Cues used by *Trigona corvina* (Apidae: Meliponini) for the location of artificial food sources: I. The role of the recruiting bee

Ingrid Aguilar & Anna den Held

Centro de Investigaciones Apícolas Tropicales, Universidad Nacional, P.O. Box. 475-3000 Heredia, Costa Rica; Tropical Bee Research Unit, Behavioural Biology, Faculty of Biology, Utrecht University, P.O. Box 80086, 3508 TB, the Netherlands

The behaviour of trained bees and recruits of *T. corvina* was studied in order to obtain more information about the recruitment process in this species and the role of the foragers to bring recruits to the available food sources. We found that significantly more recruits landed accompanied by the trained bee. When the trained bee was no longer present, some recruits still managed to locate the food source. This suggests that recruits also use other cues, such as scent marks and food odor. We conclude that the trained bee is the most important but not the only cue that is used by recruits during food source location.

Keywords: *Trigona corvina*, trained bee, recruitment

Stingless bee foragers alert nest mates concerning available profitable food sources. Although very little is known about the exact recruitment mechanism, several ways of communication about food sources have come to light.

In the simplest recruitment systems, recruits are excited inside the nest but are not provided with any information about the location of the food and search 1) alone, randomly and based on odour information, 2) by directly following the recruiter. In more complex systems recruitment takes place via exciting nest mates and the laying of a scent trail by the recruiters. This, however, does not seem to be the most complex way of recruitment in the tribe of the stingless bees. In a few recent studies (Nieh and Roubik, 1995; Nieh and Roubik, 1998; Jarau *et al*., 2000; Hrncir, 2000; Aguilar and Briceño, 2002) on *Melipona* species (*M. panamica*, *M. scutellaris*, *M. quadrifasciata* and *M. costaricensis*) it was found that the sounds and movements of returning foragers inform recruits about the location and profitability of the advertised food source.

Many aspects of the mechanism used by the species in this study, *Trigona corvina* (Apidae: Meliponini), to recruit to available food sources are still unknown. In this study on *T. corvina* we concentrated on communication about food sources outside the nest. Various ways of communication in *T. corvina* have already been found. Aguilar *et al*. (in prep.) has found that foragers of *T. corvina* guide recruits to profitable food sources by pilot flights. Significantly more recruits arrived at a feeder table accompanied by the recruiter (87.5%) than without the company of the recruiter. Other observations suggest that foragers of *T. corvina* also make use of scent marks to guide recruits to a food source (Slaa *et al*., 1997). Recruiters are thought to lay a scent trail on the vegetation between the hive and the experimental feeder. This too will be explored in this further study. Another cue that might play a role in the recruitment of *T. corvina* is the odour of the food. An experienced forager that returns to the nest brings the food odour with her and the recruits might memorise this odour and this may help them to localise the food source in the field.

The main goal of this study is to complete the knowledge about the recruitment process in *Trigona corvina.* To study whether or not recruits of *T. corvina* follow the forager to locate a food source, the following question was addressed: Do recruits of *T. corvina* localise a food source by using the presence of the recruiting forager?

MATERIAL AND METHODS

Study area and bee species

The experiments were carried out from March until August 2002 at the Centro de Investigaciones Apícolas Tropicales (10° 01' N, 84° 07' E) Heredia, Costa Rica. One colony of *T. corvina* (Apidae: Meliponini) was used. The colony was collected in El Sota, Limón province, contained about 3000 individuals and transferred to the garden of the CINAT in the beginning of 2001. *T. corvina* are predominantly black bees with a size of approximately 5 mm (Michener, 1994). They build completely exposed nests situated between the branches of trees.

Experimental set-up

Individually marked bees with water-based paint (Pelican 25 cc) were trained to feed from an artificial food source with 2.0 M peppermint scented sugar water solution. The feeder was a cardboard plate of 4 x 6 cm with a small feeding tube in the middle**,** placed at a height of 65 cm. Recruits that arrived during the experiments were collected with an aspirator. After every trial the recruits were marked blue and then released. During following trials blue recruits arriving at the experimental feeder were left out of the analysis. To avoid site bias and old recruits, we trained the bees to different places at a distance of 20 m from the nest entrance.

Two experiments were carried out. *Experiment 1* was meant to investigate the role of the presence of the trained bee. We looked at two aspects: a) short flight behaviour of one trained bee during three situations and b) landing of the recruits with or without the trained bee. During this experiment, 16 trials were carried out, eight using a scented and eight using an unscented sugar solution. For every trial a bee was trained to the feeder of either treatment (N=16 trained bees) with a total of 200 minutes of observation. The behaviour of one trained bee was recorded with respect to timing of landing, drinking, hovering and landing (at feeder or at vegetation nearby). Recruits were caught and it was recorded at what time they were collected and whether or not they arrived alone or with the trained bee in close proximity. The trained bee was not disturbed during these observations. *Experiment 2* investigated the influence of capturing the trained bee away during the final stage of recruitment and the subsequent number of recruits that arrived. During eight trials a bee was trained to a scented feeder at a distance of 20 m from the nest entrance and her behaviour was recorded as explained above. When she was repeatedly landing and hovering at the feeder, or recruits were seen nearby, she was expected to be actively recruiting. At this point she was captured with an aspirator. All recruits that landed on the feeder were also collected (using an additional aspirator). Recruits that landed before the trained bee or that had been recruited during previous trials (colour-marked) were captured and excluded from the analysis. The trained bee remained captured until there were no more recruits to be seen and was released after an average of 7:25 minutes.

Data analysis

All data were entered directly into the Excel-sheets using a laptop computer. For this an event recorder Excel-macro was used. Binomial test analysis was applied to compute p-total and to calculate significant differences between numbers of recruits that arrived with or without the trained bee under scented and unscented tests. The T-test was used to compare numbers of recruits that arrived during the catching away experiment and the Kruskal-Wallis test was used to compare the three situations of hovering and landing analysis. Results are presented as mean ± SE.

RESULTS

Experiment I-a: short flights

The behaviour of one bee during a single scented trail in which recruitment took place was examined. Table 1 shows that both the number of hoverings and the number of landings were significantly higher when more than four recruits landed (Kruskal-Wallis test, $p<0.001$). There were no differences in the number of hoverings (K-W, $p=0.227$) or landings (K-W, $p=0.747$) between situation 1 (no new bees recruited) and situation 2 (1 new bee recruited).

Table 1. (A) General information on the number of hoverings and landings during visits in three different situations. (B) Test results comparing the three situations using a Kruskal-Wallis test.

A)		Mean	SD	Mean Rank		N
Hoverings	Situation 1 (0 recruit)	0.16	0.54	4.42	47.67	81
	Situation 2 (1 recruit)	0	0	0	42.50	12
	Situation 3 (more than 4)	12	3.92	7.83	95.50	4
Landings	Situation 1 (0 recruit)	1.02	0.27	2.45	47.15	81
	Situation 2 (1 recruit)	1	0	0	46.0	12
	Situation 3 (more than 4)	9.25	2.87	5.74	95.50	4
B)		p-value				
Hoverings	All situations	<0.001				
	Situation 1 vs. Situation 2	0.227				
	Situation 1 vs. Situation 3	<0.001				
	Situation 2 vs. Situation 3	<0.001				
Landings	All situations	<0.001				
	Situation 1 vs. Situation 2	0.747				
	Situation 1 vs. Situation 3	<0.001				
	Situation 2 vs. Situation 3	<0.001				

Table 2. Binomial test values for each trial separately and for the totals. The higher the number of recruits the more significant the p-values.

Scented trials	With	W/o	Total	p-value	*Unscented trials*	With	W/o	Total	p-value
1	6	3	9	0.164	5	0	0	0	-
2	26	10	36	0.004	6	9	7	16	0.175
3	0	0	0	-	7	1	0	1	0.5
4	16	6	22	0.043	8	3	1	4	0.25
10	0	0	0	-	9	0	0	0	-
11	6	1	7	0.055	12	0	1	0	0.5
15	11	5	16	0.067	13	7	0	7	0.007
16	4	4	8	0.0273	14	1	0	1	0.5
Total	69	29	98	0.00002	*Total*	21	9	30	0.013

Experiment I-b: Trained bee guidance

We expected that if the trained bee is crucial for the recruits in order to locate the food source, fewer recruits would land when the trained bee is not present. Table 2 shows that both during the scented trials and during the unscented trials significantly more recruits landed with than without the trained bee (p=0.00002 and p=0.013, binomial test, pooled data from eight trials for scented and unscented experiments, respectively). The binomial test for each trial separately shows that where recruitment with scent took place in four out of six trials, the p-values were close around 0.05. This trend seemed only to be detectable when recruitment was high (16 recruits or more). During the 'unscented' trials there were no differences in the number of recruits (except one) that arrived with or without the trained bee. By pooling the data and taking the total number of recruits that arrive with the trained bee, compared to the total number of recruits arriving without the trained bee, we found a significant difference during both scented and unscented trials (Table 2).

Experiment II: Catching away the trained bee

Figure 1 shows that recruitment was significantly more effective during trials where the trained bee was present in comparison with trials where the trained bee was caught away and recruitment took place (T-test, Df=6, N=8, p=0.048).

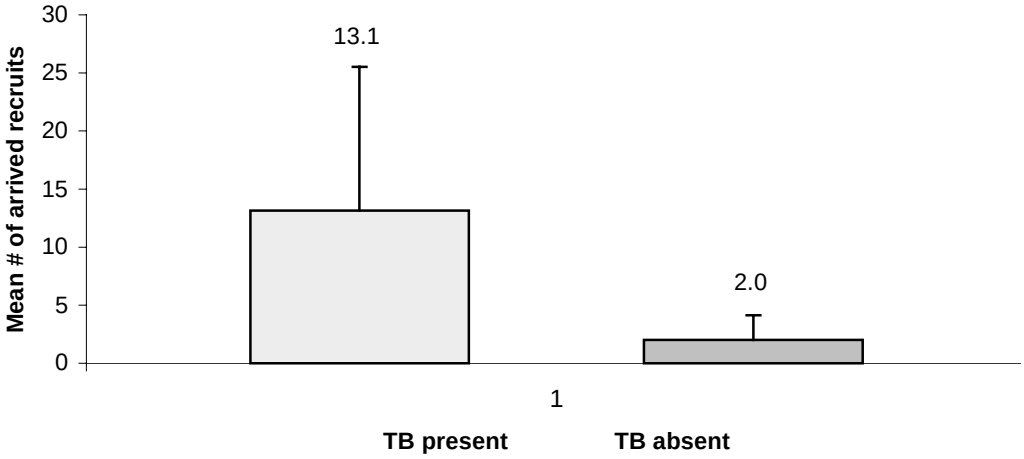

Figure 1. Mean (± SE) number of recruits that arrived when the trained bee (TB) was present or absent. TB present 13.1 ± 4.38, TB absent 2.0 ± 0.76.

DISCUSSION

Short flights

The behaviour of the trained bee significantly changes when several recruits are present. The repeated hoverings and landings by the trained bee when recruits are close to the feeder are very conspicuous and form a clear visual cue for recruits of the location of the feeder. When the bee landed during the short flights, she landed very shortly, chaotically running over the surface of the feeder. Her way of landing looked very similar to the way she lands on the vegetation while laying a scent trail. It is very likely that she is marking the feeder at this point. The pheromones she produces are deposited on the surface of the feeder, but also could be spread more widely due to the hovering behaviour. In this way she might create a cloud of pheromones that attracts the recruits. By performing the short flights, the trained bee is not only a visual cue, but additionally provides the recruits an olfactory cue created during active recruitment. This should be regarded as an extension of the scent trail, rather than a cue itself. There were no differences in the number of hoverings or landings when no recruits landed and when one recruit landed. It seems that these recruits arrive when the trained bee is not longer actively recruiting and that they are just late. If this would be indeed the case, all recruits that arrive alone should arrive directly after the recruitment-visit. This is supported by the data. So we can conclude that single recruits are latecomers and that the trained bee at this moment has already resumed exploitation.

Trained bee guidance

During all trials with scent and during all, except for one, of the trials without scent more recruits arrived accompanied by the trained bee than without the trained bee. When every trial is analysed separately we can see that these differences are not in all cases significant. This is probably because of generally low recruitment, even in scented trials**,** which makes the detection of differences more difficult. Therefore, we can see that both during the trials with and without scent significantly more recruits land accompanied by the trained bee than alone. This clearly shows that when the trained bee is present the recruits use her to locate the food source.

The fact that not all bees land together with the trained bee suggests that, when the trained bee is not present, other cues influence the navigation of the recruits. Putative scent marks placed by the trained bee could lead the recruits to the food source and during the scent trials they might be guided by the scent of the food. This would mean that the presence of the trained bee becomes of less crucial importance in the final stage of recruitment. However, controlling for these other cues is difficult without disturbing the recruitment process.

Temporary removal experiment

If the trained bee indeed is of importance for the recruits to arrive at the food source, a complete absence of the trained bee must affect the number of recruits that arrive at the source. The fact that nevertheless recruits arrive at the feeder indicates that the trained bee, although she is important, is not crucial for the recruits in order to locate the food source. They apparently make use of other cues, like food odour or scent marks. (The role of these cues will be discussed in future papers). The data indeed show that more recruits landed during the trials when the trained bee was present.

In summary, the recruits of *T. corvina* clearly make use of the presence of the trained bee. The conspicuous behaviour of the trained bee during the typical short flight when the recruits have arrived close to the feeder, together with a putative pheromone cloud, helps the recruits to locate the food source. Significantly more recruits land accompanied by the trained bee than without her, both when the trained bee is hovering elsewhere and when she has been captured.

Acknowledgements The study was realized as part of the Research Program of the Programa Regional de Apicultura y Meliponicultura (PRAM), which is financed by the Netherlands Organization for Higher Education (NUFFIC). We are also thankful to the Centro de Investigaciones Apícolas Tropicales (CINAT) of the Universidad Nacional.

REFERENCES

Aguilar, I. & Briceño, D. 2002. Sounds in *Melipona costaricensis* (Apidae: Meliponini): effect of sugar concentration and nectar source distance. *Apidologie* 33: 375-388

Hrncir, M., Jarau, S., Zucchi, R. & Barth, F.G. 2000. Recruitment behavior in stingless bees, *Melipona scutellaris* and *M. quadrifasciata*. II. Possible mechanisms of communication. *Apidologie* 31: 93-113

Jarau, S., Hrncir, M., Zucchi, R. & Barth, F.G. 2000. Recruitment behavior in stingless bees, *Melipona scutellaris* and *M. quadrifasciata.* I. Foraging at food sources differing in direction and distance. *Apidologie* 31: 81-91.

Michener, C.D. 1974. *The bee genera of North and central America (Hymenoptera:Apoidea).* Smithsonian Institution Press, Wahington DC, USA.

Nieh, J.C. & and Roubik, D.W. 1995. A stingless bee (*Melipona panamica*) indicates food location without using a scent trail. *Behav Ecol Sociobiol*. 37: 63-70.

Nieh J.C. & Roubik, D.W. 1998. Potencial mechanisms for the communication of height and distance by a stingless bee, *Melipona panamica*. *Behav. Ecol. Sociobiol*. 43: 387-399.

Slaa, E.J., Nieuwstadt, M., Pisa, L.W. & Sommeijer, M.J. 1997. Foraging strategies of stingless bees (Apidae, Meliponinae): The relation between precision of recruitment. Competition and communication. In: Richards, K.W. (ed.), *Proc.Int. Symp. On Pollination*, Acta Hort 437, pp.193-197. ISHS.