Insect. Soc. 55 (2008) 417 – 424
0020-1812/08/040417-8
DOI 10.1007/s00040-008-1021-6

Insectes Sociaux

Research article

Bumble bee olfactory information flow and contact-based foraging activation

M.A. Renner and J.C. Nieh

University of California, San Diego, Division of Biological Sciences, Section of Ecology, Behavior, and Evolution, Mail Code 0116, 9500 Gilman Drive, La Jolla, CA 92093, USA, e-mail: michelle.a.renner@gmail.com

Received 4 September 2007; revised 30 May 2008; accepted 15 July 2008.
Published Online First 11 August 2008

Abstract. Nestmate foraging activation and interspecific variation in foraging activation is poorly understood in bumble bees, as compared to honey bees and stingless bees. We therefore investigated olfactory information flow and foraging activation in the New World bumble bee species, *Bombus impatiens.* We (1) tested the ability of foragers to associate forager-deposited odor marks with rewarding food, (2) determined whether potential foragers will seek out the food odor brought back by a successful forager, and (3) examined the role of intranidal tactile contacts in foraging activation. Bees learned to associate forager-deposited odor marks with rewarding food. They were significantly more attracted to an empty previously rewarding feeder presented at a random position within an array of eight previously non-rewarding feeders. However, foragers did not exhibit overall odor specificity for short-term, daily floral shifts. For two out of three tested scents, activated foragers did not significantly prefer the feeder providing the same scent as that brought back by a successful forager. Finally, bees contacted by the successful forager inside the nest were significantly more likely to leave the nest to forage (38.6 % increase in attempts to feed from empty feeders) than were non-contacted bees. This is the first demonstration that tactile contact, a hypothesized evolutionary basal communication mechanism in the social corbiculate bees, is involved in bumble bee foraging activation.

Keywords: Foraging, *Bombus,* bumble bees, foraging activation, olfactory information flow.

Introduction

Bumble bees (Bombini, Apidae, Hymenoptera) live in social groups in which foragers act largely as independent units, unlike other social bees such as honey bees and stingless bees which can recruit to specific food locations (Dornhaus and Chittka, 2004). However, a successful bumble bee forager returning to the nest can also activate nestmates to search for food (Dornhaus and Chittka, 2001). The study of such information flow can enhance our understanding of the evolution of recruitment communication and foraging specializations in the corbiculate bees.

Olfactory information flow plays a particularly important role in social insect foraging. Many ant species use odor trails to recruit nestmates (Hölldobler and Wilson, 1990), and in some species, such as *Acromyrnex lundi,* workers learn the odor of a food fragment brought back by a nestmate, and use this olfactory information in future foraging decisions (Roces, 1990). Recruiting honey bees can scent mark food sources, carry back food-scent into the nest, and produce a recruitment pheromone inside the nest (von Frisch, 1967; Thom et al., 2007). Stingless bees also use a wide range of olfactory communication strategies, including odor trails and localized scent marks (Lindauer and Kerr, 1958).

The extent of variation between bumblebee species in olfactory foraging activation is not known, largely because researchers have studied relatively few species. Although there are over 250 species of bumble bees inhabiting different arctic, palaearctic, nearctic, and tropical habitats (Goulson, 2003), recruitment information flow has been mainly studied in one European species, *B. terrestris* (Dornhaus and Chittka, 2004), with some work on the neotropical *Bombus transversalis* (Dornhaus and Cameron, 2003). In both species, a successful forager can increase the number of nestmates exiting the nest into a foraging arena (Dornhaus and Cameron, 2003; Dornhaus and Chittka, 2004). Odor marking has been studied in a broader range of species. Bumble bee species such as *B. terrestris, B. pascuorum,* and *B. vosnesenskii* can learn to associate nestmate-deposited odor marks with a food source (Cameron, 1981;

Stout et al., 1998; Goulson et al., 2001; Stout and Goulson, 2001). In *B. terrestris*, foragers produce deposit odor marks with their tarsal glands (Schmitt et al., 1991; Stout et al., 1998). Recently, researchers have shown that bumble bee scent marks are not inherently attractive or repellent (Schmitt and Bertsch, 1990; Goulson et al., 2001), but are learned to be attractive if revisits are rewarding and repellent when the revisits are not (Saleh et al., 2007). For example, *B. impatiens* foragers can use scent marks to reject flowers that required longer handling time (Saleh et al., 2006). We therefore tested the hypothesis (H1) that *B. impatiens* foragers are attracted to odor marks that they learn to associate with rewarding food.

In *B. terrestris*, nestmates can learn the food odor brought back by recently successful foragers and search for a food source with the same odor (Dornhaus and Chittka, 1999). We term this phenomenon "resource odor specificity." However, it is not known if other species share this ability. Bumble bees do not communicate specific food location (Jacobs-Jessen, 1959; Esch, 1967; Kerr, 1969; Dornhaus and Chittka, 2004). Thus, presumably the only information a forager can provide is the odor of a visited food source (H2: resource odor specificity hypothesis) and perhaps its quality, as conveyed through intranidal (within nest) behavior.

In addition, tactile contacts between individuals in social insect colonies may be an evolutionary basal form of information flow. This behavior is seen in many social insect groups, including ants, wasps, honey bees, stingless bees, and bumble bees (Hölldobler and Wilson, 1990; Rohrseitz and Tautz, 1999; Hrncir et al., 2000; Raveret Richter, 2000). In honey bees, Rohrseitz and Tautz (1999) demonstrated that antennal contact between waggle dancers and dance followers is important for information transfer. Antennal contact can provide information about the dancer's orientation, waggle run duration, body temperature, resource scent, and whether pollen was collected (Rohrseitz and Tautz, 1999).

Contact is also important in stingless bee recruitment. Several investigators have described a jostling behavior in which a successful forager makes contact with other bees inside the nest (Lindauer and Kerr, 1958; Kerr, 1960; de Bruijn and Sommeijer, 1997; Nieh, 1998). In *M. scutellaris* and *M. quadrifasciata*, the number of jostling behaviors by a recruiting forager correlates with an increase in recruitment to a feeder (Hrncir et al., 2000). *Scaptotrigona depilis* exhibits similar recruitment behaviors. Foragers made jostling contacts and engaged in trophallaxis after returning from rewarding food sources (Schmidt et al., 2006).

However, relatively little is known about the role of tactile contact in bumble bee foraging activation. Dornhaus and Chittka (2001) reported intense intranidal contacts between successful foragers and nestmates in *B. terrestris.* This is interesting because bumble bees do not exhibit trophallaxis, and thus contact with a successful forager does not include food sampling, as occurs in ants, stingless bees, and honey bees (Heinrich, 1979). Successful *B. terrestris* foragers run in a zig-zag motion combined with fanning bouts inside the nest (Dornhaus and Chittka, 2001; Oeynhausen and Kirchner, 2001). Fanning may disperse foraging activation pheromone (Dornhaus and Chittka, 2001). Chittka and Dornhaus (1999) have qualitatively observed similar behavior in the North American species, *B. impatiens* and *B. occidentalis.* However, the exact function of intranidal contacts and zig-zag running is unknown. Such excitatory movements and tactile contacts may activate or reactivate foragers to search for food outside the nest. We term this the contact hypothesis (H3).

We tested three hypotheses: (H1) foragers can deposit attractive odor marks on food sources, (H2) nestmates prefer to visit a feeder scented with the same odor as that brought back by a successful forager, and (H3) nestmates contacted by a forager have an increased probability of leaving the nest. For these experiments, we used a modified standard foraging arena design and observation nest that allows investigators to control foraging and monitor intranidal behavior (Schmitt and Bertsch, 1990; Dornhaus and Chittka, 2001).

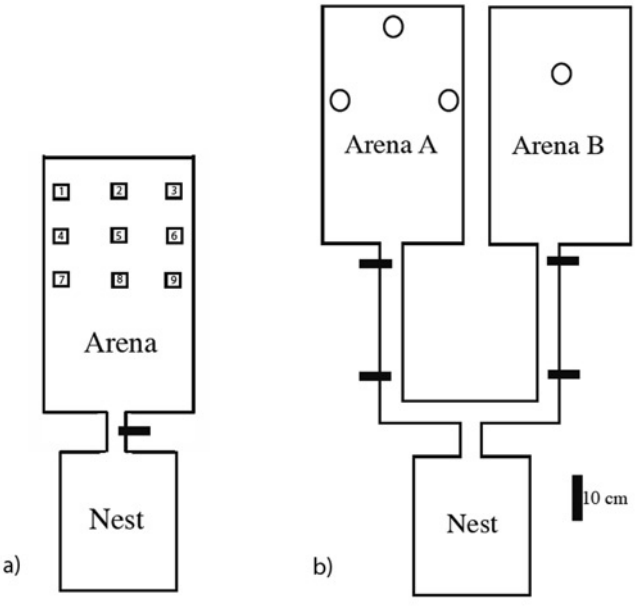

Figure 1. The flight arena and nest setups for the (a) odor marking experiment and (b) resource odor specificity experiment. The position of gates is shown as thick lines. (a) For the odor marking experiment, numbered squares show feeder positions. (b) For the resource odor specificity experiment, small circles indicate feeder position. We alternated the locations of the rewarding and test feeders between the flight arenas to control for potential arena bias.

Methods and materials

Colonies and study site

We conducted our experiments in a temperature-controlled (21°C) laboratory (N32°52.690′, W117°14.464′) on the UCSD campus, La Jolla, California, USA, and collected data from October 2005 to May 2007. *Bombus impatiens* is found throughout the Eastern United States and Canada, ranging from Ontario and Maine to Florida and west to Michigan, Illinois, Kansas and Mississippi (Heinrich, 1979), and a colony can contain up to 450 workers (Plath, 1934). We purchased our colonies from *Koppert Biological Systems* (Ann Arbor, Michigan), and *Biobest Biological Systems* (Ontario, Canada), successively using seven colonies of *B. impatiens.* The first four colonies contained approximately 200–300 individuals each and the latter three contained approximately 100 to 200 individuals each. We housed the bees in a wood nest box (32.5 x 28.4 x 15 cm, with a clear plastic cover) and allowed them to feed in one or two foraging arenas, depending upon the experiment (Fig. 1, design based upon Dornhaus and Chittka, 2001). The foraging arenas were clear plastic boxes (32 x 54 x 27 cm) with clear plastic lids inset with one mesh panel (25.5 x 21 cm) each to allow ventilation. We connected the foraging arenas to the nest boxes in different ways, depending upon the experiment (specified below).

General methods

We labeled each bee by capturing an individual and placing it in a vial (4.8 cm in height, 2 cm in diameter). Bees were chilled at 0°C for one min. To identify individuals, we attached a small numbered plastic tag (The Bee Works, Orillia, Ontario, Canada) to the thorax of foragers with cyanoacrylate adhesive (Kearns and Thomson, 2001). We placed each bee back into her nest after the glue fully dried (approximately one min).

We fed the bees 7 ml of freshly ground honey bee pollen every other day in 7 ml plastic Petri dishes inside the nest. All colonies were fed daily in the foraging arena with 1.5 M unscented analytical-grade sucrose prepared in double-distilled water. Colonies containing ≥ 100 bees received 14 ml and colonies containing ≤ 100 bees received 7 ml. If the subsequent experimental trial was to occur in less than 24 hours, we gave the colony, regardless of size, only 7 ml of sucrose solution to encourage foraging during the upcoming trial. Water was available at all times *ad libitum* from a filled 7 ml dish placed in the nest (changed every other day). We illuminated the flight arena with an incandescent lamp providing 12 hrs of light (0800–2000). Cotton was removed from around the brood cells and food pots to facilitate observations, and thus we enclosed the nest with a polystyrene foam cover to maintain colony temperatures and to exclude light when trials were not being conducted. We conducted only one trial per day to ensure an adequate amount of foraging activity for each trial. On days with no trials, bees could freely move into the foraging arena or arenas.

Scent marking experiment

We tested H1 by sequentially using four colonies (1–4), with the nest box connected to a single foraging arena (Fig. 1a). Before every trial, we connected a clean foraging arena to the nest with a vinyl tube (15 cm length, 3 cm diameter). Each feeder consisted of a transparent plastic block (3.3 x 3.3 x 1.3 cm) with a cylindrical well in the center (1.2 cm diameter, 0.5 cm deep) and yellow tape underneath the block to provide orienting color (Saleh et al., 2006). We chose this type of feeder to ensure the bees had adequate surface to deposit odor marks. To feed, a forager placed all of her legs on the feeder and extend her proboscis into the well. In the foraging arena, we placed nine of these feeders in a 24 x 16 cm grid (Fig. 1a). We used eight unrewarding feeders (containing distilled water in the training phase) and one rewarding feeder (containing unscented 1.5 M sucrose solution in the training phase). Each 1 hr trial was separated into 30 min training and 30 min experimental periods. Each 5 min in both periods, we moved the rewarding feeder to a new random position within the array. We did this to teach bees that odor marks, not feeder position, provide the most reliable information about food reward in the training period (Church, 2006, Saleh, 2007), and to prevent foragers from using information apart from feeder odor marks in the experimental period.

During the training period, we presented bees with nine feeders (one rewarding, eight not). On average, the rewarding feeder received 7.6 visits and each of the eight unrewarding feeders received 2.0 visits. In the experimental phase, we removed all remaining sucrose and water from the feeders. Thus, all feeders were empty but retained putative odor marks. We controlled access to the foraging arena with plastic shutters (Fig. 1a) and allowed only one bee at a time to enter the arena. We removed this forager with an aspirator after she chose a feeder (defined as placing her head over a feeder or touching a feeder with her proboscis). We recorded the choice of each tested bee only once (all bees were individually marked) and returned the removed bees to the nest at the end of the 1 hour trial.

Communication of resource odor experiment

For this experiment, we connected two foraging arenas, A and B, to the nest with clear tubing (5.5 cm diameter, Fig. 1b) and controlled access with plastic shutters (Fig. 1b). We used feeders with a larger capacity to maximize odor exposure (filled 7 ml plastic petri dishes with yellow tape underneath to assist visual orientation). Scented solutions contained 10 µl of almond, lemon, or peppermint extract per 30 ml of sucrose solution or water (McCormick, Hunt Valley, MD), as appropriate. We freshly prepared these scented solutions immediately before each trial. We took precautions to avoid odor cross contamination by wearing a different pair of new latex gloves and using clean instruments and feeder dishes for each odor preparation.

Odor preference control experiment. Because foragers may have different *a priori* odor preferences (different sensitivities to different odorants) and because odorant concentrations may not have been identical, we first performed a series of choice tests. We used unrewarding feeders (scented water) to avoid training bees to associate scent with a reward. We used only one foraging arena in this experiment and alternated between arena A and arena B to control for any potential arena effect (Fig. 1b). We placed three feeders in an equilateral formation (18 cm between each feeder) on a plastic tray. We filled each feeder with distilled water containing one of the three different scents and thus presented a choice among all three scents in each trial. The position of each scent was randomly selected at the beginning of each trial. Each trial lasted 1 hr. Once all feeders were in place, we allowed one bee into the foraging arena for 3 min and recorded her first choice (head or proboscis contact with a feeder). We then removed the forager with an aspirator and allowed another forager to enter the foraging arena. We held all used foragers in a box and did not return them to the nest until the end of the 1 hr trial. All bees were individually marked, and thus we tested a different bee each time. If a previously used bee entered the arena, we immediately captured her. To control for potential odor marking cues (such as cuticular hydrocarbons deposited by walking bees), we rotated the feeders counterclockwise after each forager choice within each trial. In addition, we replaced the scented water every 15 min to maintain a relatively consistent level of scent.

Communication of resource odor. We randomly determined the experimental scent for each trial (a 1 hr period in which the experiment was conducted each day) and placed one feeder filled with scented sucrose solution in the training arena. We switched the odors on a daily basis to simulate a natural situation of different floral species becoming available on different days. Only one focal forager was permitted to enter and re-enter the training arena, feed on the sucrose, and return to the nest. Throughout the 1 hr trial, we allowed this focal forager to freely go back and forth from the training arena. We used a different focal forager for each trial. We used the shutters to control access to both arenas (Fig. 1b). Five minutes after the focal forager returned to the nest for the first time, we allowed another bee (the next bee to exit the nest) to enter the choice arena. The 1 hr trial began when this first

naïve forager entered the choice arena. The setup in this arena was similar to the choice test, with a triangular formation of three scented, unrewarding (water) feeders. We numbered all bees in the colony and recorded the identity of all bees that had contact with either scented water or sucrose. Thus, we were able to record the choice behavior of naïve bees (bees that had never previously fed at a scented feeder) for a three-min period. We changed the scented water each 15 min. We only used naïve bees and recorded the choice of each naïve bee only once.

Nest contacts. We recorded all contacts between the focal forager and nestmates (recording the tag number of each contacted bee) whenever the focal bee returned from successfully foraging during a trial. We recorded the identity of all bees that left the nest to forage, defined as a bee making contact with a feeder. We counted the number of contacted and non-contacted bees and tested this against a null hypothesis expectation that contacted and non-contacted bees have an equal probability of entering the foraging arena and contacting a feeder after the return of the focal forager.

Statistical analysis

We used JMP (v. 5.1) for the ANOVAs and Microsoft Excel (v. 11.1.1) to conduct the χ^2 and G-tests. For the scent marking experiment, we used ANOVA to test the effect of time interval during a trial, colony identity, and distance of the chosen feeder from the nest on the arcsine transformed proportion of bees choosing the experimental feeder each 5 min (we used the arcsine transformation to convert the proportions from a binomial to a nearly normal distribution, Zar, 1984). We used the G-test to assess if contact with a focal bee affects naïve bees' motivation to forage as compared to non-contacted bees that made a choice. We used χ^2 tests for all other analyses.

Results

Scent marking experiment

In this experiment, we used four colonies and conducted 45 trials. We obtained 200 five-minute experimental intervals in which foragers made choices. In 70 five-minute intervals, no foragers made choices, and thus we did not include these intervals in our analysis. We scored each individual forager choice only once to avoid pseudoreplication (745 foragers used). The overall full model with all interactions is significant (*ANOVA*, full model $F_{15,185}$=3.06, p=0.0002, all interactions NS; three-factor model $F_{5,195}$=6.37, p<0.0001). There is no significant effect of time (*ANOVA,* $F_{1,195}$=2.02 , p=0.16) or feeder distance from the nest entrance (*ANOVA*, $F_{1,195}$=0.93, p=0.34) on the bees' choices. Thus, bees did not simply choose the closer feeders.

There is a significant colony effect (*ANOVA*, $F_{3,195}$=9.32, p<0.0001). Therefore, for the statistical analysis, we separated the colonies into two groups: Group 1 (Colonies 1 and 4) and Group 2 (Colonies 2 and 3, Tukey HSD, α=0.05, Q=2.59). There is no significant colony effect within Group 1 or within Group 2 (Tukey HSD, α=0.05, Q=2.59). In each group, foragers choose the rewarding feeder significantly more often than the unrewarding feeder (Fig. 2, Group 1: χ^2=18.8, df=1, p<0.001, Group 2: χ^2=388.1, df=1, p<0.001). Between groups, the difference is in the magnitude of the effect.

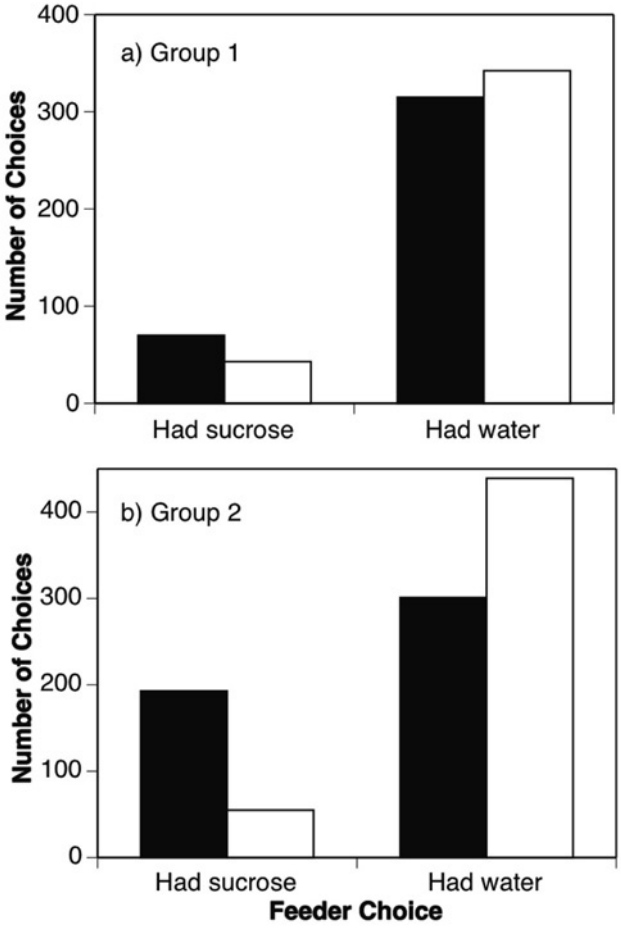

Figure 2. Forager choices in the scent marking experiment. Observed results (filled bars) and expected results (open bars) based upon an equal probability of choosing between the feeders. For both groups, significantly more bees (Group 1: p<0.001, Group 2: p<0.001) chose the previously rewarding feeder.

Feeders receiving more visitations during the odor-mark deposition phase were not more attractive compared to feeders receiving fewer visitations. In 29 half-hour training trials, we counted the number of foragers feeding on each feeder in the array. We find no significant effect of the number of feeder visits during the odor-mark deposition phase on the proportion of bees choosing the experimental feeder (Group 1: *ANOVA*, $F_{1,14}$=0.01, p=0.93, R^2<0.001; Group 2: *ANOVA*, $F_{1,12}$=1.48, p=0.25, R^2=0.11).

Resource odor specificity experiment

Control experiment. We tested 73 bees from three separate colonies for possible *a priori* scent preferences over 16 observation hours. Overall, we found that the bees show no significant preference any of the scents (χ^2=1.36, *df*=2, p=0.40, lemon: 37.7 %, almond: 24.5 %, peppermint: 37.7 %).

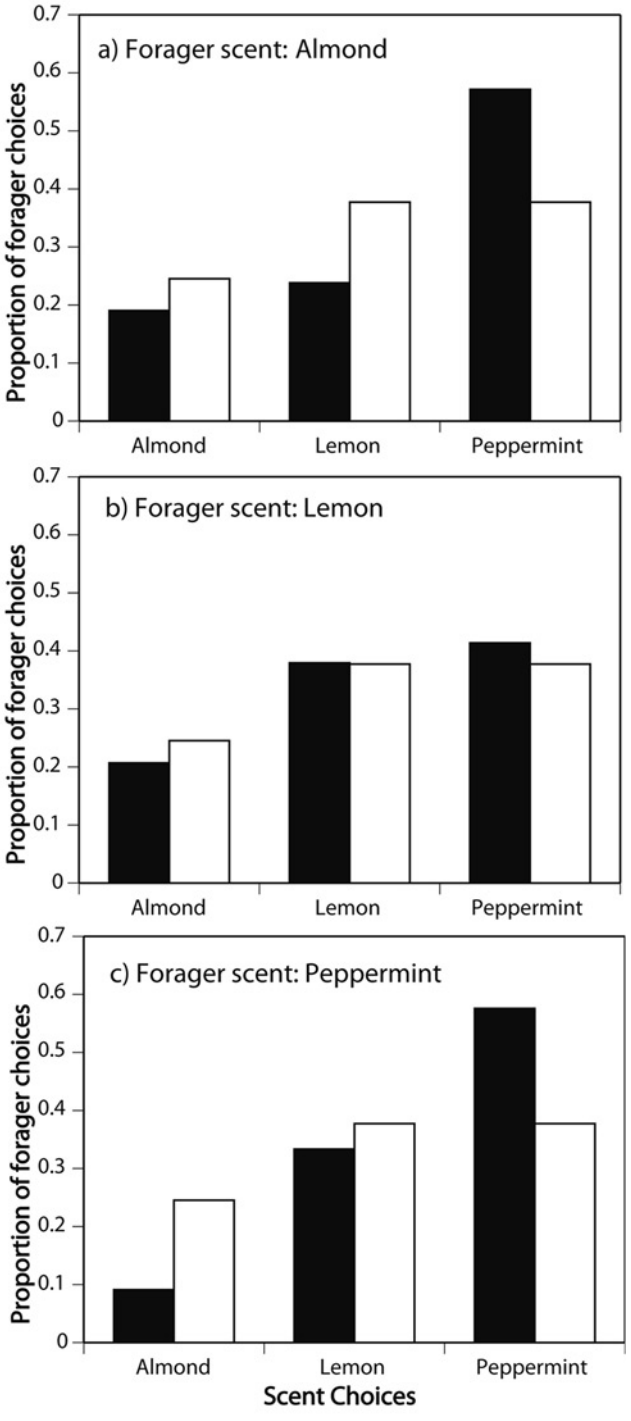

Figure 3. Results of the resource odor experiment. The bar graphs compare the observed and expected proportions of bees choosing each odor. Observed proportions are shown as filled bars. Expected proportions are calculated from the results of the scent-choice control experiments and are shown as open bars.

Communication of resource odor. We collected data during 37 hours of observation time using 47 bees. The distribution of choices do not differ significantly among the three scents (χ^2 test of independence, χ^2=3.22, df=4, p=0.52). We find significant differences in forager choices when peppermint (Fig. 3, χ^2=5.62, df=2, p=0.03) is the foraged scent, but not when the foraged scent is lemon (Fig. 3, χ^2=0.10, df=2, p=0.87) or almond (Fig. 3, χ^2=2.44, df=2, p=0.18, expected values based upon control experiment proportions for all χ^2 tests). When the focal forager brought back peppermint-scented sucrose, bees chose the peppermint-scented feeder at a higher proportion than lemon- and almond-scented feeders.

Within-nest contacts. After a successful forager entered the nest, she typically spent approximately half of her time depositing sucrose in a few honey pots. When not storing food, she ran around the nest in an erratic pattern, encountering multiple nestmates (Fig. 4). Different focal foragers did not make contact with the same nestmates upon their return to the nest. Significantly more contacted bees than non-contacted bees leave the nest and made a choice by touching one of the three feeders (G=29.5, df=6, p=0.01). Over all twelve trials, contact increased the probability of a forager leaving the nest and making a choice by an average of 38.5% over what we expected based upon feeder choice rate of non-contacted bees.

Discussion

Our results show that New World *B. impatiens* foragers share the ability of the European bumble bee, *B. terrestris*, to associate a scent mark with a rewarding food source. However, activated foragers did not exhibit odor specificity for short-term, daily floral shifts. Inside the nest, we found that direct contact between a successful forager and nestmates significantly increased the probability of the contacted nestmate going out into the foraging arena and trying to forage from a feeder. Over all trials, contact increased the probability of a forager leaving the nest and making a choice by an average of 38.5% over the departure and choice rate of non-contacted bees. This is the first evidence that physical contact between successful bumble bee forager and a nestmate increases the probability of nestmate departure.

Food source odor marking

We found that *B. impatiens* foragers could learn to associate forager-deposited odor marks with a rewarding food source. Foragers were attracted to feeders that were previously rewarding and visited by other nestmates. In our analysis, we separated the four colonies into two groups because of a significant inter-group colony effect that may be related to the respective size and life stages of these colonies. Colonies in Group 1 were older than colonies in Group 2. For example, colony 1 (group 1) contained multiple virgin queens, and thus was at a late

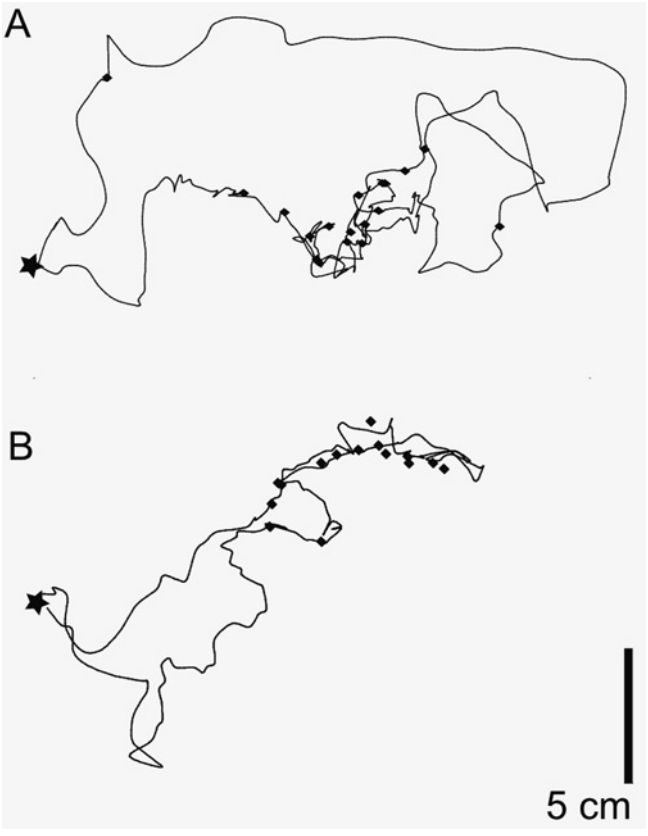

Figure 4. Paths taken by two different, randomly selected focal foragers (A and B) inside the nest after returning from a rewarding food source. Stars mark the nest entrance. Filled diamonds mark points at which the successful foragers contacted nestmates. Data obtained from digital video using Videopoint v2.5.0 software.

life stage (Heinrich, 1979). Group 1 colonies both contained less than 130 bees when we began running the trials (Colony 1 had 63 labeled bees, Colony 4 had 127 labeled bees). In comparison, the colonies in Group 2 were both large (Colony 2 had 166 labeled bees, Colony 3 had 157 labeled bees) and younger than Group 1 colonies.

The colony effect in the scent-marking experiment was not due to a greater number of visits to the feeders (during the odor-mark deposition phase) in larger as compared to smaller colonies. The colony effect may be due to a change in foraging strategies as colonies become smaller and older and thus forage less for food. It is important to note that this colony effect influenced only the degree of choice, not the bees' overall ability to choose, which is significantly biased towards the experimental feeder in both groups.

Our results agree with conclusions drawn by other researchers studying bumble bee scent marking. Cameron (1981) found that *B. vosnesenskii* foragers were attracted to rewarding and previously rewarding feeders as a result of odor marks left by previous foragers. In *B. terrestris, B. lapidaries* and *B. pascuorum*, extracts from bees' tarsal glands attracted foragers (Goulson et al., 2000). Saleh et al. (2006) demonstrated that *B. impatiens* foragers can facultatively learn to associate such odor marks to reject flowers that require longer handling time. Thus, our results support the idea that bumble bees can flexibly learn to positively or negatively associate forager-deposited odor marks depending upon their experiences at a food source.

Lack of resource odor specificity

We tested the resource odor specificity hypothesis (H2) with odors that changed on an approximately daily basis to match the natural situation of a colony that experiences different rewarding resources over time. Such exposure to multiple different floral odors over the life of the colony occurs in natural colonies (Free, 1962; Heinrich, 1976). Foragers may sense these food odors directly from nestmates or when they check honey pots for food storage levels (Dornhaus and Chittka, 2005). We were unable to complete reject H2 because nestmates exhibited a significant preference for peppermint, one out the three tested odors. The lack of preference for almond and lemon odor could have resulted from different response thresholds to these odors or from differences in odorant concentration. However, foragers had no significant *a priori* preferences for any of the tested odors in the control experiment, although we presented the odors at concentrations that bees clearly detected, eliciting strong orientation behavior (body and antennal movements) in the absence of forager-deposited odor marks. Thus, the effect of forager-carried odors on nestmate choices is relatively weak in *B. impatiens* when the odors change on a near-daily basis. A longer period of exposure may be necessary to elicit a strong foraging response. For example, Dornhaus and Chittka (1999) presented *B. terrestris* colonies with the same experimental scent for all trials, and used three colonies, each trained to only one of the three scents.

Thus, *B. impatiens* nestmates may be activated to a specific odor if it is being brought into the colony for a long period. However, our results show that foraging activation for a specific floral odor is not strong if this floral odor changes on an approximately daily basis. This lack of specificity for short-term floral shifts may be adaptive if it allows foragers to find new, unvisited food sources when the food being brought in by other foragers does not provide a long-term bonanza. Investigators have suggested that bumble bees do not need to communicate food location because each forager finds her own food (Heinrich, 1979; Dukas and Real, 1993; Kirchner and Towne, 1994). If individual foraging characterizes bumble bee foraging activation, then some species may not need to activate foragers to search for similar-smelling food sources, relying instead upon general foraging activation.

Species-specific differences may also account for the differences between our results and those of Dornhaus and Chittka (1999). *Bombus impatiens* is found in Eastern North American and *B. terrestris* inhabits Europe. In

addition, *B. impatiens* colonies have more workers on average than *B. terrestris*, but *B. terrestris* colonies have more reproductives on average than *B. impatiens* (Cnaani, 2002). Such interspecific differences may contribute to differences in foraging activation for specific resource odors or floral odor transfer within the nest.

Contacts increase the probability of nest departures

Although bumble bees do not perform trophallaxis, they evidently use other types of contact behaviors to recruit nestmates. Over all trials in this experiment, contact increased the probability of a forager leaving the nest and making a choice by an average of 39% over what we expected. Thus simple behaviors, such as contact by a successful forager, can increase the probability that the contacted bee will leave the nest to seek food.In the nest, a successful forager often visited the same honey pot multiple times during a trial. Successful foragers also ran inside the nest in a seemingly random pattern with periodic physical contact with nestmates (Fig. 4). Similarly, Jandt and Dornhaus (2006) reported that *B. impatiens* foragers distributed themselves non-randomly inside the nest in individual spatial fidelity zones. These erratic runs may be a way to increase contact with nestmates. Our observations are similar to the intranidal behaviors of *B. terrestris* (Dornhaus and Chittka, 2001).

In *B. terrestris* and *B. transversalis*, a returning forager also releases an alerting pheromone that activates the colony and results in increased forager nest exits (Dornhaus and Chittka, 2001; Dornhaus and Cameron, 2003; Granero et al., 2005). This food alerting pheromone is airborne and requires no tactile contact (Dornhaus and Chittka, 2001).

As in *B. terrestris*, *B. impatiens* releases a pheromone that increases foraging (Lin, in prep.). Although direct contact is not required to transfer food alert pheromone (Dornhaus, 2002; Dornhaus et al., 2003, Lin, in prep.), this contact behavior could facilitate pheromone dispersal. Interestingly, such contacts are also made by recruiting stingless bees (Hrncir et al., 2000) and dancing honey bees (Rohrseitz and Tautz, 1999). These intranidal contacts between successful foragers and nestmates may be one evolutionarily basal form of communication in the social corbiculate bees.

Acknowledgements

We would like to thank David Holway, David Woodruff, and anonymous reviewers whose comments have significantly improved our manuscript. We would also like to thank Anna Dornhaus for her advice on the experimental design and our wonderful research assistants, Haley Hunter-Zinck, Kim Fong, Prema Hampapur, Katie Wedemeyer, and Clarie Wainwright. This research was partially supported through the ORBS (Opportunities for Research in the Behavioral Sciences program) sponsored by NSF Grant IBN 0503468 and IBN 0545856.

References

Cameron S.A. 1981. Chemical signals in bumblebee foraging. *Behav. Ecol. Sociobiol.* **9:** 257–260

Chittka L. and Dornhaus A. 1999. Comparisons in physiology and evolution, and why bees can do the things they do. *Ciencia al Dia* **2:** 1–17

Church D.L. and Plowright C.M.S. 2006. Spatial encoding by bumblebees (*Bombus impatiens*) of a reward within an artificial flower array. *Anim. Cogn.* **9:** 131–140

Cnaani J., Schmid-Hempel R. and Schmidt J.O. 2002. Colony development, larval development and worker reproduction in *Bombus impatiens* Cresson. *Insect. Soc.* **49:** 164–170

de Bruijn L.L.M. and Sommeijer M.J. 1997. Colony foraging in different species of stingless bees (Apidae, Meliponinae) and the regulation of individual nectar foraging. *Insect. Soc.* **44:** 35–47

Dornhaus A. 2002. When do dances make a difference? Significance of honey bee recruitment depends on foraging distance. *Entomol. Gener.* **26:** 93–100.

Dornhaus A., Brockmann A. and Chittka L. 2003. Bumble bees alert to food with pheromone from tergal gland. *J. Comp. Physiol. A* **189:** 47–51.

Dornhaus A. and Cameron S. 2003. A scientific note on food alert in *Bombus transversalis. Apidologie* **34:** 87–88

Dornhaus A. and Chittka L. 1999. Evolutionary origins of bee dances. *Nature* **401:** 38

Dornhaus A. and Chittka L. 2001. Food alert in bumblebees (*Bombus terrestris*): possible mechanisms and evolutionary implications. *Behav. Ecol. Sociobiol.* **50:** 570–576

Dornhaus A. and Chittka L. 2004. Information flow and foraging decisions in bumble bees (*Bombus* spp.). *Apidologie* **35:** 183–192

Dornhaus A. and Chittka L. 2005. Bumble bees (*Bombus terrestris*) store both food and information in honeypots. *Behav. Ecol.* **16:** 661–666

Dukas R. and L.A. Real, 1993. Effects of recent experience on foraging decisions by bumble bees. *Oecologia* **94:** 244–246

Esch H. 1967. The evolution of bee language. *Scient. Amer.* **216:** 97–104

Free J.B. 1962. The attractiveness of geraniol to foraging honeybees. *J. Apicult. Res.* **1:** 52–54

Goulson D. 2003. *Bumblebees: their Behaviour and Ecology.* New York, Oxford University Press. 235 pp

Goulson D., Chapman J.W.and Hughes W.O.H. 2001. Discrimination of unrewarding flowers by bees; direct detection of rewards and use of repellent scent marks. *J. Insect Behav.* **14:** 669–678

Goulson D., Stout J.C., Langley J.and Hughes W.O.H. 2000. Identity and function of scent marks deposited by foraging bumblebees. *J. Chem. Ecol.* **26:** 2897–2911

Granero A.M., Sanz J.M., Gonzalez F.J., Vidal J.L., Dornhaus A., Ghani J., Serrano A.R. and Chittka L. 2005. Chemical compounds of the foraging recruitment pheromone in bumblebees. *Naturwissenschaften* **92:** 371–374

Heinrich B. 1976. The foraging specializations of individual bumblebees. *Ecol. Monog.* **46:** 105–128

Heinrich B. 1979. *Bumblebee Economics.* Cambridge, Massachusetts, Harvard University Press. 245 pp

Hölldobler B. and Wilson E.O. 1990. *The Ants.* Cambridge, Massachusetts, Belknap Press of Harvard University Press, 732 pp

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

Jacobs-Jessen U.F. 1959. Zur Orientierung der Hummeln und einiger anderer sozialer und solitärer Apiden. *Z. Vergl. Physiol.* **41:** 597–641

Jandt J.M. and Dornhaus A. 2006. Spatial distribution of bumble bees (*Bombus impatiens*) inside the nest: evidence for spatial fidelity zones among workers. *Proc. Int. Congr. IUSSI Washington D.C.*

Kearns C.A. and Thomson J.D. 2001. *The Natural History of Bumble Bees: a Sourcebook for Investigations.* Boulder Colorado, University Press of Colorado, 130 pp

Kerr W.E. 1960. Evolution of communication in bees and its role in speciation. *Evolution* **14:** 326–327

Kerr W.E. 1969. Some aspects of the evolution of social bees. *Evol. Biol.* **3:** 119–175

Kirchner W.H. and Towne W.F. 1994. The sensory basis of the honeybee's dance language. *Scient. Amer.* **270:** 74–80

Lindauer M. and Kerr W.E. 1958. Die gegenseitige Verständigung bei den stachellosen Bienen. *Z. Vergl. Physiol.* **41:** 405–434

Nieh J.C. 1998. The food recruitment dance of the stingless bee, *Melipona panamica. Behav. Ecol. Sociobiol.* **43:** 133–145

Oeynhausen A. and Kirchner W.H. 2001. Vibrational signals of foraging bumblebees (*Bombus terrestris*) in the nest. *Proc. Eur. Congr. IUSSI, Berlin*, pp 25–29

Plath O.E. 1934. *Bumblebees and their Ways.* New York, Macmillan Co. 201 pp

Raveret Richter M. 2000. Social wasp (Hymenoptera: Vespidae) foraging behavior. *Annu. Rev. Entomol.* **45:** 121–150

Roces F. 1990. Olfactory conditioning during the recruitment process in a leaf-cutting ant. *Oecologia* **83:** 261–262

Rohrseitz K. and Tautz J. 1999. Honey bee dance communication: waggle run direction coded in antennal contacts? *J. Comp. Physiol. A* **184:** 463–470

Saleh N. and Chittka L. 2007. Traplining in bumblebees (*Bombus impatiens*): a foraging strategy's ontogeny and the importance of spatial reference memory in short-range foraging. *Oecologia* **151:** 719–730

Saleh N., Ohashi K., Thomson J.D. and Chittka L. 2006. Facultative use of the repellant scent mark in foraging bumblebees: complex versus simple flowers. *Anim. Behav.* **71:** 847–854

Saleh N., Scott A.G., Bryning G.P. and Chittka L. 2007. Distinguishing signals and cues: bumblebees use general footprints to generate adaptive behaviour at flowers and nest. *Arthropod-Plant Interactions* **1:** 119–127

Schmidt V.M., Zucchi R. and Barth F.G. 2006. Recruitment in a scent trail laying stingless bee (*Scaptotrigona* aff. *depilis*): Changes with reduction but not with increase of the energy gain. *Apidologie* **37:** 487–500

Schmitt U. and Bertsch A. 1990. Do foraging bumblebees scent-mark food sources and does it matter? *Oecologia* **82:** 137–144

Schmitt U., Lübke G. and Franke W. 1991. Tarsal secretions mark food sources in bumblebees (Hymenoptera: Apidae). *Chemoecology* **2:** 35–40

Stout J.C. and Goulson D. 2001. The use of conspecific and interspecific scent marks by foraging bumblebees and honeybees. *Anim. Behav.* **62:** 183–189

Stout J.C., Goulson D. and Allen J.A. 1998. Repellent scent-marking of flowers by a guild of foraging bumblebees (*Bombus* spp.). *Behav. Ecol. Sociobiol.* **43:** 317–326

Thom C., Gilley D.C., Hooper J. and Esch H.E. 2007. The scent of the waggle dance. *PLoS Biol* **5:** e228

von Frisch K. 1967. *The Dance Language and Orientation of Bees.* Cambridge, Massachusetts, Belknap Press, 566 pp

Zar J.H. 1984. *Biostatistical Analysis.* Englewood Cliffs N.J., Prentice-Hall, 718 pp