

Visual and chemical cues provide redundant information in the multimodal recruitment system of the stingless bee *Scaptotrigona mexicana* (Apidae, Meliponini)

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Abstract Multimodal communication plays an important role in pollination biology. Bees have evolved multimodal communication to recruit nestmates to rewarding food sources. Highly social bees can use visual and chemical information to recruit nestmates to rich food sources. However, no studies have determined if this information is redundant or has an additive effect such that multimodal information is more attractive than either modality presented by itself to free-flying bees. We tested the effect of two modalities, forager-deposited odor marks and the visual presence of foragers, on the orientation of stingless bee (*Scaptotrigona mexicana*) recruits. Our results show that odor marks alone were significantly more attractive than multimodal information, and that multimodal information was significantly more attractive than visual forager presence alone. Given the high olfactory sensitivity and limited visual acuity of insects, odor marks likely attracted recruits over a greater distance than the visual presence of nestmates. Thus, multimodal information in *S. mexicana* is redundant, not additive, in terms of orientation to food sources.

Keywords Recruitment · Multimodal communication · Odor marks · Visual cues

Introduction

Organisms use and produce multimodal information because such information has several advantages. Different information modalities vary in how long they persist in the environment, how far they can travel, and how resistant they are to environmental noise (Bradbury and Vehrencamp, 1998; Goodenough et al., 2010). Moreover, by combining information modalities, it is possible to increase the number of messages and thus the complexity of communication (Calvert et al., 2004).

Multimodal information can provide either redundant or non-redundant information. To determine if multiple modalities provide redundant information, one can present the information to the receivers alone (as a single modality) and in combination (multiple modalities, Partan and Marler, 1999). When multiple modalities are presented, redundant information will elicit the same response as information presented through a single modality. There will be no additive effect. Such multimodal redundancy can increase information reliability in noisy environments (Johnstone, 1996), or increase the probability of information reception (Leavens, 2007). In the wasp *Vespula germanica*, visual and odor cues provided together (live wasps inside transparent, perforated containers which allowed free foragers to see and smell conspecifics) were not significantly more attractive than odor (live wasps inside dark, perforated containers) cues alone (D'Adamo et al., 2000). Thus, relative to odor cues alone, visual and odor information is redundant in *V. germanica* foraging.

Conversely, non-redundant information will elicit a different or heightened response as compared to information presented through a single modality. For example, successful foragers of the ant, *Aphaenogaster albisetosus*, use multimodal signaling to recruit to prey. An attractant

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pheromone acts over a longer distance (2 m) and a vibratory signal (stridulation) acts over a shorter range to pinpoint the prey's location (Hölldobler, 1999). Such multi-component signals are widespread in ant communication (Hölldobler, 2010). In general, multimodal signals can improve locatability, helping receivers determine the location of the signaled resource (Bradbury and Vehrencamp, 1998).

The social corbiculate bees (bumble bees, stingless bees, and honey bees) employ a variety of sensory modalities to recruit nestmates to food sources: olfactory, tactile, visual, and vibratory (Barth et al., 2008; Biesmeijer and Slaa, 2004; Dyer, 2002; Gould and Gould, 1988; Nieh, 2004). For example, stingless bees are attracted to the visual presence of nestmates (visual local enhancement) on a food source (Slaa and Hughes, 2009) and to forager-deposited odor marks (Hrncir et al., 2004; Jarau et al., 2004; Lindauer and Kerr, 1958; Schorkopf et al., 2007). However, no studies of these bees have determined if visual and olfactory information at a food source is redundant or enhances receiver responses by using multi- and single-modality tests. We, therefore, investigated the relative roles of vision (nestmate visual presence on a food source) and forager-deposited odor marks on the choice behavior of *Scaptotrigona mexicana* recruits. Our goal was to gain a better understanding of how the two primary modalities of field-based information (vision and olfaction) influence recruits' choice behavior.

Materials and methods

Study site

The experiments were carried out at the campus of El Colegio de la Frontera Sur, in the city of Tapachula, Chiapas, Mexico, from October to December 2008 and August to December 2010. We used four colonies of *S. mexicana* housed in wood boxes. These colonies were transported from an apiary in which they were kept for at least 1 year. All four colonies were considered healthy and similar in strength as determined through visual inspection of adult population, brood and food reserves by an experienced keeper of stingless bees.

Bee conditioning

Five to ten paint-marked foragers were trained to collect 2.0 M unscented sucrose solution from a feeder located 10 m north of the colony (training phase). At this distance, other feeders were placed to evaluate olfactory and visual cues (test phase), as in the direction experiments of Sánchez et al. (2004) study; their arrangement varied according to the experiment (Fig. 1). We used one colony at a time.

Feeders

Each feeder consisted of a 2 cm high \times 6 cm diameter Petri dish, with nine equally spaced holes (1 mm diameter) drilled in its lid and covered with a perforated circular piece of filter paper through which a bee could extend its proboscis to feed (2.0 M unscented sucrose solution). This dish rested on a square polystyrene foam base placed on a table. We used four different types of feeders: odor-marked only feeder (OM, a feeder with filter paper that was odor-marked), a visually enhanced feeder (VE, a feeder surrounded by five pinned dead bees washed in hexane and with a clean filter paper), an odor + visual feeder (OV, a feeder with five pinned dead bees and an odor marked filter paper) and a clean control feeder (CF, with no pinned bees and a clean filter paper).

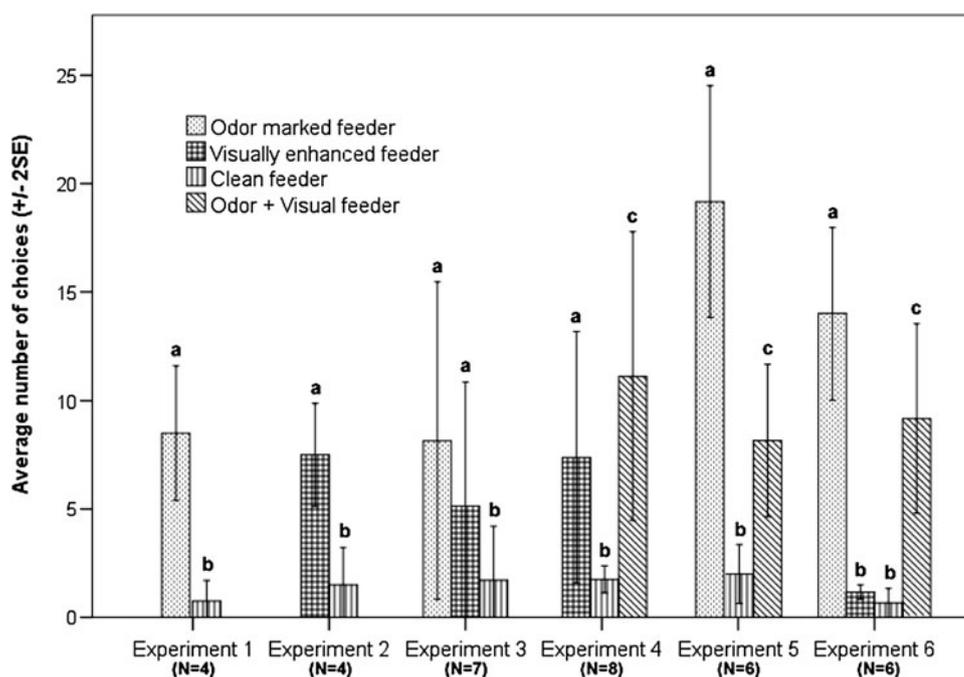
Scaptotrigona mexicana foragers deposit nestmate-attracting odor marks on rewarding food sources (Sánchez et al., 2008). To obtain an odor-marked filter paper, we placed it over an unscented sucrose feeder for one hour and collected odor marks from five bees (approximately 50 visits, Sánchez et al., 2008). Visually enhanced feeders had five bees inserted into the foam block around the feeder. To remove odors, each dried, dead bee was washed three times (each wash consisting of a 60 s immersion in hexane) and dried in an oven (40°C for 1 h) thoroughly before use. Feeders were spaced 20 cm apart, in a row perpendicular to the colony.

Experiments

We performed six experiments evaluating the choices of recruits (newcomers to the feeder). We conducted one trial per day, with each trial lasting 1 h. We trained a new set of foragers from each colony by gradually moving a training feeder from the nest entrance to the test location, 10 m from the nest. We registered only choices made by unmarked bees (newcomers). The first three experiments were designed to evaluate the effect of *individual modalities* (vision only or olfaction only): (1) OM + CF; (2) VE + CF, and (3) OM + VE + CF. In experiments 1 and 2, each bee chose between two feeders. In experiment 3, each bee chose among three feeders.

The next three experiments evaluated the effect of *multimodal information* (vision and olfaction): (4) OM + OV + CF; (5) VE + OV + CF and (6) OM + VE + OV + CF. In experiments 4 and 5, each bee chose among three feeders. In experiment 6, each bee chose among four feeders. We scored a choice as a bee landing on a feeder. To prevent the presence of other bees from influencing forager choice, a choice was only scored if no other bees were in the vicinity. To avoid pseudoreplication, all bees arriving to the experimental setup were trapped and marked with a color

Fig. 1 The average (\pm SE) number of bees that chose each of the feeders (treatments) in each experiment is shown. Within each experiment, statistically significant differences between treatments, are depicted with different letters (Dunn–Sidak multiple comparisons, where $\alpha = 1 - (1 - 0.05)^{1/c}$, based upon omnibus tests shown in Table 1). The letters only apply to comparisons made between attraction among different feeders within each experiment. The number of trials (N) for each experiment is shown



different from that used to mark the trained foragers. Thus, in our data, each choice was made by a different bee. In addition, we used unscented sucrose solution in these experiments. Thus, on odor-marked feeders, foragers’ odor marks provided the only olfactory information that recruits could use.

Statistics

Data from each experiment was analyzed separately with a Generalized Linear Model approach, assuming a negative binomial distribution and log-linking the data to the mathematical model. To further compare attraction to the different feeder types within each experiment, we applied Dunn–Sidak multiple comparison post hoc tests, where $\alpha_{DS} = 1 - (1 - 0.05)^{1/c}$. For these tests, we state that the results are significant if $P < \alpha_{DS}$.

Results

We recorded the choices of 3,034 recruits (806, 1272, 451, and 505 foragers from colonies 1–4, respectively). We allowed each bee to choose only once. Because colonies were depleted of naïve foragers after some experiments, colonies 1 and 2 were used for experiments 3, 4 and 6, and colonies 3 and 4 for experiments 1, 2 and 5.

The GLM was a good fit for all experiments. Omnibus tests (Chi-square based) were highly significant for all experiments (constructed model was significantly different to the intersection-only model) and the value/df ratio of the

Table 1 Model fitting parameters for each experiment

Experiment	Value/df ratio	Omnibus test
OM + CF	0.81	$\chi^2 = 11.85$, $df = 3$, $P = 0.008$
VE + CF	0.76	$\chi^2 = 10.39$, $df = 3$, $P = 0.016$
OM + VE + CF	1.07	$\chi^2 = 67.45$, $df = 4$, $P < 0.001$
VE + OV + CF	0.27	$\chi^2 = 65.23$, $df = 4$, $P < 0.001$
OM + OV + CF	0.41	$\chi^2 = 44.61$, $df = 4$, $P < 0.001$
OM + VE + OV + CF	0.40	$\chi^2 = 120.31$, $df = 5$, $P < 0.001$

deviance was between 0.27 and 1 (Table 1). Colony was not a significant factor (Wald Chi square < 3.35 , $df = 1$, $P > 0.05$) in experiment 1–4 and therefore was not considered in the GLMs for these experiments. Colony was a significant factor in experiment 5 (Wald Chi square = 7.89, $df = 1$, $P < 0.001$). Thus, colony was included as factor in the GLM for this experiment. In experiment 5, colonies differed in the magnitude of their attraction to the different feeders. However, the same pattern of attraction (odor-only $>$ odor + visual $>$ control, Fig. 1) was observed for all colonies.

In the single-modality experiments 1 and 2, recruits significantly preferred the odor marked (92%) or the visually enhanced (83%) feeder to the control feeder (Fig. 1; Table 1, $P < 0.05$). In the single-modality experiment 3, there was no significant difference between the number of recruits choosing the odor marked feeder or the visually enhanced feeder. However, both of these feeders were significantly preferred over the control (Fig. 1, $P < 0.017_{Dunn-Sidak}$).

In the multi-modality experiments (4, 5, and 6), recruits chose the odor marked-only feeder significantly more often than a multimodal feeder and significantly more often than a visually enhanced-only feeder (experiments 5: $P < 0.017_{\text{Dunn-Sidak}}$; experiment 6: $P < 0.013_{\text{Dunn-Sidak}}$). Thus, an odor-marked-only feeder was more attractive than any of the other options. Experiment 6 provided recruits with all four different options and showed no effect of visual-enhancement-only. There was no significant difference in attraction to the visually enhanced over the control feeder. In experiment 4, recruits chose among a multimodal feeder, a visually enhanced feeder, and a control feeder. In this case, the multimodal feeder was most attractive, the visually enhanced feeder was less attractive, and the control feeder was the least attractive (significant differences in attraction among all three feeders, $P < 0.017_{\text{Dunn-Sidak}}$).

Discussion

In *S. mexicana*, recruits use field-based information that consists of forager-deposited odor marks and the visual presence of nestmates on a food source (visual local enhancement, Sánchez et al., 2009, 2008) to reach advertised resources. This is in agreement with findings in other bee species (Nieh, 2004; Slaa et al., 2003). However, (1) odor marks were most attractive to recruits and (2) multimodal information did not have an additive attractive effect when compared to single-modality information. Thus, multimodal field-based recruitment communication is redundant in *S. mexicana*.

In our experiments, recruits consistently preferred a feeder that provided odor marks (alone or in conjunction with visual stimuli) over visual-stimuli alone and over the control feeder. In experiment 4, the multimodal feeder was more attractive than a single modality feeder (visually enhanced). The same result was found in wasps, *V. germanica* (D'Adamo et al., 2000). However, the results of experiments 5 and 6 show that the attraction to odor marks is overriding (Fig. 1). Thus, the greater attraction to the multimodal feeder in experiment 4 is most likely due to the presence of odor marks on this feeder, not due to the presence of multimodal information.

Bees are highly sensitive to odors (Hallem et al., 2006; Spaethe et al., 2007;), but have relatively limited visual acuity (Lunau et al., 2009). Thus, recruits should encounter odor marks before being able to resolve images of bees on the feeder. In the stingless bee, *Tetragonisca angustula*, foragers did not use their nest box as a visual landmark until they were approximately 1 m from the nest (Zeil and Wittmann, 1993) even though, a nest box provides a larger visual target than an individual bee. In the bumblebee, *Bombus impatiens* (which has a visual acuity somewhat

higher than that of honey bees) foragers could only resolve nectar guides separated by 1–11 mm when they were 4–45 cm away (Macuda et al., 2001). If *S. mexicana* foragers possess similar visual acuity to that of *B. impatiens*, they would likely have detected the odor marks deposited on the feeder before they could visually distinguish their nestmates. Stingless bees such as *Melipona panamica* can detect forager-deposited odor marks from 6 to 12 m away from the training site (Nieh, 1998). In *M. seminigra*, the minimum detection distance is at least 1 m away (Hrncir et al., 2004). Longer distance orientation is possible. Schmidt et al. (2003) found that *Scaptotrigona postica* recruits are attracted to forager-deposited odor marks up to 20 m away. Thus, *S. mexicana* recruits were likely exposed to odor marks over a longer distance and for a greater period of time than to visual information. This may account for their preference for an odor-marked-only feeder over a visually enhanced-only feeder.

In experiments 5 and 6, significantly more recruits chose a feeder with odor-marks-only over one with multimodal information (odor marks and visual presence of bees, Fig. 1). This might seem puzzling because one expects no difference under the hypothesis that odor marks and visual presence are redundant. However, in our experiments, the feeders were closely spaced (20 cm apart) and thus presented a patch in which portions of the patch had odor-marks-only and portions had both olfactory and visual information. In this situation, it is possible that odor-marks only were more attractive because they represented areas that were profitable to exploit, but not occupied by nestmates, reducing intracolony competition. A previous study with *S. mexicana* (Sánchez et al., 2008) showed that this behavior might be mediated by previous experience. To clarify this, future experiments could examine the innate preferences of *S. mexicana* foragers to determine if naïve forager are attracted to, avoid, or show no preferences when presented with odor marks or visual forager presence alone.

Under natural conditions, nestmates being recruited to a good patch would encounter information that acts over different spatial scales, both odor marks and the visual presence of nestmates. This multimodal information should increase the spatial precision of recruitment on different scales, drawing in recruits from several meters to investigate odor marks and then providing further, redundant confirmation (at the choice site) of the recruitment location within a patch through the visual presence of nestmates. For example, the multimodal feeder was more attractive than a single modality feeder (visually enhanced, Fig. 1). The same result was found in wasps, *V. germanica* (see Fig. 1 of D'Adamo et al., 2000) and this may be a general phenomenon in flying insects where olfaction provides longer-range information and relatively low visual acuity limits the ability to see nestmates on a resource. In general, further

studies of information redundancy and, in particular, tests of multimodal redundancy would enhance our understanding of the relative roles of chemical and visual communication in a wide variety of animals (Hebets and Rundus, 2011).

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