials, $N_{bees} = 32$), a significantly greater number of recruits (72%) chose the training feeder over the four control feeders in the *odor-marking* phase ($\chi^2_4 = 433.2$, $P < 0.0001$, Fig. 1a). In the *odor-removal* phase, the majority of recruits visited the control feeders (68.8%, $N = 22$), instead of the experimental ($G = 33.28$, $d.f. = 4$, $P < 0.001$, Fig. 1b). Thus, odor removal significantly influenced the behavior of recruits, reducing the number of bees arriving at the experimental feeder by 41%.

In the *distance* experiment (four trials, $N_{total} = 32$), recruits exhibited no significant preference for the experimental over the control feeders in the *odor marking* (40.6% chose experimental; $\chi^2_4 = 85.2$, $P < 0.001$) and in the *odor-removal* phases (40.6%, Fig. 1c, 1d). There was just a slight significant difference between the spatial distribution of recruit choices in the *odor-marking* and the *odor-removal* phases ($G = 9.49$, $d.f. = 4$, $P < 0.05$), but in both phases the same number of recruits ($n = 13$) choose the experimental feeder.

Comparing results from the direction and distance arrays at each distance, there were no significant differences between the spatial distribution of forager choices in the *odor-marking* phase (ANOVA: $F_{1.6} = 9.39$, $P = 0.04$, $\alpha_{bonferroni} = 0.01$, N.S.) and in the *odor-removal* phase (ANOVA: $F_{1.6} = 0.28$, $P = 0.61$).

3.3. *Melipona panamica* intra-patch precision experiments: directional arrays

In the directional array at 25 m from the nest (0.46° separation between each feeder relative to the nests, total array angle of 1.83° relative to the nest, eight trials, $N_{total} = 64$), most recruits (87%) preferred to visit the experimental feeder during the *odor-marking* phase ($\chi^2_4 = 2338.8$, $P < 0.0001$; Fig 2a). In the *odor-removal phase*, significantly more recruits still preferred to visit the experimental feeder but odor removal reduced the proportion of correct choices by 23% ($G = 33.36$, $d.f. = 4$, $P < 0.001$, Fig. 2b).

We next trained bees to a *directional array 75 m from the nest* (0.08° separation between each feeder relative to the nests, total array angle of 0.31° relative to the nest, four trials, $N_{total} = 32$). In the *odor-marking phase*, recruits from colony M2 significantly preferred to visit the experimental feeder (84.4%) over the control feeders ($\chi^2_4 = 535.2$, $P < 0.0001$, Fig. 3a). In the *odor-removal phase*, recruits from colony M2 did not show any preference for the experimental (59.4%) over the control feeders (Fig. 3b). Odor removal reduced the proportion of correct choices by recruits by 25% ($G = 9.83$, $d.f. = 4$, $P < 0.0001$).

3.4. *Melipona panamica* intra-patch experiments: distance arrays

In the *odor-marking phase* of the *25 m distance array* (eight trials, $N_{total} = 64$), recruits from both colonies preferred to visit the experimental feeder (85.9%) to the control feeders ($\chi^2_4 = 2246.8$, $P < 0.0001$, Fig. 2c). However, in the *odor-removal phase*, recruits did

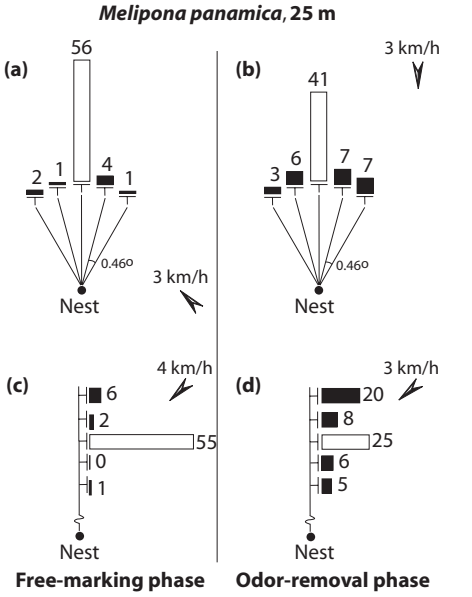

Figure 2. Number of *M. panamica* recruits in the feeder-array choice experiments performed 25 meters from the colony. Direction trials at the (a) *odor-marking* and (b) *odor-removal* phases. Distance trials at the (c) *odor-marking* and (d) *odor-removal* phases. Sample sizes given at the tops of bars. White bar: experimental feeder, black bars: control feeders, 25 m refer to the distance from experimental feeder to nest. Feeders were distanced 20 cm each other (not shown in scale). Arrowheads indicate the average wind direction for each experiment.

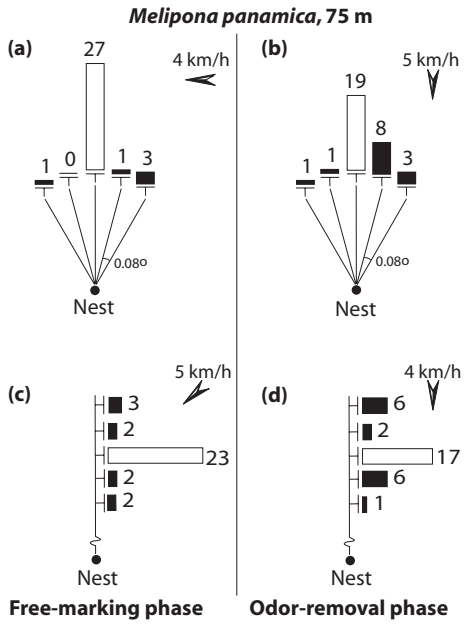

Figure 3. Number of *M. panamica* recruits in the feeder-array choice experiments performed at 75 m from the colony. Direction trials at the (a) *odor-marking* and (b) *odor-removal* phases. Distance trials at the (c) *odor-marking* and (d) *odor-removal* phases. Sample sizes given at the tops of bars. White bar: experimental feeder; black bars: control feeders, 75 m refer to the distance from experimental feeder to nest. Feeders were distanced 20 cm each other (not shown in scale). Arrowheads indicate the average wind direction for each experiment.

not show any preference for the experimental (39.1%) over the control feeders (Fig. 2d). Thus, odor-removal reduced the proportion of correct choices by recruits by 46% at 25 m ($G = 78.67$, $d.f. = 4$, $P < 0.0001$).

In the *odor-marking phase* of the *75 m distance array* (four trials, N_{bees}=32), recruits from colony M2 preferred to visit the experimental feeder (71.9%) over the control feeders ($\chi^2_4 = 345.2$, $P < 0.0001$, Fig. 3c). In the *odor-removal phase*, recruits from colony M2 did not show any preference for the experimental (53.1%) or control feeders (Fig. 3d). Thus, odor-removal reduced the proportion of correct choices by recruits by 18% at 75 m ($G = 9.83$, $d.f. = 4$, $P < 0.05$).

At each distance *within each phase*, the intra-patch spatial precision of recruits was similar in the direction and distance arrays. At 25 m, there is no significant difference between the distribution of recruits arriving at the feeder array in the *odor-marking* phases (ANOVA: $F_{1.14} = 0.05$, $P = 0.82$) or the odor-removal phases (ANOVA: $F_{1.14} = 3.75$, $P = 0.07$) of the direction and distance arrays. This was also true for comparisons of the distributions of recruits between the distance and direction arrays at 75 m (*odor-marking*: ANOVA: $F_{1.6} = 2.18$, $P = 0.19$, *odor-removal*: ANOVA: $F_{1.6} = 0.4$, $P = 0.55$).

3.5. *Melipona panamica* intra-patch precision at different distances

Between different distances *within each phase*, the distribution of recruits was similar

at the distance arrays. It was also similar for the direction experiments between different distances within each phase (*odor-marking* phases at 25 m and 75 m; direction: ANOVA $F_{1.10} = 0.15$, $P = 0.70$; distance: ANOVA $F_{1.10} = 2.90$, $P = 0.12$). Similarly, there was no significant difference in the *odor-removal* phases at 25 m and 75 m (direction: ANOVA $F_{1.10} = 0.09$, $P = 0.77$; distance: ANOVA $F_{1.10} = 1.32$, $P = 0.28$).

4. DISCUSSION

Melipona panamica is known to deposit odor marks on food (Nieh, 1998, this experiment) and our experiments now show that the same is true of *P. peckolti*. In both meliponine species, we found a high precision of recruitment in a small patch and odor marking of the food source significantly enhanced this precision, because odor removal significantly reduced directional and distance spatial precision in both species. In *P. peckolti*, directional precision decreased by 41% when forager-deposited odors were removed, but distance precision did not decrease (arrays 45 m from the nest). In *M. panamica*, odor removal decreased newcomer directional precision by 23% and 25%, respectively, at arrays located 25 m and 75 m from the nests. Odor removal decreased *M. panamica* distance precision by 46% and 18%, respectively, at arrays located 25 m and 75 m from the nests. Our results also showed that for *M. panamica*, there was no significant difference on the precision of recruit choices at the different distances tested in our experiment: 25 m and 75 m. Differences in precision may exist with greater distances among feeders and because stingless bees can have a foraging range that extends more than 1 km (Roubik and Aluja, 1983; Van Nieuwstadt and Iraheta, 1996).

In general, floral nectars contain from 5% to 80% sugar (Baker and Baker, 1983), corresponding to 0.15 M and 3.0 M (Bubnik et al., 1995). Roubik and Buchmann (Roubik and Buchmann, 1984) reported that sucrose concentrations of nectar collected by four species of *Melipona* in central Panama during the dry season (including *M. panamica* colonies studied on Barro Colorado Island) ranged from 19% (0.6 M) to 72% (2.8 M), and noted that *M. panamica* colonies did particularly well at imbibing viscous, high concentration sucrose solution. Due to competition from natural food sources, relatively high sucrose concentrations are required to elicit consistent foraging at artificial feeders, even during periods of relative food dearth (Nieh, 2004), and we therefore used 2.5 M (65% g solute per g solution).

Regarding the effects of wind on recruit choice, the average wind direction in the *P. peckolti* experiments directed feeder odors away from the nest (Fig. 1). In the *M. panamica* experiments, in those cases where the wind was blowing on average from the feeder arrays towards the nests, there should have been an equal effect on all feeders in the directional array (Figs. 2b and 3b) because the wind was oriented on average in a direct line from the central feeder to the nest. In cases where the wind blew on average from the distance arrays to the nests (Figs. 2c, d and 3c, d), we would expect a skewing of the distribution such that more recruits would choose the feeders closest to the nest. However, this did not occur (Figs. 2c, d and 3c, d). Thus wind did not appear to have a strong effect on our results, perhaps because of the small inter-feeder distances and because the winds were not very strong (3–6 km/h).

It is also possible that our odor removal manipulation did not remove all forager-deposited odors. However, foragers always landed exclusively on the filter papers that we sent out. Thus removing these filter papers removed all contact-deposited odor marks. With both tested species, this odor removal manipulation significantly reduced the spatial precision of recruit choices (Fig. 1b–d, Fig. 2b–d, Fig. 3b–d).

4.1. *Partamona peckolti* intra-patch precision and odor-marking behavior

Our results show *P. peckolti* foragers can deposit odor marks to experienced nestmates (reactivated foragers) to a food source. A significant number of foragers (55%, $N = 512$)

preferred to visit the feeder with filter paper over the previously visited feeder, demonstrating that this species can mark the food source, like other stingless bees (Jarau et al., 2002; Nieh et al., 2003; Schmidt et al., 2005).

In the *distance* precision experiment, recruits did not significantly choose the experimental feeder over the control feeders during the *free-foraging* or *odor-removal* phases. Thus, a failure to find a significant decrease in *distance* precision with odor removal is not surprising. It may be that distance accuracy is less important than directional accuracy in this species with such small distances between feeders. Future research may examine the source of the odor marks through glandular extract experiments and determine whether such odor marks are cues deposited by experienced foragers for their own reference (see Boogert et al., 2006) or signals deposited by experienced foragers for newcomers (see Schmidt et al., 2005) as well as the effect of such marks in a feeder array with a greater angular distance among feeders.

We did not observe *P. peckolti* foragers depositing odor trails from feeder towards the nest. No typical *Trigona* or *Scaptotrigona* odor-marking behavior was observed (Lindauer and Kerr, 1958; Nieh et al., 2003, 2004). We did not see bees landing and rubbing their mandibles on or around the feeder. Preliminary behavioral observations suggest that the attractive odor marks were air-borne, not requiring contact, because we observed foragers of this species flying at a low height and orienting towards the experimental feeder with the odor-marked filter paper within 10 cm of the feeder. In several cases, we observed a group of experienced foragers arriving together at the feeder. Whether this was due to their previous experience with the feeder or due to a spatio-temporal clumping enhanced by an aerial odor trail and by piloting bees is unclear. In *P. tica* and *T. corvina*, Aguilar et al. (2005) reported that experienced foragers use piloting to guide recruits to profitable food sources. The hypothesis of aerial odor trails suggested by Kerr (1969) for *Partamona* thus remains to be tested, although such a mechanism would only function in relative weak winds, at a low height (where air currents would not dissipate the odors), and not as a trail extending for hundreds of meters but as a supplemental signal, allowing recruits to more easily localize the guide bee (Michener, 1974; Roubik, unpubl. data).

4.2. *Melipona panamica* intra-patch precision

In *M. panamica* (Nieh, 1998) and other *Melipona* species (Aguilar and Sommeijer, 2001; Hrncir et al., 2004), foragers can deposit recruitment odor marks on food. However, little is known about how these odors affect the intra-patch precision of food location communication when recruits have to choose among several feeders. Our results show that such marks are important when *M. panamica* recruits have to choose among clumped food sources. Precision was quite high at the direction (≅ 88%) and distance (from 72% to 86%) experiments, when foragers were allowed to freely mark the experimental feeder. The high intra-patch precision that recruits demonstrated may allow nestmates of this species to fully exploit an already known and profitable food source, and the small scatter could allow them to verify the profitability of the unexploited food sources nearby.

Odor removal substantially reduced the precision of *M. panamica* recruitment, especially in the distance trials, when only 39% of the recruits arrived at the experimental feeder when it was located 25 m from the nest. Precision at longer distances (75 m) was not significantly different from precision at shorter distances (25 m), although odor-removal decreased distance precision by 46% at 25 m and 18% at 75 m.

Thus, in *M. panamica*, intranidal communication may provide precise information to recruits about the spatial location of a food source (Nieh, 1998, 2004; Nieh and Roubik, 1998; Nieh et al., 2003), whereas odors deposited at the food source are important to guide recruits once they are near the target (Nieh, 1998). Other mechanisms, like social enhancement and piloting flights should also be considered in this process, and should be investigated. The role of forager deposited odor

marks in spatial precision at different heights, especially at the canopy level, at greater distances from the nest and with greater distances among feeders, remains to be studied.

4.3. Origin of marks

In our study we did not investigate which glands were involved in the odor marking behavior of *M. panamica* and *P. peckolti*, but we know from previous studies (Hrncir et al., 2004; Jarau et al., 2005) that footprint pheromones originated from tarsal glands, and not mandibular glands, are important for scent marking in *M. seminigra*. For *M. panamica*, Nieh (1998) hypothesized several mechanisms that could be involved in scent marking, but excluded, based upon his results, anal droplets and mandibular glands as the source of scent marking. Thus, footprint pheromones, like in *M. seminigra*, are a possible source of the pheromones that influenced the behavior of recruits in this study and in the experiments of Nieh (1998). In the *Partamona* genus there are no studies that investigated the origin of odor marks deposited by foragers besides this study. Thus, it remains to be investigated the origin of marks in *P. peckolti* and to corroborate the possible role of tarsal glands as the source of scent marking in *M. panamica*.

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Influence des odeurs déposées par une butineuse sur la précision, à l'intérieur d'un site de butinage, du recrutement chez *Melipona panamica* et *Partamona peckolti* (Apidae, Meliponini).

Abeille sans aiguillon / marquage odorant / communication chimique / recrutement / précision intra-patch / Meliponini

Zusammenfassung – Die Auswirkung der von Arbeiterinnen hinterlegten Düfte auf die Genauigkeit der Sammlerinnenrekrutierung innerhalb von Blütenansammlungen bei den Stachellosen Bienen *Melipona panamica* und *Partamona peckolti* (Apidae, Meliponini). Wir zeigen, dass die neotropischen Stachellosen Bienen *Melipona panamica* und *Partamona peckolti* eine hohe räumliche Genauigkeit bei der Sammlerinnenrekrutierung innerhalb von Blütenansammlungen aufweisen, und dass diese stark von durch die Sammlerinnen abgegebenen Duftmarkierungen beeinflusst wird. Wir trainierten Sammlerinnen auf einen mit 2,5 M Zuckerlösung versehenen Fütterer in der Mitte einer fächerförmigen Reihung von 5 identischen Fütterern (20 cm Abstand zwischen den Fütterern) in unterschiedlichen Abständen und Richtungen vom Nest und ermittelten die Verteilung von Besuchen durch Neuankömmlinge (Bienen, die nie zuvor einen der Fütterer besucht hatten). Trainierte Sammlerinnen wurden nur zu der mittleren Futterstelle zugelassen. Die Richtungs- und Entfernungsgenauigkeit innerhalb der Ansammlungen wurde durch verschiedene Reihen von Fütterern untersucht. Jedes Experiment wurde in zwei Phasen unterteilt: *freies Sammeln*, wobei die Sammlerinnen ein Filterpapier um den Fütterer herum mit Duft markieren konnten, und *Duft entfernen*, wobei wir das Filterpapier alle 5 Minuten entfernten und durch ein neues, sauberes Papier ersetzten. Insgesamt bevorzugten sowohl bei den Versuchen zur Entfernungsgenauigkeit als auch zur Richtungsgenauigkeit bei beiden Arten signifikant mehr Rekruten die experimentellen Fütterer gegenüber Kontrollfütterern. (72 % bis 87 % an den experimentellen Fütterern, $P \leqslant 0{,}034$, Abb. 1a, c, 2a, c, 3a und 3c). Die Entfernung der von den Sammlerinnen abgegebenen Duftmarken verminderte die Genauigkeit der Rekrutierung bei *M. panamica* signifikant (−23 % bzw. −25 % weniger Neuankömmlinge bei dem Experiment mit einer Richtungsreihung, −46 % bzw. −18 % bei einer Entfernungsreihung; bei 25 m: Abb. 2b, d, und bei 75 m: Abb, 3b, d, $P \leqslant 0{,}024$) und bei *P. peckolti* (−41 % bei der Richtungsreihung, keine Änderung bei der Entfernungsreihung; bei 45 m, $P \leqslant 0{,}034$, Abb. 1b, d). Diese Ergebnisse zeigen zum ersten Mal, dass Sammlerinnen von *P. peckolti* Duftmarken abgeben, die auf Nestgenossen anziehend wirken. Bei beiden Arten scheinen Duftmarken daher wichtig zu sein, um die Rekruten auf den Besuch von bereits bekannten profitablen

Futterstellen hin zu orientieren und eine rasche Ausnutzung dieser Futterstellen zu ermöglichen.

Duftmarkierung / Rekrutierung / Stachellose Bienen / Genauigkeit in Blütenansammlungen / Meliponinen

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