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A stingless bee (*Melipona panamica*) indicates food location without using a scent trail

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Abstract The study of location specification in recruitment communication by bees has focused on two dimensions: direction and distance from the nest. Yet the third dimension, height above ground, may be significant in the tall and dense forest habitats of stingless bees. Foragers of the stingless bee *Scaptotrigona postica* recruit to a specific three-dimensional location by laying a scent trail. Stingless bees in the genus *Melipona* are thought to have a more sophisticated recruitment system that communicates distance through sounds inside the nest and direction through pointing zig-zag flights outside the nest. However, prior research on *Melipona* has not examined height communication or even established that foragers can recruit newcomers to a specific location. We used identical paired feeders to investigate recruitment to food in *M. panamica* on Barro Colorado Island, Panama. We trained foragers from an observation hive to one feeder and monitored both feeders for the subsequent arrival of newcomers. We changed the relative positions of the feeders to test for correct direction, distance, and canopy-level communication. A 40-m canopy tower located inside the forest enabled us to examine canopy-level communication. We found that *M. panamica* foragers can recruit to a specific (1) direction, (2) distance, and (3) canopy level. To test the possibility that foragers accomplish this by means of a scent trail, we placed the colony on one shore of a small cove and trained bees over 116 m of open water to a feeder located on the opposite shore. We also placed a second feeder on this shore, equidistant from the colony but 20 m from the first feeder. Significantly more

newcomers consistently arrived at the feeder visited by the foragers. *Thus foragers evidently do not need a scent trail to communicate direction.* Inside the nest, a forager produces pulsed sounds while visibly vibrating her wings after returning from a good food source. She is attended by other bees who cluster and hold their antennae around her, following her as she rapidly spins clockwise and counterclockwise. Locational information may be encoded in this behavior. However, foragers may also directly lead newcomers to the food source. Further experiments are planned to test for such piloting and other communication mechanisms.

Key words Stingless bees · Recruitment to food · Location communication · *Melipona panamica*

Introduction

Social insect colonies require considerable food intake, yet often exploit ephemeral and widely scattered food sources. Faced with such temporal and spatial constraints on their food collection activities, social insects have evolved mechanisms of forager recruitment. These recruitment mechanisms must solve the basic problem of communicating food location in a complex three-dimensional environment. Evolution has tailored a variety of solutions. For example, recruiting ants lay scent trails or lead their nestmates directly to the food source (Hölldobler and Wilson 1990). Because ants must walk upon suitable surfaces and negotiate large obstacles, the recruitment path may acquire a complex topography and include trail markers that would be difficult to communicate indirectly. Flight simplifies path specification by enabling foragers to make a “bee-line” directly to food sources. Recruiting honey bees thus specify the polar coordinates of their food sources. Their symbolic dance language communicates distance and direction; but it provides only two-dimensional

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information because it does not indicate height (von Frisch 1967). (We use the term “recruitment communication” term to refer to any recruitment mechanism that allows newcomers selectively to find the signaled food source at a specific spatial position.)

The diverse recruitment systems of stingless bees include features of ant and honey bee recruitment, but are the least understood (Lindauer and Kerr 1960; Esch 1967). In several stingless bee genera, as in ants, recruits directly follow foragers the entire distance to food sources or follow scent trails laid down by foragers (Kerr 1969, 1994). Direct leading (also known as piloting) and scent trails are the only mechanisms currently known that allow foragers to specify a three-dimensional location to recruits.

The data gathered in this study indicate that recruits of *Melipona panamica* perform a directed search to find a food source in a three-dimensional array based upon information provided by successful foragers. Furthermore, these foragers apparently do not use scent trails to communicate location.

Their complete communication mechanism remains a mystery. Prior studies (Esch et al. 1965; Esch 1967) reported that certain *Melipona* may use sound to communicate distance. However, these studies only demonstrated a correlation between the duration of sound pulses produced by successful foragers and the distance to the food source visited by the bees. They did not demonstrate that recruits use this information.

Recruitment in Meliponinae (not including the genus *Melipona*)

In this paper, we employ the recent taxonomic revision of the Apidae by Michener (1990) that divides this family into four subfamilies: Bombinae (bumble bees), Euglossinae (orchid bees), Apinae (honey bees), and Meliponinae (stingless bees).

The stingless bees are highly social bees that occupy diverse habitats in the tropical and subtropical forests of Africa, Asia, Australia, and Central and South America and include many genera known to recruit to rich food and resin sources (Roubik 1989). Of these, the genus *Melipona* contains species that apparently possess the most sophisticated methods of recruitment (Kerr 1969). However, it is useful to first review the recruitment systems of other stingless bee genera. Our knowledge of these systems is more extensive and may thus provide clues to the mechanisms employed by *Melipona*.

The approximately 35 genera of non-*Melipona* Meliponinae have developed diverse recruiting systems that exhibit at least three levels of communicative complexity. Additional levels may be uncovered as more species are studied.

1. In the simplest forms of recruitment, foragers produce weak sounds and do not provide location

information or scent mark the food source. Foragers apparently rely only upon the natural food odor, conveyed inside the nest, to attract recruits outside the nest. This strategy is reported in *Trigona silvestrii*, *T. freiremaiai*, and *T. flavicornis* (Kerr 1969).

2. At the next level, foragers of species such as *Trigona* (*Tetragonisca*) *angustula*, *Plebeia droryana*, and *P. muelleri* employ loud sounds and random zig-zag running, often colliding with other bees inside the nest (Kerr 1969). *Nannotrigona testaceicornis* and *Meliponula* (*Axestotrigona*) *tescorum* foragers also exhibit these behaviors. However, their sounds initiate a chain reaction, exciting nearby bees to buzz and causing the entire colony to chorus within 1 min (Esch et al. 1965; Kerr 1969). At levels 1 and 2, recruits presumably find the food source by following the experienced forager or randomly searching for the food's natural odor. Foragers of these species apparently do not communicate location (Kerr 1969).

3. At the highest level, foragers combine buzzing and zig-zag running with trail marking pheromones. *Scaptotrigona postica*, *S. xanthotrica*, *Trigona spinipes*, *T. trinidadensis* (= *amalthea*), *T. hyalinata*, *T. recursa*, *Cephalotrigona capitata*, *Oxytrigona tataira*, and *T. (Geotrigona) mombuca* foragers lay a scent trail by depositing secretions of their mandibular glands upon grass and leaves (Lindauer and Kerr 1960; Kerr 1960; Kerr et al. 1981). *S. postica* recruits evidently need this trail. Foragers did not successfully recruit nestmates to a feeder placed across a water gap until the researchers provided a substrate for the pheromone (a rope covered with branches) to bridge the gap (Lindauer and Kerr 1960). In *S. postica*, *S. bipunctata*, and *T. spinipes*, a scent trail alone is apparently sufficient since shifting the trail (by moving a rope with scented branches) can misdirect recruits to a novel feeder not visited by experienced foragers (Kerr et al. 1963). Such a scent trail can also be laid vertically, allowing *S. postica* foragers to recruit to a particular point in the canopy (Lindauer and Kerr 1960). A *Partamona cupira* forager may adopt an intermediate strategy. Kerr (1969, p. 141) postulates that she releases her mandibular pheromone while flying, perhaps enhancing the ability of recruits to follow her closely: in flight “the bee, excited by the finding of food, [opens] its mandibular gland orifices in such a way that the whole animal becomes enveloped in odor and releases maximal quantities of odorous material into the air.”

Thus some of the most complex stingless bee recruitment systems depend upon a scent trail. In simpler systems, recruits presumably (1) search alone and without locational information, guided only by the source's natural scent or (2) directly follow the forager the entire distance to the food source (piloting). Studies of forager behavior inside the nest have not revealed correlations suggesting the transmission of location information in any stingless bee species outside the

genus *Melipona*. All recruiting stingless bee foragers apparently produce sounds inside the nest upon returning from rich food sources. However, only *Melipona* species produce sounds that are correlated with food location (Esch et al. 1965). Specifically, the sounds of *S. postica*, *Meliponula* (*Axestotrigona*) *tescorum*, *Partamona cupira* and *Trigona* (*Frieseomelitta*) *varia* are not correlated with the distance to the food source (Esch et al. 1965). Except in the most primitive species, all recruiting foragers appear to make irregular, excitatory zig-zag movements inside the nest that may signal the presence of rich food outside the nest. To date, no studies have reported correlations between these motions and food location.

Recruitment in the genus *Melipona*

The recruitment systems of *Melipona* species are comparatively understudied, although they are potentially the most complex. Foragers of some species exhibit correlations between their recruitment behavior inside the nest and food location outside (Esch et al. 1965). Recruits of these species are assumed to search non-randomly for a food source based upon information provided by experienced foragers, but this has yet to be demonstrated (Lindauer and Kerr 1960; Esch et al. 1965).

Previous experiments failed to show the communication of distance or direction in *Melipona*. Lindauer and Kerr (1960) trained *M. scutellaris* foragers to one feeder (the experimental feeder) and placed an identical feeder in a different location (the control feeder). They trained no bees to the control feeder and captured all bees that found it. Thus no bees could signal the control feeder's location. To test for directional communication, they placed the two feeders equidistant from the nest but in opposite directions. In tests for distance communication, they placed the two feeders in the same compass direction from the nest, but at different distances. Consistently more newcomers should have arrived at the experimental feeder if they were guided by trained foragers or searched with location information. In two out of three *directional* trials, more newcomers arrived at the experimental feeder, but these differences were not significant in any of the trials ($P = 0.11, 0.21, 0.58$: probabilities are calculated from their data using a binomial distribution with $q = 0.5$ and a one-tailed test). In three out of three *distance* trials, significantly more newcomers *failed* to arrive at the experimental feeder ($P = 0.15, 0.42, 0.999$; in the $P = 0.999$ trial, significantly more newcomers arrived at the *control* feeder). Upon the basis of these few tests, one might tentatively conclude that *M. scutellaris* recruits do not acquire direction or distance information.

Kerr and Rocha (1988, p.1200) stated that *M. rufiventris* foragers have a location communication

system consisting of "a small smell trail of one, two or three marks." Kerr (1994) later extended these observations to *M. scutellaris*, *M. bicolor*, and *M. quadrifasciata*. However, the role of such marks in communication was not examined in either paper. They also did not clearly demonstrate that foragers left *scent-marks*. While "marking," foragers rubbed their metathoracic legs together, their mesothoracic and prothoracic legs against their abdomen, and finally their mandibles against the borders of a leaf or stem. As Kerr and Rocha (1988) acknowledged, this behavior closely resembles simple grooming that does not leave scent marks. They argued that the mandibular rubbing deposits pheromone because it resembles the scent-marking behavior of other stingless bees. Yet they did not chemically analyze these marks or mention if the marks attracted other foragers. Even if the mandibular rubbing deposits scent marks, the data do not convincingly demonstrate a *scent trail*. With the food source 100 m away, all marks occurred within 8 m of the feeder and were, on average, 4 m away from the feeder (Kerr and Rocha 1988). With the food source 20 m away, all marks occurred within 7.5 m of the feeder and were, on average, 2.9 m away from the feeder (Kerr 1994). In this second experiment, both *M. rufiventris* and *M. scutellaris* marked more often at points closest to the feeder: 92% of all marks occurred within 4 m of the feeder (Kerr 1994). At such close distances, these "marks" may signal the final position of the food source, but not provide a *trail* guiding recruits from the nest. For example, honey bees can release a forager attracting Nasanov pheromone at a food source, but they do not lay a *scent trail* (von Frisch 1967).

Prior studies of other *Melipona* species detailed possible mechanisms of distance and direction communication, but did not rigorously test if recruits use such information (Esch 1967; Esch et al. 1965). Esch et al. (1965, p. 149) reported that recruiting *M. quadrifasciata* and *M. merillae* foragers produce sound pulses of increasing duration with increasing distance to the food source. They performed the following playback experiment to determine if these sounds can communicate distance:

Bees from a nest of *M. quadrifasciata* were trained to visit a feeding station about 10 m from the nest. All bees were marked, and then food was removed until the bees no longer visited the feeding place. As soon as the signal for a nearby (0 to 30 m) feeding station was played from the tape recorder through a loudspeaker connected to the nest, old and new bees arrived at the 10-meter feeding place....The bees did not respond to a signal for a feeding station 300 m away or for a station at any other distance.

These results are subject to multiple interpretations because the experiments did not include a control

feeder. Experienced foragers and newcomers may have found the experimental feeder based on distance information provided by the playbacks or simply by a random search. Alternatively, the sounds may have sent experienced foragers to the experimental feeder with newcomers only following along, not using the sounds as a distance cue. Esch et al. (1965) repeated this experiment with foragers trained to a feeder 300 m from the nest. This time, no bees left the nest in response to sounds for any distance, although injecting the food's scent was sufficient to draw out experienced foragers. Thus the function of these sounds remains uncertain. Perhaps they indicate only if a food source is near or distant and must be coupled with other information to arouse foraging for distant sources.

Esch (1967) observed a potential method for the communication of direction outside the nest. He stated that *M. quadrifasciata* foragers guided recruits "in a striking zig-zag flight into the direction of their feeding place" (Esch 1967, p. 199). The recruits reportedly lost contact with the forager after 30–50 m and returned to the nest to await her return. They reached the feeder only after 20–30 guiding flights (Esch 1967).

Thus although the behavior of *Melipona* foragers suggests mechanisms for communicating food location, *Melipona* recruits have not been shown to use locational information. We therefore designed tests to determine if *M. panamica* foragers can cause recruits to search for a food source at a given three-dimensional location, reserving most questions of mechanism for future studies. We tested for the use of scent trails (1) because of reports that some *Melipona* may use scent trails (Kerr and Rocha 1988; Kerr 1994) and (2) because this communication mechanism is used by another stingless bee, *Scaptotrigona postica*, to communicate three-dimensional food location (Lindauer and Kerr 1960; Kerr et al. 1981).

Materials and methods

Study site and colony

Our study species, *M. panamica*, usually lives in tree cavities containing colonies of 500–800 adults (Roubik 1992). Colonies are found at elevations up to at least 1400 m in the moist forests and rain forests from north-west Brazil to Nicaragua (Roubik 1992). Adults are comparatively large, measuring 10 mm in length and weighing 70 mg on average (Roubik and Buchmann 1984). They have a maximum flight range of over 2 km (Roubik and Aluja 1983). We considered these features in choosing a natural site for our research and in designing our feeder arrays and experimental procedure.

These studies were conducted in the forest on Barro Colorado Island in Lake Gatun, Panama. We obtained a natural colony of *M. panamica* from the Santa Rita Ridge region (in Colón Province approximately 15 km south-west of Portobelo, Panama) and housed it inside a wooden observation hive. This hive consisted of three interconnected chambers gradually decreasing in depth (Fig. 1).

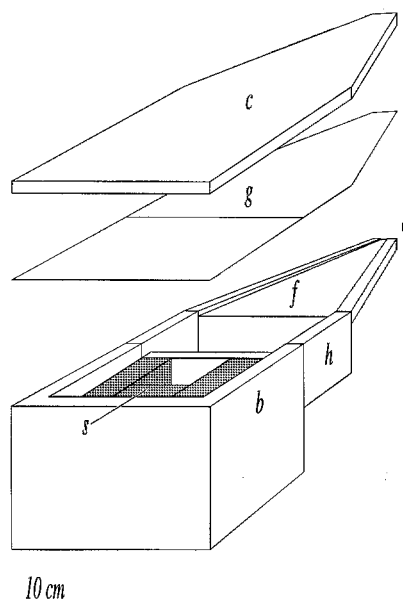


Fig. 1 The observation hive. Bees enter and exit through a single point (e). The hive is constructed of wood and covered with plate glass (g). This glass is in two sections to allow the removal of glass over the honey and pollen pots (h) and the food unloading platform (f) without disturbing the involucrum sheath that covers the brood comb and becomes attached to its glass cover. The hive cover (c) and the brood chamber (b) are lined with styrofoam (s)

The deepest chamber held the brood comb and was insulated with styrofoam boards. The second shallower chamber held honey pots and pollen pots. We periodically pipetted honey from these pots to keep the colony in a state of hunger and thereby encourage recruitment. The triangular third chamber was 6.4 mm deep and gradually tapered to a point from which a vinyl tube allowed bees to leave and enter the hive (1 cm diameter). Foraging bees unloaded their food and performed recruitment behavior in this chamber. The face of the entire hive was covered with plate glass. We placed a wooden cover insulated with styrofoam over the glass when not making observations. For the water experiments, the hive was brought to the shore of an inlet and secured inside a metal cage that was further walled with chicken wire and plastic sheets to protect the bees from animals such as coatimundis, toads, and basilisks. For all other experiments, we placed the hive inside a laboratory building and inserted the hive entrance through the window. To reduce the disturbing effects of light, we enclosed the area around the hive with a velvet curtain and covered the window with aluminum foil.

Feeder

We trained bees to a sucrose feeder consisting of a 60-ml Kimax glass recrystallization dish inverted over a clear grooved Lucite disk. Underneath this disk, we placed a circle of fluorescent paper overlaid with a black radial pattern printed onto a transparent acetate sheet. We dispensed 100 μ l of scent (either anise or lemon-extract) onto a 4.9-cm² wedge of *Whatman* filter paper placed on top of the glass dish. We placed this feeder in the center of a plastic stand sitting inside a saucer containing water (to exclude ants) fastened to a tripod. Concentrations of sucrose within the feeder varied from 0.5 to 2.75 M as appropriate to the experiment. All solutions contained 1 μ l scent/ml sugar solution.

Both the experimental and control feeders were identical. The same colored paper and scent were used for both. Both feeders contained approximately equal quantities of solution with identical

sugar concentrations. We simultaneously applied all changes to both feeders through coordination by two-way radios. Scent was periodically and simultaneously dispensed onto the filter paper from calibrated syringes at both feeders. We added scent (100 μ l) approximately once each 1.5 h.

We trained bees by first presenting the feeder near the hive entrance until a few bees began to feed and then gradually moved the feeder further away in the desired direction (technique described by von Frisch 1967). In some experiments we used a 40-m-high canopy tower sited 150 m South-east of the hive. The tower consisted of an open framework of stacked aluminum scaffolding sections each $2 \times 2 \times 1.5$ m containing internal stairs slanted at 45° through each section. This tower was mounted on a concrete base and stiffened with steel cables attached to the tower at 20 m and 40 m and running to steel eyelets driven into concrete piers on the forest floor. When training bees up the canopy tower, we used the stairs to carry the feeder slowly up the scaffolding platforms. To train bees across water, we placed the feeder inside a canoe and gradually moved across to the opposite shore.

We allowed a fixed number of foragers at the experimental feeder. We used powdered paint pigments suspended in shellac to mark each visiting forager with a unique combination of colors on the thorax and abdomen (method of von Frisch 1967). For the videotaped experiments, we marked bees with numbered tags glued on the thorax (*Opalith-Zeichenplättchen*). We censused the number of foragers arriving in each 15 min interval and captured or released additional marked foragers as necessary to maintain a constant number visiting the feeder. We captured all bees in Ziploc plastic freezer bags to limit the potential release of alarm pheromone (method discussed in Gould et al. 1970).

Data collection at the feeder

We defined newcomers as bees that were not trained to the feeder and that consequently must have found the feeder either by a random or a directed search. *We identified newcomers as bees that were either unmarked or had marks from visiting a previous, different location.* After uniquely marking all newcomers at the experimental feeder, we captured and confined them in plastic bags, releasing them only as necessary to maintain a constant number of foragers. To confirm that the newcomers arriving at the experimental feeder were from our colony, we released them at the end of each day and verified their return to the observation hive. At the beginning of the next day, we captured all returning foragers from foreign colonies and separately captured all other bees that exceeded our quota of trained foragers. (These excess bees were not counted as recruits.) We froze all bees from foreign colonies and released all bees from our colony at the end of each day. Since all bees arriving at the feeder were uniquely marked, we could distinguish between these experienced foragers and newcomers. We thus avoided killing and thereby depleting the colony of foragers. This is an important consideration as colonies of *M. panamica* typically contain only 500–800 adults (Roubik and Buchmann 1984).

We captured all bees arriving at the control feeder, but we did not release them, to prevent any bees from signaling the control feeder's position. At the end of the day, we individually marked these bees with paints or plastic tags and transferred them into a temporary hive consisting of a glass fishbowl covered with mesh and containing a pitted slab of styrofoam filled with sucrose solution. We maintained them inside this structure until we began a new set of experiments using entirely different feeder locations. After release, these bees usually found their way back into the colony. Again, we took these measures to avoid excessively depleting the colony's supply of foragers.

We assumed, but did not verify, that newcomers at the control feeder came exclusively from our colony. However, this increased the stringency of our test of H1 (more newcomers from our hive will arrive at the experimental feeder) by biasing results against a directed search.

Because of competition from natural food sources, the bees were often reluctant to recruit for our sucrose feeders, and on several days we received only two to three recruits. The data for some trials were thus collected over multiple days. However, all replicates of a given trial contain data collected over a different set of consecutive days. We ended each trial once we reached a significance level ≤ 0.05 . In the two canopy level trials that did not show significant differences, we stopped after collecting nine or ten newcomers.

Observations of behavior inside the hive

We lit selected portions of the unloading platform with a Bausch and Lomb Fiber-lite. We did not illuminate the honey pots or the brood comb. This cool, yet bright, light source did not appear to disrupt normal behavior inside the hive. Bees unloaded food, recruited, and did not try to fly towards the artificial light as they exited. Their behavior appeared identical to that observed under a bright red light-emitting diode (LED) light source (gallium-aluminum-arsenide light emitting diode) generating negligible amounts of heat with a peak intensity around 667 nm and filtered to only pass wavelengths above 640 nm (Roscolux medium red filter #27). The photoreceptors of *M. marginata* and *M. quadrifasciata* workers are insensitive to light at these wavelengths (Hertel and Ventura 1985).

To monitor forager sounds, we inserted a Knowles electret microphone (EK-3103) inside the hive at the entrance.

Hypothesis testing

We tested the following hypotheses with identical paired feeders placed in locations that varied by a single component: (1) direction, (2) distance, or (3) canopy level.

1. H0: More newcomers *do not* arrive at the experimental feeder than arrive at the control feeder (probability of a recruit arriving at the experimental feeder ≤ 0.50)
2. H1: More newcomers arrive at the experimental feeder than at the control feeder (probability of a recruit arriving at the experimental feeder > 0.50)

To test for the communication of direction, we placed both feeders the same distance from the observation hive, but in opposite directions. In the test of distance communication, we placed both feeders in the same direction from the hive, but at different distances. Finally, in the test of canopy level communication, we placed both feeders at different canopy heights but in the same direction and horizontal distance from the hive.

To test if specific recruitment in *M. panamica* requires a scent trail, we followed the strategy of Lindauer and Kerr (1960) and prevented foragers from laying a scent trail by placing the hive and feeders on opposite shores of a water gap (Fairchild Cove). The experimental and control feeders were 20 m apart and were each 116 m from the hive, separated by 10° of azimuth. These positions were dictated by the geometry of the cove, which was approximately 116 m wide, but narrowed at one end. We placed both feeders 1 m back from the water line. They were separated by vegetation of density comparable to that found in the previous distance and direction experiments.

To eliminate site bias, we swapped the positions of the control and experimental feeder in half of the trials in all experiments.

Statistical tests

For each trial, we calculated the one-tailed binomial probability of our observed distribution of newcomers at the control and experimental feeders with $p = q = 0.5$. We also pooled our data, as appropriate, to calculate the overall one-tailed binomial probability.

Results

We began by testing for the correct communication of (1) direction, (2) distance, and (3) canopy level.

Newcomers consistently arrived at the feeder in the correct direction (Fig. 2). We placed both feeders on the ground in opposite directions 100 m from the hive and found that significantly more newcomers arrived at the experimental feeder in all trials ($P \leq 0.05$ in all 6 trials, for pooled data, $P = 4 \times 10^{-8}$).

As Fig. 3 shows, recruitment to a particular distance is remarkably specific. With the experimental feeder 100 m from the hive, we placed the control feeder 5–90 m from the hive in the same direction (both feeders on the ground) and found that newcomers always arrived in significantly greater numbers at the experimental feeder ($P \leq 0.04$ in all ten trials, for pooled data $P = 8 \times 10^{-16}$). Newcomers still preferred the experi-

mental feeder even when both feeders were only 10 m apart.

In the canopy level experiments, we trained bees to a 40-m-high canopy tower located in the forest 130 m south-east of the hive. This height corresponded to the canopy top in this section of forest. Newcomers came only to the experimental feeder when we placed it at the tower base (Fig. 4). This was true regardless of whether we placed the control feeder 40, 20, or 10 m above the experimental feeder ($P \leq 0.03$ in all eight trials, for pooled data $P = 5 \times 10^{-13}$). However, with the experimental feeder at the top of the tower (40 m), we attracted roughly equal numbers of newcomers with the control feeder at 30 m (see Fig. 5, $P \leq 0.38$, for pooled data, $P = 0.32$). Newcomers thus did not discriminate between 40 and 30 m. However, significantly more newcomers were attracted to the experimental feeder (at 40 m) when we placed the control feeder at 0 m and at 20 m ($P \leq 0.03$ in all six trials, for pooled data $P = 2 \times 10^{-8}$). Newcomers could apparently discriminate between the top experimental feeder and

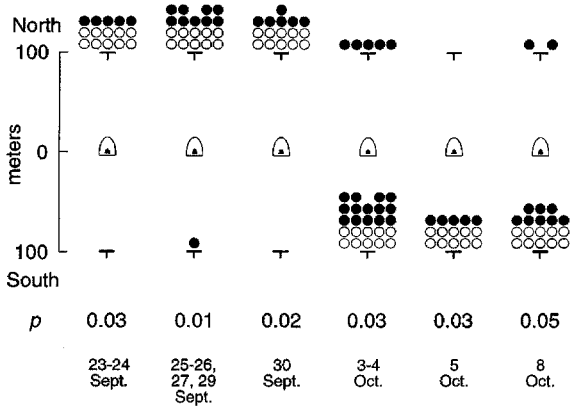


Fig. 2 *Melipona panamica* foragers can recruit to a specific direction. In all six trials, significantly more newcomers arrived at the experimental feeder ($P \leq 0.05$). The open circles represent trained foragers (denoting the experimental feeder) and the black circles represent newcomers. Both feeders are 100 m away from the hive, one directly north and one directly south. The dates and the one-tailed binomial probability for each trial are shown

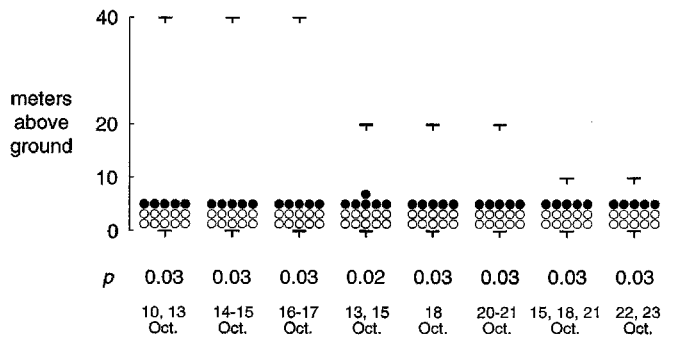
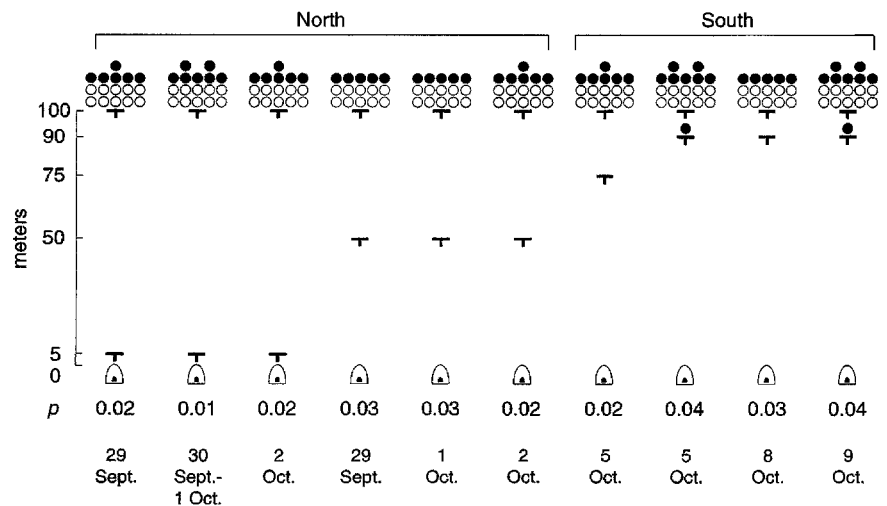


Fig. 4 *M. panamica* foragers can specifically recruit to the base of the canopy tower. The experimental feeder is at the base of the tower. The control feeder is located either 10, 20, or 40 m directly above the experimental feeder. In all eight trials, all newcomers arrived at the experimental feeder ($P \leq 0.03$). The dates and the one-tailed binomial probability of each trial are shown; symbols as before

Fig. 3 *M. panamica* foragers can recruit to a specific distance. In all 10 trials, significantly more newcomers arrived at the training feeder ($P < 0.04$). The experimental feeder is 100 m either directly north or south of the hive. The control feeder is 5, 50, 75, and 90 m away from the hive in the same direction as the experimental feeder. The dates and the one-tailed binomial probability of each trial are shown; symbols as before



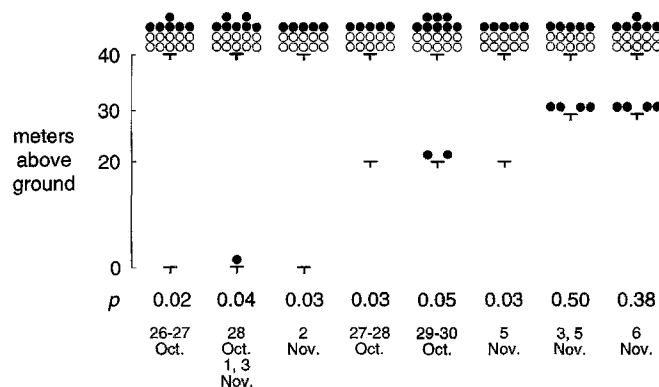


Fig. 5 *M. panamica* foragers can specifically recruit to the top of the canopy tower. The training feeder is 40 m high at the top of the canopy tower. The control feeder is located either at the base of the tower (0 m) or at intermediate heights (20 m or 30 m): six out of eight trials are significant ($P \leq 0.05$). The dates and the one-tailed binomial probability of each trial are shown; symbols as before

the lower control feeder when they were separated by either 40 or 20 m.

Thus foragers can evidently recruit newcomers to a specific direction, distance, and canopy level. In the next experiment, we tested for a communication mechanism, the scent trail, that allows the stingless bee *Scaptotrigona postica* to accomplish the same task.

In these trials, we observed that all foragers leaving the feeder flew directly over the water and thus did not leave substrate-borne scent trails. Nevertheless, as shown in Fig. 6, significantly more newcomers arrived at the experimental feeder in all six trials ($P \leq 0.03$, for pooled data, $P = 3.41 \times 10^{-13}$). Thus *M. panamica* recruits evidently do not need a scent trail to recruit hivemates to a specific location.

Some location information may be communicated inside the hive. A recruiting *M. panamica* forager exhibits a fascinating, stereotypic sequence of behav-

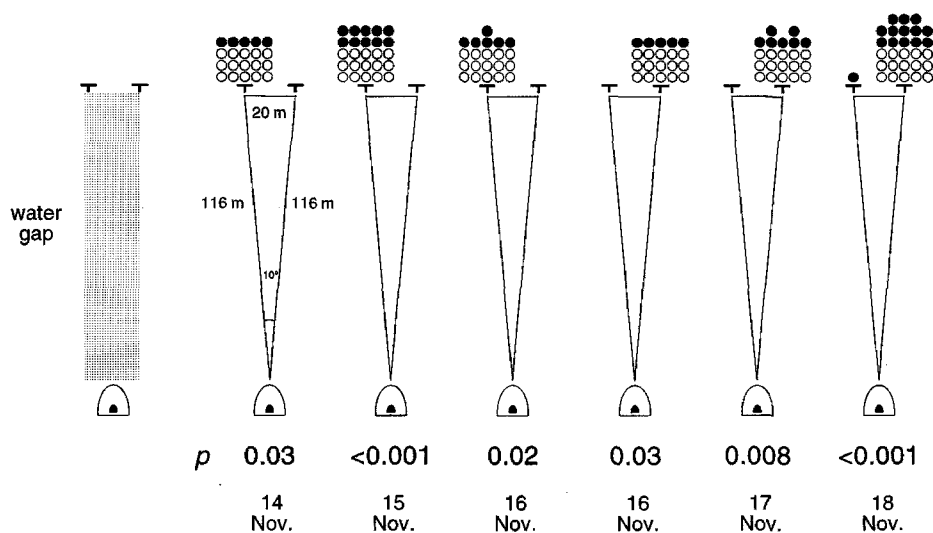
iors. Upon entering the nest, she begins to produce buzzing sounds. These appear to increase in intensity if the entrance is blocked, particularly if she must push her way into the hive. She produces pulsed sounds while searching for a bee to unload her nectar and continues sound production while unloading her gathered nectar. She folds her wings over her abdomen and vibrates them along her dorsal-ventral axis during each pulse. Foragers with waving antennae cluster around her abdomen, occasionally holding splayed antennae over her vibrating wing tips. They follow her as she spins rapidly clockwise and counterclockwise between bouts of food exchange. During these darting movements, her body pivots around the tip of her abdomen. She appears intentionally to contact other bees by changing her path if they move near. When feeding at a rich food source (2.5 m), she moves more rapidly than in the colony, buzzing while most bees spinning and running in the area in front of the food storage pots. She often continues sound production even as she exits the hive.

Discussion

M. panamica foragers can evidently communicate the location of food sources in three-dimensions without using a scent trail to indicate direction. This is remarkable because only one other species in the Meliponinae, *Scaptotrigona postica*, has been rigorously shown to communicate three-dimensional location, and it relies upon scent trails (Lindauer and Kerr 1969).

We currently have a limited understanding of the mechanisms for communicating the location of such three-dimensional coordinates. The best-studied system, the honey bee dance language, does not indicate height (von Frisch 1967). Yet such information would provide a competitive advantage to social insects

Fig. 6 *M. panamica* foragers evidently do not need scent trails in order to recruit newcomers to a specific direction. Both feeders and the hive were placed along the shore on opposite sides of a waterway. All foragers departing from the feeder were observed to fly directly across the water. In all six trials, significantly more newcomers arrived at the training feeder ($P \leq 0.03$). The dates and the one-tailed binomial probability of each trial are shown; symbols as before



foraging in the tall and dense tropical forests. Direct piloting and scent trails allow ants and some stingless bees to accurately deliver recruits to a particular three-dimensional location (Hölldobler and Wilson 1990, Lindauer and Kerr 1960). However *M. panamica* evidently does not use scent trails and other *Melipona* species reportedly do not pilot recruits directly to the food source (Esch 1967).

Esch (1967) reports that *Melipona* foragers fly in a zig-zag pattern pointing in the correct direction without leading recruits the entire distance to the food source (partial piloting). Partial piloting assumes that recruits arrive at the correct distance by separately acquiring distance information (perhaps from forager sounds) or by searching for particular scent marks deposited near the feeder.

Because the observations of Esch (1967) did not eliminate the possibility of foragers directly piloting recruits the entire distance to the food source, we will test for piloting in our next set of experiments. However, we have already made some suggestive observations. We observed that newcomers sometimes arrived at the feeder when no other bees were present. Arrival times were not necessarily clumped together as one might expect for guided bees. Furthermore, newcomers would sometimes continue to arrive for several minutes after all the marked foragers had been captured at the feeder (feeder 100 m from the colony).

Alternatively, recruits may fly in a random pattern searching for a particular olfactory or visual beacon. They may search (1) for a marking pheromone deposited at or near the food source by the forager or (2) a visual stimulus provided by foragers swirling around the feeder before they land. We will also test these hypotheses in our next series of experiments.

Although the first hypothesis provides a simple answer, it seems unlikely that a recruitment system including such a complex repertoire of sounds and motions can be entirely explained by a food-marking pheromone. *Scaptotrigona postica* foragers also scent mark the food source, however no recruits arrive at the feeder, without a scent trail (Lindauer and Kerr 1960). Since *M. panamica* forage evidently do not use a scent trail, one wonders what other mechanisms they may have evolved to solve the problem of communicating location.

The correct answer may not be simple. A recruiting forager's rich repertoire of sounds and motions suggests that information may be exchanged inside and outside the colony. Thus an intriguing mystery remains: how exactly do *Melipona* foragers communicate three-dimensional food location?

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References

- Esch H (1967) Die Bedeutung der Lauterzeugung für die Verständigung der stachellosen Bienen. *Z Vergl Physiol* 56:408–411
- Esch H, Esch I, Kerr WE (1965) Sound: an element common to communication of stingless bees and to dances of the honey bee. *Science* 149:320–321
- Frisch K von (1967) The dance language and orientation of bees (translated by Leigh E. Chadwick) Belknap, Cambridge
- Gould JL, Henerey M, MacLeod MC (1970) Communication of direction by the honey bee. *Science* 169:544–554
- Hertel H, Ventura DF (1985) Spectral sensitivity of photoreceptors in the compound eye of stingless tropical bees. *J Insect Physiol* 31(12):931–935
- Hölldobler B, Wilson EO (1990) The ants. Belknap, Cambridge
- Kerr WE (1960) Evolution of communication in bees and its role in speciation. *Evolution* 14:386
- Kerr WE (1969) Some aspects of the evolution of social bees. *Evol Biol* 3:119–175
- Kerr WE (1994) Communication among *Melipona* workers (Hymenoptera: Apidae). *J Insect Behav* 7:123–128
- Kerr WE, Rocha R (1988) Comunicação em *Melipona rufiventris* e *Melipona compressipes*. *Ciência Cultura* 40:1200–1203
- Kerr WE, Ferreira A, Mattos NS de (1963) Communication among stingless bees – additional data. *J N Y Entomol Soc* 71:80–90
- Kerr WE, Blum M, Fales HM (1981) Communication of food source between workers of *Trigona (Trigona) spinipes*. *Rev Brasil Biol* 41:619–623
- Lindauer M, Kerr WE (1960) Communication between the workers of stingless bees. *Bee World* 41:29–41, 65–71
- Michener CD (1990) Classification of the Apidae (Hymenoptera). *Univ Kansas Sci Bull* 54:75–164
- Roubik DW (1989) Ecology and natural history of tropical bees. Cambridge University Press, New York
- Roubik DW (1992) Stingless bees: a guide to Panamanian and Mesoamerican species and their hives (Hymenoptera: Apidae: Meliponinae). In: Quintero D, Aiello A (eds) *Insects of Panama and Mesoamerica: selected studies*. Oxford Science, Oxford, pp 495–524
- Roubik DW, Aluja M (1983) Flight ranges of *Melipona* and *Trigona* in tropical forest. *J Kans Entomol Soc* 56:217–222
- Roubik DW, Buchmann SL (1984) Nectar selection by *Melipona* and *Apis mellifera* (Hymenoptera: Apidae) and the ecology of nectar intake by bee colonies in a tropical forest. *Oecologia* 61:1–10

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