



# Variation in the ability to communicate three-dimensional resource location by stingless bees from different habitats

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We evaluated the ability of two Brazilian stingless bee species, *Melipona mandacaia* and *M. bicolor*, to recruit nestmates to a specific three-dimensional location. We used experimental feeder arrays and provide the first detailed evidence demonstrating that recruitment communication in *Melipona* can lead to large, rapid and highly significant increases in the number of nestmates visiting a specific location. *Melipona bicolor* and *M. mandacaia* foragers both recruited nestmates to the correct distance and direction, but differed in their ability to recruit nestmates to the correct height. These differences may relate to their respective habitats. *Melipona mandacaia* inhabits semi-arid areas of Caatinga where most food sources occur close to the ground, and its foragers evidently cannot recruit nestmates to the correct height. *Melipona bicolor*, an Atlantic rainforest species, evidently does not communicate height when the food source is at ground level, but can communicate height when the food source is at the forest canopy level (12 m high), where major food sources occur. Species-specific variation in three-dimensional location communication is intriguing because it suggests that *Melipona* may be a good model for studying the evolution of recruitment communication systems in highly social bees.

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Highly social bees (stingless bees and honeybees) can recruit nestmates to good resources such as rich nectar sources. These recruitment communication systems can be remarkably sophisticated, as exemplified by the ability of honeybees to communicate food distance and direction through the waggle dance (von Frisch 1967; Gould 1975; Michelsen et al. 1989; Dyer 2002). The goal of understanding how such recruitment communication systems have evolved has led investigators to study the stingless bees (Hymenoptera, Apidae, Meliponini), a highly diverse, monophyletic group (Michener 2000) encompassing a wide range of recruitment strategies with a broad and graded range of communication complexity (Lindauer & Kerr 1958; Kerr 1960; Esch 1967).

## Location-specific Recruitment

Researchers have paid particular attention to the stingless bee genus *Melipona* because some *Melipona* species

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may possess functionally referential communication, the ability to transform environmental information into specific, abstract coded signals (Marler et al. 1992). Correlations have been found between the distance to the food source and the duration of sound pulses produced by recruiting *Melipona* foragers inside the nest (Esch et al. 1965; Esch 1967; Nieh & Roubik 1998; Aguilar & Briceño 2002; Nieh et al., in press a). Although it is still unclear whether such sound pulses communicate distance information to recruits (Hrcir et al. 2000, 2002; Aguilar & Briceño 2002), *Melipona* are the only animals, other than honeybees, in which such potential spatial coding has been reported (Esch et al. 1965; von Frisch 1967; Nieh & Roubik 1998; Aguilar & Briceño 2002).

An important first step in evaluating location-specific recruitment is to determine its influence on colony foraging. However, it is unclear whether location-specific recruitment provides an adaptive benefit by significantly contributing to foraging in *Melipona* colonies. Nieh & Roubik (1995) reported low rates of location-specific recruitment in *M. panamica*, and the work of Biesmeijer et al. (1998) suggested that location-specific recruitment in *Melipona* may only play a limited role in colony foraging. Prior studies of *Melipona* did not focus in detail on the rate of location-specific recruitment (Lindauer & Kerr 1960; Nieh & Roubik 1995) or appear to have

used a methodology that counts both feeder-naïve and feeder-experienced bees as recruits (Jarau et al. 2000).

We therefore examined recruitment rates and recruitment efficiencies to determine whether location-specific recruitment can strongly influence foraging in *Melipona*. In addition, we address the question of scale and audience size: what is a low recruitment rate and efficiency for species that have relatively few foragers?

## Species and Habitat Differences

Habitat may have played an important role in shaping the evolution of *Melipona* recruitment systems. *Melipona* are found in extremely diverse habitats throughout Central and South America, but Brazil has the greatest *Melipona* species and habitat diversity (Schwarz 1948; Roubik 1989; Michener 2000). We therefore studied two Brazilian stingless bee species whose food-recruitment systems had not been previously examined: *Melipona mandacaia* from a semi-arid, savannah-type habitat and *Melipona bicolor* from a rainforest habitat.

*Melipona mandacaia* Smith 1863 is endemic to the Caatinga habitat in the southern portion of the state of Bahia. The Caatinga is a semi-arid ecosystem that occupies approximately 10% of Brazil and consists of relatively sparse vegetation that is, on average, 5–6 m high (Rizzini 1997).

*Melipona bicolor* inhabits the Atlantic rainforest along the southern coast of Brazil. The Atlantic rainforest has quite variable canopy heights, but averages 15 m high (Wilms et al. 1997). Two subspecies are recognized. *Melipona b. bicolor* Lepetelier 1836 is found in the Brazilian states of São Paulo, Rio de Janeiro, Espírito Santo and Minas Gerais. *Melipona b. schenky* is found in eastern Paraguay and from the Argentinian province of Misiones to the Brazilian state of Paraná, with isolated Brazilian populations in São Paulo, Rio de Janeiro and Minas Gerais (Hilário 1999). Both subspecies nest close to the soil, in the trunks of trees (Hilário et al. 2000). We studied *M. b. bicolor*, but will refer to these bees as *M. bicolor* in the remainder of this paper.

Our study therefore had three goals: (1) to determine whether recruitment to a specific food site by *Melipona* foragers can lead to large and rapid increases in the number of newcomers visiting a food source; (2) to determine whether the recruitment systems of *M. mandacaia* and *M. bicolor* allow recruits to find the advertised resource at the correct direction, distance and height; and (3) to explore whether habitat may influence location-specific recruitment communication in different *Melipona* species.

## MATERIALS AND METHODS

### Study Site and Bee Colonies

We used one colony of *M. bicolor* and two colonies of *M. mandacaia* (*M. mandacaia* colonies 1 and 2) in these experiments. The *M. bicolor* colony came from Cunha (23°05'S, 44°55'W) in the Atlantic rainforest of coastal

southern Brazil. This colony contained approximately 800–1100 workers and was initially transferred to the Universidade de São Paulo, São Paulo, Brazil for 20 days before being brought directly to our study site. Both *M. mandacaia* colonies contained 300–400 workers and originally came from the southern portion of the state of Bahia. *Melipona mandacaia* colony 1 was initially transferred to the Universidade de São Paulo, São Paulo, Brazil for 60 days before being brought directly to our study site. *Melipona mandacaia* colony 2 had lived at our study site for several years. We estimated the colony sizes from the number and size of brood combs and from daily flight activity.

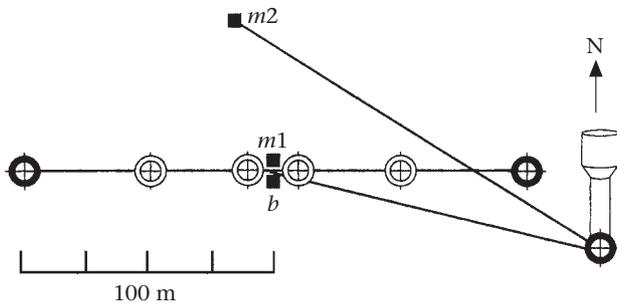
We conducted all studies at a farm, the Fazenda Aretuzina, near the town of São Simão in the state of São Paulo, Brazil (21°26.390'S, 047°34.810'W). This study site is situated in an agricultural region with patches of native forest preserved alongside the fields. A section of native Cerrado forest with a canopy height of approximately 12 m was less than 500 m from the nest sites. Floral resources exploited by stingless bees included small shrubs and large flowering trees such as *Cassia bicapsularis* (Fabaceae, Caesalpinioideae). Throughout our field season, natural pollen and nectar resources were available in the canopy and understory of the Cerrado forest.

We studied *M. mandacaia* colony 1 during our first field season from 8 August 2000 to 5 September 2000. We studied *M. mandacaia* colony 2 and *M. bicolor* during our second field season from 1 July 2001 to 29 October 2001, and briefly on 22 September 2002. The period from July to August corresponds to winter in southern Brazil and to a seasonal decrease in natural food sources that allowed us to train bees to artificial food sources more easily.

We housed *M. mandacaia* colony 1 and the *M. bicolor* colony inside the laboratory in three-chamber observation nests made of wood and covered with plate glass. The three chambers, which were of decreasing depth, respectively housed brood comb, food storage pots and recruitment activity (Nieh & Roubik 1995). We connected nests to the laboratory exterior using vinyl tubes (25 cm long, 1 cm in diameter). We housed *M. mandacaia* colony 2 outside the laboratory in a wooden nestbox (20 × 30 × 30 cm, W × L × H) placed inside a larger wooden box (30 × 60 × 40 cm) that was filled with sawdust for insulation. A wood tunnel (4 cm long, 2 cm in diameter) connected the inner box to the outside. A sheet metal top shielded with terracotta roofing tiles provided protection against rain (Nogueira-Neto 1997).

### Training and Marking Bees

We trained bees by injecting 2 ml of 2.5 M anise-scented sucrose solution (a rich food source consisting of 10 µl of anise extract per litre of sucrose solution) into the nest entrance and placing the feeder in contact with the entrance. The feeder consisted of a glass bottle (5 cm in diameter, 4.5 cm high, 65-ml capacity) filled with the same scented sucrose solution and inverted over a grooved circular plastic plate (6.7 cm diameter, 40 grooves; von Frisch 1967). After waiting for a few foragers to discover the feeder, we moved it 1 cm away, leaving a



**Figure 1.** Diagram of feeder positions and nest sites. Filled circles indicate the positions of training and control feeders. Open circles indicate positions occupied only by control feeders. Lines connect the colonies with the feeders used with each colony. The water tower (not to scale) is shown in the lower right. Filled squares mark the nest positions: *M. mandacai*a colony 1 (*m1*), *M. mandacai*a colony 2 (*m2*) and *M. bicolor* (*b*). We placed colonies *m1* (year 2000) and *b* (year 2001) in the same position in successive years, although they are offset in the diagram for clarity.

gap over which the foragers were forced to fly. We gradually moved this feeder (hereafter ‘the training feeder’) to train bees to forage at the desired location. We also trained bees to feed 12 m above ground by slowly climbing to the top of a water tower while holding the training feeder. We used acrylic paints or paint pens to mark each bee visiting the training feeder with unique colour combinations on the thorax and abdomen.

### Testing for Location Communication

To test the ability of each species to communicate resource direction, distance and height, we used feeder arrays following classic paired-feeder designs (Lindauer & Kerr 1960; von Frisch 1967). We refer to these experiments as the direction, distance and height experiments, respectively. The experiments consisted of (1) laying out paired identical feeders (one training and one control) in the appropriate dimensional axis, (2) training individually marked foragers to only one feeder (the training feeder) and (3) recording newcomer arrivals at both feeders. Control feeders were identical to the training feeders in all respects, except that any bee that landed on a control feeder was immediately captured before she could feed. We defined a ‘newcomer’ as a bee that had never previously visited a feeder at any point in its life. If foragers can communicate the tested dimension, then significantly more newcomers should arrive at the training feeder than at the control feeder. In the direction experiment, feeders were placed at the same height and distance from the subject colony but in opposite directions. In the distance experiment, both feeders were placed in the same direction and at the same height, but at different distances from the colony. Finally, in the height experiment, both feeders were at the same distance and direction, but at different heights relative to the colony. For the height experiments, we used a 12-m-high steel water tower situated 140 m southeast of the *M. bicolor* nest and *M. mandacai*a colony 1. This water tower was 173 m from *M. mandacai*a colony 2 (Fig. 1).

Each feeder set-up consisted of a 1-m high video-camera tripod bearing a 20-cm diameter yellow plastic dish to hold the sucrose feeders. In all experiments, we placed the feeders on top of fluorescent orange discs with black radial marks to assist forager orientation. We also placed a 20-cm<sup>2</sup> disk of Whatman filter paper underneath the grooved feeder plate, exposing 10 cm<sup>2</sup> of filter paper, to which we added 20 µl of scent (anise extract) each 60 min. We used two-way radios to coordinate the addition of scent in all experiments. All feeders in all experiments contained the same 2.5 M anise-scented sucrose solution (10 µl of anise extract per litre of sucrose solution).

We only allowed foragers to feed at the training feeder. We immediately and uniquely marked all foragers visiting the training feeder or captured them in one of two aspirators: one for marked foragers and one for unmarked foragers. Each aspirator consisted of two flexible vinyl tubes (1-cm inner diameter, each approximately 25 cm long) inserted into a clear plastic cylinder (7 × 8 cm). We covered the distal end of the mouthpiece tube with fine mesh and lined the cylinder walls with tissue paper to cushion aspirated bees. We uniquely marked all the unmarked captured bees at the end of each day.

In all experiments, we allowed only 20 individual foragers to feed at the training feeder. We censused the number of marked foragers visiting the feeder at 15-min intervals and removed or released marked foragers (from the marked-forager aspirator) as needed to maintain a constant number of 20 individual foragers visiting the feeder. At the control feeder, a feeder monitor used an aspirator to capture all bees as soon as they landed. Thus, bees could not recruit for the control feeder. We defined an ‘experienced forager’ as any bee that had ever visited a feeder. Thus, newcomers became experienced foragers as soon as they landed on a feeder.

Because the rate of recruitment is extremely variable, depending in part upon the availability of natural food sources, we ended each trial by specifying a fixed number of newcomers rather than a fixed time interval. The choice of sample size is clearly important. Ideally, it should be large. However, our *M. mandacai*a colonies contained only 300–400 bees and our *M. bicolor* colony contained only 800–1100 bees (both natural colony sizes), of which only a fraction were foragers. Amino acids obtained from pollen are essential for brood development (Roubik 1989). Thus, a variable but often significant portion of foragers foraged at natural pollen and nectar sources. A large per-trial sample size would therefore limit the number of experiments and trials that could be conducted. Our necessarily stringent definition of an experienced forager as any bee that had previously contacted a feeder further limited the population of potential newcomers, because a sizeable portion of foragers would become experienced foragers over the course of several experiments.

Five is the smallest sample size that allows one to distinguish a statistically significant result from the binomial probability. Therefore, in the first year, we used five newcomers to define a trial. In the second year, we increased the trial sample size to eight newcomers,

because a sample size of five had not exhausted the population of newcomers in the first year. In some trials, we simultaneously received multiple newcomers just at the end of the trial. We included these simultaneous newcomers in our data, because their inclusion did not change our conclusions about the outcome of any trial and because excluding them would have artificially changed our recruitment rate measurements.

### Recruitment Controls

Mistaking the identity of foragers at the feeder can lead to misleading results. There are three types of errors.

(1) Foragers from other colonies may also discover the food source. Thus, it is important to verify that all bees visiting the feeders come from the subject colony. We therefore individually marked all captured bees and released the bees captured at the training feeder (training feeder bees) at the end of each day. An observer waited at the nest to verify the return of each marked bee. To prevent bees that were captured at the control feeder (control feeder bees) from returning and recruiting for the control feeder, we individually marked and held all control feeder bees in a holding tank provided with a cup of cotton saturated with unscented 2.5 M sucrose solution. During all experiments, we released the control feeder bees and verified their identity only after we had moved the control feeder to a different location in order to prevent the potential communication of the capture location.

(2a) A 'recruit' is a forager who finds a food source based upon information provided by another bee from the same colony (i.e. a nestmate; Seeley 1983). However, it is usually not possible to know whether any given forager coming to the feeder has arrived because of communicated information or from a random search. Because experienced foragers have a search image of feeders they have visited, they can initiate their own search without requiring communicated information when a feeder has been moved to a different location (Biesmeijer & de Vries 2001). Thus, experienced foragers searching randomly might be equally likely to encounter control and training feeders, particularly when the feeders are only displaced by relatively short distances. This is essentially how investigators train bees to forage at a different location, by displacing the feeder for a short distance and waiting for experienced foragers to search for and find the feeder (von Frisch 1967). Therefore, counting experienced foragers as newcomers can lead to the incorrect conclusion that location communication does not occur or is particularly weak, especially when the new feeder locations are close to the old feeder locations.

(2b) A 'reactivated forager' is a bee who revisits a feeder after a hiatus. Although reactivated foragers may find the training feeder at a new location based upon communicated information, we could not distinguish this result from that of a random search (as in 2a above). It is thus essential not to count a reactivated forager as a newcomer when one is assessing the communication of food location (Lindauer & Kerr 1960; Gould 1976; Biesmeijer & de Vries 2001). We therefore considered only those foragers

who had never previously visited a feeder to be potential newcomers.

(3) 'Scouts' are foragers that search for a resource without using information from nestmates. In recruitment experiments, such scouts can lead to misleading results because they may search randomly or in a preferred foraging direction. Scouts are unmarked and thus cannot be distinguished from true recruits. We therefore performed a random discovery control experiment to measure the rate at which scouts randomly discovered the feeders. We first captured all foraging bees with aspirators. This eliminates recruitment communication from nestmates. Next, we injected 1 ml of 2.5 M anise-scented sucrose solution into the colony, thereby providing an excitatory foraging stimulus, and duplicating the normal procedure followed at the beginning of each day. Based upon the recruitment rate observed on the same or previous day, we chose a trial duration sufficient to ensure a high probability of arrivals. We conducted five random discovery control trials, one for 120 min and four for 60 min each. We conducted trials for each combination of feeder positions used during the location communication experiments.

### Statistical Analyses

Our goal was to test whether foragers could communicate the location of the training feeder. Thus, we calculated a one-tailed binomial probability to test the hypothesis ( $H$ ) that significantly more newcomers would arrive at the training feeder than at the control feeder ( $P_{\text{null}}=0.5$ ). It was appropriate to test  $H$ , because we had a reasonable expectation based upon prior studies that newcomers would be able to arrive at the correct distance, direction and height (Kerr 1960; Lindauer & Kerr 1960; Nieh & Roubik 1995; Jarau et al. 2000). For the distance experiments, the control feeder was placed closer to the nest, and thus, one might expect newcomers to have a higher probability of finding the control feeder ( $P_{\text{control}}>0.5$ ) under the null hypothesis. In this case, we adopted the more conservative approach of using  $P_{\text{control}}=0.5$ , thereby making our null hypothesis more difficult to falsify. Where explicitly stated, we also used the chi-square test and the Mann-Whitney  $U$  test. In all tests, we rejected the null hypothesis when  $P\leq 0.05$ . Averages are given as means  $\pm 1$  SD.

## RESULTS

### Recruitment Controls

In all experiments with *M. mandacai* and *M. bicolor*, all of the bees visiting the feeders came from the colony under study. Furthermore, no bees discovered any of our feeders following capture of the experienced foragers during random discovery trials (Table 1). In all five random discovery trials, the number of newcomers to both feeders significantly decreased (to zero) compared with the number of newcomers that arrived during an equal time interval before or after the control trial and

**Table 1.** Results from the random discovery experiment comparing the number of newcomers from *Melipona* colonies that discovered feeder arrays during random discovery (control trials), and during equal time periods immediately before and after random discovery and during the same time period 1 day before and after random discovery

	Random discovery				Number of newcomers/trial			
	Date	Time	Feeder locations (distance or height from colony)	Number of newcomers/trial	During equal time periods		During the same time period	
						$P^a$		$P^a$
<i>M. mandacaia</i>	12 August 2000	1415–1615	100 m E, 100 m W	0	Before	8 <0.01	Before	4 <0.05
	12 July 2001	1630–1730	Tower: 0 m, 12 m	0	Before	4 <0.05	Before	15 <0.001
<i>M. bicolor</i>	20 July 2001	1335–1435	100 m W, 50 m W	0	Before	38 <0.001	Before	9 <0.01
	23 July 2001	0930–1030	100 m E, 100 m W	0	After	8 <0.01	After	4 <0.05
	24 October 2001	1000–1100	Tower: 0 m, 12 m	0	After	4 <0.05	After	15 <0.001

<sup>a</sup> $\chi^2_1$  test.

during the same time period as the control trial on preceding and subsequent days ( $\chi^2_1 \geq 4$ ,  $P < 0.05$ ). Thus, the rate of random discovery was zero.

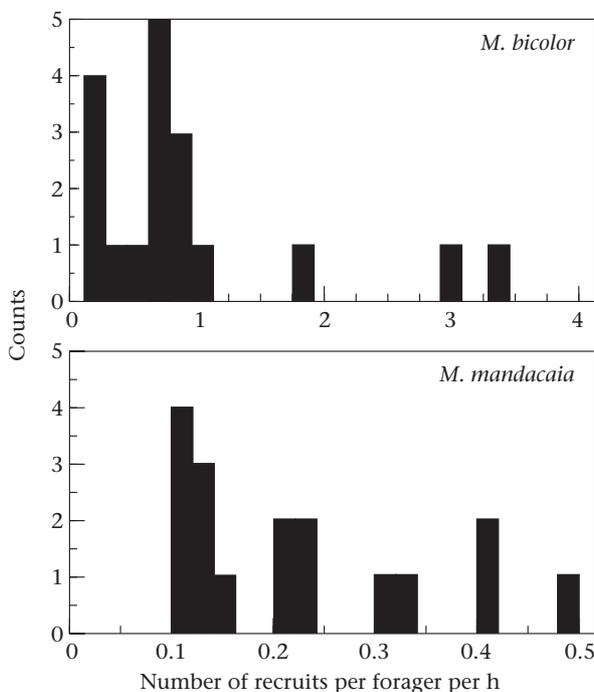
**Recruitment Rates**

We observed highly variable recruitment rates during both field seasons. The distributions of recruitment rates differed markedly between *M. mandacaia* and *M. bicolor* (Mann–Whitney *U* test:  $U=48$ ,  $N_1=17$ ,  $N_2=18$ ,  $P=0.0005$ ; Fig. 2). *Melipona mandacaia* had a mean recruitment rate of  $0.22 \pm 0.13$ , with a maximum of 0.50 and a minimum of 0.10 newcomers per forager per h (combined data from 2000 and 2001 field seasons,  $N=17$  trials). *Melipona bicolor* had a mean recruitment rate of  $0.92 \pm 0.93$ , with a

maximum of 3.43 and a minimum of 0.10 newcomers per forager per h ( $N=18$  trials). Thus, the *M. bicolor* colony recruited more rapidly than *M. mandacaia* colonies 1 or 2.

The efficiency of recruitment (number of forager returns per newcomer) was also highly variable and significantly different between *M. mandacaia* and *M. bicolor* (Mann–Whitney *U* test:  $U=94$ ,  $N_1=17$ ,  $N_2=18$ ,  $P=0.05$ ). *Melipona mandacaia* foragers returned to the feeder an average of  $74.6 \pm 34.5$  times per newcomer (maximum of 126.0 and a minimum of 25.2 feeder returns per newcomer, pooled data from 2000 and 2001,  $N=17$  trials). *Melipona bicolor* foragers made an average of  $61.7 \pm 67.5$  feeder returns per newcomer (maximum of 258.7 and a minimum of 7.8 feeder returns per newcomer,  $N=18$  trials). Thus, *M. bicolor* foragers recruited more newcomers per feeder visit than did *M. mandacaia* foragers.

Because the *M. bicolor* colony was much larger (800–1100 adults) and thus had a larger population of potential recruits than did the *M. mandacaia* colonies (300–400 adults), the differences in recruitment rate and recruitment efficiency may correspond to differences in colony size. Recruitment rates and efficiencies varied from day to day. However, on occasion, location-specific recruitment led to a rapid, large increase in newcomers, even when the number of foragers at the feeder was fixed (*M. bicolor* maximum cumulative recruitment rate observed on 20 July 2001; Fig. 3).



**Figure 2.** Frequency distribution of recruitment rates. For each species, the distribution combines data taken from all experiments. The data for *M. mandacaia* are pooled from 2000 and 2001 data.

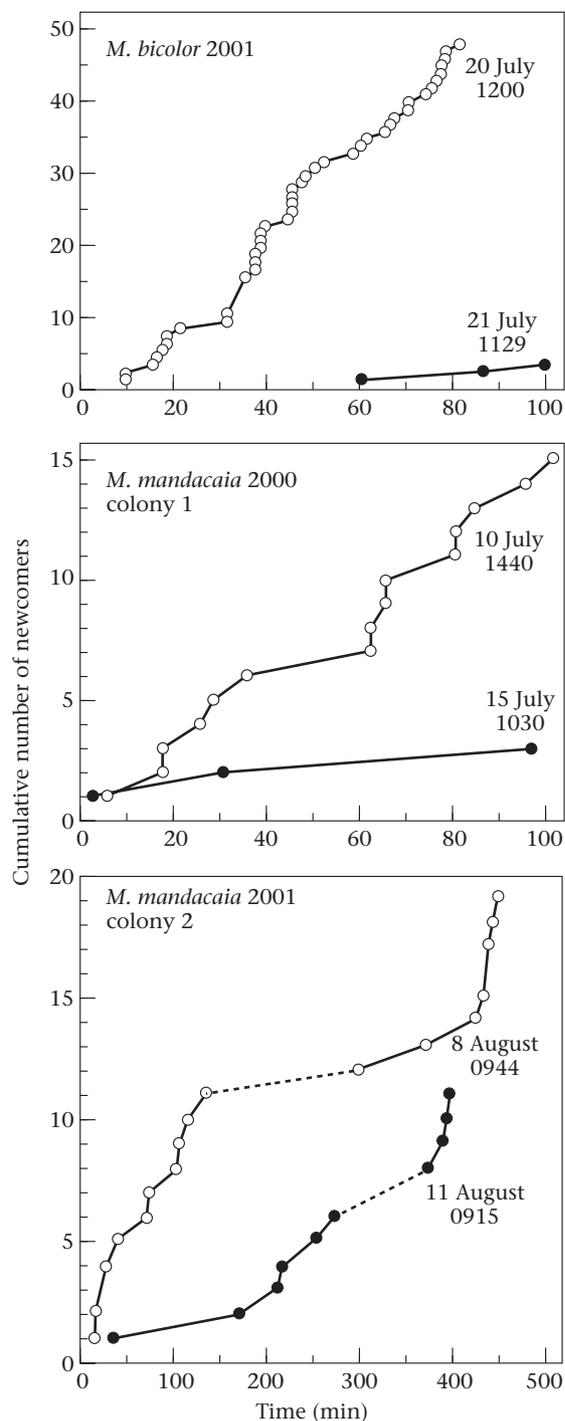
**Direction Experiments**

*Melipona mandacaia*

In the *M. mandacaia* direction trials, all newcomers arrived at the training feeder, regardless of whether it was located 100 m west or east of the colony ( $P \leq 0.03$  in all six trials; Fig. 4a). Overall, 34 newcomers arrived at the training feeder and zero arrived at the control feeder ( $P < 0.0001$ ).

*Melipona bicolor*

In all *M. bicolor* direction trials, a majority of newcomers arrived at the training feeder. In five out of six trials, significantly more newcomers arrived at the training feeder, regardless of whether it was located 100 m



**Figure 3.** Cumulative recruitment plots. The plots show trials with the highest ( $\circ$ ) and lowest ( $\bullet$ ) recruitment rates recorded for each species during 2000 and 2001. Dashed lines indicate breaks during the observation period. The X axis gives the time from the start of foraging. Dates and start times are given above each trace. For all experiments, we maintained a constant number of 20 foragers at the feeder.

west or east of the colony ( $P \leq 0.035$ ; Fig. 4a). Overall, 49 newcomers arrived at the training feeder and three arrived at the control feeder ( $P < 0.0001$ ). In the nonsignificant trial, a majority of newcomers still chose

the training feeder (six at the training and two at the control).

Thus, *M. mandacaia* and *M. bicolor* foragers were both able to communicate the direction of the food source.

## Distance Experiments

### *Melipona mandacaia*

In all *M. mandacaia* distance trials, all newcomers arrived at the training feeder, regardless of whether it was located 100 m east or west of the nest ( $P \leq 0.03$  in all four trials; Fig. 4b). A total of 22 newcomers arrived at the training feeder and zero at the control feeder ( $P < 0.0001$ ) even though the control feeder was only 10 m from the nest.

### *Melipona bicolor*

In all *M. bicolor* direction trials, a majority of newcomers arrived at the training feeder. In five out of six trials, significantly more newcomers arrived at the training feeder, regardless of whether it was located 100 m west or east of the colony ( $P \leq 0.04$ ; Fig. 4b). Overall, 45 newcomers arrived at the training feeder and three arrived at the control feeder ( $P < 0.0001$ ). In the nonsignificant trial, a majority of newcomers still chose the training feeder (six at the training and two at the control).

Thus, *M. mandacaia* and *M. bicolor* foragers were both able to communicate the distance to the food source.

## Height Experiments

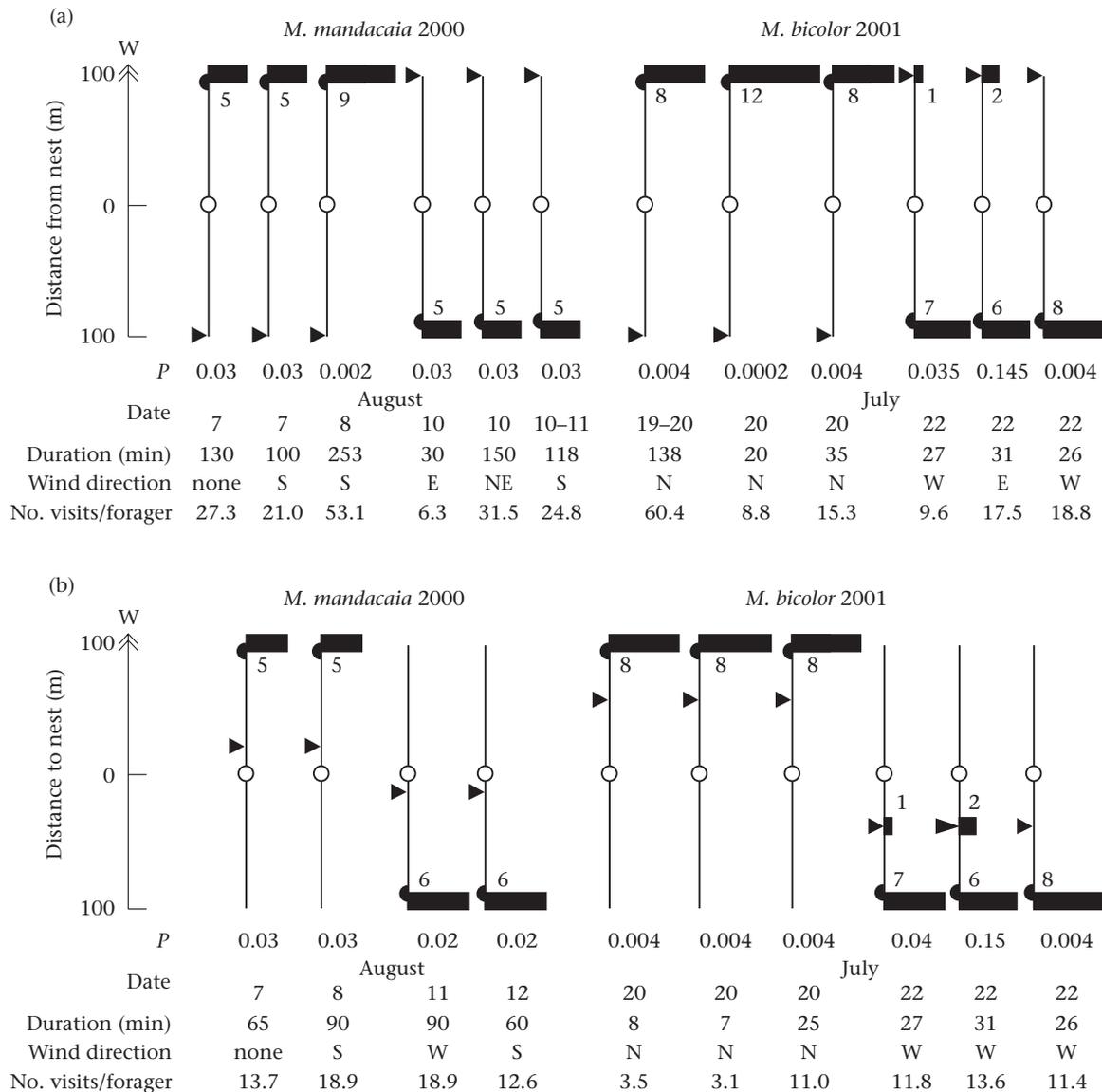
### *Melipona mandacaia*

None of the *M. mandacaia* height trials with either colony showed any significant newcomer preference for the training feeder, regardless of whether it was located 12 m high or at ground level ( $P \geq 0.15$ , 11 trials; Fig. 5). Newcomers showed no preference for a particular height. With the training feeder at ground level, 17 newcomers arrived at the training feeder and 20 arrived at the control feeder (NS). With the training feeder 12 m high, 24 newcomers arrived at the training feeder and 24 arrived at the control feeder (NS). Overall, nearly equal numbers of newcomers arrived at both feeders. A total of 41 newcomers arrived at the training feeder and 44 newcomers arrived at the control feeder.

### *Melipona bicolor*

When the training feeder was placed at the base of the water tower, *M. bicolor* newcomers showed no significant tendency to approach the training feeder versus the control feeder (three trials:  $P = 0.5$ ,  $P = 0.19$  and  $P = 0.5$ ; Fig. 5). However, when the training feeder was placed on top of the tower, all newcomers arrived at the training feeder (15 arrived at the training feeder and zero arrived at the control feeder,  $P = 0.03$  for each trial, for pooled data,  $P < 0.00004$ ).

Thus, although *M. mandacaia* and *M. bicolor* foragers were unable to communicate the height of the food source when the food source was at ground level,



**Figure 4.** Testing (a) directional and (b) distance communication. The arrowhead indicates west. Each vertical graphic unit is a modified bar graph representing one trial. The open circle indicates the nest position. A filled triangle indicates the position of the control feeder. A filled half-circle indicates the position of the training feeder. Horizontal black bars show the number of newcomers (exact values given). Absence of a bar at a feeder site indicates zero newcomers. For each trial, we show the binomial probability, the trial date, the trial duration, the wind direction and the average number of visits per forager.

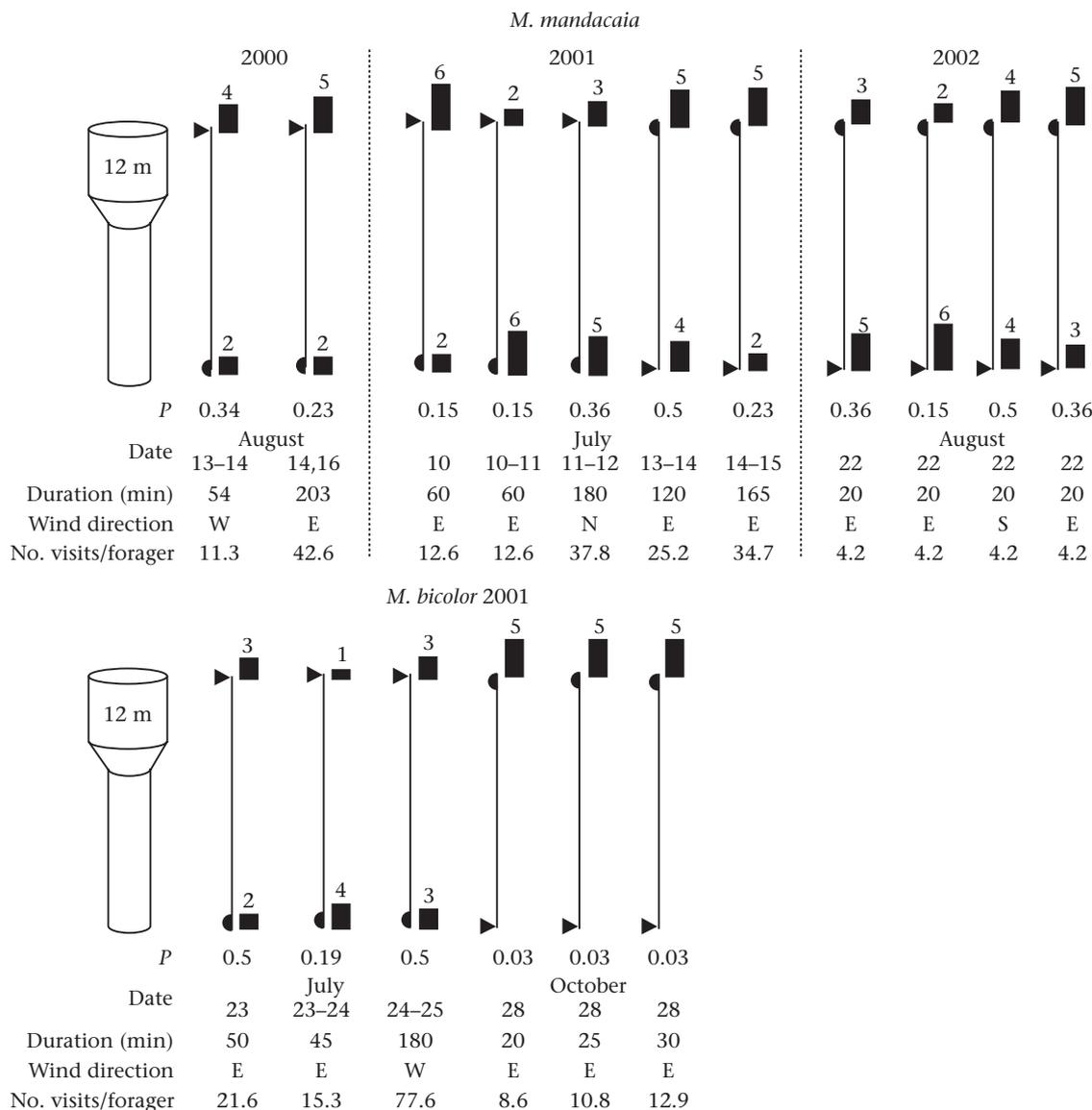
*M. bicolor* foragers differed significantly from *M. mandacaia* foragers in their ability to communicate height when the food source was at canopy level ( $\chi_1^2=15$ ,  $P=0.0001$ ).

## DISCUSSION

*Melipona mandacaia* and *M. bicolor* showed an excellent ability to recruit significant numbers of nestmates to the correct distance and direction. However, these species differed in their ability to recruit newcomers to the correct height. The recruitment system of *M. mandacaia* evidently does not indicate height, whereas the recruitment system of *M. bicolor* evidently indicates height only when the food source is at canopy level (12 m).

## Recruitment Control Experiments

The recruitment control experiments cannot completely exclude the possibility that some foragers found the feeders without receiving information from nestmates. However, the results of the distance and direction experiments are striking. Only six of 156 newcomers from both species (3.8%) came to the control feeder, even though we switched the control feeder position in all experiments to control for potential site bias. It is highly unlikely (binomial probability:  $P \ll 0.0001$ ) that randomly searching foragers matched the particular spatial patterns and temporal order in which we placed and presented the training feeder. Given these results, the six newcomers that arrived at the control location may have received



**Figure 5.** Testing height communication. The water tower is 12 m high. A filled triangle indicates the position of the control feeder. A filled half-circle indicates the position of the training feeder. Horizontal black bars show the number of newcomers (exact values given). Absence of a bar at a feeder site indicates zero newcomers. For each trial, the binomial probability, the trial date, the trial duration, the wind direction and the average number of visits per forager are also given.

some location information but became lost or did not use this information correctly.

In addition, no scouts of either species found any of the feeders during the five random discovery control trials. Even the initial stimulus of the odour and taste of the food source was insufficient to allow inexperienced bees to discover the food source. However, newcomers had no difficulty in finding the training feeder when foragers were allowed to recruit. Thus, recruitment communication, not random searching, evidently led *M. bicolor* and *M. mandacaia* newcomers to the training feeder.

### Recruitment Rates

In both *M. mandacaia* and *M. bicolor*, the rate and efficiency of recruitment was highly variable, but recruit-

ment communication, under certain conditions, led to large, rapid increases in the number of nestmates at the food source (Figs 2, 3). On 20 July 2001, 20 *M. bicolor* foragers recruited 48 nestmates within 81 min, corresponding to a recruitment efficiency of one newcomer per 14 forager visits (Fig. 3). On the following day, however, the same 20 *M. bicolor* foragers recruited only three newcomers during 86 min. We found similar changes in recruitment rates in both *M. mandacaia* colonies (Fig. 3).

Several factors affected recruitment rates to the feeder: (1) colonies were not large; (2) not all foragers recruited (thereby lowering our measured efficiencies); (3) not all recruiting foragers recruited upon each return to the nest; (4) not all bees inside the nest could be recruited; (5) competing natural food sources were available at all times

(although somewhat reduced throughout most of our field seasons); and (6) the increase in recruitment was limited by our experimental procedure, whereas under natural, uncontrolled conditions (when recruited foragers are not removed at the food source), newly recruited foragers would have returned to the nest and become additional recruiters. These factors can be simplified into three primary causes of apparently low recruitment rates: low recruiter motivation, competition from natural food sources, and using up a sizeable portion of the available foragers during the experiments.

Low recruiter motivation for an artificial food source is influenced by colony needs and by competition from natural food sources. The preference of stingless bees and honeybees for natural food sources over artificial ones provides a strong constraint on recruitment to feeders (Hubbell & Johnson 1978; Seeley 1995). This preference was especially clear at the end of our field seasons, when natural food sources became more abundant and foragers no longer showed an interest in our feeders.

In addition, we counted only feeder-naïve foragers as true newcomers, and a sizeable and increasing portion of the forager population was therefore excluded as the experiments progressed. We also trained a group of 20 new foragers each time we changed the training feeder site, and this further reduced the pool of feeder-naïve foragers and thus the potential rate of recruitment. For example, with *M. mandacaia* colony 1, we marked approximately 32–45% of the colony's total workforce (300–400 adults) during 7–15 August 2000. With the *M. bicolor* colony, we marked approximately 16–22% of the total workforce (800–1100 adults) during 19–25 July 2001.

In summary, recruitment rates to a feeder providing a rich sucrose solution can be highly variable depending upon forager motivation, availability of natural food sources and the size of the forager population. However, by providing a controlled food source, we determined that recruitment communication can lead to large, rapid increases in the number of nestmates recruited to a food source at a specific location (Fig. 3). In addition, the random discovery experiments revealed that randomly searching foragers would not have found our food source without recruitment communication (Table 1). Although recruitment communication may not be as important in colony foraging during times of food abundance, rapid and efficient recruitment may be critical during seasonal periods when food sources are scarce, and competition for available resources and risk of starvation are high (Johnson & Hubbell 1974; Roubik 1980).

For *M. scutellaris* and *M. quadrifasciata*, Jarau et al. (2000) reported much higher rates of recruitment than what we observed in *M. mandacaia* and *M. bicolor*. However, they appear to have used a different methodology, counting both feeder-experienced foragers (who previously experienced the feeder at a different location) and feeder-naïve foragers as newcomers. Although only a fraction of all bees in a colony are foragers, Jarau et al. (2000) obtained 656 newcomers over 43 days from a *M. scutellaris* colony containing approximately 400–600 adult bees and 569 newcomers over 90 days from a

*M. quadrifasciata* colony containing approximately 300–400 adult bees. The differences in methodology between our experiments and those of Jarau et al. (2000) may also explain why they initially observed nestmates randomly searching for the feeders, followed by the weak communication of distance.

In our studies, experienced *Melipona* foragers flew out early in the morning and began to search for the feeder if it was no longer in the position at which they previously experienced it. After some time, the majority found the new feeder locations and were captured. If we had counted these experienced foragers as newcomers, we would have initially recorded nonspecific location communication, followed by location-specific communication. Random searching of experienced foragers may also account for the surprising finding that *M. scutellaris* and *M. quadrifasciata* foragers did not communicate distance beyond 30 and 40 m, respectively (Jarau et al. 2000). These results are counter to the expectation that precise distance communication should be more important for more distant food sources than for nearby food sources (Seeley 1995; Weidenmuller & Seeley 1999; Dyer 2002).

The reactivation of foragers searching for food sources that they have previously experienced is an important component of colony foraging and should be considered in an overall analysis of the division of foraging labour (Biesmeijer & Toth 1998; Biesmeijer et al. 1998). However, this is a separate issue from determining whether foragers can communicate the specific location of a food source. In the latter case, only the very strict definition of a newcomer as a bee that has never previously visited a feeder may be used (Gould 1975, 1976; Biesmeijer & de Vries 2001).

## Communication Mechanisms

Recruiting *M. mandacaia* and *M. bicolor* foragers may use multimodal communication. Possible information sources include excitatory movements and sounds produced by recruiting foragers inside the nest, following a piloting bee for part of the distance to the food source, and odours. In *M. merillae*, *M. quadrifasciata* (Esch 1967), *M. panamica* (Nieh & Roubik 1998) and *M. costaricensis* (Aguilar & Briceño 2002), foragers produce sounds whose temporal characteristics are correlated with the distance to the food source. In both *M. mandacaia* and *M. bicolor*, recruiting foragers also produce sound pulses whose duration increases with increasing food distance (Nieh et al., in press a). Thus, foragers may encode distance in recruitment sounds. We are continuing to investigate the relationship between recruitment sounds and the height of the food source.

Following a forager directly to the food source appears unlikely because this mechanism should have allowed newcomers to arrive at the correct height in the height experiments. Moreover, newcomers often arrived alone, unaccompanied by experienced foragers. Piloting remains a strong possibility for the communication of direction (Esch et al. 1965) and could also indicate height through the height of the piloting flight. However, such a

mechanism for indicating height should result in specific height orientation for both high and low food sources, which is not what we observed in *M. bicolor*.

Odour orientation is likely to be important in the communication systems of both species. *Melipona mandacaia* deposits food-marking odours (Nieh et al., in press b) and *M. bicolor* may also deposit food-marking odours. Forager-deposited odours have been found in all social bee groups (von Frisch 1967; Goulson et al. 2000) and, in *Melipona*, evidence for forager-deposited odours at the food source has been found in all studies testing for such attractive odours (*M. rufiventris* and *M. compressipes*: Kerr & Rocha 1988; *M. bicolor*, *M. scutellaris* and *M. quadrifasciata*: Kerr 1994; *M. panamica*: Nieh 1998; *M. favosa*: Aguilar & Sommeijer 2001; *M. seminigra*: Jarau et al. 2002; Hrncir et al., in press).

Newcomer orientation to a combination of food-scent and forager-deposited odours may account for our height results. A height difference of 12 m is within the potential active space of *Melipona* forager-deposited odours (Nieh 1998). Thus, differences between the height-localization abilities of *M. mandacaia* and *M. bicolor* recruits may result from different orientation strategies towards odours near the food source.

### Is Height-specific Recruitment Habitat Specific?

Differences in the habitats of *M. mandacaia* and *M. bicolor* may be reflected in their recruitment systems. *Melipona mandacaia* lives in a semi-arid region with sparse, low vegetation and a very low density of trees that are only 5–6 m high, on average (Rizzini 1997). In such a habitat, the communication of height may not have much importance. *Melipona bicolor* lives in the Atlantic rainforest, where the canopy height is quite variable, but averages 15 m high (Wilms et al. 1997). *Melipona bicolor* newcomers arrived at the correct height when the training feeder was 12 m high, but arrived at both high and low feeders when the training feeder was at ground level. This result may relate to the distribution of natural food sources in the habitat of *M. bicolor*. Mass-flowering canopy trees are the most important food plants for stingless bees in the Atlantic rainforest (Wilms et al. 1996). Thus, food sources in the canopy may be richer and subject to greater competition than those at ground level (see Nagamitsu & Inoue 1997 for stingless bees in an Asian tropical rainforest). The communication system of *M. bicolor* may thus focus on the rapid and accurate localization of canopy food sources.

It is also possible that these recruitment systems contain elements reflecting their evolutionary history. The habitat-adaptation hypothesis leads to the prediction that *Melipona* species from the Caatinga will not recruit to a specific height, whereas *Melipona* species from the Atlantic rainforest will have some ability to recruit for a specific height, depending upon the location of food sources that they exploit. The evolutionary-history hypothesis allows for greater variation in location-specific recruitment strategies on the basis of shared ancestry. *Melipona* recruitment systems probably reflect both forces: current utility and evolutionary history. The rela-

tive influences of both forces may be teased apart given sufficient variation in species, habitats and mechanisms. All three elements are abundant in *Melipona*. There are at least 50 species distributed throughout South and Central America in a large diversity of habitats. We now have evidence for variation in their ability to indicate three-dimensional food location and evidence that such recruitment communication can mobilize a sizeable portion of a colony's workforce. Moreover, different species of *Melipona* may use different communication strategies, including functionally referential communication (Kerr et al. 1963; Esch 1967; Nieh & Roubik 1998). *Melipona* may therefore be a useful model for exploring the evolution of sophisticated recruitment communication systems in the highly social bees.

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### References

- Aguilar, I. & Briceño, D. 2002. Sounds in *M. costaricensis* (Apidae: Meliponini): effect of sugar concentration and nectar source distance. *Apidologie*, **33**, 375–388.
- Aguilar, I. & Sommeijer, M. 2001. The deposition of anal excretions by *Melipona favosa* foragers (Apidae: Meliponinae): behavioural observations concerning the location of food sources. *Apidologie*, **32**, 37–48.
- Biesmeijer, J. C. & Toth, E. 1998. Individual foraging, activity level and longevity in the stingless bee *Melipona beecheii* in Costa Rica (Hymenoptera, Apidae, Meliponinae). *Insectes Sociaux*, **45**, 427–443.
- Biesmeijer, J. C. & de Vries, H. 2001. Exploration and exploitation of food sources by social insect colonies: a revision of the scout-recruit concept. *Behavioral Ecology and Sociobiology*, **49**, 89–99.
- Biesmeijer, J. C., van Nieuwstadt, M. G. L., Lukács, L. & Sommeijer, M. J. 1998. The role of internal and external information in foraging decisions of *Melipona* workers (Hymenoptera: Meliponinae). *Behavioral Ecology and Sociobiology*, **42**, 107–116.

- Dyer, F. C. 2002. The biology of the dance language. *Annual Review of Entomology*, **47**, 917–949.
- Esch, H. 1967. Die Bedeutung der Lauterzeugung für die Verständigung der stachellosen Bienen. *Zeitschrift für Vergleichende Physiologie*, **56**, 408–411.
- Esch, H., Esch, I. & Kerr, W. E. 1965. Sound: an element common to communication of stingless bees and to dances of the honey bee. *Science*, **149**, 320–321.
- von Frisch, K. 1967. *The Dance Language and Orientation of Bees*. Cambridge, Massachusetts: Belknap Press.
- Gould, J. L. 1975. Honey bee recruitment: the dance-language controversy. *Science*, **189**, 685–693.
- Gould, J. L. 1976. The dance-language controversy. *Quarterly Review of Biology*, **51**, 211–244.
- Goulson, D., Stout, J. C., Langley, J. & Hughes, W. O. H. 2000. Identity and function of scent marks deposited by foraging bumblebees. *Journal of Chemical Ecology*, **26**, 2897–2911.
- Hilário, S. D. 1999. Considerações sobre a atividade de vôo das abelhas indígenas, com ênfase em *Plebeia pugnax* Moure (in lit.) e *Melipona bicolor bicolor* Lepetelier, 1836 (Apidae, Meliponinae). In: *Departamento de Zoologia, Instituto de Biociências*, p. 51. São Paulo, Brazil: Universidade de São Paulo.
- Hilário, S. D., Imperatriz-Fonseca, V. L. & Kleinert, A. d. M. P. 2000. Flight activity and colony strength in the stingless *Melipona bicolor bicolor* (Apidae, Meliponinae). *Revista Brasileira de Biologia*, **60**, 299–306.
- Hrncir, M., Jarau, S., Zucchi, R. & Barth, F. G. 2000. Recruitment behavior in stingless bees, *Melipona scutellaris* and *M. quadrifasciata*. II. Possible mechanisms of communication. *Apidologie*, **31**, 93–113.
- Hrncir, M., Jarau, S., Zucchi, R. & Barth, F. G. 2002. Dependence of sound signals in *Melipona seminigra* with factors other than food location. In: *XIV International Congress of IUSSI: the Golden Jubilee Proceedings* (Ed. by J. Billen), page 124. Sapporo: Science Council of Japan, Entomological Society of Japan, Hokkaido University Coop.
- Hrncir, M., Jarau, S., Zucchi, R. & Barth, F. G. In press. On the origin and properties of scent marks deposited at the food source by a stingless bee, *Melipona seminigra* Friese 1903. *Apidologie*.
- Hubbell, S. P. & Johnson, L. K. 1978. Comparative foraging behavior of six stingless bee species exploiting a standardized resource. *Ecology*, **59**, 1123–1136.
- Jarau, S., Hrncir, M., Zucchi, R. & Barth, F. G. 2000. Recruitment behavior in stingless bee, *Melipona scutellaris* and *M. quadrifasciata*. I. Foraging at food sources differing in direction and distance. *Apidologie*, **31**, 81–91.
- Jarau, S., Hrncir, M., Zucchi, R. & Barth, F. G. 2002. Foot print pheromones used to mark food sources by stingless bees. In: *XIV International Congress of IUSSI: the Golden Jubilee Proceedings* (Ed. by J. Billen), page 16. Sapporo: Hokkaido University Coop.
- Johnson, L. K. & Hubbell, S. P. 1974. Aggression and competition among stingless bees: field studies. *Ecology*, **55**, 120–127.
- Kerr, W. E. 1960. Evolution of communication in bees and its role in speciation. *Evolution*, **14**, 120–127.
- Kerr, W. E. 1994. Communication among *Melipona* workers (Hymenoptera: Apidae). *Journal of Insect Behavior*, **7**, 123–128.
- Kerr, W. E. & Rocha, R. 1988. Communication in *Melipona rufiventris* and *Melipona compressipes*. *Ciência e Cultura*, **40**, 1200–1202.
- Kerr, W. E., Ferreira, A. & Simões de Mattos, N. 1963. Communication among stingless bees: additional data (Hymenoptera: Apidae). *Journal of the New York Entomological Society*, **71**, 80–90.
- Lindauer, M. & Kerr, W. E. 1958. Die gegenseitige Verständigung bei den stachellosen Bienen. *Zeitschrift für Vergleichende Physiologie*, **41**, 405–434.
- Lindauer, M. & Kerr, W. E. 1960. Communication between the workers of stingless bees. *Bee World*, **41**, 29–41, 65–71.
- Marler, P., Evans, C. S. & Hauser, M. D. 1992. Animal signals: motivation, referential, or both? In: *Nonverbal Vocal Communication: Comparative and Developmental Approaches* (Ed. by M. Papoušek), pp. 66–86. Cambridge: Cambridge University Press.
- Michelsen, A., Andersen, B. B., Kirchner, W. H. & Lindauer, M. 1989. Honeybees can be recruited by a mechanical model of a dancing bee. *Naturwissenschaften*, **76**, 277–280.
- Michener, C. D. 2000. *The Bees of the World*. Baltimore, Maryland: Johns Hopkins University Press.
- Nagamitsu, T. & Inoue, T. 1997. Aggressive foraging of social bees as a mechanism of floral resource partitioning in an Asian tropical rainforest. *Oecologia*, **110**, 432–439.
- Nieh, J. C. 1998. The role of a scent beacon in the communication of food location in the stingless bee, *Melipona panamica*. *Behavioral Ecology and Sociobiology*, **43**, 47–58.
- Nieh, J. C. & Roubik, D. W. 1995. A stingless bee (*Melipona panamica*) indicates food location without using a scent trail. *Behavioral Ecology and Sociobiology*, **37**, 63–70.
- Nieh, J. C. & Roubik, D. W. 1998. Potential mechanisms for the communication of height and distance by a stingless bee, *Melipona panamica*. *Behavioral Ecology and Sociobiology*, **43**, 387–399.
- Nieh, J. C., Contrera, F. A. L., Rangel, J. & Imperatriz-Fonseca, V. L. In press a. Relationship between food location, food quality, and recruitment sounds in two stingless bees, *Melipona mandacaia* and *Melipona bicolor*. *Behavioral Ecology and Sociobiology*. doi:10.1007/s00265-003-0680-6.
- Nieh, J. C., Ramírez, S. & Nogueira-Neto, P. In press b. Multi-source odor-marking of food by a stingless bee, *Melipona mandacaia*. *Behavioral Ecology and Sociobiology*. doi:10.1007/s00265-003-0658-4.
- Nogueira-Neto, P. 1997. *Vida e Criação de Abelhas Indígenas sem Ferrão*. São Paulo: Editoria Nogueirapis.
- Rizzini, C. T. 1997. *Tratado de Fitogeografia do Brasil*. Rio de Janeiro: Âmbito Cultural.
- Roubik, D. W. 1980. Foraging behavior of competing Africanized honeybees and stingless bees. *Ecology*, **61**, 836–845.
- Roubik, D. W. 1989. *Ecology and Natural History of Tropical Bees*. New York: Cambridge University Press.
- Schwarz, H. F. 1948. Stingless bees (Meliponinae) of the Western Hemisphere. *Bulletin of the American Museum of Natural History*, **90**, 1–546.
- Seeley, T. D. 1983. Division of labor between scouts and recruits in honeybee foraging. *Behavioral Ecology and Sociobiology*, **12**, 253–259.
- Seeley, T. D. 1995. *The Wisdom of the Hive: the Social Physiology of Honey Bee Colonies*. Cambridge, Massachusetts: Harvard University Press.
- Weidenmuller, A. & Seeley, T. D. 1999. Imprecision in waggle dances of the honeybee (*Apis mellifera*) for nearby food sources: error or adaptation? *Behavioral Ecology and Sociobiology*, **46**, 190–199.
- Wilms, W., Imperatriz-Fonseca, V. L. & Engels, W. 1996. Resource partitioning between highly eusocial bees and possible impact of the introduced Africanized honey bee on native stingless bees in the Brazilian Atlantic rainforest. *Studies on Neotropical Fauna and Environment*, **31**, 137–151.
- Wilms, W., Wendel, L., Zillikens, A., Blochtein, B. & Engels, W. 1997. Bees and other insects recorded on flowering trees in a subtropical Araucaria forest in southern Brazil. *Studies on Neotropical Fauna and Environment*, **32**, 220–226.