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# 12 Chemical Communication during Food Exploitation in Stingless Bees

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## INTRODUCTION

Chemical compounds play a key role in the communication systems of many living organisms. Information-carrying molecules, known as *semiochemicals*, have been classified into several groups, depending on the identities of the sender and recipient organisms and on who benefits from the transmitted information (reviewed, e.g., in Howse et al. 1998; Wyatt 2003). In short, chemical signals that act within species, to induce either a specific behavior (*releaser pheromones*) or a developmental process (*primer pheromones*), are known collectively as *pheromones*, whereas semiochemicals that act between species are known as *allelochemicals*. There are three main types of allelochemicals, which can either (1) benefit the receiver at the cost of the sender (*kairomones*), (2) benefit the sender at the cost of the receiver (*allomones*), or (3) benefit both sender and receiver alike (*synomones*). In addition to pheromones and allelochemicals, other *chemical cues*, which are not primarily emitted for the purpose of communication, can also act as a source of information for many animals, that way influencing their behavior. Social insects are one of the best examples of a group of organisms whose daily lives depend heavily on chemical communication. Wilson (1990) estimated that for the vast majority, or if not all species of social insects, at least 90% of

communication is mediated principally by chemicals. Or, to express it in Murray S. Blum's more poetic words, "the road to insect sociality was paved with pheromones" (Blum 1974a, p. 197).

Apart from honey bees (Hymenoptera, Apidae, Apini), stingless bees (Hymenoptera, Apidae, Meliponini) are the only other group of bees that have developed an advanced eusocial colonial organization (Michener 1974). Depending on the species, the population of a stingless bee colony can range from a few dozen to many tens of thousands of adult workers (Michener 2000). As with all eusocial insects, not all individuals within the colony leave the nest at the same time to forage for food. Therefore, for a colony to survive, it is critical that a limited number of foraging workers can collect enough food, at any one time, to nourish the entire population of the nest. Important dietary components that help to sustain a colony include pollen, or animal proteins (in a few necrophageous species) to feed the developing larvae, as well as nectar or other carbohydrate sources to provide energy for adult bees. Stingless bees have evolved a variety of communication mechanisms for the transfer of information among workers, on both the nature and locality of a food source, to increase the overall foraging efficiency of the colony (reviewed in Nieh 2004; Barth et al. 2008). Chemical signals, including pheromones and allelochemicals, as well as indirect chemical cues are of great importance in this context to stingless bees.

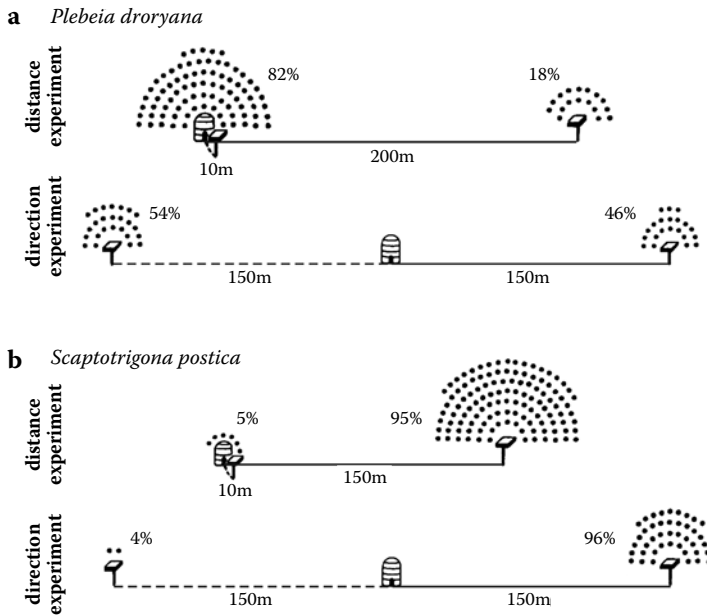
In this chapter I review the existing literature on the role of semiochemicals in the foraging ecology of stingless bees and divide the main volatile compounds into the following four categories: (1) food odors, (2) food source marking volatiles, (3) trail pheromones, and (4) the chemicals used by robber bees and casual thieves during nest plundering.

## FOOD ODORS

Food odors, such as flower volatiles or compounds emanating from carcasses, are used by many animals to detect and orient toward food sources. In social insects, foraging information can also be transferred to the colony by food scent trapped on the body of a returning worker. The importance of food odors for the recruitment of workers remains largely unstudied in stingless bees, but it has been investigated in great detail in honey bees (see Chapters 9 and 10). However, the existing data collected for some species of stingless bees indicate that food odors play an important role both for the flower constancy of individual bees and for the recruitment of fellow workers within the nest.

Slaa et al. (1998) tested the flower constancy of three species of stingless bees (*Trigona fuscipennis*, *T. fulviventris*, and *Frieseomelitta nigra*) in relation to floral scent using an experimental arrangement in which the bees were exposed to equal numbers of artificial flowers of the same color and shape, but scented with two distinct fragrances (eighteen rosewood scented and eighteen peppermint scented). In all three species, individual foragers displayed a distinct preference for a single floral scent, with 78–87% of individuals returning to flowers with the same floral scent during consecutive foraging bouts. Furthermore, most recruited individuals preferred the same floral scent as the first foraging bee to return to the nest during each experiment (Slaa et al. 1998). A similar constancy was also displayed by all three species of bees in relation to flower color. Thus, both visual and chemical cues appear to be equally important for the flower constancy of *Trigona fuscipennis*, *T. fulviventris*, and *Frieseomelitta nigra* (Slaa et al. 1998). This was also reported for *Oxytrigona mellicolor* and *Tetragona dorsalis* (Slaa et al. 2003). However, flower constancy in the latter two species significantly increased when the tested flowers differed in both color and odor (Slaa et al. 2003).

The first evidence that stingless bee foragers transmit food odor to fellow workers inside the nest, and that they use this information to locate food in the field came from an experiment conducted by Lindauer (1956) on the island of Sri Lanka. He trained foraging bees of *Trigona iridipennis* to an artificial food source scented with peppermint oil, and found that all bees that were subsequently recruited by the foragers were exclusively attracted to the same scent, and ignored another feeder scented with lavender oil placed next to it. However, when both feeders were scented with peppermint oil, the newly recruited bees no longer discriminated between the two food sources



**FIGURE 12.1** Recruitment to artificial feeders containing peppermint-scented sugar solution. The recruiting foragers were allowed to regularly collect food at the feeder at the end of the solid line, whereas any bee arriving at the control feeder at the end of the broken line was immediately captured. (a) In *Plebeia droryana*, a species that does not communicate the location of a particular food source, the majority of the recruited bees landed on the feeder closest to the nest in the distance experiment, and their distribution was almost equal in the direction experiment. (b) In *Scaptotrigona postica*, a species that precisely guides recruits to the feeder by means of pheromone spots deposited along the route between the food source and the nest, the great majority of recruits always arrived at the feeder visited by the foragers. The black dots denote individual recruits, and the percentages give their distribution. (After Lindauer and Kerr 1958. With permission.)

(Lindauer 1956). Similar results were also recorded for a second species, *Plebeia tica*, which, like *Trigona iridipennis*, lacks a precise mechanism for the communication of food localities (Aguilar 2004; Aguilar et al. 2005). In addition, 80% of the *P. tica* recruits landed on a control feeder close to the nest (5 m) when it had the same peppermint odor as the experimental feeder, which was visited by the recruiting foragers, at a distance of 50 m (Aguilar et al. 2005). This number is very similar to that reported by Lindauer and Kerr (1958, 1960) for a related species, *P. droryana*, in which 82% of the recruits landed on the scented feeder positioned closest to the nest (Figure 12.1a). However, when both feeders were positioned at equal distances from the nest (150 m), but in opposite directions, almost equal numbers of *P. droryana* recruits landed on them both (Figure 12.1a). Apparently, the recruits left the nest with a knowledge of the food scent, but searched for the source of the scent at random (Lindauer and Kerr 1958, 1960). Food odor is also likely to influence the localization of sugar water feeders by recruited bees of the species *Trigona carbonaria*, as demonstrated in a study by Nieh et al. (1999/2000). In this study more recruits landed on the scented experimental feeder, which was situated upwind from the nest and visited by the initial foragers, than on a control feeder treated with the same scent, which was placed at an equal distance, but in the opposite direction. When the experimental feeder was placed downwind, however, only a slight majority of new bees came to it. In experiments with the control feeder placed at 50 m and in the same direction as the experimental feeder (150 m), the distribution of newcomers on the two food sources was almost equal when they were both treated with the same scent. Interestingly, when unscented feeders were used, significantly more newcomers alighted at the control feeder

(Nieh et al. 1999/2000). It is not clear, in this case, whether the bees were attracted by visual stimuli associated with the feeding device (which they had never seen before), or whether the scent of the sugar water alone (Adrian Wenner, personal communication) was sufficient to attract them. However, it is clear that *T. carbonaria* recruits can use food odors brought back to the hive by their foraging nestmates to locate the same food resource in the field—although not necessarily the same individual plants. Kerr (1969) mentioned that the orientation of recruited bees of two species (*Nannotrigona testaceicornis* and *Axestotrigona ferruginea tescorum*) improved considerably when odor-impregnated filter paper was added to the feeding device visited by the recruiting foragers. However, no experimental data were presented to support this observation. Esch et al. (1965) reported that foragers of *Melipona quadrifasciata* revisited a former feeding site, 300 m from the nest, when exposed to playback recordings of sounds produced by bees returning to the hive from the same food source together with the associated food scent. However, in contrast to the food odor, the auditory cues alone were not sufficient to induce experienced bees to leave the nest (Esch et al. 1965). Although these observations are only anecdotal, without giving underlying experimental data, they suggest that the mechanism involved in quickly reactivating stingless bee foragers to visit a known food source by providing the food's odor within the nest may be similar to what is known from honey bees (see Chapter 9).

The studies reviewed above clearly demonstrate that food odor can help experienced bees to relocate a specific food source, thereby promoting constancy toward particular flowers or artificial feeders. Furthermore, recruits apparently learn the odor inside the nest and use the information when searching for food in the field. The observations that prospective recruits of *M. quadrifasciata* and *M. scutellaris*, within the nest, preferentially touch the thoraxes and abdomens of returning foragers before they take up food samples (Hrncir et al. 2000), along with the recent demonstration that workers of *M. quadrifasciata* can be conditioned to floral odors through associative learning (McCabe et al. 2007), suggest that the food odor clinging to a forager's body in connection with a delivered nectar sample (reward) is an important element for food odor learning during the recruitment process. Again, these potential communication mechanisms have been studied in much greater detail in honey bees (see Chapter 10).

## FOOD SOURCE MARKING VOLATILES

### ATTRACTIVE SCENT MARKS

The deposition of volatile organic compounds at food resources and the function of these scent marks in the foraging behavior of stingless bees have mainly been investigated through the use of artificial feeders that dispense a sugar solution. The compounds that accumulate on the feeders with each visit by a foraging bee attract both the depositor herself and other incoming bees that arrive at the food source.

To test for the possible effects of scent marking at food sources, Villa and Weiss (1990) trained foragers of *Tetragonisca angustula* to sugar water feeders of a particular color (yellow or blue). The trained bees were then exposed to two identically colored feeders, only one of which had been visited by foragers before. These experiments demonstrated that feeders, which were previously visited by foraging bees, were significantly more attractive—presumably due to chemical marks left on them. Indeed, even when the color of the visited feeder was changed, the bees still continued to prefer it to an identical, but unmarked feeder of the original color, indicating that the scent marks are more important than color in feeder constancy of *T. angustula* (Villa and Weiss 1990). The authors also found that the volatiles deposited by foraging bees were attractive to conspecifics from a different colony. Likewise, *Scaptotrigona mexicana* workers landed equally on two clean feeders, but exclusively chose scent-marked feeders, previously visited by conspecifics, when given the choice (Villa and Weiss 1990). A similar result has also been recorded for *Plebeia tica* (Aguilar 2004). *Melipona panamica* newcomers (feeder inexperienced bees) exclusively chose a marked

feeder over a clean one when they were placed 1 m apart, regardless of whether the food itself was scented, when they arrived while experienced foragers were present on the feeder (Nieh 1998). The newcomers' choice in these experiments likely was influenced by visual local enhancement (see Chapter 8), but it only decreased to ca. 91% of landings on the used feeder when the experienced foragers were caught prior to testing the newcomers' choice (Nieh 1998). Likewise, a significant majority of *M. mandacai* (Nieh et al. 2003b) and *M. rufiventris* (Nieh et al. 2004a) foragers had chosen a putatively odor marked paper disc from a previously visited feeder over a clean control paper in two-feeder-choice experiments that excluded visual local enhancement. In *Trigona corvina* a significant majority of the foragers chose a previously used (scent-marked) feeder over a clean feeder, too, when the odors were deposited by the same bee that was tested, by its nestmates, or by foragers of a different conspecific colony (Boogert et al. 2006). However, feeders previously visited by foragers of another species, *Melipona beecheii*, did not attract *T. corvina* workers (Boogert et al. 2006). By contrast, *T. spinipes* foragers, which were significantly more attracted to a feeder bearing their nestmates' scent marks over a feeder scent-marked by *Melipona rufiventris* workers when both were presented at the *T. spinipes* training site, preferred the heterospecifics' odor over their own scent marks at the *Melipona* feeding site only 2 m away (Nieh et al. 2004a). Furthermore, *T. spinipes* aggressively drove away *M. rufiventris* and took over the food sources. Nieh et al. (2004a) interpreted these findings as olfactory eavesdropping used by *T. spinipes* to locate resources that had been encountered by competitors (see Chapter 8). They further speculated that olfactory eavesdropping could have driven the evolution of a concealed symbolic communication of food location inside the nest in some stingless bee species (i.e., *Melipona*), and that such referential location information replaced odor trail information. This, however, would imply that pheromone trails are the ancestral mode of location communication in stingless bees, which is unlikely. For example, many species within the genera *Scaptotrigona* and *Trigona*, which are derived rather than basal taxa (Michener 2000; Costa et al. 2003), use trail pheromones to communicate the precise location of a food source to their nestmates. Furthermore, a conclusive demonstration that any stingless bee species indeed uses a referential location information communicated inside the nest to locate a food source is still lacking to this day (Hrncir et al. 2006; Barth et al. 2008; see also Chapter 11).

More detailed studies investigating the influence of the number of forager visits at a food source on the subsequent choice behavior of the bees (experienced foragers and newly arriving individuals) have been carried out with *Melipona seminigra* (Hrncir et al. 2004) and *Nannotrigona testaceicornis* (Schmidt et al. 2005). Artificial feeders only became attractive to *M. seminigra* foragers after bees had landed and fed on them on forty occasions, after which up to 72% of the bees subsequently tested chose the used feeder over a clean control feeder. Under similar experimental conditions, the number of visits necessary to attractively mark a feeder for *N. testaceicornis* foragers was only twenty. In this species approximately 86% of the bees chose the marked feeder when it was presented alongside a clean control feeder (Schmidt et al. 2005). In both species, the feeders previously visited by bees attracted more newcomers than clean feeders, even when the food used was itself scented. However, discrimination between the two food sources was significantly higher when both feeders contained unscented sugar solution. Apparently the bees were attracted by the food odor itself, but the information from the deposited scent marks was not lost. The attractiveness of feeders that had been visited by foragers on twenty occasions was also demonstrated for *Scaptotrigona aff. depilis* (Schmidt et al. 2003). In this species the majority of bees always landed on the putatively marked feeders rather than on clean control feeders in two-feeder-choice experiments, regardless of whether the sugar solution was 0.75 M, 1.5 M, or 3 M, and whether the feeders were separated by a distance of 20 or 170 cm. *Scaptotrigona* bees recruit nestmates by means of scent trails, which lead them toward the food (Lindauer and Kerr 1958, 1960; Kerr et al. 1963; Schmidt et al. 2003; see below). The attractive scent marks at the feeders, which apparently differ from the actual trail pheromone markings, can help the bees to locate the endpoint of the odor trail. This conjecture is based on the observation that scent-marked feeder plates displaced from their original food site—and set up 20 m beyond it or half the way toward the nest—still attracted the bees, whereas clean feeders

did not (Schmidt et al. 2003). The high precision of recruitment to a particular food source found in *Scaptotrigona* aff. *depilis*, where 97.5–100% of the recruited bees arrived at the feeder visited by the recruiting foragers, likely can be explained by the combination of a scent trail and the attractive food-marking volatiles (Schmidt et al. 2003). A similarly high precision in the arrival of bees at used and potentially marked artificial food sources was found in *S. mexicana*, both at a larger scale with control feeders placed 5 and 10 m away from the experimental feeder and in a within-patch situation with the control feeders arranged 0.5 and 1 m around it (Sánchez et al. 2004, 2007). It seems likely that scent marks left on the experimental feeder by the recruiting foragers, as well as visual cues caused by the foragers' presence while taking up sugar solution (local enhancement; see Chapter 8), accounted for the attraction of the newly arriving bees to the particular experimental feeder in *S. mexicana*. Scent marks deposited by bees on a sugar water feeder also accounted for the precise recruitment of experienced foragers to this food source when it was presented together with four unvisited, clean feeders in *Melipona panamica* and *Partamona peckolti* (Contrera and Nieh 2007).

The attractiveness of the scent marks deposited by foragers at feeders persisted for 70 min in *Melipona mandacaia* (Nieh et al. 2003b). Feeders marked by *M. seminigra* attracted bees for up to 2 h, and their active range was restricted to approximately 1 m (Hrncir et al. 2004). When the distance between the scent-marked feeder and a control feeder was 1.5 m or greater the bees' choice behavior did not significantly differ from their choice between two clean feeders. Experiments with *M. panamica*, using similar sugar solution feeders, showed that the effective radius of forager-deposited scent marks lay somewhere between 6 and 12 m (Nieh 1998). Probably the larger radius of attraction in *M. panamica* was due to a much larger quantity of accumulated scent on the feeders. Nieh (1998) allowed twenty foragers to freely visit the feeders for at least 30 min prior to the experiments (which probably resulted in a minimum of 250 visits), whereas a fixed number of forty forager visits was allowed in the case of *M. seminigra* (Hrncir et al. 2004).

## REPELLENT SCENT MARKS

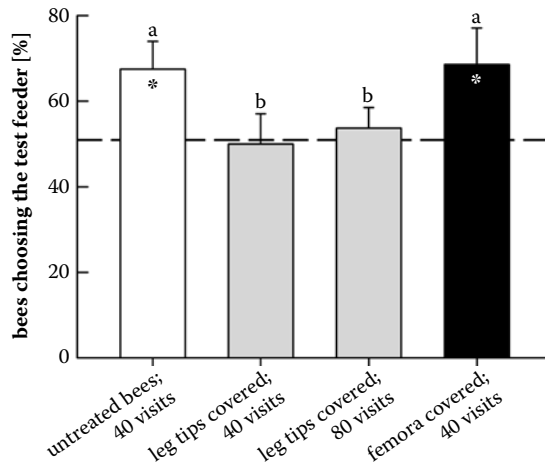
Studies carried out with bumble bees (Chapter 13), honey bees (Chapter 9), and the social sweat bee *Halictus aerarius* (Yokoi and Fujisaki 2007; Yokoi et al. 2007), as well as with a few species of solitary bees (e.g., *Anthophora plumipes*, Gilbert et al. 2001; *Anthidium manicatum*, Gawleta et al. 2005), have demonstrated that scent marks left on natural flowers (as opposed to artificial feeders) by foraging bees can be used by subsequent visitors to quickly reject a depleted food source, which is likely to significantly increase foraging efficiency. In stingless bees the only observations of workers foraging on natural flowers, within the context of scent marking, were reported for *Trigona fulviventris* (Goulson et al. 2001). Individuals that foraged on *Priva mexicana* (Verbenaceae), which has small tubular flowers, accepted ca. 76% of the flowers that had not been visited by other bees for at least 45 min. By contrast, only 40% of flowers were landed on and probed if they had been visited by another bee within the previous 2 min. Recently visited flowers were still rejected even when the depleted nectar was artificially replenished, using nectar from other flowers. This, therefore, indicates that the bees' decisions were based on the presence or absence of scent marks rather than direct assessment of the reward (Goulson et al. 2001). However, in the case of the plant species *Byrsonima crassifolia* (Malpighiaceae), foragers of *T. fulviventris* equally accepted both recently visited and unvisited flowers, which are simple in structure and have easily accessible nectaries (Goulson et al. 2001). Therefore, although *T. fulviventris* workers apparently can detect and respond to scent marks when foraging, they do not necessarily use such information when flower handling time is short and visiting depleted flowers not costly, as might be the case with *B. crassifolia*. This conjecture is supported by recent experiments with bumble bees foraging on artificial flowers carried out by Saleh et al. (2006). The authors found that the bees responded to scent marks more strongly (higher rejection rate) when they were encountered on long flowers—which require a longer handling time—than on short flowers.

Another interesting observation of a repellent effect of volatiles deposited by bees was reported by Nieh et al. (2004a). This study demonstrated that workers of *Melipona fulviventris* show a strong aversion to feeders that had previously been visited by foragers of *Trigona spinipes*, which is known to aggressively defend food sources. The avoidance behavior suggests that *M. fulviventris* can detect interspecific scent marks at food sources, and probably retreats from them to avoid encounters with aggressive competitors. Whether this avoidance behavior is a general response to foreign odors or the result of prior experience with *T. spinipes* remains to be determined (Nieh et al. 2004a).

## ORIGIN AND CHEMICAL COMPOSITION OF SCENT MARKS

Kerr and Rocha (1988) hypothesized that the volatiles used to mark food sources by foragers of *M. rufiventris* and *M. compressipes* come from the anal liquids that the bees sometimes excrete at or near a sugar water feeder after food uptake. However, this conjecture was only based on the observation of defecation behavior, without demonstrating that bees are actually attracted by the anal droplets. Aguilar and Sommeijer (1996, 2001) subsequently observed that in *M. favosa* the percentage of foragers that deposited anal droplets on and around sugar solution feeders, as well as the total number of droplets, increased in relation to the distance between the food source and the nest. The total numbers of anal droplets deposited by *M. favosa* foragers that fed on sugar solutions of both low and high concentrations were similar (Aguilar and Sommeijer 2001). In *M. panamica*, however, the deposition rate increased at feeders containing less concentrated sugar solution, i.e., at poor food sources (Nieh 1998). In both species, the volume of the droplets decreased as the sugar concentration increased. In *M. mandacaia*, the foragers deposited twice as many droplets when feeding at 1.25-M sugar solution feeders than at 2.5-M solution feeders (Nieh et al. 2003b). As argued by Nieh et al. (2003b), the putative scent from the anal excretions would therefore be stronger for low-quality food sources than for high-quality food resources. This seriously undermines the hypothesis that anal droplets function as attractive food-marking substances. Nevertheless, Aguilar and Sommeijer (2001) found that the visitation rate of *M. favosa* foragers at feeding tables with anal droplets was higher than that at clean feeders, and that a larger proportion of bees (ca. 63%) landed on a feeder with anal droplets than on a simultaneously offered clean control feeder. However, the authors did not exclude the possibility that other volatiles, in addition to anal excretions, were also deposited on the feeders when the bees were foraging. Therefore, the results of this study have to be interpreted with caution. In two other studies that controlled for other potential sources of attractant, workers showed no preference for feeders baited with anal deposits over clean feeders (*M. panamica*, Nieh 1998; *M. seminigra*, Hrncir et al. 2004). Compounds excreted from a bee's anus thus can be excluded as the source for attractive food-marking odors. In the same way, the sugar water upon which the bees have fed, as well as their mandibular gland secretions, can also be excluded (Nieh 1998; Hrncir et al. 2004). Nieh et al. (2003b) reported that *M. mandacaia* foragers were attracted to feeders baited with anal droplets collected on filter papers and to feeders baited with filter papers simply held below the abdomen of foragers—without even touching them. They assumed that these papers were impregnated with a “ventro-abdominal odor” of a rather mystic origin. However, the preference for the marked papers is only significant if the data for all experiments are pooled, as within the original study (Nieh et al. 2003b). Separate comparisons, on the data recorded from each of the individual experiments (e.g., with chi-square tests; data given in Table 1 in Nieh et al. 2003b), reveal a nonrandom forager distribution for only one of the five experiments that were conducted using both types of scent marks. Therefore, the conclusion drawn by Nieh et al. (2003b) that *M. mandacaia* foragers are attracted to abdominal droplets and ventro-abdominal odors should be taken with caution.

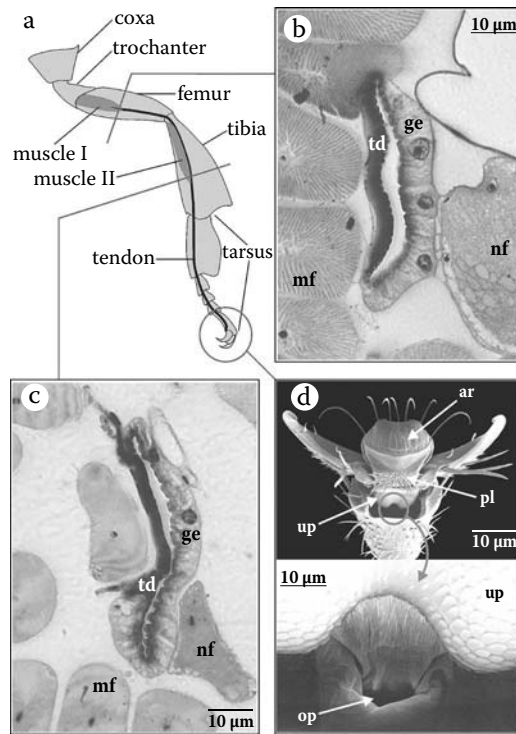
Compelling evidence for the importance of footprint secretions in the food-marking context—and against any other potential origin of the volatiles—comes from experiments carried out with *M. seminigra* by Hrncir et al. (2004). These authors covered the tips of the foragers' legs with nail polish, thus sealing any potential gland opening on the legs' distal segments. As a consequence of



**FIGURE 12.2** Choice behavior of *Melipona seminigra* workers between a clean (unvisited) sugar solution feeder and an identical feeder, which was previously visited by nestmates that were either untreated (open bar; N = 12 experiments) or had their leg tips (gray bars; N = 4 each) or femora (black bar; N = 4) covered with nail polish. The bars and whiskers give means + 1 SD. Asterisks indicate statistically significant differences in the proportion of bees choosing the previously visited feeder compared to the proportion of bees choosing one of two identical and clean feeders during a series of control experiments ( $50.8 \pm 1.2\%$ ; N = 6; dashed line = mean); different letters denote significant differences between bars (one-way ANOVA and Tukey pair-wise comparisons; minimum significance level  $P < 0.05$ ). Obviously, the foragers were unable to leave attractive volatiles at the feeder when their leg tips were covered, which likely is due to the sealing of glands that open there and account for the deposition of footprint substances. (After Hrnčir et al. 2004. With permission.)

this treatment, the feeders the foragers had visited did not become attractive to other bees even after forty or eighty visits (Figure 12.2). By contrast, feeders visited forty times by untreated foragers, or by bees with their femora covered with nail polish, were significantly preferred over clean feeders by newly arriving bees (Figure 12.2; Hrnčir et al. 2004). These experiments clearly demonstrated that the volatiles deposited while a bee is sitting or running on a feeding plate are secreted from glands within their legs that open at the legs' tips, rather than from any other part of the bee, including the general cuticle surface.

The most obvious glands that could account for the secretion of footprint substances are the tarsal glands, which are situated in the fifth tarsomeres of a hymenopteran's legs (Dahl 1885; Arnhart 1923). However, a histological study on the species *M. seminigra* has shown that these glands lack any openings to the outside (Jarau et al. 2005), as is the case in other stingless bees (Cruz-Landim et al. 1998), honey bees (Lensky et al. 1985; Federle et al. 2001), and bumble bees (Pouvreau 1991). Therefore, they are unlikely to be involved in the production of chemical footprints (Jarau et al. 2005). The contradiction between the apparent use of attractive footprint secretions by *M. seminigra* foragers at food sources on the one hand and the lack of openings of the tarsal glands on the other was resolved by the discovery of a different system of glands within the bees' legs (Jarau et al. 2004b). Each claw retractor tendon, which runs from a leg's femur through its tibia and tarsus and connects to the base of the pretarsus, has specialized glandular epithelia within the femur and tibia (Figure 12.3). The glands' products are secreted into the tendons, which form hollow tubes and serve as the excretory canals leading to the legs' tips, where they are secreted as footprints. Feeding tables baited with extracts of the tendon glands, dissected from *M. seminigra* foragers, attracted the bees in the same way as feeders naturally marked by foragers (Jarau et al. 2004b), thus providing strong evidence that the secretions of these glands account for the attraction of bees to footprints.

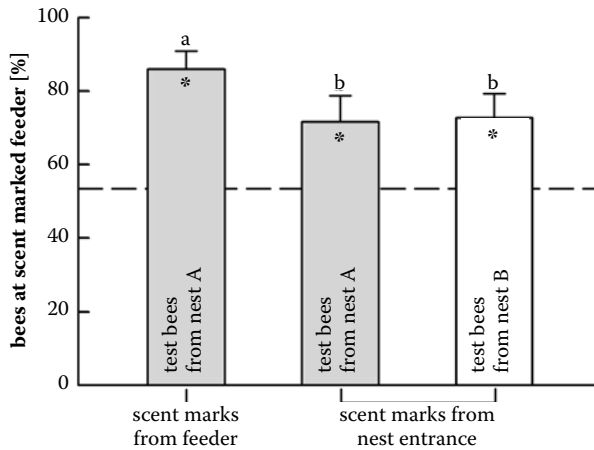


**FIGURE 12.3** The tendon gland of *Melipona seminigra*, which produces the footprint secretions that are released at a leg's tip. (a) Diagram of a metathoracic leg illustrating the location of the tendon and its associated muscles. (b, c) Cross sections through the tendon gland in the leg's femur and tibia showing the cuticular part of the hollow tendon (*td*) and the large cells of the glandular epithelium (*ge*). (d) Ventral view of the fifth tarsomere and the pretarsus (top) and of an enlargement (bottom) of the opening (*op*) of the tendon at the base of the unguitactor plate (*up*). Further abbreviations: *ar*, arolium; *mf*, muscle fibers; *nf*, nerve fibers; *pl*, planta. See color insert following page 142. (After Jarau et al. 2004b. With permission.)

The chemical structures of the compounds that are deposited by stingless bees at food sources have so far been elucidated for only one species (*Melipona seminigra*). The scent marks collected from artificial feeders that were previously visited by foragers consisted of 12 alkanes, 8 alkenes, 1 methyl alkane, and 1 aldehyde (Jarau et al. 2004b). The main compounds, each constituting  $\geq 10\%$  of the total amount of the identified volatiles, were pentacosane, heptacosane, and the corresponding alkenes 7-(*Z*)-pentacosene and 7-(*Z*)-heptacosene. The same compounds were also detected in extracts collected from the tendon glands of *Melipona seminigra* (see above) as well as from its last tarsomeres. These extracts also contained a further forty-one compounds, mainly esters, acids, and methyl alkanes (Jarau et al. 2004b). Interestingly, the hydrocarbons identified from *M. seminigra* scent marks are similar to the compounds reported from bumble bee scent marks (e.g., Schmitt et al. 1991; Eltz 2006; Saleh et al. 2007), which have a similar effect on the behavior of foraging bees (see Chapter 13).

### FOOD-MARKING CUES OR TRUE SIGNALS?

One important question that arises from the discovery that bees are attracted to food sources that have been previously visited by other foragers is whether the deposited substances are true signals or merely cues. Signals have been specifically shaped by natural selection to broadcast information and are released on purpose, whereas cues can contain useful information, but have not especially



**FIGURE 12.4** Choices of *Nannotrigona testaceicornis* workers between a feeder bearing scent marks and a clean (unmarked) but otherwise identical feeder. The scent marks originated from bees of nest A that either landed and sat on the feeder 25 m away from the colony in order to collect sugar solution, or simply ran over it directly in front of the nest's flight hole. The bars and whiskers give means + 1 SD (N = 6 experiments each). Asterisks indicate statistically significant differences in the proportion of bees choosing the scent-marked feeder compared to the proportion of bees choosing one of two identical and clean feeders during a series of control experiments ( $53.4 \pm 3.6\%$ ; N = 6; dashed line = mean); different letters denote significant differences between bars (one-way ANOVA and Tukey pair-wise comparisons; minimum significance level  $P < 0.05$ ). Apparently, the attractive volatiles are neither nest specific nor specifically deposited while foraging at a food source. (After Schmidt et al. 2005. With permission.)

been molded by natural selection to convey them (Seeley 1989). Such cues are incidentally left on the substrate by foragers, and the accumulating odor could then be interpreted by other bees as, for example, an indicator for the presence of food.

Schmidt et al. (2005) recently investigated this question in *Nannotrigona testaceicornis*. In experiments with artificial food sources the bees had to choose between two feeder plates: one that was clean and unvisited by bees and one (which was optically identical and contained the same sugar solution) that was scent-marked by other bees. Regardless of whether the scent marks were deposited by twenty foragers that collected food from the test feeder or twenty bees that simply ran over its surface to reach the entrance to their nest, the bees significantly preferred the marked feeder over the control (Figure 12.4). A significantly larger proportion of bees chose the feeders marked at feeding sites than feeders marked by workers returning to their nest, but this difference can probably be explained by variation in the total amounts of deposited volatiles between the two locations. Whereas each forager spent ca. 20 s on the feeder, the time required for a bee to cross it at the entrance to the nest was less than 4 s. There is good reason to assume that the approximately fivefold time the bees spent on the feeders compared to the nest entrance plates has resulted in the deposition of more scent. The compounds left on the substrate at the nest entrance, presumably as footprints, were not nest specific, but also attracted conspecific bees of another colony (Figure 12.4; Schmidt et al. 2005). The substances that bees apparently leave on any substrate simply by walking on it, regardless of the behavioral context, which act as an attractant for other bees, thus are cues that carry the information only incidentally. This conclusion can also explain the observation, reported by Nieh et al. (2003b), that *M. mandacai* foragers deposited attractive volatiles on rich food sources (2.5-M sugar solution), to which they recruited nestmates, as well as on poor food sources (1.25 M), to which recruitment did not occur. Recent experiments have demonstrated that in bumble bees, too, the chemical compounds left on substrates are passive cues rather than deliberately deposited signals (Saleh et al. 2007; Wilms and Eltz 2008; see also Chapter 13).

Additional evidence that food-marking volatiles are context-dependent cues rather than fixed signals containing specific information on the nature or quality of a food resource comes from recent experiments conducted with *Scaptotrigona mexicana*. Foragers of this species learned to positively or negatively associate scent marks with the quality of the food in choice experiments using artificial feeders (Sánchez et al. 2008). When the bees had to choose between two feeders, one of which was scent-marked and the other a clean control, they landed on both at the same frequency, when each feeder contained the same rewarding sugar solution. However, the bees showed a preference for the clean feeder when the contents of the scent-marked feeder were replaced with pure water (no sugar reward). The opposite occurred when the rewards were switched, so that only the scent-marked feeder contained sugar (Sánchez et al. 2008). Likewise, *Melipona scutellaris* foragers also use volatiles to avoid revisiting feeders that are depleted after a single visit (Roselino et al. 2007), but they are attracted to scent-marked feeders if they contain an even higher concentrated sugar solution during a second visit (Rodrigues et al. 2008). Apparently, stingless bees can learn to associate forager-deposited volatiles with the quality of a food resource and adapt their feeding behavior during subsequent visits. Volatiles deposited by bees can, therefore, provide information on a food source that can be quickly and effectively learned in the same way as other floral cues, such as flower odor, color, or shape (Menzel 1999). Recently, the ability to learn and predict food reward levels from volatile food markings has also been demonstrated in bumble bees (Saleh and Chittka 2006; Witjes and Eltz 2007; see also Chapter 13), and may represent a general feature of the foraging behavior of bees.

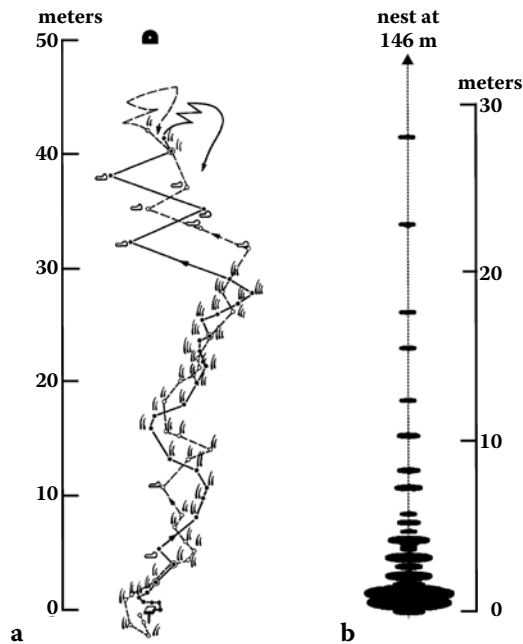
## TRAIL PHEROMONES

The use of trail pheromones, which guide recruited foragers from the nest to a food resource, has been observed in several species of stingless bees within the genera *Trigona*, *Scaptotrigona*, *Geotrigona*, *Cephalotrigona*, and *Oxytrigona* (Lindauer and Kerr 1958, 1960; Kerr 1960, 1969, 1973; Kerr and Cruz 1961; Kerr et al. 1963, 1981; Hebling et al. 1964; Cruz-Landim and Ferreira 1968; Blum et al. 1970; Johnson 1987; Noll 1997; Schmidt et al. 2003; Nieh et al. 2003a, 2004b; Jarau et al. 2004a, 2006; Sánchez et al. 2004; Aguilar et al. 2005; Schorkopf et al. 2007). This remarkable behavior belongs to the most precise and effective communication mechanisms for the exploitation of food found within the social bees (Lindauer and Kerr 1958, 1960; Jarau et al. 2003). As a result of the high numbers of workers that quickly and precisely gather at a particular food source, following its discovery by scouts, some species can easily compete with the recruitment efficiency of honey bees (Lindauer and Kerr 1958, 1960). Furthermore, trail pheromone communication allows the precise recruitment of bees not only at a particular direction and distance from the nest (Figure 12.Ib), but also to a particular height above ground level (Lindauer and Kerr 1958, 1960; Nieh et al. 2004b). Height, which is not communicated in the honey bees' dance language (von Frisch 1965), is an important dimension for stingless bees that live in tropical forests.

Kerr and Rocha (1988) and Kerr (1994) also proposed the use of short odor trails by *Melipona* bees, based on observations that foragers of several species (*M. rufiventris*, *M. compressipes*, *M. scutellaris*, *M. bicolor*, and *M. quadrifasciata*) sometimes landed and walked on leaves (for several centimeters) on their return flights from a feeding station. However, so far there is no proof that the foragers deposit scent and that recruited bees search for scent marks when orienting toward a food source. Furthermore, the infrequent occurrence of interrupted return flights from a food source in *M. scutellaris* and *M. quadrifasciata* (0.25% and 0.62%, respectively, of ca. 6,000 observations per species) found by Hrncir et al. (2000) points to the insignificance of this behavior for the guidance of recruits to a food source in *Melipona* bees. Likewise, the actual existence and use of an "aerial odor tunnel," which is assumed to be created by *Melipona* (Kerr 1994) and *Partamona* (Kerr 1969) foragers through the release of a pheromone during their flight toward a food source and followed by recruited nestmates, has yet to be scientifically demonstrated.

## SCENT MARKING BEHAVIOR AND THE SPATIAL DISTRIBUTION OF PHEROMONE MARKS

The deposition of trail pheromone marks, even when deposited at the food source itself, markedly differs from the passive transfer of odor cues to food sources described above. It is always connected with a very conspicuous behavior exhibited by the foragers that deposit the scent marks. This behavior was first described in detail by Lindauer and Kerr (1958, 1960), who trained foragers of *Scaptotrigona postica* to feed at sugar solution feeders at a distance of 50 m from the nest. The trained bees returned several times to the feeders to collect food and flew directly back to the nest without showing any special behavior. After a couple of visits, however, the bees appeared excited, briefly left the feeding table in a hectic flight, alighted on it again, flew up and landed on nearby blades of grass or sticks, and finally left in the direction of the nest. On their return flight they again landed on leaves or sticks at intervals of 1–2 m. About 8 m before reaching the nest they usually turned around and flew back to the food (Figure 12.5a). Occasionally, a forager also entered the nest, presumably to alert and recruit nestmates. The hectic behavior displayed by the foragers is clearly connected with the deposition of scent marks that are subsequently used by the recruits to find the food: When the nest and feeder were placed on the opposite sides of a small lake, denying the bees any solid substrate for the deposition of pheromones, no newly recruited bees found the food resource (Lindauer and Kerr 1958, 1960). Recruitment took place, however, when the foragers



**FIGURE 12.5** Spatial distribution of pheromone marks deposited (a) by *Scaptotrigona postica* foragers on stones and ground-proximate vegetation and (b) by *Trigona hyalinata* workers along a rope with leaves fastened to it at 1 m intervals, which extended for 100 m from the food source toward the nest (the leave-bedecked rope was the only possibility for bees to deposit scent marks during the experiment). Whereas recruiting foragers of *S. postica* deposit pheromone marks along most of the distance covered by their return flight to the nest (two forager flights shown; dots denote a scent mark, arrows indicate the flight direction), the scent marks are most concentrated at the food source and along the first few meters toward the nest in *T. hyalinata* (area of ellipses correspond to the number of scent marks deposited, the smallest ones being equal to 1 mark; in sum, 103 marks are shown). The food sources (sugar solution feeding tables) were located at 0 m; note the different scales and distances to the nest in (a) and (b). The black hive symbol in (a) denotes the location of the bees' nest. (a, after Lindauer and Kerr 1958; b, after Nieh et al. 2003a. With permission.)

were provided with a substrate in the form of a rope decorated with twigs and leaves tightened over the water surface, where they could land and deposit pheromone marks on their way back to the nest (Lindauer and Kerr 1958, 1960).\* A rapid change from normal feeding to the hectic scent marking behavior of recruiting foragers has also been observed in *Scaptotrigona* aff. *depilis* (Schmidt et al. 2003), *S. mexicana* (Sánchez et al. 2004), *Trigona fulviventris* (Johnson 1987), *T. hyalinata* (Nieh et al. 2003a), *T. recursa* (Jarau et al. 2004a), and *T. spinipes* (Nieh et al. 2004b). To deposit scent marks, foragers of *T. recursa* run for a short duration of about 0.6 s across the substrate, thereby covering a distance of approximately 1 cm (Jarau et al. 2004a). An equally short duration (ca. 0.7 s) of scent mark deposition was also reported for *T. hyalinata* and *T. spinipes* (Nieh et al. 2003a, 2004b).

The trail of scent observed in the species *S. postica*, which extended for almost the entire distance from a food source toward the nest (Lindauer and Kerr 1958, 1960), appears not to be the general rule in stingless bees.\* In the species *T. amalthea*, distribution of scent marks was non-uniform, with the highest concentration of scent deposited on the food source and on the first 5 m of the trail toward the nest, and with larger intervals between the marks thereafter (Kerr et al. 1963). A similar distribution of scent marks could have accounted for the correct approach of *S. postica* recruits at a feeder located at the most distant end of the trail made by their nestmates (Kerr et al. 1963). Likewise, Johnson (1987) reported that foragers of *T. fulviventris* concentrate the scent marks they deposit at the food source or within 1 m around it (51% and 43% of 277 observed scent marking events, respectively). Only 6% of the scent marks were deposited on leaves 1–2 m away from the feeder in the direction of the nest. Thus, *T. fulviventris* does not lay an entire trail of pheromone spots from a food source to the nest—at least when the food resource is within 20 m of the nest (Johnson 1987). Instead, the bees concentrate the odor marks around the food source itself. More recently, Nieh et al. (2003a, 2004b) studied the distribution of scent marks in two further *Trigona* species. During their experiments the trained foragers could only deposit their pheromone on leaves, which were attached, at 1–m intervals, along ropes that extended from the feeders to the nest in an otherwise unnatural environment (a terrace paved with tiles). The authors found that both species laid only short trails and deposited the majority of their scent marks on the feeding table itself or only a few meters away from it. In *T. hyalinata*, the scent marks were mainly deposited within 5 m of the feeder and formed a gradient of decreasing scent concentration that extended for a maximum of 27 m from the food source to the nest, which was situated 146 m away (Figure 12.5b; Nieh et al. 2003a). Likewise, *T. spinipes* foragers deposited trail scent marks at a maximum distance of 29 m from the food source in the direction of the nest, which was up to 225 m away, with 95% of the marks placed within 3 m of the feeder (Nieh et al. 2004b). The much larger concentration of scent marks close to the feeders resulted in a polarization of the trails. Foragers of both species used the odor gradient to orient toward the end of the trail and preferred a feeder at this position compared to a feeder within the trail or at the end of the trail that was closest to the nest, even if the trails (on the ropes) were displaced from their original positions (Nieh et al. 2003a, 2004b). In *T. spinipes* the concentration of the scent marks was also highest on and around the feeder when it was placed on top of a 12-m-high water tower, with a decreasing number of scent marks laid toward the tower's base (Nieh et al. 2004b). Recruited *T. spinipes* workers were attracted to the scent trail for 8 min (Kerr et al. 1963) to 25 min (Nieh et al. 2004b). A similar time span (ca. 15 min) during which recruits followed scent trails was found in *Scaptotrigona postica* and *S. bipunctata* when the deposition of further scent spots by foraging bees was experimentally hindered (Lindauer and Kerr 1960; Kerr et al. 1963). However, under normal conditions, trails are rapidly reinforced by the scent deposits of recruiting bees (Schmidt et al. 2006), and are likely to remain effective over much longer periods of time. In *S. postica* and *T. corvina* the orientation of newly recruited bees along a scent

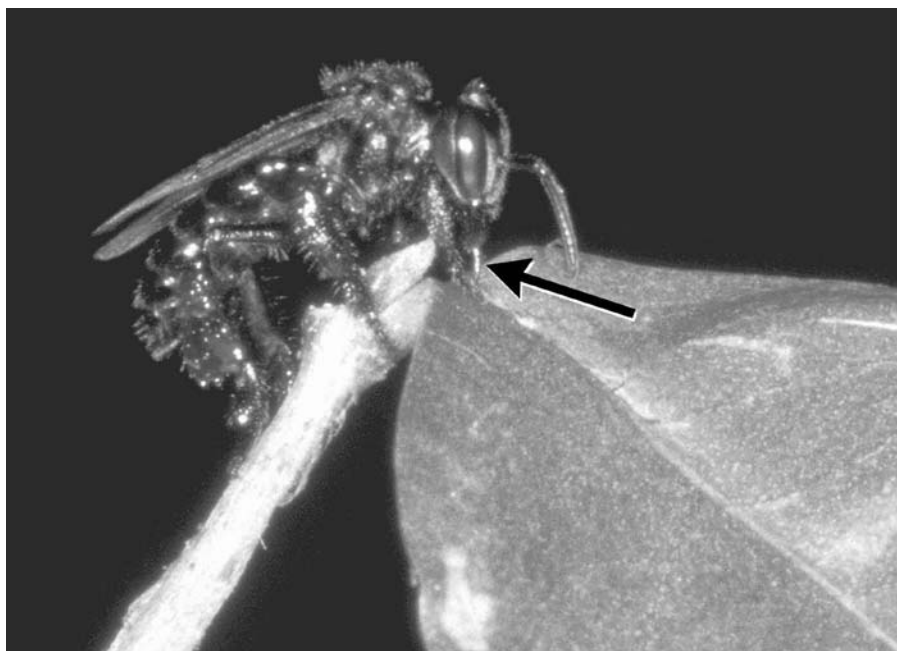
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\* Dirk Louis P. Schorkopf and colleagues recently repeated the lake experiments with *S. postica*, and gathered strong evidence that an entire scent trail is not imperative for the recruitment success of this species (Schorkopf, personal communication). The publication of their results, not yet available at the preparation of this chapter, likely will change our view of stingless bee trail pheromones.

trail probably is aided by the recruiting foragers, which were observed to guide small groups of bees on their first flight to the food source (Lindauer and Kerr 1958, 1960; Aguilar et al. 2005).

### GLANDULAR ORIGIN OF TRAIL PHEROMONES

The origin of the pheromones deposited by stingless bees when marking scent trails was long considered to be their mandibular glands. This idea has been perpetuated by virtually every textbook or review article dealing in some way with stingless bees, trail pheromones, or animal behavior (e.g., Blum 1970, 1974b; Wilson 1971; Blum and Brand 1972; Shorey 1973; Michener 1974; Wille 1983; Haynes and Birch 1985; Free 1987; Roubik 1989; Morgan 1990; Traniello and Robson 1995; Alcock 2001; Wyatt 2003; Nieh 2004). This is astounding since no experiment, within the primary literature, has ever demonstrated that the trail pheromone of any stingless bee species is secreted from its mandibular glands (see below). In fact, in their seminal study on stingless bee recruitment, Lindauer and Kerr (1958, 1960) had already reported to the contrary, that *Scaptotrigona postica* recruits *never* followed experimental trails, made from mandibular gland secretions, when these trails deviated away from the bees' natural scent trail! However, because the bees somehow "examined" mandibular gland marks when the authors placed them between the natural scent marks deposited by foragers, and because a scent marking bee alights on the substrate and runs a short stretch on it with her mandibles opened, Lindauer and Kerr (1958, 1960) concluded that the trail pheromone is produced in the mandibular glands. In several later studies, researchers merely assumed that trail pheromones in stingless bees are produced in their mandibular glands without conducting specific experiments, such as bioassays that employed artificial scent trails, to test this assumption (Kerr and Cruz 1961; Kerr et al. 1963; Cruz-Landim and Ferreira 1968; Blum et al. 1970). The most recent study heavily influenced by the "mandibular gland trail pheromone paradigm" was published by Nieh et al. (2003a). These authors presented extracts at feeding tables, prepared from mandibles with their appendicular glands plus additional tissues pulled out of the heads of *T. hyalinata* foragers, and observed the behavior of newcomers approaching them. For about 8 min the arriving bees showed aggressive behavior, even attacking the vials containing the extracts, and did not land (Nieh et al. 2003a). Only after this deterrent effect had ceased did the bees show a preference for the baited feeders when compared to unbaited control feeders. Although these findings stand in clear contrast to the observed synchronization of the foragers' scent marking behavior and the newcomers' landing at the feeders—and despite the fact that bees never attack food sources naturally scent-marked by foragers prior to landing—Nieh et al. (2003a, p. 2194) concluded that recruiting *T. hyalinata* foragers "evidently use mandibular gland secretions to create the short odour trail to guide nest-mates." As argued by Jarau et al. (2004a), however, it is likely that the repellent effect of the extracts prepared by Nieh et al. (2003a) was caused by mandibular gland compounds, whereas the attraction of bees after 8 min can probably be attributed to compounds from the bees' labial glands that had been unknowingly extracted by the authors due to the crude method of mandibular gland dissection. In fact, neat preparation and extraction methods are imperative for any study on the role of gland secretions. Indeed, when mandibular and labial glands were carefully separated, Jarau et al. (2004a) found in experiments with *T. recursa* that extracts from the former gland were purely repellent, whereas extracts from the latter were clearly attractive to bees arriving at artificial feeders. In choice experiments with two feeders, one baited with gland extract and the other with pure solvent (control), ca. 84% of the bees landed on the feeder bearing labial gland components, whereas in the case of the mandibular gland treatment, 73% of the bees chose the control (Jarau et al. 2004a). Importantly, the attraction of recruited bees to labial gland secretions was not limited to the food source alone. When artificial scent trails were created using labial gland secretions, large numbers of newly recruited bees deviated from their natural scent trails in order to follow them, whereas mandibular gland extracts and pure solvent pentane (control experiments) had no effect (Jarau et al. 2006). Therefore, the trail pheromone of *T. recursa* clearly is produced in its labial glands. Recently, it was unequivocally shown, by similar artificial scent trail bioassays, that the trail pheromones



**FIGURE 12.6** A forager of *Trigona recursa* lands on a leaf in order to deposit a pheromone mark from her labial glands on her way back from a food source to the nest. The arrow points to the extended glossa, at the base of which the glands' excretory duct opens to release their secretions. (Photograph by Stefan Jarau.)

of *T. corvina* (Dambacher 2006; Dambacher et al. 2007), *T. spinipes* (Schorkopf et al. 2007), and *Scaptotrigona pectoralis* (Hemmeter 2008) are also produced in the foragers' labial glands. In all three species none of the recruited bees followed artificial scent trails made of mandibular gland extracts, whereas labial gland extracts induced trail-following behavior in a significantly larger proportion of bees than the solvent control (*T. corvina*, 51% vs. 0.5% [labial vs. solvent]; *T. spinipes*, 90% vs. 10% [different experimental setup]; *S. pectoralis*, 35% vs. 0.5%). Indirect evidence for the importance of labial gland secretions for foraging workers also comes from a histological study published by Cruz-Landim and Puga (1967), who found that the labial glands of *S. postica* develop with age and are most developed and productive within the oldest bees (i.e., the foragers).

The labial gland secretions are released at the base of a bee's glossa (Snodgrass 1956; Cruz-Landim 1967). To deposit a pheromone mark from these glands, foragers of *T. recursa* extend their mouthparts before landing on the substrate and then rub their glossa against the surface as they run across it (Figure 12.6; Jarau et al. 2004a). Nieh et al. (2003a, 2004b) reported that foragers of *T. hyalinata* and *T. spinipes* also frequently extended their proboscises when depositing a scent mark. Interestingly, Simpson and Riedel (1964) found that honey bee workers are able to discharge their labial gland secretions even when their tongues are almost completely retracted. Assuming that stingless bees probably have a similar method of discharge, foragers may be able to apply their labial gland secretions to the substrate by rubbing the dorsal surface of the almost completely retracted proboscis, which is held between the mandibles. As already suggested by Jarau et al. (2004a), this behavior could have misleadingly been interpreted as "rubbing the mandibles on the substrate" in earlier studies.

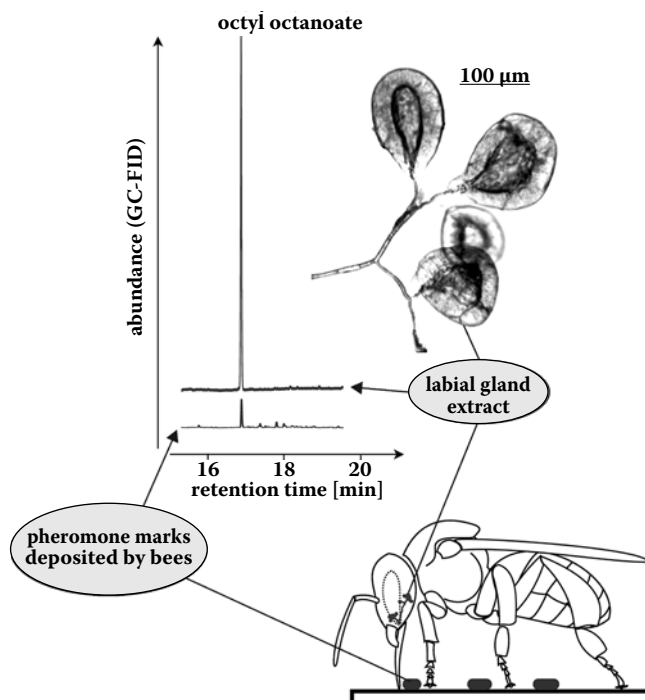
### CHEMICAL STRUCTURES OF TRAIL PHEROMONE COMPOUNDS

The first bioassays with a synthetic compound, which aimed to verify its function as a trail pheromone in a stingless bee, were reported by Blum et al. (1970). These authors tested the reaction

of *Geotrigona subterranea* workers toward small wooden blocks treated with citral, which was identified as the main compound of their mandibular glands (constituting ca. 95% of the detectable volatiles). When the citral source was placed on a feeding table, all the arriving bees landed on the wooden block, grasped it lightly, and only after releasing it crawled to the syrup and fed. When citral-impregnated blocks were placed on the ground halfway between the nest and a feeder placed only 80 cm away from it, the bees interrupted their flight in order to alight on them (Blum et al. 1970). The authors' conclusion that citral must be regarded as the primary trail pheromone employed by *G. subterranea* workers because it acts as a powerful attractant is, however, questionable. Real trail-following behavior was not demonstrated by the experiments, and grasping the food source by the arriving bees prior to feeding does not occur at resources naturally scent-marked by foragers. In addition, Blum et al. (1970) also found a clear alarm reaction of the workers when a high concentration of citral was placed at the entrance to a nest. It seems likely that the observed behavior of the bees toward the less concentrated citral sources placed 40 cm away from the nest or at the feeding table was a somehow weaker alarm reaction. Blum (1970) and Blum and Brand (1972) cite unpublished experiments in which workers of *Scaptotrigona tubiba* and *S. postica* were strongly attracted to benzaldehyde alone or in combination with 2-tridecanone and 2-pentadecanone, respectively, and conclude that these chemicals function as critical volatile elements of the trail pheromones. However, they do not give details on how and where the tests were conducted, and do not describe whether benzaldehyde and the ketones induced trail-following behavior rather than mere attraction. Later, Kerr et al. (1981) trained approximately forty workers of *T. spinipes* to a sugar solution feeder at a distance of 36 m from their nest. When the authors reinstalled the feeder on the following day and simultaneously provided an artificial scent trail made of drops of synthetic 2-heptanol, the main compound identified from head extracts of *T. spinipes*, six bees arrived and landed on the feeder within 20 min. Kerr et al. (1981) deduced from this result that 2-heptanol guided these bees to the food source, and thus may be the major constituent of the trail pheromone of *T. spinipes*. However, as already argued by Jarau et al. (2004a), it is much more likely that the six workers visiting the feeder on the second day were not newly recruited bees that were attracted by the 2-heptanol trail, but inspectors (Biesmeijer and de Vries 2001) that spontaneously checked the feeding site they had visited the day before. In fact, the ability of foragers to remember the location of a food source and the time of day when it is available, along with the demonstration that the bees make use of their memory to revisit known feeding sites, was reported for honey bees 80 years ago (Beling 1929) and, more recently, has also been shown for several species of stingless bees (*Melipona quadrifasciata* and *Partamona cupira*, Freitas and Raw 1996; *Trigona amalthea*, Breed et al. 2002; *M. scutellaris*, *M. seminigra*, *M. compressipes*, Schorkopf et al. 2004).

Recently, compounds from the trail pheromones of three *Trigona* species were identified by means of both (1) chemical analyses of extracts prepared from foragers' labial glands, the source of trail pheromones in stingless bees (see above), and (2) bioassays verifying the function of single synthetic compounds or compound mixtures as releasers of actual trail-following behavior in newly recruited bees. The main compound of labial gland extracts prepared from *T. recursa* foragers is hexyl decanoate, which makes up about 72% of its constituents (Jarau et al. 2006). Artificial scent trails that branched away from *T. recursa*'s natural scent trails and which were baited with this ester diverted a significant number of recruits from their original flight direction, whereas control trails made of pure solvent did not. However, hexyl decanoate alone was less effective at releasing trail-following behavior than the natural labial gland extract, indicating that the entire trail pheromone of *T. recursa* is a blend containing additional constituents that enhance the activity of the signal that is dominated by this specific ester (Jarau et al. 2006). Octyl decanoate, hexyl decanoate, isopropyl octadecenoate (double bond position unknown), and two unidentified compounds were the next most abundant volatiles in the labial glands of *T. recursa*, followed by a variety of minor constituents (Jarau et al. 2006). It is not yet known which of these compounds act synergistically with hexyl decanoate to release full trail-following behavior of this species. Dambacher (2006) and Dambacher et al. (2007) analyzed labial gland extracts prepared from *T. corvina* and also carried

out gaschromatographic analyses coupled to electroantennographic detection (GC-EAD) with these extracts and worker antennae. Of the twelve compounds that released a detectable response in the chemoreceptors on the bees' antennae (indicating that the bees can perceive them), seven could be identified (one terpene ester and six carboxylic acid alkyl esters). The main component in the labial gland extracts was octyl octanoate, which did not release trail-following behavior in recruited *T. corvina* workers along artificial scent trails when it was applied alone (Dambacher 2006). A synthetic blend of five of the identified compounds (octyl hexanoate, octyl octanoate, geranyl octanoate, decyl octanoate, and octyl decanoate; making together ca. 83% of the detected volatiles), which were mixed in approximately natural relative proportions, did, however, successfully lead the bees along experimental scent trails (Dambacher 2006; Dambacher et al. 2007). Thus, the trail pheromone of *T. corvina* is a blend of esters, with its main component alone being inactive. Interestingly, *T. spinipes* differs from *T. recursa* and *T. corvina* in that a single compound, octyl octanoate, is just as effective at inducing trail-following behavior in food-searching bees as the natural labial gland extract (Schorkopf et al. 2007). In experiments that used these odors to create artificial trails branching off the bees' natural path, about 90% of the recruits followed them rather than a trail that was simultaneously provided and marked with the pure solvent pentane. Not surprisingly, the main compound of labial gland extracts prepared from *T. spinipes* foragers was identified as octyl octanoate (making ca. 74% of the volatile constituents). This compound was also detected on substrates previously scent-marked by bees (Figure 12.7). Furthermore, mandibular gland extracts, which completely failed to induce trail-following behavior in recruited bees, contained no trace of octyl octanoate (Schorkopf et al. 2007).



**FIGURE 12.7** The trail pheromone compound of *Trigona spinipes*, octyl octanoate, is deposited on the substrate by recruiting foragers. Chemical analyses by means of gas chromatography (GC-FID, signals shown) and gas chromatography coupled to mass spectrometry (GC-MS, not shown) revealed that octyl octanoate is produced in the bees' labial glands and constitutes about 74% of the total amount of their secretion's volatile components. (After Schorkopf et al. 2007. With permission.)

**TABLE 12.1**  
**Trail Pheromone Compounds Identified from Labial Gland Secretions of Stingless Bees<sup>a</sup>**

Species	Compound	Molecular Formula	Molecular Weight	Estimated Boiling Point <sup>b</sup>	Reference for Trail Bioassays
<i>Trigona corvina</i>	Octyl hexanoate	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	228	280°C	Dambacher (2006)
	Octyl octanoate	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	320°C	
	Geranyl octanoate	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280	360°C	
	Decyl octanoate	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	360°C	
	Octyl decanoate	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	360°C	
<i>Trigona recursa</i>	Hexyl decanoate	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	320°C	Jarau et al. (2006)
<i>Trigona spinipes</i>	Octyl octanoate	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	320°C	Schorkopf et al. (2007)

<sup>a</sup> Only components are considered that have been identified by means of both chemical analyses and behavioral bioassays with synthetic compounds. For a critique on the remaining literature reports of proposed trail pheromone compounds, see the text.

<sup>b</sup> Boiling points are roughly estimated by the rule of thumb “number of C atoms × 20°C.”

To summarize, all trail pheromone compounds so far identified from gland extracts of stingless bee foragers that have also proved to be effective in triggering trail-following behavior belong to the chemical class of carboxylic acid alkyl esters, with the exception of one terpene ester (Table 12.1). The compounds have similarly low molecular weights (MW 228 to 284), estimated boiling points of between 280°C and 360°C, and show medium to low polarity. These properties are likely to make the volatility of these esters well suited for attracting bees over long distances. For example, one of the most frequently occurring compounds in moth sex pheromones is (*Z*)-9-tetradecenyl acetate (Schulz 2001), which belongs to the same chemical class of compounds and has a similar molecular weight (MW 254). Its ideal physicochemical behavior as a volatile signal is well known.

The similarity of the properties of the single compounds of trail pheromone blends may be important to maintain species or nest specific compositions (including relative proportions) following their application to the substrate by foragers. The same compounds, as well as other esters, were also found in labial gland extracts of *Scaptotrigona pectoralis* (Hemmeter 2008) and in head extracts of several other species of stingless bees (Kerr et al. 1981; Francke et al. 1983, 2000; Johnson et al. 1985; Engels et al. 1987), some of which communicate the location of food sources to their nestmates by means of scent trails. The function of single compounds or mixtures of the detected esters has not yet been investigated by means of bioassays in these species, but from the results obtained from *Trigona* species it may be predicted that at least some of them may be used for scent trail communication too. This assumption, however, awaits examination in future studies.

### NEST SPECIFICITY OF TRAIL PHEROMONES

Despite the potential importance of nest specific trail pheromones for competitor avoidance and resource partitioning, only a few studies have addressed the question of whether trail pheromones in stingless bees are primarily, or even exclusively, followed by the nestmates of a trail-laying forager. Lindauer and Kerr (1958, 1960) described an experiment in which foragers from two different nests of *Scaptotrigona postica* were placed beside each other and trained to feed at separate sugar solution feeders in such a way that the paths of their respective scent trails crossed. After recruitment took place for 1 h they recorded the colony identity of the bees at the feeders and found that workers from both nests collected sugar solution at the two food sources. However, because all bees were allowed to return to their nest after food uptake throughout the entire experiment, it cannot be

**TABLE 12.2**  
**Nest Specific Effectiveness of Trail Pheromones in Two Species of Stingless Bees**

	Percentage of Recruits That Followed Artificial Trails Made of:		
	Own Nest's Labial Gland Extract	Foreign Nest's Labial Gland Extract	Solvent
<i>Scaptotrigona pectoralis</i>	34.7 ± 21.5 <sup>a</sup> (472)	12.4 ± 5.9 <sup>b</sup> (766)	0.6 ± 1.4 <sup>c</sup> (238)
<i>Trigona corvina</i>	51.1 ± 24.5 <sup>a</sup> (1,338)	6.8 ± 7.3 <sup>b</sup> (625)	0.4 ± 1.1 <sup>b</sup> (265)

*Note:* The percentage of the recruited bees that followed artificially baited scent trails that branched off from the bees' natural pheromone trails is given as mean ± 1 SD (100% = total number of recruits that followed the natural plus the artificial trails). Different letters denote statistically significant differences in the proportion of bees of a given species misled by the artificial trails (U-tests and Bonferroni corrections; overall  $P < 0.05$ ). Values in parentheses give the numbers of the tested bee individuals. (Data for *S. pectoralis* are taken from Hemmeter 2008; data for *T. corvina* from Dambacher 2006.)

absolutely excluded that foragers from both nests recruited to either feeder. Recently, the nest specific effectiveness of labial gland extracts was clearly demonstrated in *Trigona corvina* (Dambacher 2006; Dambacher et al. 2007) and *S. pectoralis* (Hemmeter 2008). In both species, labial gland extracts from nestmates were more attractive to recruits than those made from foragers from a foreign nest (Table 12.2). This effect was more evident in *T. corvina*, where the proportion of bees diverted by the foreign labial gland extracts did not differ statistically from experiments using pure solvent. In both species foragers taken from different nests could clearly be separated by means of principal component- and discriminant function analyses based on the composition of the compounds contained in their labial glands (Dambacher 2006; Dambacher et al. 2007, Hemmeter 2008). This demonstrates the nest specific relative compositions of the glands' secretions and explains their nest specific effectiveness.

The observation that *S. pectoralis* recruits are more likely to follow conspecific scent trails made by foragers of a foreign nest than is the case in *T. corvina* likely can be explained by differences in the foraging strategies and aggressiveness of these two species. *T. corvina* is an aggressive group foraging species, and encounters with foragers from other nests always lead to costly combats between the foragers of the involved colonies (Johnson 1983; Slaa 2003). Thus, the avoidance of scent trails laid by *T. corvina* foragers of foreign nests, instead of following them in order to reach the food source already discovered by them, is advantageous, because it diminishes intraspecific competition leading to the death of many of a colony's foragers. As a consequence, natural selection likely has favored *T. corvina* colonies whose foragers did not follow foreign trails. *S. pectoralis* workers, on the other hand, forage peacefully at resources alongside other bees without displaying any aggression (Johnson 1983; Slaa 2003). Therefore, following the trail of a foreign nest is not as disastrous in this species as it would be in *T. corvina* (for similar findings in ants, see Chapter 8).

## CHEMICALS INVOLVED IN NEST PLUNDERING

The species belonging to the genera *Lestrimelitta* in South and Central America and *Cleptotrigona* in Africa have evolved a highly specialized mode of food acquisition. They are exclusively cleptobiotic. Instead of visiting flowers they raid the nests of other stingless bee species, or even honey bee colonies, in order to steal food along with other resources, such as building materials and stored resins, which are then transported back to their own nests (Müller 1874; Portugal-Araújo 1958; Sakagami and Laroca 1963; Bego et al. 1991; Sakagami et al. 1993; Nogueira-Neto 1997; Quezada-Euán and González-Acereto 2002). The workers of these robber bees have shiny and sparsely haired

bodies, and the structures for pollen collection (corbicula, penicillum, rastellum) are reduced, which presumably is related to the robbing behavior and the lack of flower-visiting habits (Michener 2000). Apparently, robber bees prefer to raid the nests of certain host species. Quezada-Euán and González-Acereto (2002) observed that *Lestrimelitta niitkib* on the Yucatan Peninsula in Mexico mainly raided the nests of *Frieseomelitta nigra* and *Nannotrigona perilampoides*. In Brazil and Panama *L. limao* preferentially attacked nests of several species of *Scaptotrigona*, *Nannotrigona*, and *Plebeia* (Sakagami and Laroca 1963; Roubik 1989; Bego et al. 1991; Sakagami et al. 1993). Less frequently, *Lestrimelitta* also invades conspecific nests, colonies of *Melipona*, *Paratrigona*, and *Tetragonisca*, or hives of *Apis mellifera* (Sakagami and Laroca 1963; Sakagami et al. 1993; Quezada-Euán and González-Acereto 2002). In Angola, Portugal-Araújo (1958) observed raids of *Cleptotrigona cubiceps* mainly on *Hypotrigona braunsi* colonies.

Typical raids, which usually do not entirely destroy the victim's nests, involve several dozen or hundreds of robber bees that invade a host colony within minutes, then take over and guard the nest entrance while simultaneously removing building materials, larval provisions from the younger brood combs, and food from the storage pots. Within the nest, most host workers and the queen retreat to the older brood combs or to the nest's periphery where they remain immobile, while host foragers that return from the field do not enter their nest. Researchers who have witnessed the raids of *Lestrimelitta* and *Cleptotrigona* have all noticed a strong, lemon-like odor emanating from the entrances of attacked nests, which are occupied by the robber bees—especially during the early phase of a raid (Portugal-Araújo 1958; Sakagami and Laroca 1963; Wille 1983; Bego et al. 1991; Sakagami et al. 1993; Nogueira-Neto 1997; Quezada-Euán and González-Acereto 2002). This strong odor emanates from the bees' mandibular glands, which are filled with secretions, particularly in workers more than 15 days old (Cruz-Landim and Camargo 1970). The two main components of *Lestrimelitta limao*'s mandibular gland secretion were identified as the two stereoisomers of citral, i.e., geranial and neral, in a ratio of ca. 2:1 (Blum 1966). In head extracts of the same species, several additional components belonging to the chemical classes of alcohols, acetates, esters, carboxylic acids, ketones, and hydrocarbons (probably extracted from the cuticle) were also identified (Wittmann et al. 1990; Francke et al. 2000). However, due to the method of extracting whole worker heads, the exact glandular origin of these compounds remains unknown. Citral may be used by the first robber bee foragers arriving at the entrance of a host nest both as a pheromone to attract further nestmates and, in high concentrations, as an allomone that irritates the host workers and disrupts their defensive organization (Blum et al. 1970; Michener 1974; Roubik 1989).

In bioassays with citral-impregnated wooden blocks that were introduced into the nest entrances of different bee colonies, Blum et al. (1970) found that workers of *Paratrigona subnuda*, *Plebeia droryana*, and *Nannotrigona testaceicornis* became highly agitated. Furthermore, the organized colonial activities of the nest ceased, and many bees left the hive and did not return for about 20 min. Likewise, in *Scaptotrigona pectoralis*, *S. mexicana*, and *Trigona fulviventris*, living or freshly killed *L. limao* workers, extracts prepared from their heads, or pure citral presented in front of the nest mainly released strong excitement in the bees at the entrance and hindered returning foragers to enter the colony (Weaver et al. 1975). The reaction of the test colonies to citral in these experiments indeed resembles the observed behavior of host workers during natural raids by *Lestrimelitta* (Sakagami and Laroca 1963; Sakagami et al. 1993). By contrast, workers of *Frieseomelitta varia* started to cover a citral-impregnated wooden block with resin 3 min after its introduction to the nest entrance, and *S. postica* guards removed the citral source from their nest (Blum et al. 1970). Wittmann (1985) found that the guards of *Tetragonisca angustula*, which constantly hover in front of the entrance of their nest, immediately attack live *L. limao* workers brought into their frontal visual field, and hypothesized that the unique defense behavior of this species evolved under the pressure of *Lestrimelitta* attacks. The robber bees are apparently recognized and distinguished from returning *T. angustula* foragers by their specific odor bouquet, with citral and 6-methyl-5-hepten-2-one acting as key volatiles that trigger the defense reaction (Wittmann et al. 1990). Additional minor compounds identified from head extracts of *L. limao* had a synergistic effect when added to the citral and 6-methyl-5-hepten-2-one mixture, but

did not release attacks in *Tetragonisca* guards when presented alone (Wittmann et al. 1990). Markedly different reactions to citral were also found in two *Melipona* species. In *M. quadrifasciata*, colonial activities totally broke down, and many workers left the nest and did not return as long as citral was present near or in the nest entrance, whereas *M. rufiventris* workers were apparently indifferent to citral (Blum et al. 1970; Pompeu and Silveira 2005). Interestingly, a colony of *M. rufiventris* was also observed to successfully repel a real attack by *L. limao* (Pompeu and Silveira 2005). The species-specific differences in the reactions to citral may contribute to the known host preferences of the cleptobiotic bees (Blum et al. 1970, Michener 1974), but raids against host nests may also depend on their conditions, with weak colonies being more susceptible to attacks (Pompeu and Silveira 2005). In relation to both of these ideas, an interesting but as yet unanswered question is how robber bee foragers select the colonies they are about to attack. It could be that *Lestrimelitta* and *Cleptotrigona* workers have an innate preference for specific host species, but they may also be attracted to host odors they already know from previous raids, or even learn to avoid odors from species where they had experienced severe resistance by guards. There is good reason to believe that the volatiles emanating from the host nests are important for their localization by robber bees and probably also for their selection. Future research, monitoring which species are raided by single *Lestrimelitta* or *Cleptotrigona* colonies over longer periods of time, could help to answer this question.

The chemical compounds that account for the disruption of the social organization within raided nests remain to be identified. Citral seems to be a good candidate (Blum et al. 1970; Michener 1974), but its typical lemon-like odor was absent in most host colonies housed in observation boxes that were opened during robber bee attacks by Portugal-Araújo (1958) and Sakagami et al. (1993). Therefore, other volatiles that are released during raids should also be considered instead of citral. Furthermore, Sakagami et al. (1993) found indications that citral, which they released at the entrance of *Scaptotrigona* nests that were raided by *L. limao* workers, triggered the retreat of the robber bees and the mass return of host workers. Thus, citral probably signifies a “robber retreat message” to end a raid, rather than serving host disruption.

Aside from the obligate robber bees, several species belonging to the genera *Melipona*, *Scaptotrigona*, *Trigona*, *Tetragona*, *Tetragonisca*, and particularly *Oxytrigona* occasionally steal food from nests of other stingless bees and from colonies of *Apis mellifera* (von Ihering 1904; Schwarz 1948; Roubik 1983, 1989; Bian et al. 1984; Roubik et al. 1987; Rinderer et al. 1988; Nogueira-Neto 1997; Souza et al. 2007). *Oxytrigona tataira* is even regarded as a pest species by beekeepers in certain areas in northeastern Brazil (Souza et al. 2007). The mandibular glands of *Oxytrigona* workers are extensively developed (Kerr and Cruz 1961), and their caustic secretions are primarily used as a powerful defense against vertebrate predators due to the skin burns they produce when chewed into the cutis (Kerr and Cruz 1961; Wille 1961, 1983; Michener 1974). Head extracts of *O. mellicolor* and *O. daemonica* (Bian et al. 1984; Roubik et al. 1987) as well as mandibular gland extracts of *O. mediorufa* (Cruz-López et al. 2007) are unique in relation to other stingless bees due to the large numbers and high quantities of saturated and unsaturated mono- and diketones (2-heptanone, heptan-2,5-dione, 3-hepten-2-one, 5-hepten-2-one, *E*- and *Z*-3-hepten-2,5-dione, nonan-2,5-dione, 5-nonen-2-one, *E*- and *Z*-3-nonen-2,5-dione) they contain, in addition to several other compounds (hydrocarbons, carboxylic acids, carboxylic acid alkyl esters). Rinderer et al. (1988) conducted bioassays with several synthetic compounds, which were applied to rubber septa and introduced into the observation hives of honey bees, and found that *E*-3-hepten-2,5-dione and *E*-3-nonen-2,5-dione had a clear, but localized repellent effect on the bees on the comb. Furthermore, the honey bees' defensive behavior was significantly reduced when the diketones were presented in the nest compared to the presentation of clean rubber septa or septa treated with 2-heptanone, 2-decanone, 3-hepten-2-one, pentadecane, or with various acetate esters (all compounds previously identified from *Oxytrigona* head extracts; Bian et al. 1984). The effect of *E*-3-hepten-2,5-dione and *E*-3-nonen-2,5-dione on honey bee workers thus is similar to the observed absence of organized resistance against *Oxytrigona* foragers that invade *Apis* colonies in order to rob their resources (Bian et al. 1984). The latter observation is interesting, because a ketone, 6-methyl-5-hepten-2-one,

was also identified from *Lestrimelitta limao* head extracts (Wittmann et al. 1990; Francke et al. 2000), where it may serve the same function, i.e., as a repellent or appeasement allomone released inside host nests during raids (see above).

## CONCLUDING REMARKS

The studies reviewed on the preceding pages clearly show that chemicals derived from food sources or released by foragers are of the utmost importance for the localization and exploitation of food, as well as for the recruitment of stingless bees. Orientation toward food odors by foraging animals is surely an ancestral characteristic that can be found throughout the entire animal kingdom. Beyond this, in eusocial insects the odor of a food source, which is passed on from one worker of a colony to others, and which then biases the food-searching behavior of these individuals in the field, also functions as social information. However, we are only just beginning to understand the importance of food odors in this context, which is also true of odor cues left by visitors at food sources that influence a forager's decision whether to visit them. Exciting new studies with stingless bees and bumble bees have shown that the message conveyed by these volatiles is not fixed but depends on an individual bee's past experience. Since foraging efficiency is increased by the use of these chemical cues, similar mechanisms are also likely to be at work in other insects—social and solitary species alike. However, this remains to be studied.

Among all social insects whose workers forage on the wing, some species of stingless bees are unique by depositing pheromone spots on solid substrates at and around food sources, as well as along the route back to their nest. These pheromone marks are true signals involved in recruitment communication and strongly enhance the attraction of the recruits to a particular food source, increasing the efficiency of its exploitation. However, the use of pheromones to create continuous scent trails has to be questioned, as recent studies provide accumulating evidence for short odor trails that are basically deposited in the immediate vicinity of the food. Instead, the pheromone marks may function as a “strong and extended food odor,” which is easily encountered by recruits that search for it after leaving the nest. It can also contain information about the identity (at both the species and colony level) of other foragers that exploit a resource, which in turn could influence a forager's decision whether to land and feed on it. Only recently we have identified the actual glands that produce these pheromones and studied the chemical structure of some of the compounds. This, finally, allows us to address more specific questions. For example: How do recruits orient toward the deposited pheromones? Or how does the specific pheromone composition of foragers from different nests contribute to competitor avoidance? We already have a clearer idea of the roles played by volatile chemicals in many aspects of the foraging ecology of stingless bees, but there can be no doubt that many intriguing details await discovery in years to come.

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