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ORIGINAL ARTICLE

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Polarized short odor-trail recruitment communication by a stingless bee, *Trigona spinipes*

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Abstract Polarized odor-trail communication, in which a receiver can orient towards the correct endpoint from within the trail, is documented in relatively few animals and is poorly understood, although such directionality could significantly enhance resource localization. Among animals, stingless bees exhibit the unique behavior of depositing long substrate-borne odor trails that assist the orientation of flying nestmates to a specific three-dimensional food location. However, relatively little is known about the spatial structure of such odor trails, particularly vertical trails, and whether these trails are polarized to indicate the correct terminus. We show that a stingless bee, *Trigona spinipes*, can rapidly recruit nestmates in large bursts to a food source at a specific distance, direction, and height. In conjunction with a major recruitment burst, foragers deposited odor marks that attracted nestmates for up to 20 min. Surprisingly, these odor marks formed a short odor trail instead of a complete odor trail extending from the feeder to the nest (the classic description of a meliponine odor trail). The length of the odor trails varied between different feeder locations with different colonies, from a minimum of 3 m to a maximum of 29 m. The odor marks formed a polarized trail that newcomers followed to the end with the most concentrated odor marks (the feeder), even when the entire odor trail was rotated 180° and clean test feeders were set out at locations that foragers had never previously fed at. Thus locale odor or the potential communication of food location inside the nest do not account for the ability of newcomers to find the correct terminus. This result provides the first strong evidence for odor-trail polarization in social insects.

Keywords Recruitment · Stingless bee · Three-dimensional location communication · Polarized short odor trail

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Introduction

The localization of ephemeral resources in a changing environment presents a significant challenge to animals, who therefore rely on several sensory modalities, including olfaction, to find mates and food sources (Bradbury and Vehrencamp 1988; Grasso 2001). In both cases, correct directional orientation is essential. Thus, a common feature of olfactory orientation is reliance upon an odor plume followed to its highest concentration, the odor source (Greenfield 2002). Animals can also orient towards substrate-deposited olfactory trails, but orientation towards the correct trail direction from within a trail (trail polarity) remains poorly understood and has been documented in relatively few species. A trail is odor-polarized if an animal, placed within the trail, can determine the correct direction to follow from olfactory information alone (Bradbury and Vehrencamp 1988). Polarity is most relevant if an animal discovers a trail section and needs to know which direction to proceed. For example, there is evidence that males of certain snakes and lizards can follow, in the correct direction, female odor trails (Ford and Low 1984; Vitt and Cooper 1985; Cooper and Vitt

1987). There is no evidence that ants deposit or can follow polarized odor trails, although the correct direction to the resource may be determined via other sensory modalities (Hölldobler and Wilson 1990). Likewise, bumblebees are known to deposit odor trails used by walking individuals near the nest (Cameron and Whitfield 1996; Chittka et al. 1999), although there is no evidence for bumblebee odor-trail polarization.

Meliponine odor trails

Stingless bees are unusual in that some species create substrate-deposited odor-trails to guide flying nestmates to food sources (Lindauer and Kerr 1960; Kerr 1972, 1973; Kerr et al. 1981). This strategy may therefore combine elements of odor-plume and odor-trail communication. However, relatively little is known about these meliponine odor trails—how far they extend, how long they persist, and how they are used. Lindauer and Kerr (1958) first demonstrated the existence of meliponine odor trails by stretching a leaf-bedecked rope over a pond. *Scaptotrigona postica* foragers deposited odor marks on the rope and leaves, and successfully recruited only when the rope, a substrate for odor marks, was present. After the odor-marked rope was moved to a new location, the trained foragers continued to visit the old site, but no newcomers (untrained nestmates) arrived at the old site. A control experiment showed that newcomers did not visually orient to the rope to find the feeder (Lindauer and Kerr 1958). Odor trails extending from the nest to the food source, sometimes for great distances (900 m for *Trigona trinidadensis*, also known as *T. amalthea*), were subsequently reported for several species of stingless bees (Kerr 1960).

Meliponine species deposit odor trails to specify the precise three-dimensional location of food sources, resources that can be seasonally scarce, highly sought after, and distributed throughout the forest canopy (Johnson and Hubbell 1974; Roubik 1982; Nagamitsu and Inoue 1997; Eltz et al. 2001, 2002). Stingless bees are largely tropical (Michener 2000) and thus their food sources can occur in canopies with a significant height dimension. Stingless bee odor trails therefore serve an essential function, but as indicators of valuable resources, they are also subject to selective pressures imposed by competition. There is evidence that some aggressive meliponine species use olfactory eavesdropping to exploit the resources discovered by other stingless bees (Johnson 1974; Nieh et al. 2004). The recent finding of short odor trails, tightly coupled with temporally pulsed recruitment, suggests that counter-eavesdropping strategies may have evolved to minimize odor-trail conspicuousness in space and time (Nieh et al. 2003a). For example, *T. hyalinata* foragers deposit a short odor trail beginning at the food source and ending a short distance away from the food source (maximum of 27 m), despite the nest being located 146 m away. Thus the odor trail of this species does not extend the entire distance from the nest to the food source, as has been previously reported for stingless-bee odor trails (Lindauer and Kerr 1958; Kerr 1972).

Little is known about such short odor trails, particularly their spatial structure. Kerr (1960) reported average distances between meliponine odor marks, but the detailed spatial structure of odor trails has not been examined except in the work of Lindauer and Kerr (1958), which provides five examples of foragers depositing multiple marks beginning from a feeder located 1 m above the ground and extending (along the ground) to within 12 m of the nest. There are no published data on the spatial structure of vertical odor trails.

Odor-trail polarity

Lindauer and Kerr (1958) hypothesized that stingless-bee odor trails might increase in concentration at the food source to indicate the exact food location. Kerr et al. (1963) introduced the term "polarity" to describe this effect, and experiments show that *S. postica* (Kerr et al. 1963), *T. hyalinata* (Nieh et al. 2003a), and *S. depilis* (Schmidt et al. 2003) newcomers will ignore a feeder placed within the odor trail, between the nest and the training feeder. Thus, newcomers evidently have a way to determine the correct endpoint. In *T. hyalinata*, they may use an olfactory concentration gradient (Nieh et al. 2003a). However, it is possible that newcomers orient towards the precise locale odors at the training feeder, receive information about the location of the training feeder inside the nest, or orient towards the endpoint odors alone (Schmidt et al. 2003). Thus demonstrating that significantly fewer newcomers arrive at a control feeder placed within a trail does not conclusively show odor-trail polarization. A primary goal of our study was, therefore, to demonstrate odor-trail polarization in a way that excludes the possibility of locale odors and distance communication inside the nest.

We chose to study *T. spinipes* (also known as *T. ruficrus*, Lindauer and Kerr 1958, 1960) because foragers of this species reportedly deposit odor trails (Kerr 1972, 1973; Kerr et al. 1981). In general, an odor trail should allow foragers to recruit nestmates to the correct 3-dimensional resource location, but odor-trail orientation to a 3-dimensional location has only been studied in preliminary experiments (Lindauer and Kerr 1958). Thus our objectives were to: (1) determine if *T. spinipes* is able to communicate 3-dimensional food location, as studied with feeder arrays, (2) determine the detailed structure of horizontal and vertical odor trails; and (3) test odor-trail polarity.

Methods

Study site, feeders, and training

We used three natural colonies of *T. spinipes*: two at the Fazenda Aretuzina (21°26.387S, 47°34.884 W), a ranch near the town of

São Simao, and one at the Universidade de São Paulo in the city of São Paulo, both locations in the state of São Paulo, Brazil. The Fazenda Aretuzina is 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, and bees exploited floral resources provided by small shrubs and large flowering trees such as *Cassia bicapsularis* (Fabaceae, Caesalpinioideae) at the Fazenda. The university site is largely urban, with some grassy lawns and small corridors of secondary growth forest. The São Simao and São Paulo sites are isolated by 256 km. We used colony 1 from July to September 2002, colony 2 in March 2003, and colony 3 from August to September 2003. Colonies 1 and 3 were approximately 15 m above the ground, located in trees, and colony 2 was 6 m above the ground and attached to the side of a building. In separate years, we trained colonies 1 and 3 to a feeder on a terracotta-tile plaza (63 m×38 m) located 190 m SW of colony 1 and 255 m NW of colony 2. We trained colony 2 to feeders on a grass lawn 20 m and 115 m SE of the colony. Colonies may contain up to 150,000 workers (Michener 1974), although Almeida and Laroca (1988) suggested that sizes of 5,500 workers are more typical.

During all experiments, we measured weather conditions at the feeder sites (temperature, humidity, wind direction, wind speed), in the shade, with Kestrel 4000 weather stations (model NK0840).

We measured the surface temperatures of leaves and the terracotta-tile plaza with a Raytek Phototemp MX6 non-contact infrared thermometer (close focus model). Each feeder consisted of a glass bottle (5 cm diameter, 4.5 cm height, 65 ml) inverted over a transparent plastic, grooved circular plate (6.7 cm diameter, 40 grooves, design in von Frisch 1967) on a 20-cm-diameter yellow plastic dish supported by a 1-m-high tripod.

We trained bees to the feeder with 1.0 M unscented sucrose solution, switching to a 2.5 M unscented sucrose solution once we reached the final feeder position (method of von Frisch 1967). Paint pens were used to individually mark the thoraces of bees visiting the feeder. We verified the identity of the initially trained marked foragers by moving the feeder back to each colony and confirming that foragers flew directly from the feeder into the colony entrance. We censused the number of bees visiting the feeder each 15 min and allowed only 15 individually marked foragers to visit at any given time. An *experienced forager* is any forager that has visited a feeder at any location at any time. A *newcomer* is a nestmate who has never previously visited a feeder and is thus unmarked. Thus newcomers became experienced foragers as soon as they landed on a feeder. When training foragers to a new location, we trained foragers from the old location to the new location, waited until they had recruited 15 newcomers, and then captured the old foragers, and set up the control feeder to begin a new trial.

We immediately captured all newcomers in aspirators and, after each trial, confined them in a screened cage (L×W×H= 24 cm×12 cm×12 cm) provided with sugar solution. To facilitate the transfer of bees, a clear vinyl tube (2 cm inner diameter, 10 cm length) was inserted through the mesh into the cage. They were only released after all experiments with a particular colony. Thus unmarked bees could not return and be recounted at the feeder. Furthermore, the immediate capture of newcomers excluded the possibility that more than 15 experienced foragers could freely forage and recruit for the feeder in any given trial. All experienced foragers were marked and all experienced foragers not in use were kept inside the cage. During a 15-min trial, the number of experienced foragers visiting the feeder could have decreased to less than 15, but our censuses showed that this did not occur. Because we worked with colonies in different years (forager lifespan is generally less than 60 days, Roubik 1982) and at different sites (256 km separation), released bees from one colony could not affect the results of experiments with other colonies. To release the bees and thereby verify newcomer identity, we placed the cage containing the captured newcomers directly beneath the colony under study. We then unplugged the tube inserted into the cage, allowed the bees to gradually escape, and observed their flight paths. A separate observer using binoculars (Zeiss Night Owl 7X45 B T*P*) monitored potential aggression at the nest entrance.

Testing 3-D location communication (experiment 1)

To test the communication of distance, direction, and height, we used feeder arrays (Lindauer and Kerr 1960; von Frisch 1967). The experiments consisted of laying out two identical feeders (training and control) in the appropriate dimensional axis, training 15 individually marked foragers to only one feeder (the training feeder), and recording newcomer arrivals at both feeders. Each trial lasted for 15 min, a period in which sufficient newcomers would generally arrive to test the communication of food location. The inter-trial interval was 45 min, a period of time sufficient for odor marks deposited by foragers to lose their attractant effect (see Results) and for the rate of newcomer visitation (once all foragers had been removed from the feeder and thus prevented from recruiting) to drop to zero.

If foragers can indicate the tested dimension, then significantly more newcomers should arrive at the training feeder (von Frisch 1967). The *control feeder* was identical in shape, size, color, and sucrose-solution concentration to the training feeder, but all bees landing on the control feeder were immediately captured before they could feed. The *distance* experiment consisted of both feeders placed in the same direction and height, but at different distances from each colony (Table 1). The *direction* experiment consisted of feeders placed in different directions at the same distance and height from each colony (feeder-to-feeder separation of 40 m). For the *height* experiment, we trained foragers to a 12-m-high steel water tower located 40 m north of colony 3, training bees to the top of the water tower by slowly climbing up the attached ladder and carrying the feeder up the tower while marked foragers visited (Lindauer and Kerr 1958; Nieh et al. 2003b). We controlled for potential site bias by alternating the positions of the control and training feeders in the distance, direction, and height experiments. After training bees to a new location, we captured and confined all bees that had experienced the feeder at the previous location until the end of all experiments with each colony.

Odor-mark communication

Spatial pattern of odor marking

Stingless bees prefer to odor-mark prominent vegetation (Kerr 1972). We therefore used cotton ropes draped with leaves to study and manipulate odor trails (methods in Lindauer and Kerr 1958; Kerr et al. 1981; Nieh et al. 2003a). With all colonies, we recorded the spatial pattern of odor marks on a 60-m-long rope unless the feeder was located less than 60 m from the colony. With colony 2, we placed the feeder 20 m away and used a 20-m rope. With the water-tower experiments of colony 3, we used a 29-m rope. Using tripods, we elevated the ropes 1 m above the ground and pointed them towards the nests. We fastened leaves of the vine *Serjania grandiflora* (Sapindaceae) at 1-m intervals along the rope. This native species is common in the Cerrado forest located near our Fazenda field site. With colony 1, we also placed identical parallel ropes 1 m to each side of the central rope. Observers positioned at 10-m intervals watched departing foragers and recorded the distances and times at which foragers landed to deposit odor marks. To observe odor-marking on the 12-m-high water tower, we hung a rope with leaves spaced each 1 m from the top to 1 m above the tower base. From the base, the leaf-bedecked rope continued an additional 29 m towards colony 3, supported by tripods 1 m above the ground. To record odor-mark locations in the water-tower experiment, we placed observers at the tower top, tower base, and spaced each 10 m on the ground in the direction of the nest (three ground observers). At all locations, we set out the ropes and observed odor-marking for 30 min per day for 5 days to examine the spatial pattern of odor-marking. With colony 1, we conducted extended observations of up to 450 min per day on 5 additional days, and used this data to examine temporal patterns of recruitment and odor-marking.

Testing odor-mark attraction (general methods)

To bioassay the attraction of *T. spinipes* to putative forager-deposited odor marks, we tested the ability of foragers to choose an odor-marked feeder (experiments 2–4). Each feeder-choice trial consists of a 30-min *collection* phase followed by a *test* phase. In the collection phase, we placed the collection feeder at the training site connected to a 29-m-long rope pointing towards the nest. For 30 min, we allowed foragers to putatively odor-mark the rope and, in experiment 2, a ring of Whatman number 1 filter paper (5.5 cm inner diameter, 12 cm outer diameter) was placed around the collection feeder. A paper ring of this size was sufficient to insure that foragers positioned their entire bodies over the filter paper while feeding. To obtain the control paper and rope, we placed an identical ring of paper for 30 min and a separate leaf-bedecked rope around an identical but unvisited feeder 30 m east of the collection feeder (both monitored to insure that no bees visited them). At the end of the collection phase, we captured all foragers with the aspirator, and sealed the training feeder inside a plastic bag. Immediately after the collection phase, we began the test phase by setting out two identical, clean feeders and manipulating the odor-marked papers or the odor trail, as appropriate to the experiment. Test-phase feeders were identical to the collection-phase feeder. Bees could only differentiate between the two feeders based on olfactory cues. Due to high recruitment rates (sometimes >100 newcomers per hour), *T. spinipes* newcomers continued to arrive up to 35 min after the end of a collection phase. We captured all newcomers and counted only newcomers that arrived individually (to eliminate the effect of local enhancement, the attraction of newcomers to the visual presence of other bees, Slaa et al. 2003) during these experiments. Once used, we washed all feeders in a strong detergent, rinsing thoroughly with hot water, followed by two washes of 95% ethanol. We air-dried the glass for at least 3 h before reuse. Even without washing, this time interval is more than sufficient for odor marks to completely evaporate. Odor marks deposited on the feeder lost their attractiveness after 20 min (see Results). After each trial, we discarded the gloves and all paper and plastic items used in the trial.

Experiment 2: attractiveness of odor marks on the feeder alone

To determine the attractiveness of putative odor marks on the food source, we used colony 1 and set out two identical, clean test feeders separated by 20 cm, both perpendicular to the nest-feeder direction. We removed the putatively odor-marked rope. We then placed the paper rings containing the putative odor marks around the test feeders, swapping the test-feeder positions each 5 min (35 min test phase) to control for potential site bias. The experimenter stood centered behind both feeders to avoid blocking access to the feeders and biasing the bees towards a given direction.

Experiment 3: attractiveness of the odor trail alone

To determine the attractiveness of the putative odor-trail alone, we performed a displacement experiment, shifting the putatively odor-marked rope (29 m long) 18° to the left or right of the original training feeder (Fig. 1, feeder to feeder separation of 18 m, 15 min test phase). We did not use odor-paper rings. We sealed, but did not remove the training feeder. We placed the control rope on the opposite side of the putatively odor-marked rope and placed test feeders at the distal ends of both ropes (Fig. 1).

Experiment 4: odor-trail polarity

To determine if the putative odor trail was polarized, we displaced and rotated the rope by 180° such that the original training feeder (now sealed) was located in the center of the odor trail, and both ends of the rope (at which we placed the test feeders) were now located at new positions where foragers had never previously experienced a feeder (Fig. 1, 15 min test phase). Thus orientation to either test feeder was solely influenced by the putative odor trail and not by locale odor or by any potential communication of distance inside the nest. To perform the rotation, assistants picked up the ends of the rope and ran counterclockwise while keeping the rope taut. In this experiment, we did not use odor-paper rings.

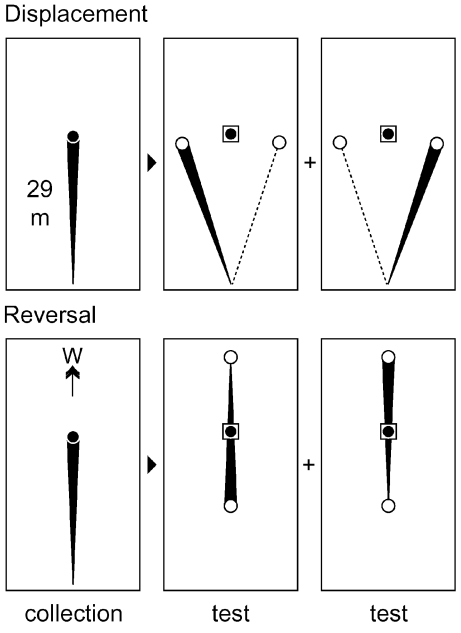

Fig. 1 Design of the displacement and reversal experiments. The collection and test phases are shown. The *elongated triangle* represents the putatively odor-marked rope. A *black circle* indicates the training feeder and *white circles* indicate the test feeders. A *square* denotes the sealed training feeder during the test phases

Video analysis

To examine odor-marking behaviors in detail, we used a Canon XL-1 NTSC digital video camera (30 frames per second) and filmed *T. spinipes* foragers approaching, depositing odor marks on a leaf attached to the feeder, and departing (leaf tip centered in the frame). We analyzed behaviors with iMovie v2.1.1 and Videopoint v2.0.3 software on a Macintosh PowerBook G4 computer.

Statistical analysis

We used Microsoft Excel vX and JMP v5.0.1.2 software to analyze our data. In the feeder-choice experiments, we calculate a two-tailed binomial probability (B.P.) based upon the null hypothesis that randomly orienting foragers will arrive equally at both feeders (P=0.5). We use the χ^2 test to determine if the maximum length of odor trails varies by trial at each distance with each feeder. We use the Wilcoxon test to analyze forager velocity and acceleration and to test for differences between the spatial distributions of odor marks. To compare the spatial distributions of different trials, we calculated the distribution of odor marks within 0.5-m bins from 0 m to 20 m with colony 1 and 0 m to 29 m with colonies 2 and 3 (reflecting the maximum length of the ropes used with each respective colony). Where appropriate, we apply a Bonferroni correction to carry out tests at a critical α"-level, where α"=0.05/k and k equals the number of tests with repeated resampling of the data (Sokal and Rohlf 1981). In such cases, we report the α"-level with the P-value, and only consider the test significant if $P \leq \alpha$". Regression is used to test the temporal relationship between the timing of odor-marking and recruitment in *T. spinipes*. ANOVA is used to

investigate the relationship between weather and odor-trail length. We report all averages as mean±1 standard deviation.

Results

Recruitment to a specific 3-D location (experiment 1)

We studied recruitment with colonies 1 and 3. In both colonies, *T. spinipes* foragers rapidly recruited large numbers of nestmates. In the example shown (a case in which recruitment was allowed to continue for 80 min, Fig. 2), 15 foragers recruited up to 16 newcomers/min for a total of 89 newcomers within 20 min to a feeder 190 m from colony 1. *Trigona spinipes* newcomers found the advertised feeder at the correct distance, direction, and height (Table 1). To control for potential site bias, we alternated the positions of the control and training feeders in all experiments. In all nine *distance* trials, significantly more newcomers (88% overall) arrived at the training feeder, regardless of whether we placed the control feeder 30 m, 15 m, or 2 m closer to the nest than the training feeder (pooled B.P., $P<0.00001$). In all eight *direction* trials, the majority of newcomers (98% overall) arrived at the correct direction (pooled B.P., $P<0.00001$). Finally, in all 16 height trials, a majority of newcomers (99% overall) arrived at the training feeder, regardless of whether the training feeder was at the base or the top of the 12-m-high tower (pooled B.P., $P<0.00001$). Thus *T. spinipes* foragers can recruit nestmates to the correct 3-dimensional location.

To verify the identity of newcomers, we released captured newcomers underneath the subject colony. Approximately 90% of foragers flew directly into the nest entrance and were accepted without aggression by bees inside the nest. Thus at least 90% of newcomers came from the colony under study. Some of the bees appeared disoriented after their confinement and flew in circular flight paths for long periods, disappearing into the foliage. Approximately 10% of marked foragers previously verified as coming from the study colony (but then held inside the cage) and 10% of unmarked foragers (captured newcomers) exhibited this behavior, suggesting that cage confinement, not colony identity, contributed to their flight behavior.

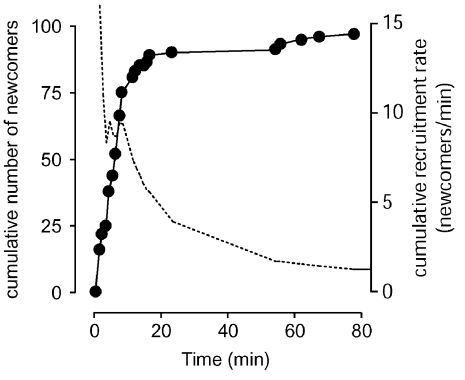

Fig. 2 Example of *T. spinipes* cumulative newcomer recruitment to a feeder limited to 15 experienced foragers. *Solid line* with *filled circles* indicates the cumulative number of newcomers. *Dashed line* gives the cumulative recruitment rate. At 10:04 a.m. on 3 September 2002, the feeder containing 1.0 M sucrose solution was set out. After 15 experienced foragers trained to the feeder on the previous day had arrived (but no newcomers), we raised the sucrose concentration to 2.5 M at 10:19 a.m. and began to receive newcomers

Odor-marking

Experiment 2: attractiveness of odor marks on the feeder

After feeding, experienced foragers occasionally landed and deposited putative odor marks on the feeder, leaves, or ropes (leaf temperature=31.4±2.2°C, $n=34$). Bees never landed on the hot, terracotta-tile plaza (tile temperature=52.4±2.8°C, $n=34$). We removed the rope to begin each trial. During the first 20 min of all three trials, significantly more foragers (average of 73%) chose the feeder with the putatively odor-marked filter paper over the control feeder (B.P., $P\leq0.0002$ for each trial, Fig. 3a). In the subsequent 15 min of each trial, an average of 48% of foragers chose the experimental feeder (B.P., $P\geq0.43$ for each trial, Fig. 3a). Thus foragers deposited odor marks on the feeder that remained attractive for approximately 20 min ($n=477$ newcomers for all trials).

Experiment 3: attractiveness of odor marks on the rope

In all six trials of the displacement experiment (Fig. 1), significantly more newcomers (75% overall) arrived at the feeder indicated by the putatively odor-marked rope (pooled B.P., $P<0.00001$, Table 2, Fig. 3b). In this experiment, attraction to the odor trail persisted for approximately 10 min.

Experiment 4: polarity of odor marks on the rope

In all seven trials of the reversal experiment (Fig. 1), significantly more newcomers (66% overall) arrived at the feeder indicated by the end of the rope closest to the training feeder, regardless of where this end was positioned and even though none of the test feeders were located at positions visited by experienced foragers (pooled B.P., $P<0.00001$, Table 2, Fig. 3b). Correct polarity orientation persisted for at least 15 min.

Odor-mark deposition

Odor-marking consisted of foragers walking for 0.68±0.49 s (video data, maximum=2 s, minimum=0.13 s, $n=19$) and rubbing the substrate with their proboscises. All landing foragers touched the substrate with their proboscises, which were generally distended before

Table 1 Nestmate recruitment to a specific three-dimensional location during 15-min trials. In the direction experiment, the feeders are both 190 m from colony 1 or both 225 m from colony 3. In the height experiment, the feeders are both 40 m from colony 3

Experiment	Trial	Colony	Date	Experimental location	Control location	Wind direction	Average wind speed (m/s)	Average recruitment rate (newcomers/min)	% at exp feeder	No. newcomers at		B.P. 2-tailed
										Exp	Ctrl	
Distance	1	1	01 Sep 02	190 m	160 m	W	1.0	1.2	83	15	3	0.00754
	2	1	01 Sep 02	190 m	160 m	W	1.0	1.4	76	16	5	0.02660
	3	1	01 Sep 02	190 m	175 m	W	1.0	0.7	100	11	0	0.00098
	4	1	03 Sep 02	190 m	188 m	W	0.9	0.7	100	10	0	0.00195
	5	1	03 Sep 02	190 m	188 m	W	0.3	0.5	100	8	0	0.00781
	6	3	25 Aug 03	225 m	195 m	W	0.6	1.1	82	14	3	0.01273
	7	3	25 Aug 03	225 m	195 m	W	0.5	1.1	82	14	3	0.01273
	8	3	25 Aug 03	225 m	210 m	W	0.8	0.6	100	9	0	0.00391
	9	3	25 Aug 03	225 m	223 m	W	0.5	0.5	100	8	0	0.00781
								Totals	88	105	14	<0.00001
Direction	1	1	02 Sep 02	SW	S	W	0.7	0.5	88	7	1	0.07031
	2	1	02 Sep 02	SW	S	W	1.2	0.5	100	8	0	0.00781
	3	1	02 Sep 02	S	SW	W	1.0	1.2	94	17	1	0.00014
	4	1	02 Sep 02	S	SW	W	0.4	1.2	100	18	0	<0.00001
	5	1	02 Sep 02	S	SW	W	0.6	1.1	100	16	0	0.00003
	6	3	25 Aug 03	NW	SW	W	1.1	0.5	100	8	0	0.00781
	7	3	25 Aug 03	SW	NW	W	0.6	1.1	100	16	0	0.00003
	8	3	25 Aug 03	SW	NW	W	1.0	1.2	100	18	0	<0.00001
								Totals	98	108	2	<0.00001
Height	1	3	20 Aug 03	Base	Top (12 m high)	NE	0.6	1.0	93	14	1	0.00098
	2	3	20 Aug 03	Base	Top	E	0.7	0.4	100	6	0	0.03125
	3	3	20 Aug 03	Base	Top	E	2.8	8.8	100	132	0	<0.00001
	4	3	20 Aug 03	Base	Top	E	1.3	8.8	100	132	0	<0.00001
	5	3	27 Aug 03	Base	Top	NE	1.0	0.3	100	5	0	0.06250
	6	3	27 Aug 03	Base	Top	E	1.0	0.3	100	5	0	0.06250
	7	3	27 Aug 03	Base	Top	SE	0.9	2.7	100	41	0	<0.00001
	8	3	27 Aug 03	Base	Top	SE	0.2	2.1	100	31	0	<0.00001
	9	3	20 Aug 03	Top	Base	E	0.6	1.1	100	16	0	0.00003
	10	3	20 Aug 03	Top	Base	NE	0.6	5.3	100	80	0	<0.00001
	11	3	20 Aug 03	Top	Base	NE	0.8	8.9	99	132	2	<0.00001
	12	3	20 Aug 03	Top	Base	NE	1.2	0.9	100	13	0	0.00024
	13	3	20 Aug 03	Top	Base	E	0.4	6.1	100	91	0	<0.00001
	14	3	21 Aug 03	Top	Base	NE	2.5	1.5	82	18	4	0.00434
	15	3	21 Aug 03	Top	Base	E	1.0	7.5	83	113	0	<0.00001
	16	3	21 Aug 03	Top	Base	E	1.0	0.9	83	14	0	0.00012
								Totals	99	843	7	<0.00001

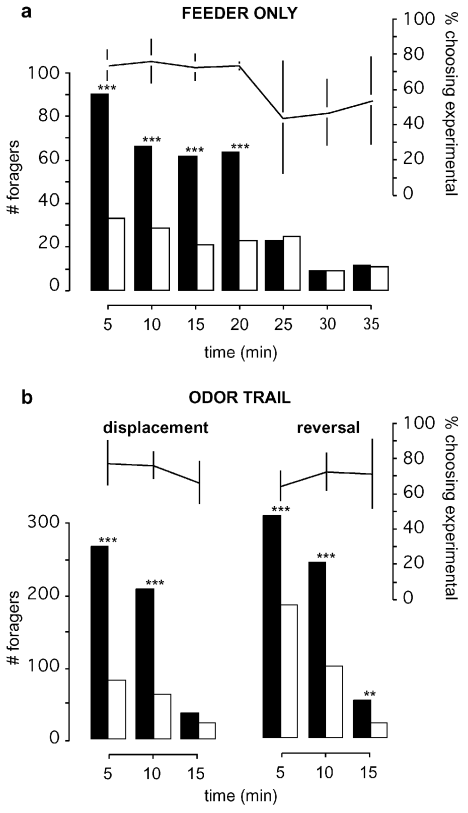

Fig. 3a, b Attraction of *T. spinipes* newcomers to odor marks deposited by nestmates (colony 3). *Filled bars* give the number of foragers choosing the test feeder with the odor marks or the test feeder positioned by the end of the odor trail that was closest to the training feeder. *Unfilled bars* give the number of foragers choosing the test feeder with no odor marks or positioned by the end of the odor trail that was closest to the nest. Percentage of foragers choosing the experimental feeder shown in the plot with standard deviation bars (***P<0.0001, **P=0.0004 B.P) **a** Attraction to odor marks on the feeder alone (pooled data from three trials) **b** Attraction to the short odor trail, displacement and reversal experiments (pooled data from Table 2)

landing, and maintained proboscis contact for 98.5% of the time that they were on the substrate (proboscis contact: 0.67±0.49 s, maximum=2 s, minimum=0.13 s, n=19). Thus foragers deposited odor marks rapidly, taking only a fraction of a second per mark, and proboscis contact may be important in mark deposition. Foragers traveled rapidly between marking events when depositing multiple marks (0.27±0.19 m/s, n=11). Foragers landed with a velocity of 0.27±0.39 m/s and departed with a velocity of 0.58±0.72 m/s within 10 cm of the mark. Departure velocity was significantly higher than landing velocity (Wilcoxon test, Z=4.15, n_{land}=143, n_{depart}=74, P<0.0001). Foragers landed with a deceleration of 8.17±11.8 m/s^2 and departed with an acceleration of 17.43±21.54 m/s^2. Departure accelerations were significantly higher than the absolute magnitude of landing decelerations (Wilcoxon test, Z=4.15, n_{land}=143, n_{depart}=74, P<0.0001).

Odor-trail temporal structure

Recruitment and odor-marking occurred in temporal bursts (Fig. 4a). We define a major recruitment burst as the arrival of more than one newcomer per minute. We define a major odor-marking burst as the deposition of more than one odor mark per minute (as a total of all odor marks deposited by the 15 trained foragers). The start and stop times of major odor-marking bursts were tightly coupled with the start and stop times of major recruitment bursts (Fig. 4b, linear regression, R^2=0.997, $F_{1,18}$=8122, P<0.00001). Odor-marking and recruitment occurred at a low rate throughout the observations; however, major odor-marking bursts preceded major recruitment bursts by 1.3±6.4 min and ended 4.8±6.2 min before the end of major recruitment bursts.

Odor-trail spatial structure

Comparisons between trials. Within each colony at each feeder distance, there was no significant variation in the spatial structure of odor trails over the 30-min observation period (conducted once a day) in which we recorded odor-trail spatial structure. Odor marks retain their attractiveness for approximately 20 min (Fig. 3a), and thus the 30-min observation period corresponded well to the maximum natural period over which receivers could detect temporally grouped odor marks. Using Wilcoxon tests to compare paired data, we found no significant effect of trial on odor-mark spatial distributions with colony 1 ($P\geq$0.59, $Z\geq$−0.58, $n_{\text{distance bins}}$=41, 10 tests); with colony 2 (for all 4 feeder locations, $P\geq$0.59, $Z\geq$−0.535, $n_{\text{distance bins}}$=59, 40 tests); or with colony 3 ($P\geq$0.69, $Z\geq$−0.447, $n_{\text{distance bins}}$=59, 10 tests). In all tests, k=4 and α'=0.0125. Moreover, there was no significant effect of trial on the maximum trail length at each feeder location with each colony ($P\geq$0.13, $\chi^2_4 \leq$7.19, 6 tests). We therefore pooled the data from all five trials at each feeder location with each colony.

Horizontal marking. The odor marks formed a short odor trail (Figs. 5, 6), beginning a maximum of 29 m away from the feeder, with 95% of forager odor marks placed within 3 m of the feeder and 52% of marks placed on the feeder (pooled data, n=295 marks, average mark location=0.89±2.36 m from the feeder). Approximately half (45%) of marking foragers produced multiple marks (average of 1.55±0.67 marks, maximum=6 marks, minimum=1 mark, Figs. 5a–c, 6b). Only 16% of colony 2 foragers produced multiple marks; however, the average number of marks deposited by a marking forager was similar to colony 1 (115 m feeder: 1.29±0.61 marks; 20 m feeder: 1.34±0.69 marks; for both distances, maximum=3 marks, minimum=1, Fig. 5b).

We examined the effect of feeder distance upon the length of the odor trail (Fig. 5b). There is no significant difference between the spatial distributions of odor marks deposited for feeders at different distances from colony

Table 2 Attractiveness and polarization of the short odor trail: odor-trail displacement and reversal experiments (colony 3)

Experiment	Trial	Date	Experimental location	Control location	Wind direction	Average wind speed (m/s)	Average recruitment rate (newcomers/min)	% at exp feeder	No. newcomers at		B.P. 2-tailed
									Exp	Ctrl	
Displacement	1	21 Aug 03	NW	SW	E	0.3	5.8	71	62	25	0.00009
Displacement	2	21 Aug 03	NW	SW	E	0.3	9.3	69	97	43	0.00001
Displacement	3	22 Aug 03	NW	SW	NE	1.2	14.0	68	143	67	<0.00001
Displacement	4	26 Aug 03	SW	NW	E	0.3	2.0	87	26	4	0.00006
Displacement	5	22 Aug 03	SW	NW	-	0	10.9	91	149	14	<0.00001
Displacement	6	26 Aug 03	SW	NW	E	0.5	3.2	71	34	14	0.00552
							Totals	75	511	167	<0.00001
Reversal	1	22 Aug 03	Closer to nest	Farther from nest	N	0.3	16.2	64	155	88	0.00002
Reversal	2	24 Aug 03	Closer	Farther	N	1.1	5.8	63	55	32	0.01783
Reversal	3	26 Aug 03	Closer	Farther	-	0	2.0	77	23	7	0.00522
Reversal	4	26 Aug 03	Closer	Farther	S	0.5	2.5	89	34	4	<0.00001
Reversal	5	22 Aug 03	Farther	Closer	NE	1.2	17.3	68	176	83	<0.00001
Reversal	6	24 Aug 03	Farther	Closer	E	0.8	13.2	63	125	73	0.00027
Reversal	7	26 Aug 03	Farther	Closer	S	0.9	3.8	65	37	20	0.03314
							Totals	66	605	307	<0.00001

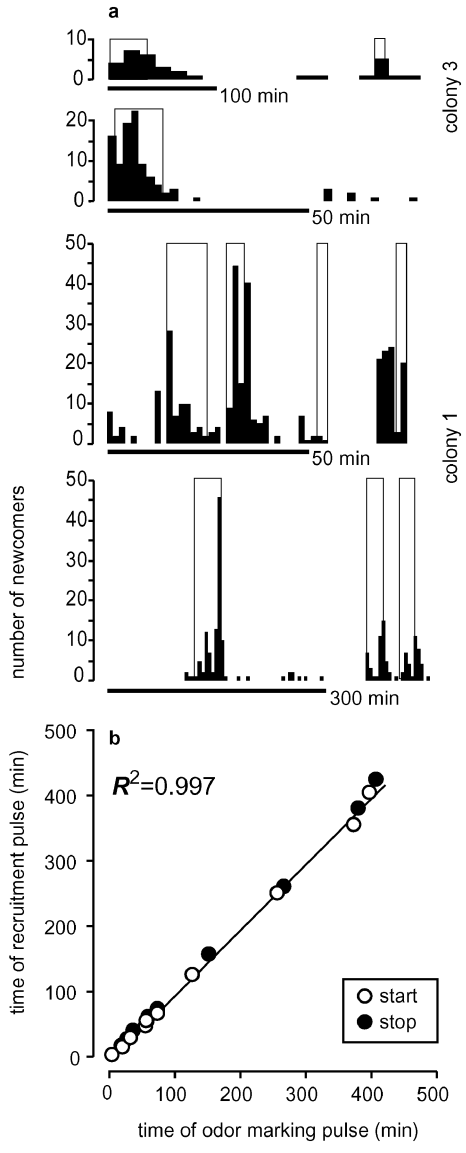

Fig. 4 **a** Distribution of recruitment and odor-marking over time beginning with the start of odor-marking observations on each day (four examples). Extended daily observations with colony 1. Recruitment occurs in pulses (*filled bars*), and the timing of major recruitment pulses is synchronized with odor-marking (*unfilled bars*). Time scales shown below each plot **b** Timing of major recruitment pulses is tightly linked to odor-mark deposition. Start and stop times of recruitment and odor-marking pulses are plotted. Data from five trials. Linear regression line shown

2 (Wilcoxon test, Z=−1.05, $n_{\text{distance bins}}$=59, P=0.27, Fig. 5b,d). However, there is a significant difference between the spatial distributions of odor marks deposited for feeders 225 m (Fig. 5c,d) and 40 m (Fig. 6b) from colony 3 (Wilcoxon test, Z=−2.801, $n_{\text{distance bins}}$=59, P=0.005).

Analysis of the pooled data (consisting of five feeder positions located on the ground) shows no significant relationship between odor-trail length and distance of the feeder from the nest (ANOVA, $F_{1,3}$=2.72, P=0.20) In addition, there is no significant relationship between

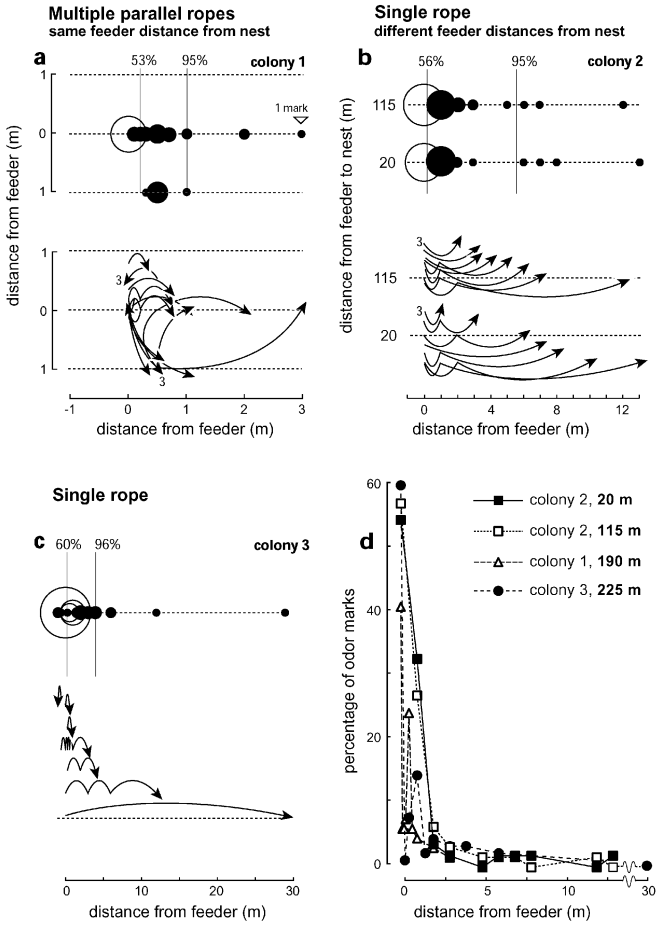

Fig. 5a–d Short odor trails created by recruiting foragers. *Circle* area corresponds to the number of marks. *Large circles* are *unfilled* to avoid obscuring other odor marks. The percentage of marks within set distances is shown. The values closest to 50% and 95% were chosen, but marks were not evenly distributed and thus we have given exact values rather than interpolate between marks. *Dashed lines* indicate ropes. Odor marks shown above. Deposition sequence shown below. Paths vertically displaced to reveal the marking pattern. *Numbers* indicate multiple runs with the same path. **a** Multiple parallel ropes (n=55 marks, recruitment rate=15 recruits/min, nest is 190 m from feeder) **b** Short odor trails deposited on a single rope for feeders at different distances from the nest (n=63 and 91 marks for the 115-m and 20-m feeders respectively, recruitment rate=12 recruits/min) **c** Short odor trail deposited on a single rope by colony 3 foragers (n=66 marks, recruitment rate=8.6 recruits/min, nest is 225 m from feeder) **d** Spatial distribution of odor marks at feeders placed at different distances from the colonies (summary of data from panels **a**–**c**)

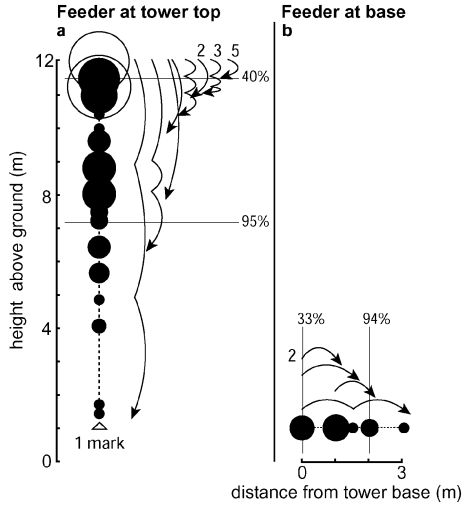

Fig. 6a, b Short odor trail created by recruiting foragers during 150 min. The nest is 40 m from the tower. *Circle area* corresponds to the number of marks. *Numbers* above paths indicate the number of marking runs with the same path **a** Feeder at tower top (264 marks, 5.0 recruits/min) **b** Feeder at tower base (18 marks, 2.9 recruits/min)

odor-trail length and wind speed, temperature, or humidity (data pooled in 30-min intervals to reflect variations in weather conditions: ANOVA, $F_{1,23} \leq 1.91$, $P \geq 0.18$, 3 tests)

Vertical marking. Foragers also marked vertically when the feeder was at the top of the water tower (Fig. 6a), producing a short odor trail that extended from 1.5 m above the ground to the top of the tower (10.5 m length). While marking, 5.6% of foragers produced multiple marks for an average of 1.06±0.41 marks/forager. Foragers did not deposit any marks along the ground (in the horizontal plane) when the feeder was on the top of the tower, even though a rope stretched from the base of the tower towards the nest. However, foragers did deposit a short odor trail (3 m long) in the horizontal plane when a feeder was placed at the base of the water (Fig. 6b). In terms of length and the distribution of odor marks along that length, there is a significant difference between the spatial distribution of odor marks deposited on the top and the base of the water tower (Wilcoxon test, Z=−3.32, $n_{\text{distance bins}}$=59, P=0.0009). During these odor-marking observations, the wind velocity and relative humidity at the top of the water tower were significantly higher than at the bottom of the tower (top 1.50±0.95 m/s, bottom 1.02±0.79, Wilcoxon test, Z=−3.86, n=98, P=0.0001; top 36.9±6.6% relative humidity, bottom 36.5±6.1%, Wilcoxon test, Z=−2.68, n=98, P=0.007). The temperature was also significantly higher at the base of the tower than at the top of the tower (top 29.4±2.2°C, bottom 29.9±1.7°C, Wilcoxon test, Z=−3.95, n=98, P<0.0001).

Discussion

Trigona spinipes foragers recruited a large number of nestmates in temporal bursts to a rich food source at a specific distance, direction, and height. Foragers oriented to nestmate-deposited odor marks but, surprisingly, deposited a short odor trail instead of a complete odor trail extending from the feeder to the nest. Classically, stingless-bee odor trails are described as extending from near the nest to the food source, and should therefore provide efficient guidance to recruited nestmates. Such odor trails are also characterized by a fairly regular spacing between odor marks (Lindauer and Kerr 1958). Thus an odor trail

extending for only a short distance from the food source and in which odor marks are more concentrated near the food source suggests a different mechanism of olfactory communication. The short odor trail deposited by *T. spinipes* foragers evidently facilitated recruitment to the correct distance, direction, and height (Tables 1, 2, Fig. 3). Interestingly, a significant majority of newcomers were also able to detect and orient towards the correct terminus, even when this was rotated by 180° and shifted to locations that experienced foragers had never previously fed at. Thus *T. spinipes* foragers can deposit a polarized short odor trail.

Recruitment rate

Competition plays an important role in stingless-bee foraging, and recruitment bursts that provide an overwhelming aerial attack and facilitate the exclusion of competitors may be a hallmark of aggressive stingless-bee foraging (Johnson 1974; Johnson and Hubbell 1974; Hubbell and Johnson 1978; Roubik 1978, 1980; Gill et al. 1982; Johnson and Hubbell 1987; Biesmeijer et al. 1999). Although we maintained a constant rate of feeder visitation, *T. spinipes* foragers recruited in bursts (a maximum of 17.3 newcomers per minute, Table 2) alternating with relatively long periods (up to 100 min) of no recruitment (Fig. 4a). Kerr (1973) reported similar variation in the rate of recruitment during hour-long time blocks. Large recruitment bursts also characterize the recruitment system of the aggressive stingless bee *T. hyalinata* (Roubik 1989; Nieh et al. 2003a). However, stingless bees with relatively non-aggressive recruitment strategies, such as *Melipona fasciata*, *M. scutellaris*, and *M. quadrifasciata*, appear to recruit at a more constant rate (Biesmeijer and Ermers 1999; Jarau et al. 2000, 2003). A similar division of strategies exists among ants, in which aggressive species such as the army ant, *Eciton burchelli*, recruit in large masses, and non-aggressive species tend to recruit in smaller groups (Hölldobler and Wilson 1990).

Newcomer identity

It is possible that approximately 10% of newcomers came from a non-subject colony because 10% of newcomers did not immediately return to the nest entrance of the subject colony following release, after long confinement in the holding cage. Observers lost track of these bees in the foliage. However, 10% of marked foragers previously verified as having come from the subject colony, but then held inside the holding cage, also did not immediately return to the nest entrance of the subject colony. Thus the effects of confinement, not colony identity, may account for this behavior. Nonetheless, it is important to consider how the potential presence of foragers from other colonies could have affected our results. In the collection of odor marks and the observations of individual odor-marking behavior and spatial and temporal patterns in odor-marking, we used marked foragers that were verified as coming from the subject colony. The effect of potential newcomers from non-subject colonies is therefore limited to newcomer choices in experiments testing the communication of food location, the attractiveness of odor marks, and the temporal patterning of recruitment. In all trials testing the spatial communication of food location, we observed strong, consistent preferences for the training feeder over the control feeder (n=119, 110, and 850 newcomers in the distance, direction, and height experiments respectively, Table 1). In experiments testing the attractiveness of odor marks, the sample sizes were even larger (n=477, 678 and 912 newcomers in the feeder only and odor trail, and odor-trail reversal experiments Fig. 3, Table 2). A 10% reduction in these sample sizes or shift in the choices of newcomers would not change the results of these experiments. Lastly, we observed a characteristic pulsed pattern of mass recruitment that a 10% reduction in newcomer arrivals would not have changed significantly (Fig. 4).

Odor-mark deposition and attraction

Several species of stingless bees, including *Scaptotrigona postica*, *Scaptotrigona bipunctata*, *Scaptotrigona xanthotricha*, *T. capitata*, *T. hyalinata*, and *T. recursa*, deposit attractive glandular secretions to mark food sources (Lindauer and Kerr 1958; Kerr et al. 1963; Nieh et al. 2003a; Jarau et al. 2004). We observed a stereotyped behavior, landing and proboscis-licking on the feeder and leaves, that was well correlated with major recruitment bursts (R^2=0.997, Fig. 4b). On average, major odor-marking bursts preceded major recruitment bursts by 1.3±6.4 min and ended 4.8±6.2 min before the end of major recruitment bursts.

Prior to depositing an odor mark, foragers appeared agitated, feeding only briefly before alighting and feeding again. This behavior is consistent with previous descriptions of *T. spinipes* odor-marking (Kerr 1972, 1973) and with the odor-marking behavior of other meliponine species (Lindauer and Kerr 1958; Schmidt et al. 2003; Jarau et al. 2004). Kerr et al. (1981) collected odor marks deposited in this way onto branch-laden wires that they then displaced, and attracted *T. spinipes* foragers up to 11 min after odor-mark deposition. In our experiment, odor marks deposited by *T. spinipes* foragers on the feeder attracted foragers for up to 20 min. Our video analysis suggests that proboscis contact may facilitate the transfer of glandular secretions onto the substrate, as proposed for the transfer of attractive labial-gland secretions by *T. recursa* (Jarau et al. 2004). *Trigona spinipes* foragers may also deposit attractive tarsal-gland secretions during the walking behavior, although in *M. seminigra* such secretions require a much longer deposition period or multiple deposition events (40 visits) and persist for a far longer period of time (2 h) than *T. spinipes* odor marks (Hrncir et al. 2004).

A short odor trail

The detailed spatial structure of short odor trails is perhaps their most interesting characteristic because it provides a mechanism for odor-trail polarization and because relatively little is known about the structure of meliponine odor trails.

Horizontal trails

T. spinipes foragers from both colonies created a short odor trail that extended a maximum of 29 m from the feeder in the direction of the nest (in the case of colony 3, located 225 m away, Fig. 5d). We tested the attractiveness of odor marks deposited on the food source separately from odor marks comprising the odor trail (Fig. 3). Odor marks deposited at both locations attracted newcomers (Fig. 3), and thus the landing and mouthpart-rubbing behaviour of foragers, the only contact that foragers had with the rope, does deposit attractive odor marks. However, it remains to be conclusively shown that all such landing and mouthpart-rubbing events actually deposit attractive odor marks. The actual length of the odor trail may therefore be shorter and the density of odor marks less than what we have measured. Thus the spatial distribution of odor marks in this short odor trail is quite different from descriptions of complete meliponine odor trails extending from near the nest towards the feeder. Lindauer and Kerr (1960) reported that trail-laying species did not deposit odor marks until they were at a threshold distance from the feeder [a minimum of 10–20 m for *T. postica*, 35 m for *T. ruficrus* (a synonym for *T. spinipes*), 25 m for *T. capitata*, and 2.7 m for *T. mombuca*] and then deposited marks up to 7.5 m from the nest.

Observer error or rope length is unlikely to account for the lack of more distant marks. In our experiment, we positioned observers at 10 m intervals to watch for mark deposition, and the ropes extended 20 m away from the feeder when the nest was 20 m away (colony 2), 29 m when the nest was 40 m away from the water tower (colony 3), and 60 m away from the feeder in all other cases. With the 20-m rope (colony 2, Fig. 5b), the odor trail was only 13 m long, and with the 29-m rope (colony 3 located 40 m away), the odor trail was only 3 m long (Fig. 6b). In all other cases, the odor trail extended for a maximum of 29 m along a 60-m rope (Fig. 5c). Thus, with 60-m horizontal ropes, the odor trail took up only 29±25% of the total rope length. To provide the clearest possible view of odor-marking, we chose sites with no vegetation or closely cropped grassy areas without trees or shrubs. In the water-tower experiments, the rope extended over a grass field for 29 m and the remaining 11 m to the nest passed through dense vegetation. However, the furthest odor mark was deposited 3 m from the base of the tower and the observers could clearly see and visually follow foragers as they flew away from the feeder towards the nest.

Kerr (1972) observed a *T. spinipes* forager deposit five odor marks (10 m, 15 m, 40 m, 50 m, and 90 m away from a feeder) during four separate trips back to the nest (located 90 m away). We are unable to account for the discrepancy between these results and our data, unless *T. spinipes* foragers sometimes deposit odor marks at greater distances. However, both our data and the data of Kerr (1972) reveal a similar pattern of decreasing mark density with increasing distance from the food source. Except for the 20-m feeder, our ropes did not extend the entire distance to the nest, and thus it is possible that *T. spinipes* foragers marked only near the feeder and near the nest (a bimodal mark distribution). Nonetheless, such bimodal marking behavior has not been observed in other *T. spinipes* studies (Kerr 1972, 1973; Kerr et al. 1981), was not observed in our 20-m feeder experiment (in which the rope extended the entire distance from the feeder to the nest, Fig. 5c), and has not been reported in any stingless-bee species (Lindauer and Kerr 1958).

Vertical trails

It is striking that the odor trail deposited up the water tower (when the feeder was 12 m high) contained no horizontal component, but stretched from 1.5 m to 12 m vertically, even though the same rope bedecked with leaves continued from the base of the tower towards the nest (Fig. 6a). For these observations, we used colony 3, located 15 m above the ground and 40 m south of the water tower. However, when the feeder was at the base of the tower, foragers odor-marked along the horizontal portion of the rope for 3 m. The rate of odor-marking was much higher for a feeder on top of the tower (1.76 marks/min) than one at the base of the tower (0.12 marks/min). Weather may play a role in this difference because wind velocity, relative humidity and temperature were significantly different at the top of the tower than at the base. In particular, increased wind velocity (higher by an average of 0.47 m/s at the top of the water tower than at the base, $P<0.0001$) is known to decrease the active space of odor marks (Bradbury and Vehrencamp 1988) and may thus have contributed to the greater rate of odor-marking, if foragers adapt odor-marking to environmental conditions. This would be an interesting point to investigate.

Variations in odor-trail spatial structure

There is variation in the maximum length of odor trails between different colonies and with the same colony at different feeder distances. In some cases, there was a significant relationship between feeder distance from the nest and maximum odor-trail length (Figs. 5, 6), but there is no significant overall relationship when the data from all colonies and feeder locations (on the ground) are considered. We also found no significant relationship between weather conditions and the length of odor trails deposited on the ground. At each feeder location with

each colony, there was no significant effect of trial on the spatial distribution of odor trails or the maximum length of odor trails. Replicates were conducted sequentially and may thus capture a more homogenous set of environmental and colony conditions. Thus it is unclear what factors control the spatial density and maximum length of odor trails deposited for food sources on the ground, although the variation in spatial structure is interesting and deserves further study with a greater number of feeder distances under uniform ecological conditions. In particular, the significant correlation between weather conditions and rate of odor-marking at the tower site suggests a tuning of odor-trail deposition to maintain signal strength.

In addition, these experiments constrained foragers to mark along either horizontal or vertical ropes and are thus a simplification of the natural situation of a field with vegetation of fairly uniform height or, in the case of the water tower, where food sources such as tall canopy trees are isolated within such fields. The natural *T. spinipes* colonies that we used live in such an environment at the Fazenda Aretuzina. However, meliponine trail-marking within a dense forest canopy that provides multiple levels of potential substrates should also be studied if the difficulty of making observations in dense foliage can be overcome.

Odor-trail polarization

Given the shortness of *T. spinipes* odor trails, it is relevant to consider the utility of trail polarization. A polarized short odor trail can assist the rapid orientation of recruits to a specific spatial point, an important task for *T. spinipes*, which engages in combat and can thereby extirpate other bees (Cobert and Willmer 1980; Cortopassi-Laurino and Ramalho 1988; Gallo et al. 1988; Sazima and Sazima 1989; Martinez and Bullock 1990; Ramalho et al. 1994; Silva et al. 1997; Nieh et al. 2004) and birds from food sources (Willmer and Corbet 1981; Gill et al. 1982; Barbosa 1999). Such rapid and precise spatial orientation could assist group attacks on larger animals. In the absence of combat, the value of short odor-trail polarization for spatially diffuse food sources remains to be clarified.

Recently, Schmidt et al. (2003) showed that *Scaptotrigona depilis* is able to find the odor-marked feeder, even when it is displaced away from the putative odor trail or into the putative odor trail. The ability of newcomers to reach the correct endpoint may also be due to differences in the concentration of odor marks deposited on the target or different compounds deposited at the endpoint as opposed to the odor trail (Lindauer and Kerr 1960). We hypothesize that the odor-mark concentration gradient provides one source of polarization information. Experienced foragers deposited the most marks directly on the feeder and these odor marks decreased sharply in density with increasing distance from the feeder (Fig. 5d). This concentration gradient could enable newcomers to distinguish between the start of the odor trail and its terminus, located at the food source. The odor-trail reversal experiment (Figs. 1, 3b) demonstrates that the short odor trail is polarized and can influence newcomer choice independently of locale odor or the potential communication of distance information inside the nest. Foragers odor-marked the entire length of the rope (29 m, Fig. 5c). However, in all trials, significantly more newcomers oriented towards the clean test feeder placed at the end of the odor trail originally closest to the training feeder (Fig. 3b, Table 2) regardless of the positions of the test feeders, and even though these feeders were placed in locations never previously visited by bees. Newcomers did not orient towards locale odors or via information potentially obtained inside the nest. This reversal experiment therefore provides the first strong evidence for meliponine odor-trail polarization.

Evolutionary implications

Why do certain meliponines use complete odor trails and others use short odor trails? Olfactory eavesdropping may play a role. *T. spinipes* foragers can use olfactory eavesdropping to detect the attractive odor marks deposited by foragers of a competing bee, *M. rufiventris*, and then attack, overwhelm, and take over the food source (Nieh et al. 2004). In the face of such a selective pressure, counter-eavesdropping strategies should evolve (Guilford and Dawkins 1991, 1993), and this could lead to the evolution a short odor-trail strategy that may be less conspicuous than a complete, longer odor trail. This hypothesis should be experimentally tested.

The cost of a short odor trail may be that it provides less guidance. However, it is striking that the deposition of marks a maximum of 29 m away from the feeder was sufficient to assist the rapid orientation of a substantial number of nestmates (over 2,669 newcomers from 3 colonies in 2002 and 2003). Thus a complete odor trail leading from the nest to the feeder is not necessary to recruit a large number of *T. spinipes* newcomers to a specific 3-dimensional location (Table 1). In future studies, we plan to examine the intranidal behavior of recruiting *T. spinipes* foragers to determine the mechanisms used to assemble and perhaps even guide a group of foragers towards the feeder. Such information may increase our understanding of the advantages and tradeoffs of short and complete odor-trail communication.

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