Spitting out information: Trigona bees deposit saliva to signal resource locations

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Stingless bees of the species Trigona spinipes (Fabricius 1793) use their saliva to lay scent trails communicating the location of profitable food sources. Extracts of the cephalic labial glands of the salivary system (not the mandibular glands, however) contain a large amount (approx. 74%) of octyl octanoate. This ester is also found on the scent-marked substrates at the feeding site. We demonstrate octyl octanoate to be a single compound pheromone which induces full trail following behaviour. The identification of the trail pheromone in this widely distributed bee makes it an ideal organism for studying the mechanism of trail following in a day flying insect.

Keywords: trail pheromone; communication; stingless bees; foraging; salivary glands

1. INTRODUCTION

Pheromone trails play an essential role in the exploitation of food resources in populous and eusocially organized insect species (Hölldobler & Wilson 1990; Pasteels & Bordereau 1998; Wyatt 2003). The communication of highly profitable resources is particularly advantageous when these are limited and several species are competing for them (Johnson & Hubbell 1974; Schaffer et al. 1984; Nagamitsu & Inoue 1997; Slaa et al. 1997). So far, in-depth studies of pheromones used to mark trails showing the way towards a food source have almost been exclusively carried out in insect species with non-flying foragers. Here, we examine the pheromone used by a stingless bee (Trigona spinipes) whose foragers are known to lay scent trails guiding nestmates to a food source (Lindauer & Kerr 1960; Nieh et al. 2004).

The genus Trigona includes some of the most populous species among the stingless bees (Michener 1974; Wille 1983) and species with highly efficient mechanisms of food source communication (Lindauer & Kerr 1960; Kerr 1963; Jarau et al. 2003; Nieh et al. 2004), including the known or assumed use of pheromones. However, only little is known about the glandular origin of trail pheromones and their chemical composition in stingless bees.

Foragers of T. spinipes do protract their glossae to rub it on the substrate during presumed scent trail marking (Nieh et al. 2004). Our aims were to find out whether (i) T. spinipes indeed deposits saliva when landing for scent marking and (ii) the saliva contains any attractive substance or blend of substances representing the pheromone, which induces trail following behaviour. After having found evidence for the presence of such a pheromone we wanted to identify (iii) the active component(s) and (iv) their actual glandular origin. For this purpose, we tested the effectiveness of the synthetic form of the assumed pheromone in behavioural experiments and compared it with that of the natural saliva.

2. MATERIAL AND METHODS

The experiments were carried out on the Ribeirão Preto Campus of the University of São Paulo (Brazil) between November 2004 and March 2006. We studied seven nests of T. spinipes (Fabricius 1793; APIDAE, Meliponini). Photographs of field studies are given in the electronic supplementary material.

(a) Trail following bioassays

The artificial scent trails (T1, T2; 10 m long; figure 1) were laid at distances of 60–180 m from the nest, mimicking the natural scent laying pattern (Nieh et al. 2004). Feeders contained highly profitable (50% w/w), unscented sugar solution. The amounts of either labial gland extract or octyl octanoate (pentane solution) used for the artificially laid scent trails, increased with the distance (values in metres given in brackets) to the branching point (Bp, figure 1) in the following order (bee equivalents dissolved in pentane): 0.0 (at the Bp, 0 m), 0.05 (1 m), 0.1 (3 m), 0.15 (5 m), 0.2 (7 m), 0.3 (9 m) and 0.9 (at the feeder, 10 m). For control trails, the same amounts of solvent were applied. The distance of the recruitment feeder (RF) from the feeders of trails T1 (open circle) and T2 (filled circle) and the Bp was 10 m. In T. spinipes, the scent trails become less effective after 20 min (Nieh et al. 2004). We, therefore, renewed the artificial...
scent trails every 20 min. Any bee landing on the feeder of T1 or T2 was captured. When the newcomer (unmarked) bees landed on the feeder, there were no other individuals present. In this way, we avoided potential effects of local enhancement (Slaa et al. 2003). All the newcomers were marked with colour and included in the statistical analysis. Thus, every bee included in the statistics was only used once, avoiding pseudoreplication. Fifteen colour marked foragers were allowed to forage at RF to ensure the recruitment of newcomer bees. Any other bee landing on RF was removed from the experiment.

(b) Statistics
For normally distributed data of equal variance, we used the one-way ANOVA to test for significant differences in the percentages of bees that had landed on either of the two tested feeders. Tukey tests were applied for the pairwise multiple comparisons.

(c) Chemical analysis
Approximately 90% of the supposed scent trail markings in T. spinipes (Nieh et al. 2004) are deposited within 1 m from the food source (in most cases more than 50% at the feeder.
Octyl octanoate trail versus solvent trail: the trail made of an equivalent amount of solvent (ANOVA, n of a total of Z300). Likewise, the synthetic compound and the natural extract proved to be equally attractive when presented subsequently. We conclude that octyl octanoate represents the trail pheromone in T. spinipes.

4. DISCUSSION

More than 25 years ago, Kerr et al. (1981) pioneered meliponine scent trail communication biology by testing the effect of 2-heptanol (found in cephalic extracts of worker bees) when laid out to form an artificial scent trail in Trigona bees. Unfortunately, the results of the only one experiment done can easily be interpreted as providing evidence against 2-heptanol being a food source trail pheromone. The main reasons for this were already discussed earlier (Jarau et al. 2004). Our findings together with those of Jarau et al. (2004, 2006) demonstrate that it is the labial glands which produce trail pheromones in scent path laying stingless bees. Extracts of the labial glands are also highly attractive to newcomer bees in Trigona recurva, who follow artificial scent trails made of labial gland extracts as well (Jarau et al. 2004). While we present evidence for a single compound pheromone in T. spinipes, T. recurva a blend of compounds not yet fully identified is necessary to induce the full intensity of trail following behaviour (Jarau et al. 2006). A particularly important component of this blend is hexyl decanoate, another ester of similar volatility as octyl octanoate. Obviously, different species of stingless bees use different pheromone compounds for communicating food source locations even when closely related to each other phylogenetically. This makes sense in situations when several species of stingless bees compete for the same resource and use similar foraging strategies and ways of communicating. Both species, T. recurva and T. spinipes, indeed occur sympatrically and both feed on nectar. By
showing that octyl octanoate represents the trail pheromone or at least its most significant component in *T. spinipes*, a widely distributed species of South America, we not only found a very promising model organism to experimentally study the significance of scent trails for the exploitation of food sources in stingless bees, but also a species to investigate the behavioural and neurobiological mechanisms of three-dimensional trail following in a day-flying insect.

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REFERENCES


NOTICE OF CORRECTION

The corresponding author’s e-mail addresses are now correct and figure 1 is presented in the correct form.

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