

## A stingless bee marks the feeding site in addition to the scent path (*Scaptotrigona aff. depilis*)

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**Abstract** – *Scaptotrigona depilis* uses a scent trail to guide newly recruited bees to a food source. (i) Behavioral experiments show an additional chemical marking at the food source. The bees had to choose between an unused feeder and a feeder, at which their nestmates had fed. 71 to 86% of the bees chose the used feeder where the foragers had left attractants. The used feeder also attracted bees when it was moved away from its original site to a new site halfway along the scent path or 20 m beyond it. (ii) The localization of a food source by *S. depilis* is very precise with regard to both direction and distance. When control feeders were 1.7 m, 8.5 m, and 17 m away from the experimental feeder (at 50 m from the nest) 97.5–100% of the recruits chose the experimental feeder where the foragers were feeding. When positioned beyond the used feeder the control feeder remained unvisited. We conclude, that markings left at the used feeder represent particular end point tags and differ from scent path markings.

**stingless bee / *Scaptotrigona* / scent marking / recruitment**

### 1. INTRODUCTION

The use of chemical signals in stingless bees to scent a trail for the guidance of newly recruited nestmates to a food source was observed in species of the genera *Trigona*, *Scaptotrigona*, *Cephalotrigona*, *Geotrigona*, and *Oxytrigona* (Lindauer and Kerr, 1958, 1960; Kerr, 1960; Kerr and Cruz, 1961; Kerr et al., 1963; Blum et al., 1970; Kerr et al., 1981; Johnson, 1987; Kerr, 1994). Species of the genus *Scaptotrigona* (Apidae, Meliponinae) are surprisingly efficient in scent trail recruitment, when the number of bees recruited to a food source in a given time (Lindauer and Kerr, 1958, 1960; Jarau et al., 2003) and the precision of food source

localization (Lindauer and Kerr, 1958; Kerr et al., 1963) are taken as measures. Aiming at a better understanding of the chemical signals used for scent trail marking, the contents of *Scaptotrigona*'s mandibular glands (Luby et al., 1973) and head extracts (Franke et al., 1983) were analyzed. However, no data are available demonstrating the actual use and behavioral relevance of the components identified. The morphology of the mandibular glands, which may play a significant role in scent marking (Lindauer and Kerr, 1958, 1960; Blum et al., 1970), is well known (Nedel, 1960; Cruz-Landim and Akahira, 1966; Cruz-Landim and Ferreira, 1967; Cruz-Landim and Ferreira, 1968). The mandibular glands are well developed in young bees but

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degenerate with age. There is, however, no conclusive evidence as yet that they are indeed used for communication by foraging bees.

Our present knowledge of scent trail marking and following is still based on the experiments by Lindauer and Kerr (1958, 1960) and Kerr et al. (1963). According to these studies scent trails are a prerequisite for successful recruitment in *Scaptotrigona*. The foraging bees land every 2–5 m on their way back to the nest to secrete the scent marks, which then serve the newcomers (recruited in the nest) as guideposts on their way out to the food source (Lindauer and Kerr, 1958, 1960). The scent marks' source was postulated to be the mandibular glands, because the scent marks smelled like the dissected glands and because the bees ran 3 mm along the edge of a leaf or stone or blade of grass, rubbing their mandibles on the respective surface while scent marking (Lindauer and Kerr, 1958, 1960).

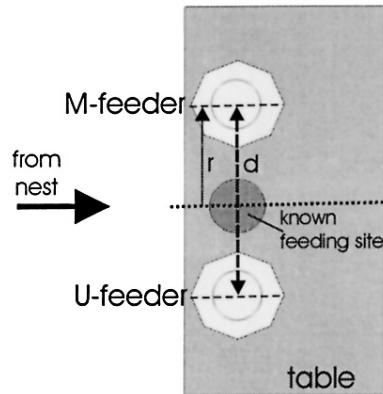
In contrast with *Scaptotrigona*, bees of the genus *Melipona* do not lay scent trails (Lindauer and Kerr, 1958; Nieh and Roubik, 1995; Hrnčir et al., 2000). However, scent beacons at the food source have been described for several species (Aguilar and Sommeijer, 1996, 2001; Nieh, 1998). For *Melipona seminigra* Hrnčir et al. (2003) recently demonstrated the chemical marking of the food source itself with attractants secreted at the bees' tarsi.

Here we ask whether scent path laying species mark the feeding site as well. Accordingly, the present study examines (1) whether *Scaptotrigona* aff. *depilis* Moure 1942, a species using scent trails to guide recruits to a food source, specifically scent marks the feeding site in a way different from scent path laying, (2) whether feeding site marks remain effective when spatially separated from their original site, and (3) whether feeding site marks specifically tag the end point of a scent path.

## 2. MATERIALS AND METHODS

### 2.1. Study site and animals

All experiments were performed on the USP-Campus at Ribeirão Preto, São Paulo, Brazil, between July 2001 and February 2002. We used two colonies of *Scaptotrigona* aff. *depilis*



**Figure 1.** Choice experiments. The bees had to choose between two feeders arranged equidistantly to the previous known feeding site (now without feeder). The two feeders were the M-feeder (marked by foragers when used at the known feeding site) and the U-feeder (unmarked, not used before), whose distance ( $d = 2r$ ) from each other was 20 cm and 170 cm, respectively. Table and feeders are not drawn to scale.

(Camargo, personal communication). The colony used for choice experiments and displacement experiments was kept in a wooden nest box inside the laboratory. For localization experiments we used another naturally occurring colony, living in a cavity inside a stonewall.

In all experiments and training phases we used feeders as described in Jarau et al. (2000). The feeders were placed on laboratory stools as feeding tables (height 60 cm, diameter 35 cm) except for the choice experiments. All items of the setup were alcohol cleaned (ethanol, 95%) before each experiment, except the feeders defined as “marked” by the foraging bees. The feeders rested on a vial (height = 35 mm) within a petri dish, which was filled with water to ward off ants.

### 2.2. Choice experiments to demonstrate whether the feeding site is marked chemically

*Training and foraging phase.* We trained several bees to the midline on the front edge of a table (height 75 cm, length 2 m, width 50 cm) at a point 18 m to the South of the bees' nest using  $0.75 \text{ ML}^{-1}$  unscented sugar water. The bees were allowed to feed ad libitum at this site for at least 45 minutes, after which time the site was defined as “the known feeding site” (Fig. 1). At the end of the training phase we marked four bees with a colored dot, whereas all other bees coming to the feeding

site were captured using a plastic suction tube. The training feeder was then replaced by an alcohol cleaned feeder containing  $0.75 \text{ ML}^{-1}$ ,  $1.5 \text{ ML}^{-1}$ , or  $3 \text{ ML}^{-1}$  unscented sugar water, depending on the test series. The four foragers were now allowed to feed at the clean feeder until together they had completed 20 visits. They were then captured and kept separate from the previously captured bees. The same feeder was now defined as M-feeder (hypothetically assuming that it was *marked* by the four foragers).

*Choice phase.* The M-feeder was shifted either to the left or to the right of the known feeding site by distance  $r$  along the front edge of the table (Fig. 1). At the same distance  $r$  but on the opposite side of the known feeding site we placed an unused, alcohol cleaned, but otherwise identical feeder defined as U-feeder (*unmarked*). Now the previously captured bees (except the four foragers) were released at the nest's entrance. They continued their search for food at the feeding site already known to them. At their previous feeding site, however, there was no food anymore. Instead the bees had to make a choice between the M-feeder and the U-feeder. The experiment started when the first bee landed on one of the feeders and lasted for 20 minutes. All bees landing at the M-feeder or at the U-feeder were captured. Only those bees were counted which landed when no other bee was at the feeder, to avoid attraction by the feeding bees. The positions of the M-feeder and the U-feeder were exchanged every five minutes in order to avoid side biasing.

For  $d = 2r = 20 \text{ cm}$  we carried out three test series using  $0.75 \text{ ML}^{-1}$ ,  $1.5 \text{ ML}^{-1}$ , and  $3 \text{ ML}^{-1}$  sugar water, respectively. Two test series were performed for  $d = 170 \text{ cm}$ , with  $1.5 \text{ ML}^{-1}$ , and  $3 \text{ ML}^{-1}$  sugar water, respectively.

*Control experiments.* The bees had the choice between two unused and alcohol cleaned feeders containing  $1.5 \text{ ML}^{-1}$  unscented sugar water (otherwise setup identical to that described above) and  $20 \text{ cm}$  or  $170 \text{ cm}$  ( $d$ ) apart from each other. All bees landing on the feeders during 20 min were captured. For the reasons given earlier a bee was only counted, if at the same time no other bee was at the feeder. The position of the two feeders again was exchanged every five minutes. In case of a preference (which was never significant) for one of the two feeders, the "preferred" feeder was defined as A-feeder and the other one as the B-feeder.

### 2.3. Experiments to examine whether marks remain effective on displaced feeders

The bees were trained to a distance of 25 m or 50 m from the nest. The training feeder was then

replaced by an alcohol cleaned feeder containing  $3 \text{ ML}^{-1}$  unscented sugar water in order to initiate the bees' free recruitment, where all the bees were allowed to return to the nest and to visit the food source without any interference by the observer for at least 45 min. From then on we defined this feeding site as "the original feeding site". An additional food source was then presented at "the experimental site" offering  $3 \text{ ML}^{-1}$  unscented sugar water. Furthermore,  $3 \text{ ML}^{-1}$  sugar water was offered throughout the whole experiment at the original feeding site in order not to endanger the maintenance of the scent path. (i) In the first test series the original feeding site was at 25 m from the nest. The experimental site was at a distance of only 13 m from the nest (between the nest and the original feeding site), i.e. halfway along the scent path; (ii) in the second test series the original feeding site was at a distance of 50 m from the nest. The experimental site was at a distance of 70 m from the nest, i.e. 20 m beyond the original feeding site (beyond the scent path).

*Experimental procedure.* Two feeders were positioned equidistant to the feeding table's midline ( $d = 20 \text{ cm}$ ) at the experimental site. One of them was the recently used and emptied feeder taken from the original feeding site (where the bees had fed ad libitum). This feeder was defined as M-feeder, assumed to be *marked* by foraging bees. The other feeder was a fresh, alcohol cleaned feeder, defined as U-feeder (*unmarked*). Both feeders were filled with  $3 \text{ ML}^{-1}$  unscented sugar water. The experiment started when we placed the M- and the U-feeder onto the feeding table at the experimental site. It lasted for 20 min during which every bee at the feeders was captured. Again we only counted bees if at the same time no other bee was at the feeder. The feeders' positions were exchanged every five minutes.

*Control tests.* For (i) and (ii) we performed a control test series to show how many bees find an additional feeding site due to the feeders' visual appearance, but without any chemical signals. Two unused, alcohol cleaned feeders containing  $3 \text{ ML}^{-1}$  unscented sugar water were presented on an otherwise identical setup. Every bee landing on a feeder was captured and counted.

### 2.4. Experiments to demonstrate the precision of food source localization

Using  $0.5\text{--}0.75 \text{ ML}^{-1}$  unscented sugar water several bees were trained to a feeder mounted on a feeding table in a specific direction and at a specific distance (at 50 m) from the nest. We marked one to three bees (foragers) with a colored dot on their thorax. All the other bees were captured using a plastic suction tube and kept in a jar until the end of

the experiment. After the marked foragers had collected at the training feeder and had returned to their nest three times the training feeder was put away in an airtight plastic dish and replaced by the alcohol cleaned experimental feeder filled with 3 ML<sup>-1</sup> unscented sugar water. An identical control feeder containing 3 ML<sup>-1</sup> unscented sugar water was placed on a feeding table in a different direction or at a different distance from the nest, as described by Jarau et al. (2000). The foragers were allowed to feed at the experimental feeder for 2 hours. All the newly recruited bees at both of the feeders were captured, counted, and the time of their arrival noted. The respective distances between the experimental and the control feeder were 1.7 m, 8.5 m, and 17 m (Fig. 5) depending on the test series.

## 2.5. Statistical analyses

For all statistical procedures SigmaStat 2.0 and SigmaPlot 2000 were used. As the number of bees visiting the feeders varied among the experiments due to environmental factors and the colony's condition, we used the percentage of the total number (N) of bees ( $n$  at U-feeder +  $n$  at M-feeder = N = 100%) to describe the distributions of bees at the feeders. The sample size always was 6 experiments per test series, except for the localization experiments with the control feeder 17 m in front and 17 m beyond the experimental feeder, where the sample size was 4 experiments. In the case of a normal distribution (Kolmogorov-Smirnov-test), the data are presented as the mean percentage ( $\pm$  SD) of bees, whereas the median percentage (1.quartil/3.quartil) of bees is given when the K-S-test failed. In control experiments the numbers of bees at the A-feeder and at the B-feeder were compared applying the  $\chi^2$ -test. The distribution of bees at the feeders in choice experiments, when bees were choosing the M-feeder, was compared by the Student t-test with the distribution of bees in the control tests when bees were "preferring" the A-feeder among two identical clean feeders. In all the other test series we applied the Student t-test or the  $\chi^2$ -test in case of normally distributed data or, when the equal variance test failed, the Mann Whitney rank sum test. For non-parametric comparisons we used the  $\chi^2$ -test and the Mann Whitney rank sum test.

## 3. RESULTS

### 3.1. Behavior at the feeding site

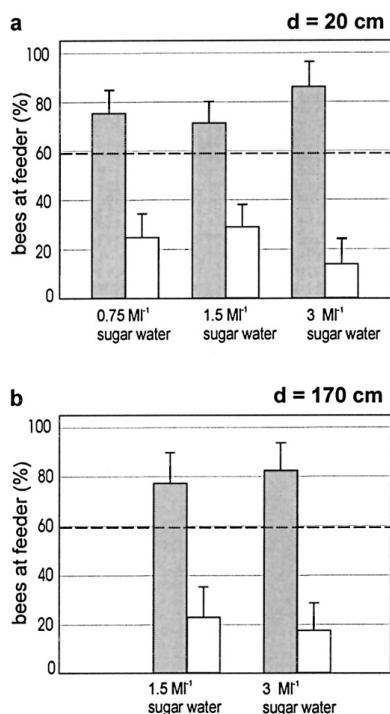
The time spent by *S. depilis* at a feeder containing 3 ML<sup>-1</sup> sugar water varied from 1

to 61 seconds (N = 6; n = 292). The mean time which each individual bee (N = 6) spent at the feeder varied between  $13 \pm 10$  s to  $30 \pm 13$  s. We noticed a behavior of the foragers, which resembled the "nervous" behavior of *Scaptotrigona* at the feeding site described by Lindauer and Kerr (1958, 1960). The bees often rapidly changed their feeding behavior from one single food intake per visit to interrupted visits. They stopped drinking after a few seconds to fly up and in a circle around the feeding site (within approx. 1 m). Then they landed again at the feeder to drink for another few seconds. This behavior was repeated two to four times. The mean duration of uninterrupted feeding times was  $26 \pm 10$  s (n = 150). Each feeding time during interrupted visits lasted for  $13 \pm 8$  s (n = 142). The obvious behavior of scent path marking was never observed at the feeders or beyond the feeding site, but could be seen clearly on the bees' way from the feeding site to the nest. When at the feeders the bees did not rub their mandibles on any items nor did they deposit substances visible to the human observer like anal droplets.

### 3.2. Chemical marking at the feeding site

In all the choice experiments the bees strongly preferred the used M-feeder. In most of the control tests, when the bees had to choose between two identical clean feeders, more bees landed on one of them. However, the distributions of bees at the "preferred" control feeder (A-feeder) and at the B-feeder never differed from each other significantly ( $\chi^2$ -test,  $P \geq 0.05$ ). When the distance  $d$  between the feeders was 20 cm,  $58.9 \pm 7.7\%$  of the returning bees chose the A-feeder. When  $d$  was 170 cm a mean percentage of  $59.3 \pm 4.2\%$  landed at the A-feeder (Fig. 2). In all cases the percentage of bees choosing the used M-feeder significantly exceeded the percentage of bees choosing the A-feeder in control tests ( $P \leq 0.05$ ) (Fig. 2). This is taken as evidence that the bees do mark their feeding site chemically and thereby attract nestmates searching for food.

When the distance between the M-feeder and the U-feeder was  $d = 20$  cm the percentage of bees at the M-feeder was  $75.8 \pm 9.6\%$ , with 0.75 ML<sup>-1</sup> sugar water. The percentage was



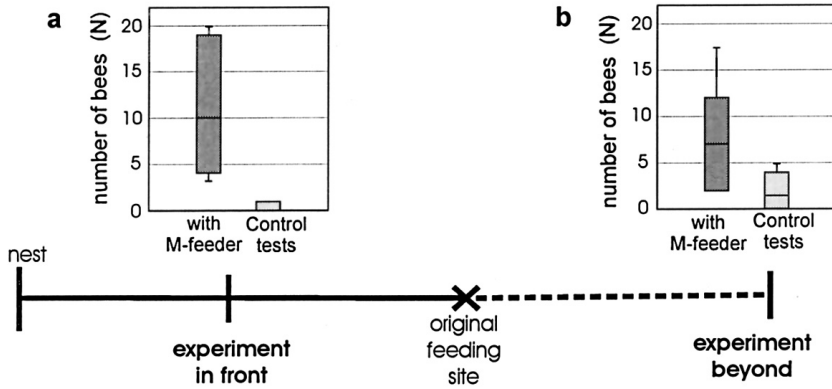
**Figure 2.** Mean percentage (+ 1 SD) of bees landing at the (*marked*) M-feeder (grey bars) and at the (*unmarked*) U-feeder (white bars) during choice experiments (100% = bees at M-feeder + bees at U-feeder). The dashed line represents the mean percentage of bees in control tests “preferring” one out of two identical clean feeders. In all cases the percentage of bees at the M-feeder significantly exceeded that of the control group ( $P \leq 0.05$ ). (a)  $d = 20$  cm. The percentage of bees preferring the M-feeder during experiments with 0.75 ML<sup>-1</sup>, 1.5 ML<sup>-1</sup>, and 3 ML<sup>-1</sup> sugar water, respectively, differed significantly from the percentage of bees at the U-feeder ( $P \leq 0.001$ ). (b)  $d = 170$  cm. The percentage of bees landing at the M-feeder during experiments with 1.5 ML<sup>-1</sup> and with 3 ML<sup>-1</sup> sugar water was significantly higher than the percentage at the U-feeder ( $P \leq 0.001$ ).

71.1 ± 8.9%, when we used 1.5 ML<sup>-1</sup> sugar water, and 86.1 ± 10.2% with 3 ML<sup>-1</sup> sugar water (Fig. 2a). These values significantly exceed the percentages of the control group ( $P \leq 0.05$ ). When the distance between M-feeder and U-feeder was  $d = 170$  cm, 77.1 ± 12.7% of the returning bees chose the M-feeder containing 1.5 ML<sup>-1</sup> sugar water. 82.3 ± 11.4% of the bees chose it when 3 ML<sup>-1</sup> sugar water was offered (Fig. 2b).

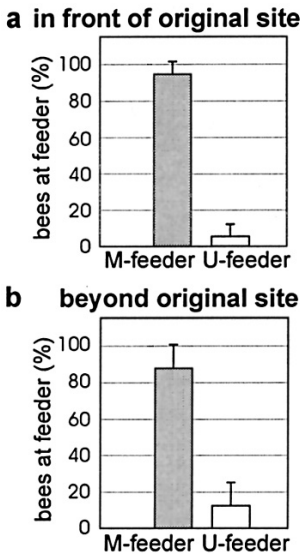
Again, significantly more bees came to the M-feeder than came to the “preferred” feeder in the control tests ( $P \leq 0.01$ ).

### 3.3. Feeding site marks remain effective when spatially separated from their original site

The used feeder still attracted bees when it was displaced from the original feeding site. (i) The bees recognized the M-feeder as the goal of their search, when it was positioned halfway along the scent path to the original feeding site together with the unused U-feeder. When the used M-feeder was moved away from its original feeding site at 25 m to our experimental site at 13 m from the nest a median number of 10 (4/19) bees alighted during 20 minutes of observation (Fig. 3a). As many as 94.7 ± 6.8% of these bees landed on the used M-feeder. This is significantly more than on the clean U-feeder ( $\chi^2$ -test,  $P \leq 0.001$ ) and underlines the persisting attraction of the used feeder (Fig. 4a). In the control tests using two clean feeders, only the visual appearance, but not chemical cues, could have attracted the bees to the additional feeding site halfway along the scent path (Fig. 3a). No bee, or only one bee, (median N = 0; 0/0.75) landed during 20 minutes of observation. The difference in the number of bees landing on the feeders in experiments, which included the M-feeder, and in control tests with two unused feeders is highly significant ( $P \leq 0.01$ ). (ii) Its chemical markings also attracted the bees when the used M-feeder was 20 m beyond the original feeding site together with a clean U-feeder. A median number of 7 (2/12) bees appeared within 20 min of observation (Fig. 3b). Again the mean percentage of bees landing at the M-feeder (87.9 ± 12.8%) significantly exceeded the mean percentage of bees at the U-feeder ( $\chi^2$ -test,  $P < 0.001$ ) (Fig. 4b). In control tests, hardly any bees alighted within 20 min (median N = 1.5; 0/4). Again the difference from the number of bees landing in experiments with the used feeder was significant ( $P < 0.05$ ) (Fig. 3b). The efficiency of the attractants did not differ significantly between the test series halfway along and 20 m beyond the scent path, either in regard to the number of bees alighting at the feeders or in regard to the percentage of bees landing at the



**Figure 3.** Moving the used M-feeder away from the original feeding site attracted bees to the respective new experimental site. (a) During experiments with the used M-feeder and unused U-feeder at 13 m from the nest in front of the original feeding site (25 m) significantly more bees alighted at the feeders (dark bars) than in control tests (light bars) where two unused feeders were presented ( $P \leq 0.01$ ). (b) 20 m beyond the original feeding site (50 m) again significantly more bees appeared at the feeders in experiments with the M-feeder and the U-feeder than during control tests with two clean feeders ( $P \leq 0.05$ ). The bold line represents the scent path between nest and original feeding site. The dashed line represents the 20 m gap between the end of the scent path and the experimental site beyond it. The distances between the nest and the original feeding site and the experimental sites are not drawn to scale, because the distances to the original feeding site were different in a (25 m) and b (50 m).



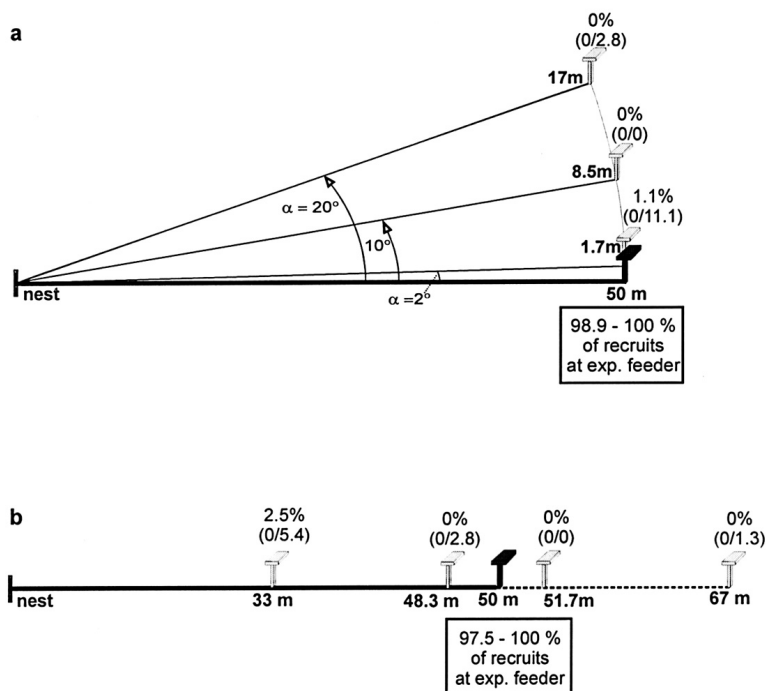
**Figure 4.** Mean percentage (+ 1 SD) of bees landing at the (marked) M-feeder (grey bars) and (unmarked) U-feeder (white bars) when the used M-feeder was moved away from the original to a new feeding site 13 m in front (a) and 20 m beyond (b) the original one. The bees strongly preferred the M-feeder ( $P \leq 0.001$ ) over the U-feeder, indicating that the feeding site markings remained effective at some distance from the feeder’s original site.

M-feeder ( $P = 0.05$ ). Hence the markings remained effective regardless of their new position away from their original site. The fact that the bees still recognized the M-feeder as the goal of their search suggests that the markings at the feeder are particular attractants which differ from the scent path markings.

### 3.4. Precise localization of the food source

Of what use could an additional attractant at the food source proper be? Experiments on the precision of food source localization seem to provide an explanation. Foragers of *S. depilis* showed an impressively precise recruitment of their nestmates to the experimental feeder (Fig. 5). Group foraging was occasionally observed. However, in all of the experiments only about 8% of the arrivals at the feeder were groups of 3–20 bees, whereas all the other recruited bees arrived singly. There was no indication of group effects.

In all our experiments the number of recruits arriving at the used experimental feeder was significantly higher than the number of recruits at the control feeder (both



**Figure 5.** Precision of food source localization. **(a) Direction:** during these experiments foragers were feeding at the experimental feeder 50 m (see black table) from the nest and recruiting their nestmates. Control feeders (grey tables) with sugar water of the same concentration were offered 1.7 m, 8.5 m, or 17 m laterally to the experimental feeder at the same distance. The angles ( $\alpha$ ) between the direction from the nest to the experimental feeder and from the nest to the control feeder were  $2^\circ$ ,  $10^\circ$ , and  $20^\circ$ , respectively. Recruits nearly exclusively arrived at the experimental feeder (see inset which gives the range of median percentages of bees arriving at the experimental feeder). The median percentage (1.quartil/3.quartil) of recruits landing at the control feeders is given above each of the three feeding tables. It was significantly smaller than that at the experimental feeder ( $P \leq 0.01$ ) in all cases. The bold line indicates the scent path between the nest and the experimental feeder. **(b) Distance:** here the experimental feeder was 50 m from the nest. Control feeders were offered at different distances but in the same direction from the nest. The median percentage (1.quartil/3.quartil) of recruits is shown above each control feeding table (see grey tables). In all cases significantly more bees landed at the experimental feeder (see black table) than at the control feeder ( $P \leq 0.01$ ). Inset: range of median percentages of bees landing at the experimental feeder. The bold line represents the gap beyond the scent path. The distances between the experimental feeder and the control feeder were 17 m and 1.7 m, respectively. Note that not a single bee came to the control feeder 1.7 m beyond the experimental feeder and only one bee to the control feeder 17 m beyond it.

containing  $3 \text{ ML}^{-1}$  sugar water) ( $\chi^2$ -test,  $P \leq 0.01$ ). Even when the control feeder was as close as 1.7 m to the left or to the right of the experimental feeder, and at the same distance of 50 m from the nest, a median percentage of 98.9 (88.9/100)% of the recruits landed at the experimental feeder which the foragers were using. Under these conditions the angle  $\alpha$  between the direction from the nest to the experimental feeder and to the control feeder

measured  $2^\circ$  only (Fig. 5a). With the control feeder 8.5 m ( $\alpha = 10^\circ$ ) laterally to the experimental feeder (both 50 m from the nest) only 3 bees in only one experiment landed on the control feeder and no bees at all in all other experiments. All other recruits (range = 98–100%) landed on the experimental feeder. With the control feeder 17 m to the left or to the right of the experimental feeder ( $\alpha = 20^\circ$ ) a median percentage of 0 (0/2.8)% of the

recruits flew to the control feeder and 100 (97.2/100)% found the experimental feeder. This impressively precise localization of the food source with regard to its direction is equalled by the precision with which the bees handled food distance (Fig. 5b). When the control feeder was 33 m away from the nest along the scent path only 2.5 (0/5.4)% of the recruits landed on the control feeder, although it was nearer now to the nest than the food source used by their foraging nestmates. As many as 97.5 (94.6/100)% of the recruits still followed the scent path to its end at 50 m and landed at the experimental feeder. In the experiments with the control feeder 1.7 m in front of the experimental feeder (i.e. control feeder nearer to the nest) only twice did a single recruit choose the nearer food source whereas the vast majority of the recruits (range = 94.3–100%) landed on the experimental feeder. When the control feeder was 17 m beyond the experimental feeder, i.e. beyond the scent path, no bee or only one bee (in 1 out of 4 experiments) found the control feeder (median percentage 0; 0/1.3%). The most compelling results were those of the experiments with the control feeder located 1.7 m behind the experimental feeder. In all of these experiments all (100%) of the recruits landed on the experimental feeder, but not a single bee on the control feeder. This impressive precision in food source localization suggests that the markings at the feeding site tag the end point of a bee's foraging trip.

#### 4. DISCUSSION

From our results we draw two conclusions. 1. *S. depilis* scent marks its food source and does this in a way different from the laying of a scent path. 2. These scent marks function as effective attractants, and are interpreted as tags particularly marking the end point of the path to the food source.

##### 4.1. Chemical markings at the feeding site

Our choice experiments demonstrated attractant scent marks left at the food source

by *S. depilis* which are reminiscent of those recently described in *M. seminigra* (Hrncir et al., 2003). When comparing the two species, the scent marking of *S. depilis* seems to be more efficient than that of *M. seminigra*. In the latter species 20 landings of the foragers did not affect the choice of the bees between the marked and the unmarked feeder. In contrast to *S. depilis* at least 30 landings by the foragers were necessary to attract recruits to the used (and marked) feeder. In *S. depilis* the percentage of bees choosing the marked feeder after 20 forager landings (71 to 86%) always exceeded the percentage found for *M. seminigra*, even after as many as 200 landings ( $d = 20$  cm; 68%).

Our results also bring to mind attractants left at food sources by bumblebees and honeybees (Butler et al., 1969; Ferguson and Free, 1979; Schmitt and Bertsch, 1990; Schmitt et al., 1991). In these cases the attractants are "footprint" substances, secreted at the bees' tarsi. In stingless bees attractants on the feeding site itself were studied by Aguilar and Sommeijer (1996, 2001). They suggested that anal droplets serve as chemical cues for foraging *Melipona favosa*. Nieh (1998) tested attractants at the feeding site in *M. panamica*, but the source of the scent beacon remained unclear. Hrncir et al. (2003) excluded the possibility of anal droplets for *M. seminigra* and showed that instead "footprint" substances are the relevant signals. Our present work did not aim at the identification of the scent marks' origin. However, our experiments do exclude (i) the deposition of attractant substances into the sugar water itself (we changed the sugar water when moving the M-feeder away from the original feeding site) and (ii) the involvement of anal droplets which were not deposited by *S. depilis*.

According to Lindauer and Kerr (1958) foragers of *Scaptotrigona postica* started scent marking near the feeding site and on their way back to the nest (scent path) after only 11 visits to the food source. In our choice experiments four foragers fed at the food source until they completed a total of 20 visits which amounts to an average of five visits per bee. In no case did the number of visits per bee exceed 9. We conclude that the bees started to mark the food source right after its discovery.



#### 4.2. Particular attractants differing from scent path marks

In *S. depilis* the feeding site marks at the M-feeder remained attractive even when presented both halfway along the scent path or as much as 20 m beyond the end of the scent path and thus far away from the original feeding site. We interpret our findings to imply that the used feeder had been marked with specific attractants representing the end point of the scent path. Even when displaced from their original site these marks retained this function.

Lindauer and Kerr (1958, 1960) demonstrated the necessity of the scent path for successful recruitment in *Scaptotrigona* by training foragers along a lake's shore. When flying from the feeding site to the nest over a body of water the foragers could not lay a scent path. Accordingly, no recruits came to the feeding site. The lack of a scent path over a length of approximately 20 m made it impossible for recruits to find the feeding site. Our own displacement experiments demonstrated that the M-feeder (already used and obviously marked by the foragers) attracted the bees even when it was 20 m beyond the original feeding site and thus 20 m away from the scent path. Such high attraction can hardly be explained by scent path marks, even less so, as the foragers never showed the behavior typical of scent path marking at the feeder or beyond the original feeding site.

Further evidence for the existence of particular feeding site marks comes from our localization experiments and the remarkable precision with which *S. depilis* finds a food source (Fig. 5). When the control feeder was placed 1.7 m beyond the used feeder not a single bee landed on the control feeder. Our results suggest that the marks left at the feeding site serve as end point tags, which are hardly ever surpassed by newly recruited bees.

Natural food sources are more scattered than the food sources offered in our experiments. Obviously, ignoring still unvisited flowers near the flowers already exploited would be of little use for a colony. Such behavior is indeed unlikely to occur because the scent of flowers is an important cue in the communication between the forager and its recruits (Lindauer, 1956; Lindauer and Kerr, 1960; Biesmeijer and Ermers, 1999). In order

to exclude this kind of communication only unscented food was presented in the present study. In both *Trigona carbonaria* (Nieh et al., 2000) and in *Melipona seminigra* (Hrncir et al., 2003) it has been suggested that active chemical marking of the feeding site is of greater importance on unscented food sources than on scented food sources.

#### 4.3. Ecology of foraging strategies

The remarkably precise localization of a food source in scent path laying stingless bees, as shown by several authors (Lindauer and Kerr, 1958, 1960; Kerr et al., 1963; Hubbel and Johnson, 1978) and confirmed by our present work, seems to contrast with less precise localization of food sources in non-scent path laying bees of the genus *Melipona* (Lindauer and Kerr, 1958, 1960; Kerr et al., 1963; Nieh and Roubik, 1995; Nieh and Roubik, 1998; Jarau et al., 2000). In *Melipona* sp. the majority of recruits chooses the food source nearest to the nest, be it the used experimental feeder or the unused control feeder (Jarau et al., 2000). The strategy of an initial random search employed by *Melipona* species (Jarau et al., 2000, 2003) increases their foraging success, and non-scent trail laying species may encounter many food sources in a very short time (Jarau et al., 2003; Slaa et al., 1997). In honeybees various adaptations to environmental conditions are known (e.g. von Frisch, 1967). When resources are plentiful and arrayed in small patches the recruitment system is less precise (i.e. recruits follow fewer dances and their orientation is less focused) than when the resources are scarce. In the latter case the precision of the recruitment system is highly important for the colony's food acquisition (Visscher and Seeley, 1982; Seeley and Visscher, 1988; Seeley et al. 1991, Waddington et al., 1994). Scent trail laying stingless bees, on the other hand, seem to be particularly good at the exact localization of a food source and its exploitation until it is depleted (Hubbel and Johnson, 1978). Furthermore, scent trail recruitment facilitates group foraging, which enables bees to monopolize dense patches of highly rewarding resources with sufficient food for many bees (Johnson and Hubbel, 1975; Hassel and Southwood, 1978; Hubbel and Johnson, 1978). According

to Jarau et al. (2003), up to 1682 recruits of *S. depilis* came within 4 hours to exploit a single artificial food source such as those used in our study. As expected from the difference in foraging strategies, the marks left by *S. depilis* at the feeding site are more attractive than those of *M. seminigra* (Hrncir et al., 2003). In our experiments *S. depilis* followed its scent path and perceived the marks left at the feeder as the end point of the foraging trip.

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**Résumé – Une abeille sans aiguillon (*Scaptotrigona aff. depilis*) marque la source de nourriture en plus de la piste odorante.** De nombreuses espèces d'abeilles sans aiguillon laissent une piste odorante pour indiquer le chemin d'une source de nourriture (Lindauer et Kerr, 1958). Jusqu'à présent on ignore si le marquage odorant des pistes constitue le seul indicateur chimique utilisé par les abeilles pour trouver des sources de nourriture. Nous montrons ici que *Scaptotrigona aff. depilis* (Apidae, Meliponinae) Moure 1942 dépose une piste odorante mais laisse en outre un marquage chimique à la source de nourriture. Lorsque des abeilles en quête de nourriture ont le choix, à une source de nourriture connue, entre un nourrisseur M auquel des membres de la colonie ont déjà butiné, et un nourrisseur U identique mais inutilisé (Fig. 1), 71 à 86 % d'entre elles choisissent le nourrisseur M. Visiblement les abeilles qui avaient butiné au nourrisseur M l'avaient marqué avec des substances attractives (Fig. 2). On suppose que ces substances se différencient de celles de la piste odorante car les abeilles présentes sur le nourrisseur ne montraient jamais le comportement typique du dépôt de piste odorante. En outre les marquages à la source de nourriture par les abeilles en quête de nourriture ont été reconnus même lorsque le nourrisseur utilisé avait été déplacé de l'endroit d'origine (Figs. 3 et 4). La question se pose de savoir quelle fonction possède ce marquage supplémentaire. Pour cela nous avons fait des expériences dans lesquelles une à trois abeilles butinaient à un nourrisseur M éloigné de 50 m du nid et recrutaient des membres de la colonie. Des nourrisseurs identiques placés soit dans une autre direction soit à une autre distance servaient de témoin (nourrisseur U). Durant les deux heures d'observation toutes les nouvelles

arrivantes (recruées) ont été capturées et comptées aussi bien sur le nourrisseur M que sur le U. Les recrues trouvaient toujours presque toutes (98–100 %) avec une précision impressionnante le nourrisseur M où s'étaient abreuviées les butineuses (Fig. 5). Par contre il n'y avait que très peu ou pas du tout d'abeilles sur les nourrisseurs U qui étaient éloignés latéralement de 1,7 m, 8,5 m ou 17 m. Même lorsque le nourrisseur U était plus près du nid que le nourrisseur M de 1,7 m ou 17 m, seulement 0 à 2,5 % des abeilles atterrirent sur le nourrisseur U. De toute évidence les marquages chimiques au nourrisseur sont reconnus par les abeilles comme point d'arrivée de leur quête de nourriture. Comparée à *Melipona seminigra*, qui ne dépose pas de piste odorante mais laisse des marquages semblables à la source de nourriture (Hrncir et al., 2003), l'attractivité des marquages est plus forte chez *S. depilis*. Cela se voit par le nombre de visites nécessaires pour un marquage efficace (*M. seminigra* > 30 ; *S. depilis* < 20).

## *Scaptotrigona* / abeilles sans aiguillon / marquage odorant / recrutement

**Zusammenfassung – Eine stachellose Biene markiert die Futterstelle zusätzlich zum Duftpfad (*Scaptotrigona aff. depilis*).** Viele Arten von stachellosen Bienen legen Duftpfade als Wegweiser zu Futterquellen (Lindauer und Kerr, 1958). Ob diese Duftpfadmarken die einzigen chemischen Wegweiser bei der Nahrungssuche dieser Bienen sind, ist bisher nicht bekannt. In der vorliegenden Arbeit wird gezeigt, dass die einen Duftpfad legende Biene *Scaptotrigona aff. depilis* Moure 1942 an ihrer Futterstelle zusätzliche chemische Markierungen hinterlässt. Wenn die futtersuchenden Bienen an einer schon bekannten Futterstelle die Wahl zwischen einem Futterschälchen hatten, an dem ihre Nestgenossinnen bereits getrunken hatten, und einem identischen unbenutzten Futterschälchen (Abb. 1), dann wählten 71 bis 86 % von ihnen das erstere. Offensichtlich hatten die zuvor am Futterschälchen trinkenden Bienen dieses mit attraktiven Substanzen markiert (Abb. 2). Diese Substanzen unterscheiden sich vermutlich von denen des Duftpfades: An der Futterstelle zeigten die Bienen niemals ihr für das Legen des Duftpfades typisches Verhalten. Weiterhin konnten die Markierungen am Futterschälchen von den futtersuchenden Bienen auch dann erkannt werden, wenn das benutzte Futterschälchen sich nicht mehr am ursprünglichen Ort befand (Abb. 3, Abb. 4). Es stellt sich die Frage, welche Funktion eine zusätzliche Markierung der Futterstelle hat. Dazu führten wir Experimente durch, in welchen ein bis drei Bienen an einem Futterschälchen 50 m vom Nest entfernt sammelten und ihre Nestgenossinnen rekrutierten. Zur Kontrolle befanden sich identische Futterquellen entweder in einer anderen Richtung oder einer anderen Entfernung vom Nest. Während

der zweistündigen Beobachtungszeit wurden alle neuankommenden Bienen (Rekruten) sowohl am benutzten Futterschälchen als auch am Kontroll-Futterschälchen gefangen und gezählt. Stets fanden fast alle Rekruten (98–100 %) mit beeindruckender Genauigkeit das Futterschälchen, an dem die Sammelbienen tranken (Abb. 5). Dagegen flogen nur wenige oder gar keine Bienen zu den unbenutzten Futterschälchen, die 1,7 m, 8,5 m bzw. 17 m seitlich davon entfernt standen. Auch wenn das Kontroll-Futterschälchen 1,7 m bzw. 17 m näher am Nest stand als das benutzte Futterschälchen, landeten nur 0–2,5 % der Bienen an diesem. Bei einer Position des Kontroll-Futterschälchens jenseits des bereits benutzten Futterschälchens besuchten die Bienen ausschließlich letztere. Die chemischen Markierungen am Futterschälchen werden von den Bienen offensichtlich als Endpunkt ihrer Futtersuche erkannt. Im Vergleich zu *Melipona seminigra*, die keine Duftpfade legt, aber ähnliche Markierungen an der Futterstelle hinterlässt (Hrncir et al., 2003), ist die Attraktivität der Markierungen bei *S. depilis* stärker. Dies folgt aus der Zahl der Bienenbesuche, die für eine effektive Markierung erforderlich sind (*M. seminigra* > 30, *S. depilis* < 20).

### Stachellose Biene / *Scaptotrigona* / Duftpfade / Rekrutierung

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