Animal Behaviour 85 (2013) 225–232

Contents lists available at SciVerse ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Learnt information in species-specific ‘trail pheromone’ communication in stingless bees

Christian Reichle [a], Ingrid Aguilar [b], Manfred Ayasse [a], Robert Twele [c], Wittko Francke [c], Stefan Jarau [a,*]

[a] Institute for Experimental Ecology, University of Ulm, Ulm, Germany
[b] Centre for Tropical Bee Research (CINAT), National University of Costa Rica, Heredia, Costa Rica
[c] Institute for Organic Chemistry, University of Hamburg, Hamburg, Germany

ARTICLE INFO

Article history:
Received 19 July 2012
Initial acceptance 20 September 2012
Final acceptance 15 October 2012
Available online 28 November 2012
MS. number: 12-00557

Keywords:
odour learning
pheromone
recruitment communication
Scaptotrigona spp.
scent trail
signature mixture
stingless bee

Specificity in biological signalling systems is often important to keep information private. Foragers of several species of stingless bees deposit chemical marking signals to guide nestmates to food sources. The markings show species- and colony-specific compositions and primarily attract a bee’s nestmates. An interesting question is whether the bees innately recognize specific trail markings or learn their particular composition from nestmates. To investigate this question, we tested whether *Scaptotrigona pectoralis* and *Scaptotrigona subobscuripennis* workers taken from their mother colonies and workers that emerged from combs transferred to foster colonies of the congeneric species are attracted to the marking compounds of workers from their natal colony or from the foster colony. A significant majority of workers were attracted to extracts prepared from foragers of the nest they inhabited, regardless of whether this was the original mother or the congeneric foster colony. Thus, the preference of stingless bee workers for specific food-marking scent mixtures is not innate, but is influenced by the odour they experience within their colony. Despite marked differences in the chemical composition of the scent marks in labial gland secretions of the two investigated species they also shared some main components. We hypothesize that recruitment trail information in stingless bees is composed of one or a few key pheromone compounds acting in conjunction with an additional signature mixture that is species and colony specific and must be learnt by recruited workers.

The transmission of species-specific information in biological signalling systems is important in some situations, for example for finding a conspecific mate by means of sex pheromones, but not in others, such as in the responses elicited by alarm pheromones (Wyatt 2003). Privacy is also advantageous for scent marks deposited by social insect foragers at or around a food source in order to communicate its location to their nestmates but not to foragers of other colonies. Not surprisingly, social insects have thus evolved species- and colony-specific chemical recruitment signals allowing them to avoid interactions with competitors at resources (Traniello 1980; Akino & Yamaoka 2005; Jarau 2009; Jarau et al. 2010, 2011; John et al. 2012).

The chemical compounds used for marking by social stingless bees are mixtures of carboxylic acid alkyl- and terpenyl esters secreted from the foragers’ labial glands (Jarau et al. 2004, 2006, 2010, 2011; Schorkopf et al. 2007; Barth et al. 2008; Jarau 2009; Stangler et al. 2009; Lichtenberg et al. 2011). At a resource, bees deposit the scent marks that attract additional foragers they had recruited within the nest, thereby enhancing their domination at and quick exploitation of the resource (Lindauer & Kerr 1958, 1960; Jarau et al. 2003; Lichtenberg et al. 2010). Complete scent trails laid between a food source and the nest are not needed for successful recruitment (Nieh et al. 2003, 2004a). Rather, just a few chemical markings at the food sources provide sufficient information for recruits to find them (Schorkopf et al. 2011). None the less, the deposited compounds may potentially be detected and exploited by workers from neighbouring conspecific colonies, or even from other species with similar food requirements, that eavesdrop on the information provided (Wyatt 2003; Nieh et al. 2004b; Slaa & Hughes 2009). Competition for food is generally high among social insect colonies. Thus, eavesdropping may be a beneficial strategy to find new food sources with reduced search effort and increased foraging efficiency (Slaa & Hughes 2009). However, eavesdropping may impose costs not only for the individuals that originally deposited the food-marking signals but also for the eavesdroppers themselves. This is particularly true for social insect species that aggressively defend resources and for which the resulting fights lead to the death of many workers, such as in the stingless bees *Trigona corvina* or *Trigona hyalinata* (Jarau et al. 2010;

---

* Correspondence: S. Jarau, Institute for Experimental Ecology, University of Ulm, Albert-Einstein-Allee 11, 89081 Ulm, Germany.
E-mail address: stefan.jarau@uni-ulm.de (S. Jarau).

0003-3472/$38.00 © 2012 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.anbehav.2012.10.029

Lichtenberg et al. 2011). Thus, ‘private’ communication channels by means of species- and colony-specific compositions of chemical food markings that attract only a depositor’s own nestmates are advantageous. Jarau et al. (2010, 2011) indeed found that scent trails of *T. corvina* and *Scaptotrigona pectoralis* are colony specific in terms of their effectiveness in triggering trail-following behaviour in recruited bees. The chemical specificity of the scent marks from different colonies arises from differences in the quantitative proportions of its single components (Jarau 2009; Jarau et al. 2010; John et al. 2012). Recently, we found that foragers of *S. pectoralis* that were exposed to a specific bouquet of scent mark compounds during recruitment in the nest were subsequently attracted to it when searching for food in the field (Reichle et al. 2011). This was even true when the scent-marking secretion from foragers of a foreign conspecific colony were presented, indicating that recruited bees learn the composition of the chemical components specific to a colony within the nest (Reichle et al. 2011).

Whether learning also plays a role in species-specific scent mark recognition and when and how the bees learn their colony’s specific composition of the food site marking secretion remain interesting questions that need to be answered. The bees might learn odours from labial gland secretions of adult bees in the larval stage from the food they encounter within the brood cells, provided that it contains the crucial compounds, and then show a preference for the respective odour blend later in life as adults. The influence of preimaginal learning of olfactory cues on an individual’s host-searching behaviour as adult has already been demonstrated for another hymenopteran insect, the parasitoid *Hyssopus pallidus* (Gandolfi et al. 2003). Alternatively, bees may learn the chemical components as adults only after emergence from the brood cells; for example, guards of the eusocial sweat bee *Lasioglossum zephyrum* learn the odour of their nestmates and use it as a recognition template to discriminate kin from nonkin (Buckle & Greenberg 1981; Greenberg 1988). Stingless bees mass provision their brood cells, close them after an egg has been laid by the queen, and larvae and pupae develop without any direct contact with the adult individuals of a colony. Therefore, it should be possible to distinguish between the two alternatives for odour learning, that is, during either the immature or the adult stage, as well as learning by innate recognition.

In the present study, we investigated whether workers of *S. pectoralis* and *Scaptotrigona subobscuripennis*, which emerged from brood cells that we transferred to a colony of the respective congeneric species, showed a preference for their own species’ scent marks or for the foreign odour bouquet of their nestmates from the foster colony. We predicted that workers living in a foreign colony would show preferences for the foreign pheromone if the bees had learnt the respective odour blend after they emerged from the brood cells. If the bees still preferred their own pheromone, we would expect an underlying innate preference, learning of the odour bouquet from the food store in the cells or learning of their own gland secretion’s composition. In addition to the bioassays we analysed the composition of the labial gland secretions from foragers of the two studied species. In particular, we asked whether scent marks of bees that spent their adult life in the nest of a foreign species resembled the secretion of their congeneric nestmates or retained its species-specific composition.

METHODS

Bee Nests and Study Site

For this study we used two nests of *S. pectoralis* and two nests of *S. subobscuripennis* (Hymenoptera, Apidae, Meliponini). The two species occur sympatrically in Costa Rica and can be easily distinguished from each other by their body coloration: orange in *S. pectoralis* (Fig. 1a) and black in *S. subobscuripennis* (Fig. 1b). The nests were collected in the surroundings of Atenas, Alajuela Province, Costa Rica, and transferred to the Centre for Tropical Bee Research (CINAT) of the National University in Heredia, Costa Rica (9°58.377′N, 84°07.754′W), where the experiments were carried out between January and June 2010. Each colony was kept in a wooden nestbox and the bees had free access to the outside. The experiments comply with the current laws of Costa Rica and Germany.

Nest Manipulation and Behavioural Bioassays

Transfer of brood combs

Prior to the bioassays we transferred brood combs containing larvae and pupae of *S. pectoralis* into a colony of *S. subobscuripennis* and vice versa. Thus, for both species we obtained bees that emerged from brood cells and lived in their mother colony, as well as bees that grew up in a foster nest of the respective congeneric species after emerging from their cells. Newly emerged bees of both species were accepted by the workers of their foster colonies. A few weeks after the introduction of the combs they were observed to work as foragers at the nest entrances. Owing to the distinct colour differences between the two species (Fig. 1) the introduced bees could easily be distinguished from the nests’ native workers.

Test substances for bioassays

We prepared extracts of the cephalic parts of the labial glands that produce the food-marking signals (Jarau 2009) from foraging bees that were collected at the entrance of their nests and killed by

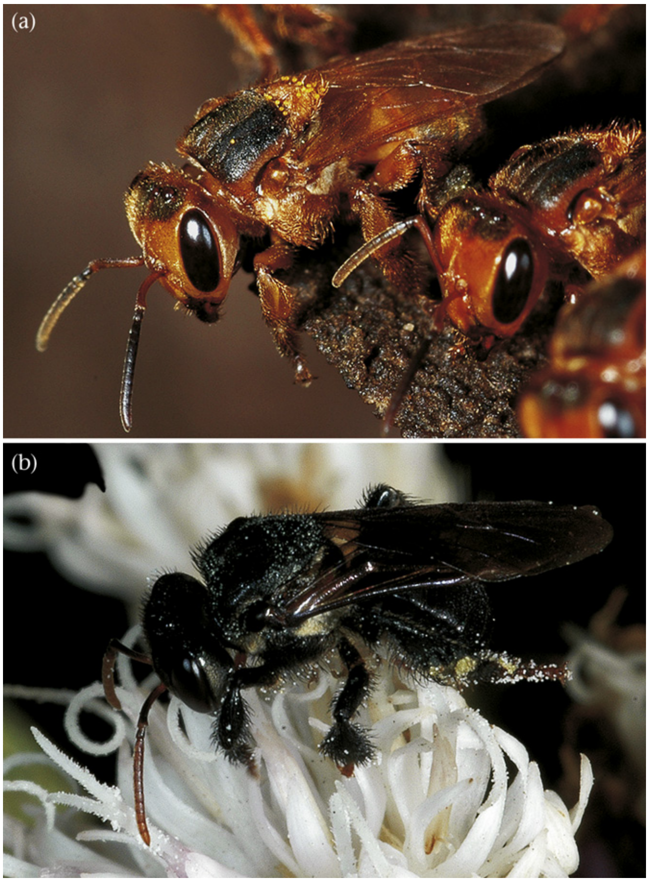

Figure 1. The two stingless bee species used for this study: (a) *Scaptotrigona pectoralis* and (b) *Scaptotrigona subobscuripennis*.