ORIGINAL PAPER

# Tactile learning in resin foraging honeybees

Michael Simone-Finstrom · Joel Gardner ·
Marla Spivak

**Abstract** Honeybees harvest and use plant resins in a mixture called propolis to seal cracks and smooth surfaces in the nest architecture. Resins in the nest may be important in maintaining a healthy colony due to their antimicrobial properties. This study had two main objectives: (1) Provide initial insight on the learning capabilities of resin foraging honeybees; (2) analyze the sensitivity of resin foraging honeybees to tactile stimuli to elucidate its possible role as a mechanism behind resin foraging. The first objective provides insight into the phenotype of these bees as compared to other forager types, while the second creates a starting point for further work on behavioral mechanisms of resin foraging. Using tactile proboscis extension response conditioning, we found that resin foragers learned to associate two different tactile stimuli, the presence of a gap between two plates and a rough sandpaper surface, with a sucrose reward significantly better than pollen foragers. The results of differential tactile conditioning exhibited no significant difference in the ability of resin foragers to discriminate between smooth and rough surfaces as compared to pollen foragers. We also determined that the sucrose response thresholds (SRTs) of returning resin foragers were lower compared to returning pollen foragers, but both resin foragers and pollen foragers learned a floral odor equally well. This is the first study to examine SRTs and conditioning to tactile and olfactory stimuli with resin foraging honeybees. The results provide new information and identify areas for future research on resin collectors, an understudied foraging phenotype.

**Keywords** Apis mellifera · Propolis · Proboscis extension response · Conditioning · Response thresholds

Communicated by M. Giurfa

M. Simone-Finstrom (✉)
Department of Ecology Evolution and Behavior,
University of Minnesota,
100 Ecology Building,
St. Paul, MN 55108, USA
e-mail: simo0377@umn.edu

J. Gardner · M. Spivak
Department of Entomology, University of Minnesota,
219 Hodson Hall,
St. Paul, MN 55108, USA

## Introduction

The mechanisms that regulate the onset of foraging division of labor in honeybees (Apis mellifera) have been widely studied over the years (reviewed by Page and Erber 2001). However, research has largely been restricted to nectar and pollen foraging and has generally neglected other foraging tasks, such as resin foraging. Bee species around the world collect resins from the vegetative apices and other resin secreting structures of various plants (Roubik 1989; Crane 1990). Resin foraging honeybees return to the nest with a resin load on their corbiculae, and the resin is unloaded by other bees, mixed with wax, and placed within the nest interior (Meyer 1956). When the resin is used within a honeybee colony, it is known as propolis. Feral honeybee colonies, which typically nest in tree cavities, line the entire nest interior with a thin layer of this resinous mixture, creating a "propolis envelope" (Seeley and Morse 1976). The use of propolis, and particularly this propolis envelope, may have significant benefits for general immunity, colony health, and protection against hive diseases and parasites (e.g., Garedrew et al. 2002; Bastos et al. 2008; Simone et al. 2009; Simone-Finstrom and Spivak 2010).

While resin collection and use by honeybees is likely quite important in maintaining healthy colonies, little research has been conducted on this subject (for a recent review, see Simone-Finstrom and Spivak 2010). Only one known study over the last 50 years has attempted to determine some of the mechanisms involved in the control of resin use behavior. Nakamura and Seeley (2006) sought to determine how resin foragers decide whether to continue or to stop foraging for resins. This study revealed much about the behaviors of resin foragers and provided insight into the role these resin foraging bees play within a colony (Nakamura and Seeley 2006; reviewed in Simone-Finstrom and Spivak 2010). It is still unclear at the most basic level how individuals assess the need for resins in the nest and decide to start foraging. This is a particularly interesting question since there is no food reward associated with resin foraging.

Resin, as propolis, is typically deposited along all inner surfaces of a tree cavity by feral colonies to smooth the walls prior to comb construction, seal cracks and crevices, and reduce the size and number of nest entrances (Seeley and Morse 1976). This propolis "envelope" with its antimicrobial properties provides important immune benefits to honeybees (Simone et al. 2009; Simone-Finstrom and Spivak 2010). In modern hives, honeybees will smooth any rough surfaces on hive walls and reduce entrances, but largely deposit propolis on the edges of the wooden frames on which they build comb (Simone-Finstrom and Spivak 2010). Since the nest interior is mostly dark, bees mainly rely on non-visual senses to detect stimuli within the nest environment. A number of factors may stimulate bees to collect and apply resins, including the presence of light, air currents, humidity changes, and tactile cues in surfaces of the nest cavity. Recognizing that all of these stimuli may be important, we decided to begin our investigation by determining if resin foragers are particularly sensitive to tactile stimuli such as gaps, crevices and rough surfaces. The bees' antennae are an integral tool for this type of information assessment (Erber and Pribbenow 2001; Johnson 2008). Bees, and specifically some resin handlers and foragers, have been noted to detect crevices by inserting the antenna into gaps in nest architecture (Nakamura and Seeley 2006). It is possible that foragers determine the need for resins based on this kind of tactile stimuli. This idea holds true for other social insects, like some species of paper wasps (*Polistes fuscatus*) and termites (*Nasutitermes costalis*, *Coptotermes formosanus*) that have been noted to detect nest damage and select building sites using antennation (Jones 1980; Downing and Jeanne 1990; Downing 1992; Lee et al. 2008).

The ability of honeybees to detect and learn various tactile stimuli is well-established (i.e., Kevan and Lane 1985; Erber et al. 1998; Scheiner et al. 2005). Much of this research has been done using classical conditioning of the proboscis extension response (PER). For PER conditioning, a bee is restrained and presented with a tactile stimulus. During the presentation the bee is given a sugar reward to elicit the PER. The tactile stimulus is presented repeatedly after specific time intervals. When the bee has learned to associate the sugar reward with the tactile stimulus, she will extend her proboscis in anticipation of the coming reward. Much of this research has been done comparing nectar and pollen foragers or with pollen foragers alone and typically use a series of small metal plates with indented grooves serving as the tactile stimulus (Erber et al. 1998; Scheiner et al. 1999, 2001, 2005). While tactile information may be relevant for pollen and nectar foragers with respect to identifying appropriate food sources (as suggested by Kevan and Lane 1985), resin foragers may directly use tactile information within the nest environment to initiate resin foraging behavior.

This study had two main objectives: (1) provide initial insight on the learning capabilities of resin foraging honeybees; (2) analyze the sensitivity of resin foraging honeybees to tactile stimuli in order to elucidate its possible role as a mechanism behind resin foraging. The former would open a window into the phenotype of these bees as compared to other forager types, while the latter may provide a starting point for further work on this important, yet understudied, group of bees.

The initial step was to conduct the first studies on PER conditioning with resin foragers. Specifically, we aimed to determine if resin foragers are more sensitive to the presence of a gap as compared to pollen foragers, and if any observed differences would also be seen for conditioning to an odorant. We followed this by performing an analysis of sucrose response thresholds (SRTs) of resin and pollen foragers, as a bee's SRT is correlated with learning performance (e.g., Scheiner et al. 1999, 2001). Finally, we utilized differential tactile conditioning using a smooth and rough substrate to determine if resin foragers are better able to discriminate between tactile cues as compared to pollen foragers. This is the first study to examine the SRTs and conditioning to tactile and olfactory stimuli with resin foraging honeybees.

## Materials and methods

### Animals

General methods for proboscis extension response conditioning to the tactile stimulus followed those by Erber et al. (1998). Similar methods were used for conditioning to the odor, but followed those previously used (Masterman et al. 2000, 2001). Resin and pollen foragers were collected from

colonies by closing the entrance of the colony and trapping the bees in cages as they returned with their respective corbicular loads. Pollen foragers were chosen for these experiments because they have been shown to learn tactile stimuli better than nectar foragers on average (Scheiner et al. 1999, 2001). Therefore, if differences exist between resin foragers and good learners (i.e., pollen foragers), we would expect an even stronger, and more robust, difference between resin foragers and average learners (i.e., nectar foragers).

Bees were brought into the laboratory, chilled on ice and then restrained in soda straws with duct tape. After restraining, all bees were fed 2 μl of 2 M sucrose solution by touching the antennae with the solution to elicit proboscis extension for feeding. Bees that did not respond with proboscis extension in this initial feeding were not used in the learning trials. To ensure that the individuals for testing would be acclimated to the restraints and not satiated prior to the learning trials, the bees were placed in a humidified incubator at 34°C for 1.5 h. For the sucrose response threshold assay, restrained bees were fed water until satiation before being held in the incubator. After these bees were removed from the incubator, each bee was tested for its response to water. Any bee that extended its proboscis to water was allowed to drink until satiation to control for effects of thirst per previous experiments (Goode et al. 2006).

PER conditioning to tactile stimulus

In summer of 2008, active resin foragers ($n=83$) and pollen foragers ($n=88$) were collected from six unrelated colonies. Bees from one or two colonies were tested per day over a 3-month period (July through August). Approximately zero to four resin foragers could be collected over a 20–30-min period, and due to relative rarity of resin foragers, sample sizes among colonies were not equal. Just after the bees were restrained in the laboratory (as described above), their eyes and ocelli were occluded with black enamel paint to prevent visual inputs from interfering with learning based on tactile stimulation. To perform the learning trials, an individual bee was presented with a 1 mm gap created between two 3 mm×4 mm smooth copper plates. Therefore, the entire surface area including the two plates and the gap was 4 mm×7 mm. A gap, rather than an etched plate, was used in this study to simulate a stimulus that would more likely induce resin use in colonies since gaps less than 6.35 mm are generally filled with propolis rather than wax (Crane 1990). At the start of each trial, a bee touched the tactile stimulus with the antennae for 2–3 s, and then was fed with 0.4 μL of a 2 M sucrose solution for 2 s by touching the antenna with the sucrose solution to elicit the proboscis extension. The rare bees that spontaneously responded to first presentation of the tactile stimulus by extending the proboscis were removed from testing. If the proboscis touched the plates, they were cleaned with a cotton swab dipped in 70% ethanol and wiped dry. Six trials with a 5-min inter-trial interval were completed for each bee, and in each trial bees were rewarded with sucrose solution after the presentation of the tactile stimulus. The responses of each bee to the presentation of the tactile stimulus prior to receiving the reward were recorded as a 0 (no response) or a 1 (response). If a bee responded to the presentation of the tactile stimulus by extending her proboscis, then that bee had learned the association between the stimulus and the reward.

Since the collected data for each trial was categorical (1 or 0 indicating a response/learning or not for each bee at a given trial) and describes learning over time, the data were analyzed using a logistic growth curve analysis specifically described for associative learning data sets comparing two groups (see Hartz et al. 2001). PROC GENMOD in SAS (ver. 9.2) was used to produce generalized estimating equations (GEE) for the effect of forager type and colony.

PER conditioning to odorant

In July 2009, active resin foragers ($n=29$) and pollen foragers ($n=29$) were collected at the entrances of four unrelated colonies as described above. The floral odorant geraniol was used as the stimulus for PER conditioning following methods used previously in the lab (e.g., Masterman et al. 2000, 2001).

To perform the learning trials, a bee was placed in a constant neutral airflow for 30 s followed by the presentation of the odorant. During the 5-s odorant delivery, a syringe with 0.4 μL of a 2 M sucrose solution was touched to the bee's antennae to condition the bee to the sugar reward for 2 s. This was repeated for six trials with an inter-trial interval of 7 min. For each trial, we recorded whether or not the bee responded with a proboscis extension both when the odor was presented and the sucrose. The few bees that responded to geraniol prior to conditioning were not tested further.

The data was analyzed in the same manner as for the tactile PER conditioning using a logistic growth curve analysis specifically described for associative learning (see Hartz et al. 2001).

Sucrose response threshold assay

SRTs were determined for resin and pollen foragers in June 2009. Active resin foragers ($n=42$) and pollen foragers ($n=41$) were collected at the entrances of five unrelated colonies, different from those used in 2008, and restrained in the lab as described previously.

Sucrose concentrations tested were 0.1%, 0.3%, 1%, 3%, 10%, and 30% (w/v). To determine SRTs, a drop of each concentration was touched to the antennae in ascending order over a series of six trials with 5 min between trials. Upon presentation with the sucrose solution, if the bee responded by extending the proboscis, then it was scored as a 1. If no response occurred, the bee received a score of zero for that concentration. The lowest concentration that elicits a full proboscis extension response is a direct measure of an individual bee's SRT. The sucrose concentrations were transformed to log10 values creating a linear response relationship, following previously used protocols (Goode et al. 2006). The median SRTs for resin foragers and pollen foragers were compared using a Wilcoxon rank sum test.

Differential tactile conditioning

Differential tactile conditioning was utilized to determine if resin foragers were more able to discriminate between two tactile cues as compared to pollen foragers. Our choice to use this method was twofold: we have previously used this method for olfactory discrimination (Masterman et al. 2000, 2001) and this is a novel method for use with tactile discrimination. For the tactile stimuli, we needed to use two distinctly different materials since this type of learning trial is inherently more difficult than a simple learning acquisition test. As shown by Erber et al. (1998) bees exhibit a great deal of generalization between stimuli. Preliminary trials using the same gap from the learning trials versus other variants of the metal plates (e.g., smooth and etched plate) were unsuccessful; the bees were not able to discriminate well between any combinations. However, we found the bees were able to discriminate well between smooth and rough textured paper. The conditioned or rewarded stimulus (CS+) was a 2 cm square of general purpose sandpaper (3 M©, 60 coarse). The unconditioned or unrewarded stimulus (CS−) was a 2 cm square of the reverse, smooth side of the sandpaper. Since honeybees use propolis to smooth hive walls (Simone-Finstrom and Spivak 2010), a rough surface is potentially another biologically relevant stimulus with respect to resin foraging.

To perform the differential conditioning tests, active resin foragers ($n=26$) and pollen foragers ($n=29$) were collected at the entrances of three unrelated colonies in late August and early September 2009, and harnessed as in the odorant-conditioning experiment. To prevent the bees from seeing and using possible visual cues, the experiment was carried out in a dark room lit only by red light. Preliminary tests found that performing this experiment under red light was equivalent to occluding the bees' eyes and ocelli with black enamel paint (data not shown). Bees were presented with either the CS+ or CS− and allowed to feel it for 4 s. If

they were presented with the CS+, they were then given 0.4 µL of a 2 M sucrose reward. This was done in a pseudo-random sequence of 18 trials with each stimulus being presented a total of nine times with a 7-min inter-trial interval (presentations of each CS were in the following order: (+, −, −, +, −, +, +, −, +, −, −, +, −, +, +, −). A new square of sandpaper was used for each trial to prevent accumulation of possible olfactory cues transmitted by the bees. Proboscis extension responses were recorded for each presentation.

To analyze the data, discrimination scores (DS) were calculated for each individual by subtracting the total number of responses to the CS− (unconditioned stimulus) from the total number of responses to the CS+ (conditioned stimulus). A DS of 0 would indicate that the individual responded equally to both the CS+ and CS− and therefore did not discriminate between the two. A positive number ranging to 8 would mean that the individual responded more often to the CS+ than to the CS− suggesting that discrimination occurred. We also determined the DS for the final presentations of the CS+ and CS−, to examine differences between discriminatory abilities at a point where the individuals had significant experience with the two stimuli. DS data were analyzed using a Wilcoxon rank sum test and learning curves to the CS+ were analyzed using the previously described logistic growth curve analysis.

## Results

Tactile PER conditioning

The learning curves (Fig. 1) show the percentage of bees that learned to associate a 1-mm gap created between two 3 mm×4 mm copper plates with a sucrose reward at each trial for both forager types. Logistic growth curve analysis, used for accurate determination of differences in learning (see Hartz et al. 2001), indicated that resin foragers ($n=83$, average of 14 bees per six colonies) learned at a significantly higher rate compared to pollen foragers ($n=88$, average of 15 bees per six colonies) based on the negative value for the trial×forager type parameter estimate ($p=0.031$; refer to Table 1 for GEE model parameter estimates), and that there was no colony effect ($p=0.64$; refer to Table 1). The number of non-learners across forager types was not significantly different (Fisher's exact test: $p=0.9$), with 21.7% of the resin foragers and 34.1% of the pollen foragers not exhibiting any learning after six trials.

Olfactory PER conditioning

Logistic growth curve analysis based on the learning curves (see Fig. 2) indicated that resin foragers ($n=29$, average of
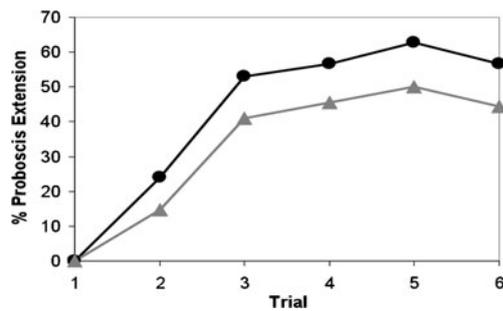
Fig. 1 Percentage of bees that responded to the tactile stimulus, a 1-mm gap created between two 3 mm×4 mm smooth copper plates, with a proboscis extension response at each trial for resin foragers (*filled circle*) and pollen foragers (*filled upright triangle*). Based on logistic growth curve analysis (Table 1 and see text) resin foragers ($n=$ 83, average of 14 bees per six colonies) learned at a significantly higher rate compared to pollen foragers ($n=88$, average of 15 bees per six colonies; $p=0.031$)



Fig. 2 Percentage of bees that responded to geraniol, the olfactory stimulus, with a proboscis response at each trial for resin foragers (*filled circle*) and pollen foragers (*filled upright triangle*). There were no significant differences between the rate of learning ($p=0.71$; Table 2) between resin foragers ($n=29$, average of seven bees from each of four colonies) and pollen foragers ($n=29$, average of seven bees from each of four colonies)

seven bees per four colonies) and pollen foragers ($n=29$, average of seven bees per four colonies) learned the odorant at equivalent rates ($p=0.71$; Table 2). There was a significant colony effect ($p=0.01$; Table 2), with one of the four colonies having lower learning rates for both pollen and resin forgers as compared to the other colonies. However, this difference did not influence overall differences due to forager type.
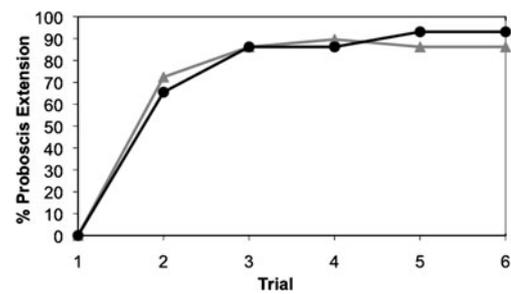
Sucrose response threshold assay

The SRTs were identified for each bee by determining the lowest concentration (transformed to log10 values) that elicited a full proboscis extension response. The median SRT for resin foragers ($n=42$, average of eight bees per five

colonies) was −1 (lower quartile, −1; upper quartile, −1) and ranged from −1 to −0.52). The median SRT for pollen foragers ($n=41$, average of eight bees per five colonies) was −1 (lower quartile, −1; upper quartile, −1) and ranged from −1 to 1.48. Most bees of both forager types responded to the lowest concentration of sucrose, resulting in the low SRTs. However, the range of SRTs was greater in pollen foragers as compared to resin foragers, which resulted in resin foragers having significantly lower SRTs on average than pollen foragers based on a Wilcoxon rank sum test ($Z=2.03$, $p=0.043$; Fig. 3).

Differential tactile conditioning

To analyze the ability of resin foragers and pollen foragers to discriminate between a smooth and a rough (sandpaper)

Table 1 Results of the logistic growth curve analysis of the responses of resin and pollen foragers to tactile PER conditioning to a gap between two metal plates (Fig. 1)

| Parameter | Estimate | Standard error | $p$ |
|---|---|---|---|
| Intercept | 2.83 | 0.30 | <0.0001 |
| Trial | −0.39 | 0.08 | <0.0001 |
| Colony | −0.13 | 0.09 | 0.16 |
| Trial×colony | −0.01 | 0.02 | 0.64 |
| Trial×forager type | −0.12 | 0.06 | 0.03 |

The $p$ value for each parameter is the probability that the respective parameter is zero. The intercept value is the point at which the logistic curve turns upward. The trial value is the rate at which resin foragers learn. The trial×forager type is the difference between the rate of learning between the two groups. A negative value indicated that pollen foragers learned significantly more slowly than resin foragers

Table 2 Results of the logistic growth curve analysis of the responses of resin and pollen foragers to olfactory PER conditioning to the odorant geraniol (Fig. 2)

| Parameter | Estimate | Standard error | $p$ |
|---|---|---|---|
| Intercept | 0.49 | 0.83 | 0.56 |
| Trial | 0.61 | 0.50 | 0.22 |
| Colony | 1.38 | 0.70 | 0.05 |
| Trial×colony | −1.08 | 0.42 | 0.01 |
| Trial×forager type | −0.07 | 0.20 | 0.71 |

The $p$ value for each parameter is the probability that the respective parameter is zero. The intercept value is the point at which the logistic curve turns upward. The trial×forager type is the difference between the rate of learning between the two groups

tactile surface a DS was calculated for each bee by subtracting the total number of responses to the CS− (unconditioned stimulus) from the total number of responses to the CS+ (conditioned stimulus). One non-responder, a resin forager, and one colony with low sample size (five bees) were removed from all analyses. For this analysis, the DS could range from −9 to 8. A higher positive number would indicate that the bees discriminated well, while a negative value would be indicative of poor discrimination. The median DS was 1.5 (lower quartile, 1; upper quartile, 3) for resin foragers ($n=26$, average of eight bees per three colonies) and 1 (lower quartile, 0; upper quartile, 3) for pollen foragers ($n=29$, average of eight bees per three colonies). These DS values were not significantly different (Wilcoxon rank sum, $Z=0.69$; $p=0.48$).

We followed the initial test by calculating the DS for resin and pollen foragers using just the final presentations of both the CS+ and the CS− when the ability to discriminate between the stimuli should have been most apparent. Using these final two trials, the DS could range from −1 to 1. A DS of 1 would indicate that the bee responded only to the CS+ and thus exhibited discrimination between the two stimuli. A DS of 0 would indicate that the bee extended its proboscis to both the CS+ and the CS− and therefore was unable to discriminate between the two stimuli. Resin foragers had a higher median DS compared to pollen foragers (1 [lower quartile: 0, upper quartile: 1] versus 0 [lower quartile: 0, upper quartile: 1]), respectively), but this difference was non-significant (Wilcoxon rank sum, $Z=1.79$; $p=0.072$).

However resin foragers had a significantly steeper learning curve to the CS+ based on logistic growth curve analysis ($p=0.042$; Table 3), meaning they more readily learned to associate the CS+ of rough sandpaper to the sucrose reward compared to pollen foragers (Fig. 4a, b). This result confirms results of the earlier tactile conditioning experiment using a gap as the CS+.
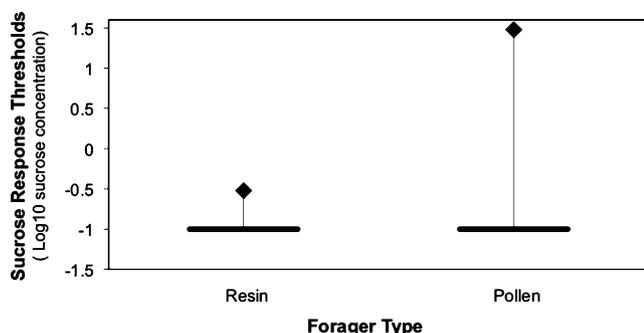


**Fig. 3** Median SRT. Resin foragers ($n=42$, average of eight bees per colony) had significantly lower SRTs on average as compared to pollen foragers ($n=41$, average of eight bees per colony; $p=0.043$). The *bold horizontal lines* represent the medians and the maximum values are represented by the *diamonds* (see text for quartile values)

**Table 3** Results of the logistic growth curve analysis of the responses of resin and pollen foragers to the CS+ (rough paper) during differential tactile PER conditioning (Fig. 4)

| Parameter | Estimate | Standard error | $p$ |
|---|---|---|---|
| Intercept | 1.46 | 0.79 | 0.06 |
| Trial | −0.74 | 0.30 | 0.01 |
| Colony | 0.02 | 0.34 | 0.96 |
| Trial×colony | 0.09 | 0.12 | 0.48 |
| Trial×forager type | −0.23 | 0.11 | 0.045 |

The $p$ value for each parameter is the probability that the respective parameter is zero. The intercept value is the point at which the logistic curve turns upward. The trial value is the rate at which resin foragers learn. The trial×forager type is the difference between the rate of learning between the two groups. A negative value indicated that pollen foragers learned significantly more slowly than resin foragers

## Discussion

### Learning by resin foragers

The results yielded new information about the resin forager phenotype and demonstrate areas for further research on the stimuli that honeybees use to initiate resin foraging. Specifically, we found that resin foragers have lower SRTs and learned to associate two tactile stimuli (a gap and a rough surface) with a sucrose reward at a significantly faster rate as compared to pollen foragers. In contrast, resin foragers did not learn an olfactory stimulus (the floral odor geraniol) better than pollen foragers. When presented with the tactile challenge of discriminating between a rough and smooth surface using a discrimination conditioning assay, resin foragers had a higher discrimination score than pollen foragers by the last presentation of each stimulus, but the difference was not significant ($p=0.07$). Honeybees typically generalize a great deal across tactile stimuli (Erber et al. 1998), and so this learning task in particular is quite difficult. It is possible that using other types of tactile stimuli could enhance the differences seen between forager types.

Differences in response thresholds, learning, and discrimination between bees of different forager type have been shown previously for nectar and pollen foragers (i.e., Scheiner et al. 1999, 2001, Drezner-Levy et al. 2009), but this is the first study to examine sucrose responsiveness and learning with resin foraging honeybees. Various studies have determined that pollen foragers on average learn tactile and olfactory cues better than nectar foragers due to their typically lower SRTs (Scheiner et al. 1999, 2001, 2004). It has been hypothesized that these differences in SRTs between the different types of foragers (namely water,
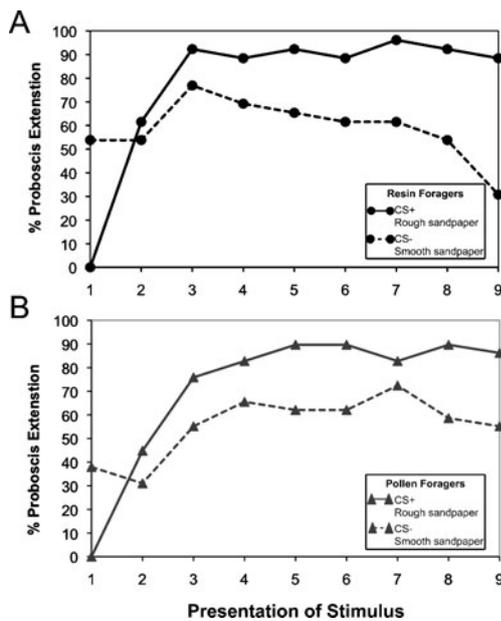
**Fig. 4** Tactile discrimination conditioning by resin foragers (**a**, *filled circle*) and pollen foragers (**b**, *filled upright triangle*). Percent proboscis extension response (% PER) over 18 trails (nine trials for each tactile stimulus), when rough sandpaper was the CS+ and smooth sandpaper was the CS−. Sample sizes: resin foragers, $n=26$, average of eight bees per three colonies and pollen foragers, $n=29$, average of eight bees per three colonies. Based on a logistic growth curve analysis (see Table 3 and text), resin foragers learned the CS+ at a faster rate compared to pollen foragers ($p=0.046$)

nectar, and pollen) have a strong influence on reinforcing division of labor within a hive and ensuring that the colony has the appropriate number of foragers for each task (Page and Erber 2001). SRTs can vary with age, foraging experience, and season (Pankiw and Page 1999; Pankiw et al. 2001; Scheiner et al. 2003). We found that the SRTs of returning resin foragers were slightly but significantly lower compared to returning pollen foragers. We do not know the extent to which the resin forager SRT is modified by genetics and/or recent foraging experience. Resin foragers have been noted to switch to pollen, nectar, and water foraging (Meyer 1956; Nakamura and Seeley 2006) and it would be interesting in future studies to test the SRT of a marked resin forager after she has shifted to a different resource.

The lower response thresholds of resin foragers could have contributed to the differences in learning ability that we observed. If the sensitivity to low concentrations of sucrose was the sole mechanism underlying learning performances of the bees in this experiment, we would have expected resin foragers to learn the odorant more effectively as well. If in fact SRTs were not responsible for the differences seen in this experiment, this could raise a host of new questions on the mechanisms behind individual differences in learning. Investigations of the pollen-hoarding phenotype and that behavioral syndrome began with studies on SRTs and learning of selected lines of bees (e.g., Page et al. 1998; Pankiw and Page 1999; Pankiw et al. 2001; Roussel et al. 2009). These experiments provided much insight into the mechanisms of foraging for specific resources, and account for much of the empirical evidence for the theories behind division of labor among the foraging task force. Future experiments to better understand the mechanisms underlying the resin forager phenotype would similarly be benefited using strains of bees selected for high and low resin collection, much like the lines of high and low pollen-hoarding bees.

Cues for resin foraging

Based on our results, resin foragers were better able to learn two tactile stimuli (a gap and a rough substrate when each was presented as the CS+) as compared to pollen foragers. This finding was based on a large number of bees collected from a total of nine unrelated colonies over the course of two years of study. This study provides the first experimental insight on the possible cues used by resin foragers in initiating resin foraging. While other social insect species have been noted to be able to detect abnormalities of the nest structure and build around or fix them, this has not been studied to our knowledge in honeybees. Termites (*Zootermopsis nevadensis* and *Zootermopsis angusticollis*) have been noted to lay trails around material with sharp edges or crevices that have been introduced into the nest and actively recruit individuals to build around and bury the material (Stuart 1967). Other termite species (*N. costalis* and *C. formosanus*) are thought to use their antennae to scan the interior nest walls as a way to explore building sites (Jones 1980; Lee et al. 2008). The paper wasp *P. fuscatus* has also been known to base building decisions on antennation of various parts of the nest (Downing and Jeanne 1990; Downing 1994). But unlike these cases, in honeybees, a specialized set of foragers possibly use tactile information to determine whether or not to initiate foraging for a specific nest building material. Resin foragers may actively or passively patrol the nest interior sensing various tactile stimuli in order to make foraging decisions, as it appears pollen foragers do (Calderone and Johnson 2002; Johnson 2008). Keeping in mind that honeybees can substitute wax for resin and propolis (Meyer 1956; Crane 1990), it is particularly interesting to investigate the mechanisms behind why and how they collect resin.

Conclusions

This study is just the starting point for a new set of research questions on the sensory and behavioral mechanisms

underlying resin collection. It would be interesting to do further studies explicitly testing the learning capabilities of resin foragers as compared to other forager types (namely nectar and pollen foragers that are typically studied). This paper was a first glimpse into general learning by resin foragers and more detailed studies could be done to better understand these bees. It would be particularly beneficial to conduct a subsequent study on sucrose response thresholds and various learning tasks (e.g., Giurfa 2007) by matching resin foragers and other bees with similar thresholds. Additionally, the exploration of alternative tactile stimuli, particularly for future discrimination assays may provide further insight into the discriminatory abilities of these foragers. Future experiments using non-restrained bees in field and observation colonies should be also conducted to further examine the role that tactile stimuli may play in resin foraging and use.

Non-tactile cues are also likely to be involved in initiating resin-related behaviors in honeybees. Antennation alone could not only serve to detect the presence of rough surfaces or gaps but it is possible that bees do this to acquire chemosensory information concerning the presence or absence of propolis (de Brito Sanchez et al. (2007) suggests that honeybees can taste resin-related compounds). More likely, the presence of moving air or odors and changes in relative humidity within the nest may also initiate resin foraging behaviors (Crane 1990). Studies of building behavior in some other social insects suggest the importance of these cues. For example, leaf-cutter ants (*Acromyrmex ambiguus*) in laboratory nests will plug tunnels with leaf material when dry air is released into it (Bollazzi and Roces 2007). Termites (*Z. nevadensis* and *Z. angusticollis*) have also been shown to seal off gaps in the nest in relation to outside air currents (Howse 1966). These cues should be investigated, as they are possibly not mutually exclusive from tactile cues in stimulating resin foraging.

## References

Bastos EMAF, Simone M, Jorge DM, Soares AES, Spivak M (2008) *In vitro* study of the antimicrobial activity of Brazilian and Minnesota, USA propolis against *Paenibacillus larvae*. J Invertebr Pathol 97:273–281

Bollazzi M, Roces F (2007) To build or not to build: circulating dry air organizes collective building for climate control in the leaf-cutting ant *Acromyrmex ambiguus*. Anim Behav 74:1349–1355

Calderone NW, Johnson BR (2002) The within-nest behaviour of honeybee pollen foragers in colonies with a high or low need for pollen. Anim Behav 63:749–758

Crane E (1990) Bees and beekeeping. Cornell Univ, Press, Ithaca

de Brito SG, Ortigão-Farias JR, Gauthier M, Liu F, Giurfa M (2007) Taste perception in honeybees: just a taste of honey? Arthropod-Plant Inte 1:69–76

Downing HA (1992) Hole repair and the influence of learning on nest repair in the paper wasp, *Polistes fuscatus* (Hymenoptera: Vespidae). J Insect Behav 5:459–468

Downing HA (1994) Information analysis by the paper wasp, *Polistes fuscatus*, during nest construction (Hymenoptera, Vespidae). Insect Soc 41:361–377

Downing HA, Jeanne RL (1990) The regulation of complex behaviour in the paper wasp, *Polistes fuscatus* (Insecta, Hymenoptera, Vespidae). Anim Behav 39:105–124

Drezner-Levy T, Smith BH, Shafir S (2009) The effect of foraging specialization on various learning tasks in the honey bee (*Apis mellifera*). Behav Ecol Sociobiol 64:135–148

Erber J, Pribbenow B (2001) Antennal movements in the honeybee: How complex tasks are solved by a simple neuronal system. In: Cruse H et al (eds) Prerational intelligence: adaptive behavior and intelligent systems without symbols and logic, vol 1. Kluwer, Netherlands

Erber J, Kierzek S, Sander E, Grandy K (1998) Tactile learning in the honeybee. J Comp Physiol 228 A 183:737–744

Garedrew A, Lamprecht I, Schmolz E, Schricker B (2002) The varroacidal action of propolis: a laboratory assay. Apidologie 33:41–50

Goode K, Huber Z, Mesce KA, Spivak M (2006) Hygienic behavior of the honey bee (*Apis mellifera*) is independent of sucrose responsiveness and foraging ontogeny. Horm Behav 49:391–397

Giurfa M (2007) Behavioral and neural analysis of associative learning in the honeybee: a taste from the magic well. J Comp Physiol A 193:801–824

Hartz SM, Ben-Shahar Y, Tyler M (2001) Logistic growth curve analysis in associative learning data. Anim Cogn 4:185–189

Howse PE (1966) Air movement and termite behaviour. Nature 210:967–968

Johnson BR (2008) Global information sampling in the honeybee. Naturwissenschaften 95:523–530

Jones RJ (1980) Gallery construction by *Nasutitermes costalis*: polyethism and the behavior of individuals. Insect Soc 27:5–28

Kevan PG, Lane MA (1985) Flower petal microtexture is a tactile cue for bees. Proc Nat Acad Sci 82:4750–4752

Lee SH, Bardunias P, Yang RL (2008) Behavioral response of termites to tunnel surface irregularity. Behav Process 78:397–400

Masterman R, Smith BH, Spivak M (2000) Brood odor discrimination abilities in hygienic honey bees (*Apis mellifera* L.) using proboscis extension reflex conditioning. J Insect Behav 13:87–101

Masterman R, Ross R, Mesce K, Spivak M (2001) Olfactory and behavioral response thresholds to odors of diseased brood differ between hygienic and non-hygienic honey bees (*Apis mellifera* L.). J Comp Physiol A 187:441–452

Meyer W (1956) "Propolis bees" and their activities. Bee World 37:25–36

Nakamura J, Seeley TD (2006) The functional organization of resin work in honeybee colonies. Behav Ecol Sociobiol 60:339–349

Page RE Jr, Erber J (2001) Levels of behavioral organization and the evolution of division of labor. Naturwissenschaften 89:91–106

Page RE Jr, Erber J, Fondrk MK (1998) The effect of genotype on response thresholds to sucrose and foraging behavior of honey bees (*Apis mellifera* L.). J Comp Physiol A 182:489–500

Pankiw T, Page RE Jr (1999) The effect of genotype, age, sex, and caste on response thresholds to sucrose and foraging behavior of honeybees (*Apis mellifera* L.). J Comp Physiol A 185:207–213

Pankiw T, Waddington KD, Page Jr RE (2001) Modulation of sucrose response thresholds in honeybees (*Apis mellifera* L.): influence of genotype, feeding, and foraging experience

Roubik DW (1989) Ecology and natural history of tropical bees. Cambridge University Press, Cambridge

Roussel E, Carcaud J, Sandoz JC, Giurfa M (2009) Reappraising social insect behavior through aversive responsiveness and learning. PLoS ONE 4:e4197

Scheiner R, Erber J, Page RE Jr (1999) Tactile learning and the individual evaluation of the reward in honeybees (*Apis mellifera* L.). J Comp Physiol A 185:1–10

Scheiner R, Page RE Jr, Erber J (2001) The effects of genotype, foraging role, and sucrose responsiveness on the tactile learning performance of honeybees (*Apis mellifera* L.). Neurobiol Learn Mem 76:138–150

Scheiner R, Barnert M, Erber J (2003) Variation in water and sucrose responsiveness during the foraging season affects proboscis extension learning in honey bees. Apidologie 34:67–72

Scheiner R, Page RE Jr, Erber J (2004) Sucrose responsiveness and behavioral plasticity in honey bees (*Apis mellifera*). Apidologie 35:133–142

Scheiner R, Kuritz-Kaiser A, Menzel R, Erber J (2005) Sensory responsiveness and the effects of equal subjective rewards on tactile learning and memory of honeybees. Learn Mem 12:626–635

Seeley TD, Morse RA (1976) The nest of the honeybee (*Apis mellifera* L.). Insect Soc 23:495–512

Simone-Finstrom M, Spivak M (2010) Propolis and bee health: the natural history and significance of resin use by honey bees. Apidologie. doi:10.1051/apido/2010016

Simone M, Evans JD, Spivak M (2009) Resin collection and social immunity in honey bees. Evolution 63:3016–3022

Stuart AM (1967) Alarm, defense and construction behavior relationships in termites (Isoptera). Science 156:1123–1125