

Olfactory eavesdropping by a competitively foraging stingless bee, *Trigona spinipes*

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Signals that are perceived over long distances or leave extended spatial traces are subject to eavesdropping. Eavesdropping has therefore acted as a selective pressure in the evolution of diverse animal communication systems, perhaps even in the evolution of functionally referential communication. Early work suggested that some species of stingless bees (Hymenoptera, Apidae, Meliponini) may use interceptive olfactory eavesdropping to discover food sources being exploited by competitors, but it is not clear if any stingless bee can be attracted to the odour marks deposited by an interspecific competitor. We show that foragers of the aggressive meliponine bee, *Trigona spinipes*, can detect and orient towards odour marks deposited by a competitor, *Melipona rufiventris*, and then rapidly take over the food source, driving away or killing their competitors. When searching for food sources at new locations that they are not already exploiting, *T. spinipes* foragers strongly prefer *M. rufiventris* odour marks to odour marks deposited by their own nest-mates, whereas they prefer nest-mate odour marks over *M. rufiventris* odour marks at a location already occupied by *T. spinipes* nest-mates. *Melipona rufiventris* foragers flee from *T. spinipes* odour marks. This olfactory eavesdropping may have played a role in the evolution of potentially cryptic communication mechanisms such as shortened odour trails, point-source only odour marking and functionally referential communication concealed at the nest.

Keywords: eavesdropping; olfaction; competition; aggression; evolution of communication

1. INTRODUCTION

Eavesdropping plays a significant role in the evolution of complex and sophisticated communication networks in animals ranging from songbirds to fishes (McGregor 1993; Stowe *et al.* 1995; Oliveira *et al.* 1998; Peake *et al.* 2002; Whitfield 2002) and may play a role in shaping bee communication (Nieh 1999). Signals that are sent over long distances or leave extended spatial traces are susceptible to eavesdropping (McGregor 1993), and thus some extirpating stingless bee species (Hymenoptera, Apidae, Meliponini) are hypothesized to use interceptive olfactory eavesdropping (intercepting signals intended for other receivers (Peake 2004)) to detect valuable food sources discovered by other bees (Kerr *et al.* 1963), whereas other species may detect the pheromones of aggressive meliponine species to avoid costly conflicts with superior competitors (Johnson 1974; Hubbell & Johnson 1978).

Such olfactory eavesdropping could have contributed to the evolution of concealed communication inside the nest (Nieh 1999), and thus to the evolution of functionally referential communication (the ability to abstractly encode environmental information into signals understood by receivers (Marler *et al.* 1992; Blumstein 1999)) as referential location information replaced odour trail information

(Nieh 1999). Several species of stingless bees use extended odour trails to communicate food location (Kerr 1960). Recently, an intermediate strategy was described for a stingless bee, *Trigona hyalinata*, which uses a short odour trail extending only a short distance from the food source towards the nest (Nieh *et al.* 2003a). Other meliponine species, including those that may use functionally referential communication, do not use odour trails and odour mark the food source alone (Nieh & Roubik 1995; Hrnčir *et al.* 2000; Nieh *et al.* 2003c). Intriguingly, honeybees odour mark only the food source and use functionally referential communication, encoding food location through a waggle dance at the nest (Esch *et al.* 2001; Dyer 2002).

Stingless bees are all highly social (Michener 2000), occupy environments where food resources are seasonally scarce and can be highly sought after, and recruit nest-mates to these resources (Johnson 1974; Roubik 1982; Eltz *et al.* 2001, 2002; Liow *et al.* 2001). To help guide nest-mates during recruitment, certain species deposit odour trails beginning near the nest and extending to the food source (Lindauer & Kerr 1958). Kerr (1960) reports a *T. amalthea* odour trail extending for 900 m, and the foraging ranges of many meliponine species are thought to extend for at least several hundred metres (Roubik & Aluja 1983; Van Nieuwstadt & Ruano 1996). Such odour trails would create a long but relatively narrow active space and could be detected by scout bees whose search paths intersected the active space, with the probability of intersection increasing with trail length. The cross-sectional

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active space of meliponine odour trails has not been measured, although odours deposited at a feeder by *Melipona panamica* foragers can attract nest-mates over distances of 6 to 12 m (Nieh 1998).

It is not known if interspecific meliponine eavesdropping actually occurs. Kerr *et al.* (1963) suggested that *Scaptotrigona xanthotricha* may orient towards *S. postica* odour marks. They trained colonies of *S. postica* and *S. xanthotricha* to separate feeders placed such that the odour trails from both colonies crossed. Two out of 122 *S. postica* foragers arrived at the *S. xanthotricha* feeder, whereas 28 out of 124 *S. xanthotricha* foragers arrived at the *S. postica* feeder. These results are suggestive, but it is unclear how many foragers would have arrived at the feeder of the other species in the absence of odour marks. Sensitivity to interspecific odour marks may also work to the advantage of the excluded, allowing frequently attacked species to detect aggressive species before serious attacks begin. Johnson (1974) noted that *T. fulviventris* foragers appeared reluctant to land on food sites previously visited by the aggressive species, *T. fuscipennis* (Johnson & Hubbell 1974). However, it is not clear if any stingless bee can be attracted to or repulsed by the food-marking odours deposited by an interspecific competitor.

We therefore chose to study the highly aggressive species *T. spinipes*, which defends and usurps food sources from carpenter bees, Africanized honeybees and other stingless bees (Cobert & Willmer 1980; Cortopassi-Laurino 1982; Cortopassi-Laurino & Ramalho 1988; Gallo *et al.* 1988; Sazima & Sazima 1989; Martinez & Bullock 1990; Ramalho *et al.* 1994; Silva *et al.* 1997), six species of passeriform birds (Barbosa 1999) and several species of hummingbirds (Willmer & Corbet 1981; Gill *et al.* 1982). *Trigona spinipes* foragers use cephalic glandular sections to deposit odour trails and to odour-mark food sources (Kerr 1972, 1973; Kerr *et al.* 1981).

Trigona spinipes is a foraging generalist (Barbola *et al.* 2000) and inhabits diverse habitats, ranging from the cerrado (neotropical savannah) to tropical forests throughout South America (Schwarz 1948; Roubik 1989; Barros Henriques 1997). Throughout its range, *T. spinipes* constructs large external nests of mud, resin and wax, usually placed above the ground in large trees (Roubik 1989). Colonies range in size from 5000 to over 100 000 workers (Michener 1974; Wille 1983; Almeida & Laroza 1988), and thus *T. spinipes* form some of the largest stingless bee colonies in the world (Roubik 1989).

Our goal was to determine whether *T. spinipes* uses olfactory eavesdropping to find and subsequently take over food sources from another species. We focus on the aggressive interaction between *T. spinipes* and *M. rufiventris* (Lepelletier 1835; Moure 1975), a moderately aggressive stingless bee that may odour-mark food sources and can pillage stingless bee nests for cerumen (wax), propolis and honey (Kerr & Rocha 1988; Kerr 1994), but generally does not attack or harass other species of stingless bees on floral resources (Rocha 1970; Souza 1978; Breed & Page 1991). Both species occur in Amazonia, Brazil (Roubik 1983; Brown & Albrecht 2001), and we observed *T. spinipes* harassing and attacking *M. rufiventris* on natural food sources at our field site.

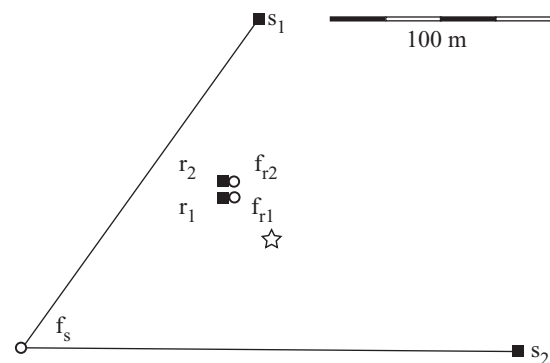


Figure 1. Research site plan. Squares indicate colonies. Open circles indicate feeder sites. Lines connect colonies to the feeder sites used with each colony. The star denotes the global positioning system reference point: 21°26.387' S, 47°34.884' W.

2. MATERIAL AND METHODS

(a) Study site, feeders and training

We used two colonies of *T. spinipes* (s_1 and s_2 , ca. 8000 bees per colony) in trees and two colonies of *M. rufiventris* (r_1 and r_2 , 500–700 bees per colony) in hives at a ranch near São Simão in the state of São Paulo, Brazil, from August to September in 2002 and 2003. We studied the following pairs of colonies: 1 (s_1 , r_1); 1 (s_1 , r_2); 2 (s_2 , r_1); and 2 (s_2 , r_2) for a total of six trials per experiment.

We trained 20 individually marked foragers from each *M. rufiventris* colony to feeders located 2 m east of each colony (f_r) (figure 1). We then trained 20 individually marked foragers from each *T. spinipes* colony to ~~identical feeders located 4 m east of each *M. rufiventris* colony~~ (f_s). After one trial at f_s and one trial at f_r , we captured and removed all *T. spinipes* and *M. rufiventris* foragers that had been trained to the feeders, and then trained a new group of foragers from each colony. We verified that all trained foragers came from the pair of colonies under study by watching them enter their respective colony entrances. With each *T. spinipes* colony, we used aspirators (Nieh 1998) to capture all foragers at the feeders and verifying for 3 h that no further foragers arrived at any feeder location before training foragers from the second colony. With *M. rufiventris*, we used wire mesh (applied in the evening after all bees had returned to their nest) to seal the entrance to the colony that was not under study.

Each feeder consisted of a small glass bottle (5 cm diameter, 4.5 cm high, 65 ml) inverted over a grooved plastic base 6.7 cm in diameter (methods of Von Frisch 1967). To facilitate forager orientation, we placed a disc of yellow paper 6.7 cm in diameter underneath all feeder bases. Each feeder contained unscented 2.5 M sucrose solution (Tautz & Sandeman 2003) and was placed on a grey plastic dish 20 cm in diameter supported by a 1 m high tripod.

We define an experienced forager as any bee that has previously visited a feeder. All other foragers were newcomers. We used paint pens to individually mark the thoraces of bees visiting each feeder (Nieh *et al.* 2003a). We allowed a fixed number of foragers to visit the feeder, and censused the number of marked training-feeder foragers each 15 min, capturing or releasing marked foragers to maintain a constant number of recruiters to ensure a more controlled rate of recruitment. Marked and unmarked bees were captured in separate aspirators. At the end of each day, we released marked bees captured at the training feeder and froze all unmarked *T. spinipes* foragers captured at

both feeders. Thus unmarked bees could not return and be recounted at the feeder (Biesmeijer & de Vries 2001). To avoid depleting the much smaller *M. rufiventris* colony of foragers, we marked and released all captured unmarked *M. rufiventris* foragers at the end of each day and verified that they returned to the colony under study.

(b) Odour mark collection

We collected putative *M. rufiventris* odour marks, *T. spinipes* odour marks and blanks that were not odour marked. To collect odour marks, we allowed 20 individually marked foragers to feed for 30 min while standing on a ring of Whatman number 1 filter paper (5.5 cm inner diameter, 12 cm outer diameter) placed around the collection feeders (f_s , f_{r1} and f_{r2}). To obtain blanks, we placed a ring of paper for 30 min around an identical, but unvisited, feeder containing the same unscented sucrose solution 15 m west of the collection feeder. A monitor observed that no bees visited this feeder. We did not use filter paper that contacted the sucrose solution or any odour-marked paper that contacted foragers other than those of the subject colony. Experimenters wore disposable gloves and used clean forceps and bags to handle the filter papers (methods in Nieh *et al.* 2003c).

(c) Testing the attractiveness of odour marks

We tested the attractiveness of odour marks to *T. spinipes* newcomers and *M. rufiventris* experienced foragers in a 25 min paired-feeder assay. We conducted only one assay at a time, at a single location. At the beginning of each trial, we placed odour-marked and non-odour-marked filter papers inside a clean, sealed plastic bag and covered the training feeder with a plastic cylinder (taking care not to trap any foragers). After 5 min (filter paper transport time), we offered newcomers a choice between two identical, empty, clean feeders. The last odour marks were thus deposited a minimum of 5 min before presentation. Around each feeder, we placed one ring of filter paper. We placed feeders 8.5 cm to right and left of the original feeding site and captured bees as soon as they landed.

Owing to high recruitment rates (more than 100 newcomers per hour), *T. spinipes* newcomers continued to arrive during this test phase even though all foragers, including the experienced foragers, were immediately captured before they could land, and no bees were therefore allowed to return to the nest to recruit. We counted only individual choices made by newcomers in the absence of other bees. All *T. spinipes* newcomers were frozen or held inside a cage (left inside a closed room at the Fazenda) for the duration of the experiments. We released marked *T. spinipes* foragers at the end of each trial to recruit a new set of foragers. In feeder choice experiments with *M. rufiventris*, we tested the orientation of experienced *M. rufiventris* foragers (out of a pool of 120 individually marked foragers from each colony), releasing them at the end of each trial.

Before testing the attraction of *T. spinipes* to feeders at the *M. rufiventris* sites (f_{r2} and f_{r1}), we captured all *M. rufiventris* foragers and sealed the *M. rufiventris* colony. We also exchanged the positions of both feeders every 5 min to eliminate site bias, continuously monitored wind direction, and shifted the feeders so that the axis connecting both feeders was always perpendicular to the wind direction.

(d) Competitive exclusion

When the odour-mark attraction experiment had been completed, we removed the *T. spinipes* feeder (f_s) and allowed *M. rufiventris* foragers to feed at either f_{r1} or f_{r2} . We filmed the

Table 1. Orientation of *Melipona rufiventris* foragers towards odour marks (versus blanks) deposited by nest-mates on the feeder. Each trial lasted 15 min and feeder positions were swapped every 5 min.

trial	colony	number of foragers choosing		
		experimental	control	two-tailed B.P.
1	1	13	0	0.0002
2	1	15	0	< 0.000
3	1	21	0	≪ 0.00001
4	1	34	1	≪ 0.00001
5	2	17	0	< 0.0001
6	2	22	1	≪ 0.0001

M. rufiventris feeder from above to record how *T. spinipes* discovered and then excluded *M. rufiventris* during 9 min trials. In separate trials, we zoomed in to film 25% of the feeder and recorded *T. spinipes* individual feeding durations (defined as forager proboscis in sucrose solution). At the end of each trial, we captured and froze all *T. spinipes* foragers at the *M. rufiventris* feeders.

(e) Video analysis

To analyse and compare forager choice and orientation behaviour in detail, we filmed with a Canon XL-1 NTSC digital video camera (30 frames s^{-1}) positioned above the feeders (paired choice experiment) or feeder (competitive exclusion). We captured the data into an Apple PowerBook G4 computer using IMOVIE v. 3.0.3 and used VIDEOPOINT v. 2.1 to measure the path length, velocity and deceleration of *T. spinipes* foragers orienting towards the feeders (measured within 28 cm of the paired feeders). In the competitive exclusion experiment, we counted the total number of bees on the feeder, the number feeding and the number fighting every 20 s (fighting as defined by Johnson (1974)).

(f) Statistical analyses

We use the χ^2 -test to analyse the recruitment control trials. In two-feeder experiments, we calculate probabilities from a two-tailed binomial distribution with $p = q = 0.5$ (binomial probability, B.P.). We use Mann-Whitney *U*-tests to analyse the flight orientation data and regression, and ANOVA for the competitive exclusion data. All averages are reported as mean \pm 1 s.d.

3. RESULTS

(a) *Melipona rufiventris* odour marking

Melipona rufiventris foragers deposited attractive odour marks on the feeder (table 1). In all six trials, significantly more *M. rufiventris* foragers chose the feeder with the filter paper on which their nest-mates had formerly fed, over the control feeder with no odour marks ($p \leq 0.0002$).

(b) *Trigona spinipes* flight orientation

Once we covered the *T. spinipes* training feeder, *T. spinipes* foragers immediately began to search over an increasingly wider area for an available food source. In this way, many *T. spinipes* foragers found and oriented towards the *M. rufiventris* feeder sites. Figure 2a shows that *T. spinipes* newcomers strongly preferred *T. spinipes* odours marks to *M. rufiventris* odour marks at the *T. spinipes* feeder site (f_s). However, *T. spinipes* newcomers searching

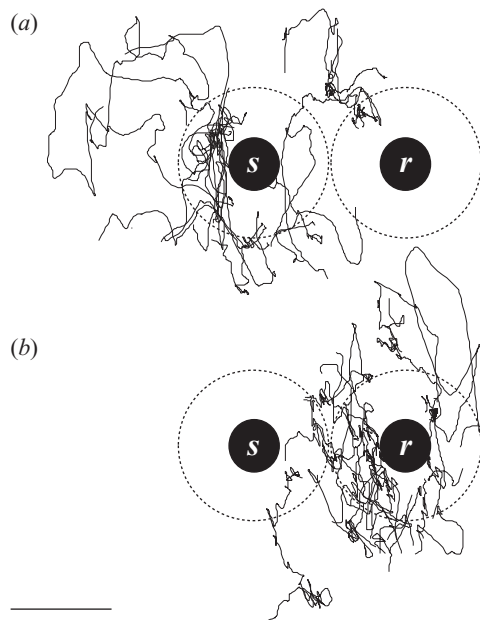


Figure 2. Flight paths of *Trigona spinipes* newcomers orienting to odour marks: choice of *T. spinipes* (s) or *Melipona rufiventris* (r) odour marks. Filled circles denote the feeder bottles. Dashed circles indicate the filter papers. Preferential orientation (a) to *T. spinipes* odour marks at the *T. spinipes* feeder site (21 foragers) and (b) to *M. rufiventris* odour marks at the *M. rufiventris* feeder site 1 (20 foragers). Feeder positions were swapped every 5 min. Pooled results shown (six trials lasting 10 min each). Scale bar, 10 cm.

for food at the *M. rufiventris* feeder site (f_{r1}) strongly preferred *M. rufiventris* odour marks to *T. spinipes* odours marks (figure 2b). Flight orientation behaviour was quite similar at both sites. There is no significant difference between the length (0.215 ± 0.149 m), average velocity (0.201 ± 0.108 m s⁻¹) or average deceleration (6.03 ± 3.23 m s⁻¹ s⁻¹) of *T. spinipes* orientation flight paths at f_s or f_{r1} (Mann–Whitney $U \geq 182$, $n_1 = 20$, $n_2 = 21$, $p \geq 0.47$).

(c) *T. spinipes* attraction to odour marks

Figure 3 shows the differential responses of *T. spinipes* newcomers in greater detail. At their own feeder site (f_s), significantly more *T. spinipes* newcomers preferred the odour marks of their nest-mates to those of *M. rufiventris*, even after 20 min had passed (overall $p \ll 0.0001$). The proportion of newcomers choosing *T. spinipes* odour marks steadily decreased with time as the odour marks evaporated (figure 3b).

At the *M. rufiventris* feeder sites (f_{r1} and f_{r2}), *T. spinipes* newcomers preferred *M. rufiventris* odour marks to those of their nest-mates within the first 15 min, with no preference exhibited thereafter (figure 3b, overall $p \ll 0.0001$). When given a choice of *M. rufiventris* odour marks and blanks (no odour marks), *T. spinipes* newcomers preferred *M. rufiventris* odour marks within the first 20 min (overall $p \ll 0.0001$). In both cases, attraction to the odour marks steadily decreased with time as the odour marks evaporated.

Thus *T. spinipes* newcomers could clearly distinguish between *T. spinipes* odour marks and *M. rufiventris* odour marks, but strongly preferred *M. rufiventris* odour marks when searching away from f_s . Experienced *T. spinipes*

foragers (marked bees) showed the same strong preferences, and during the 25 min of each attraction experiment, 77.5% chose *T. spinipes* odours over *M. rufiventris* odours at f_s , 75% chose *M. rufiventris* odours over *T. spinipes* odours at f_{r1} and f_{r2} , and 72.5% chose *M. rufiventris* odours over blanks at f_{r1} and f_{r2} (two-tailed B.P., $p = 0.0001$, $n = 80$ foragers per attraction experiment).

(d) *M. rufiventris* aversion

Melipona rufiventris foragers showed a strong aversion to *T. spinipes* odour marks (figure 3c), avoiding these marks at all tested time intervals and preferring *M. rufiventris* odour marks (99.6%, overall $p \ll 0.0001$) or even a blank paper with no odour marks (100%, overall $p \ll 0.0001$).

(e) Competitive exclusion

During the six exclusion trials (figure 4), the total number of *T. spinipes* foragers on the feeder and the number feeding significantly increased with time (ANOVA: $F_{1,26} \geq 23.3$, $p < 0.0001$), and the number fighting significantly decreased with time (ANOVA: $F_{1,26} = 9.5$, $p = 0.005$) as the number of *M. rufiventris* foragers decreased on the feeder. The total number of *M. rufiventris* foragers on the feeder, the number feeding and the number fighting significantly decreased with time (ANOVA: $F_{1,26} \geq 11.9$, $p < 0.002$). As *T. spinipes* foragers successfully took over the feeder, the amount of time that *T. spinipes* foragers spent individually feeding also increased (figure 4b; ANOVA: $F_{1,17} = 11.8$, $p = 0.003$). In all trials, *T. spinipes* foragers won and successfully excluded all *M. rufiventris* foragers (figure 4a).

4. DISCUSSION

Trigona spinipes foragers can detect interspecific odour marks deposited to advertise food sources to conspecifics. When searching for new feeding sites not already occupied by nest-mates, *T. spinipes* newcomers preferred *M. rufiventris* odour marks to nest-mate odour marks and displayed the same flight orientation behaviour towards nest-mate-deposited and interspecific marks (figure 2). In the competitive exclusion experiment, *T. spinipes* newcomers immediately began fighting if *M. rufiventris* foragers occupied the feeder, behaving as extirpators and displaying all four levels of aggression categorized by Johnson (1974): threats to intense grappling followed by decapitations. In all six trials, this strategy was successful and *T. spinipes* quickly won control after driving away or killing all *M. rufiventris* foragers (figure 4).

The strong aversion shown by *M. rufiventris* foragers towards *T. spinipes* odour marks (figure 3c) supports the hypothesis that some species may detect interspecific odour marks to avoid confrontations rather than to exploit discoveries of other bees (Johnson 1974; Hubbell & Johnson 1978). This avoidance response is in sharp contrast to the preferences of *M. rufiventris* foragers choosing between *M. rufiventris* odour marks and no odour marks. In this case, almost no foragers (1.6%) chose the blank (table 1). Moreover, the aversion was evident the first time that each *M. rufiventris* colony experienced *T. spinipes* odour marks (figure 3c; 100% avoidance of *T. spinipes* odours versus blanks in all trials). Whether this aversion is a result of prior experiences with *T. spinipes* foragers at other sites, is specific

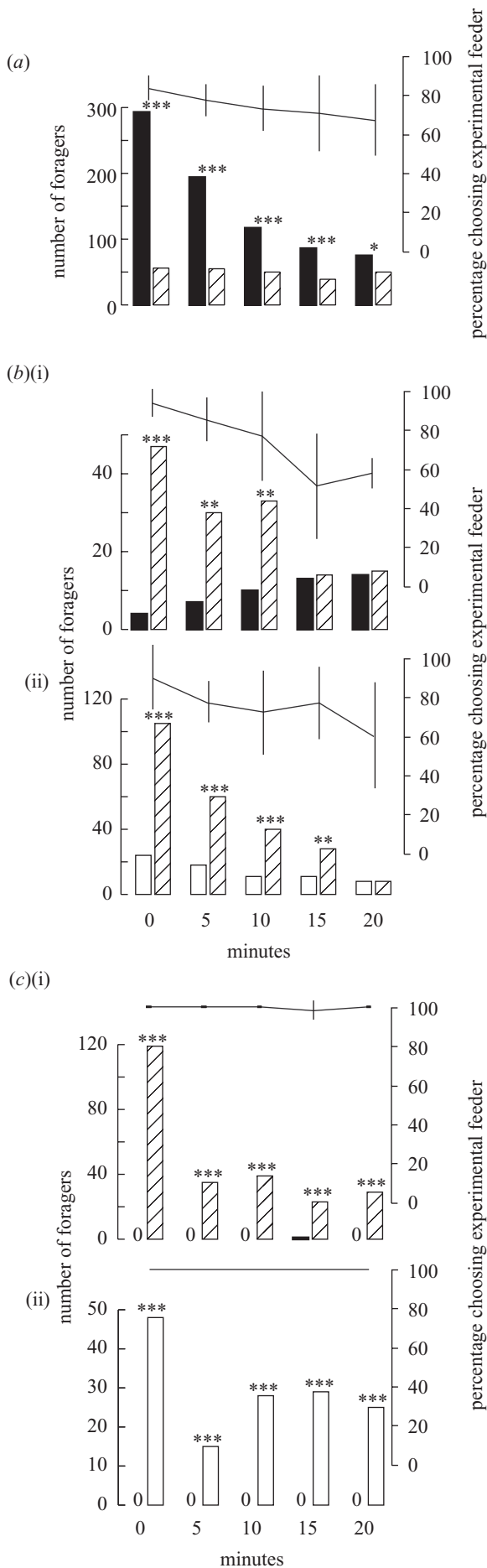


Figure 3. Testing attraction to interspecific odour marks (each plot shows pooled data from six trials). Bars show the number of foragers choosing a particular odour type (black bars, *Trigona spinipes* odour; hatched bars, *Melipona rufiventris* odour) or control (white bars). The line plot with s.d. bars shows the average percentage of foragers choosing the experimental feeder. Two-tailed B.P.: *** $p < 0.0001$, ** $p < 0.001$, * $p = 0.05$. Attraction of *T. spinipes* newcomers (a) to odour marks at the *T. spinipes* feeder site and (b) to odour marks at the *M. rufiventris* feeder sites: (i) shows choice between *T. spinipes* and *M. rufiventris* odour marks; (ii) shows choice between *M. rufiventris* and no odour marks. (c) Aversion of *M. rufiventris* foragers to *T. spinipes* odour marks (zero values shown with a '0'): (i) shows choice between *T. spinipes* and *M. rufiventris* odour marks; (ii) shows choice between *T. spinipes* and no odour marks.

to *T. spinipes* odour marks or is a general response to foreign odour marks remains to be determined.

(a) Newcomer identity

The colony identity of *T. spinipes* newcomers that were frozen was not verified, but it is unlikely that these foragers came from non-subject colonies because we did not observe conspecific fighting or stereotyped fleeing behaviour, as occurs when *T. spinipes* foragers from different colonies meet on a food source (P. Nogueira-Neto, personal communication). Moreover, *T. spinipes* experienced foragers that were directly verified as coming from the colonies under study (the marked foragers), showed the same pattern of odour choice exhibited by *T. spinipes* newcomers at the *T. spinipes* and *M. rufiventris* feeder sites.

(b) Attraction by other means?

It is important to consider alternative explanations for our results. Could *T. spinipes* newcomers orienting towards other sources of information, aside from *M. rufiventris* odours, account for our data? We counted only *T. spinipes* newcomers that had never previously experienced a feeder, counted only bees that arrived individually and made a choice in the absence of other bees, and provided identical feeders that we rotated every 5 min during the test phases. Thus visual orientation to other bees (local enhancement; see Slaa *et al.* (2003)) and potential directional biases do not account for our results. Exchanging the positions of both feeders every 5 min also eliminated the possibility of biases as a result of minute differences (less than 17 cm) in locale odours.

Because we used unscented sucrose solution and provided empty feeder bottles during the test phases, *T. spinipes* newcomers could not have oriented to sucrose solution odours. *Trigona spinipes* newcomers may have been attracted to the *M. rufiventris* feeder sites by nest-mate odours when offered a choice between *T. spinipes* odours and *M. rufiventris* odours. However, *T. spinipes* newcomers were equally attracted to these sites when presented with a choice between *M. rufiventris* odours and blanks (figure 3b). In addition, significantly more *T. spinipes* newcomers preferred to land on *M. rufiventris* odour marks in the first 15 min of all trials and never showed a preference for *T. spinipes* odours at any time interval during any trial at the *M. rufiventris* feeder sites (f_{r1} and f_{r2}).

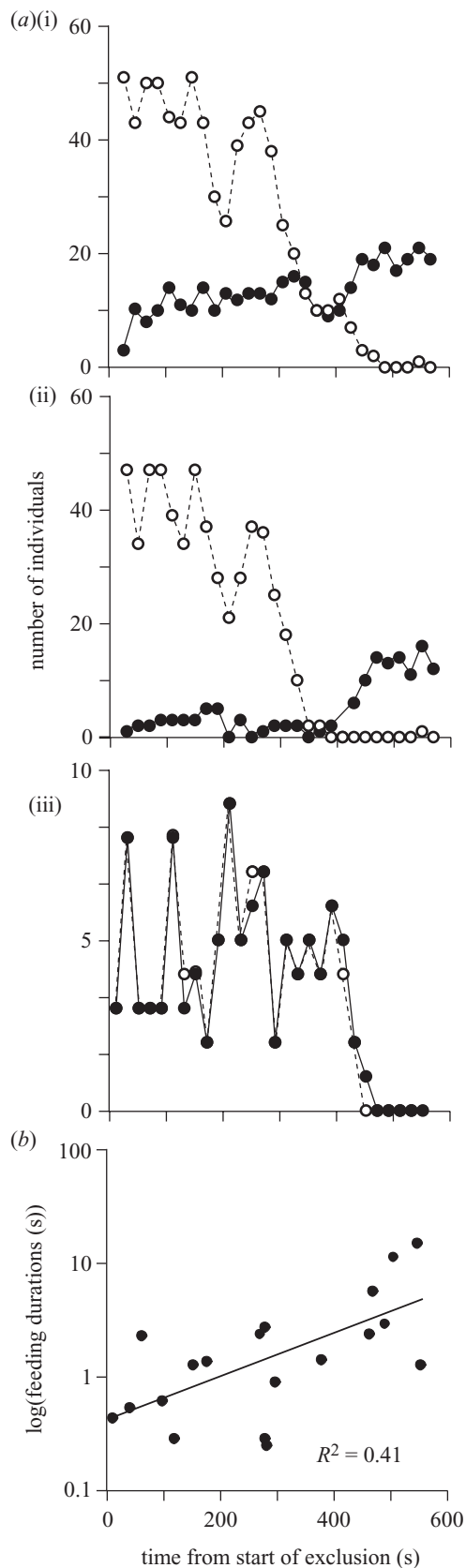


Figure 4. (a) *Trigona spinipes* competitively excludes *Melipona rufiventris* from the *M. rufiventris* feeder (pooled data from six trials). (i) On feeder; (ii) feeding; and (iii) fighting. Filled circles indicate *T. spinipes* foragers. Open circles indicate *M. rufiventris* foragers. (b) Relationship between *T. spinipes* individual feeding durations and the time from the start of exclusion (pooled data from six close-up video trials, log regression line shown).

It is possible that one aspect of locale odour, the odour of feeder assistants, drew some newcomers to f_{r1} and f_{r2} . However, feeder assistants positioned themselves equidistant to both feeders, and thus locale odour does not account for the strong preferences of *T. spinipes* newcomers for *M. rufiventris* odour marks at f_{r1} and f_{r2} .

Finally, both *M. rufiventris* feeder sites were closer to the *T. spinipes* colonies than the *T. spinipes* feeder (figure 1). Thus *T. spinipes* scouts searching for new food sources may have arrived at f_{r1} and f_{r2} without necessarily orienting to the *M. rufiventris* odours. Nonetheless, once they arrived, *T. spinipes* foragers displayed strong, consistent and highly significant preferences for *M. rufiventris* odours at the *M. rufiventris* feeder sites.

(c) Inability to distinguish odours?

Could *T. spinipes* be unable to distinguish between conspecific and interspecific odour marks? *Trigona spinipes* foragers odour mark with cephalic gland secretions (Kerr 1972, 1973; Kerr *et al.* 1981). The source of *M. rufiventris* odour marks is unknown, but other species of *Melipona* odour mark with tarsal gland secretions and anal droplets (Nieh *et al.* 2003c; Hrncir *et al.* 2004a). At all locations, each species was clearly able to distinguish between its odour marks and those of the different species (figure 3). Moreover, postulating no differences in odour-mark composition, but differences in quantity, does not consistently account for the preferences observed. For example, if both species produced odour marks with the same chemical composition, but *M. rufiventris* produced them in greater quantity, *T. spinipes* foragers should have preferred *M. rufiventris* odour marks at all feeder sites. This was not the case (figure 3a,b). Conversely, if *T. spinipes* odour marks are chemically identical to *M. rufiventris* odour marks, but are produced in greater quantity, *T. spinipes* foragers should have preferred their own odour marks at all sites. This also did not occur (figure 3a,b).

(d) Evolutionary implications

In bees, olfactory signalling provides an opportunity for eavesdropping: a reasonable strategy because new feeding sites should be sought once old sites become exhausted or have sufficient labour allocated (Waddington & Holden 1979; Seeley 1995). Exploiting the discoveries of other species (Johnson 1974) by orienting to their communication signals could provide a ready means to find rich new food sources. Floral resources are generally more scattered and yield individually poorer rewards than our *ad libitum* feeders; however, dense inflorescences provided by large blooming tropical trees (such as *Cassia bicapsularis* at our field site) can provide a rich food source that takes time to fully exploit and may thus be rewarding to eavesdroppers. In addition, several meliponine species, including *T. spinipes*, can discover and raid weaker bee colonies, extremely rich food sources that are highly sought after and sometimes contested with other raiders (Sakagami *et al.* 1993; Nogueira-Neto 1997).

If olfactory eavesdropping exerted a selective pressure, less conspicuous odour-marking strategies should have evolved. Stingless bees use a range of olfactory recruitment strategies: complete odour trails, short odour trails and point-source marking of the food source alone (Lindauer & Kerr 1958; Nieh & Roubik 1995; Nieh *et al.* 2003c).

Point-source marking is still susceptible to eavesdropping (figures 2 and 3), but may be less conspicuous than complete odour trails. It is thus relevant to consider the adaptive value of these strategies with regard to eavesdropping.

In particular, some meliponine species may use functionally referential communication (Nieh 2004). Although the existence of such communication has not been conclusively demonstrated in stingless bees (Hrncir *et al.* 2004b), studies have found correlations between distance and even the height of food sources (Nieh & Roubik 1998) and the temporal structure of recruitment sounds produced inside the nest in several species (Esch *et al.* 1965; Esch 1967; Aguilar & Briceño 2002; Nieh *et al.* 2003b). Interestingly, these same species, when they have been examined, all appear to use point-source odour marking (Nieh 1998; Hrncir *et al.* 2000, 2004a; Esch *et al.* 2001), and the only other highly social bees, honeybees, also use point-source odour marking in conjunction with the referential waggle dance (Esch *et al.* 2001; Dyer 2002). Honeybees evolved in tropical habitats (Michener 2000) in which they probably competed and still compete with aggressive stingless bees (Nagamitsu & Inoue 1997). Thus it remains unclear what forces have driven the evolution of functionally referential communication system in bees, but eavesdropping could have contributed to the evolution of location information encoded and transmitted at the well-defended nest (Nieh 1999).

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