

Chemical Signals in Bumble Bee Foraging

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Summary. Foraging bumblebees (*Bombus vosnesenskii*) deposit a substance on rewarding flowers which assists in discrimination between rewarding and non-rewarding flowers in a controlled laboratory environment. Discrimination occurs while the bee is on a flower; workers probe rewarding flowers as well as empty ones that have rewarded in the recent past, but they do not probe flowers that have had no reward. Recognition is not the result of honey contamination left on the flower by the bee during feeding. The deposit is only slightly soluble in water or ethyl alcohol but is very soluble in pentane.

Introduction

The use of chemical signals in food collection is a widespread phenomenon among honeybees and stingless bees (Johnson and Hubbell 1974; von Frisch 1967; Ribbands 1955) and has recently been observed in the solitary *Anthophora* (Frankie and Vinson 1977). Yet this behavior has not been observed in primitively eusocial bees (Michener 1974). I have found that the bumblebee (*Bombus vosnesenskii*) leaves odors on rewarding flower models and thereby can distinguish these from non-rewarding models. Until now it has been assumed that no communication about floral resource occurs among bumblebees (Brian 1952; Free, in Butler 1951). I have found that

1) bumblebee workers discriminate between rewarding and non-rewarding flower models when on a model. They probe only models associated with rewards, including those that are presently rewarding and those that have been rewarding in the recent past. The latter are probed only for several visits after the reward ceases.

2) this behavior occurs with honey rewards which have odor, and sucrose rewards, which are odorless;

3) the apparent recognition cue is chemical, with some evidence that the cue is deposited on a rewarding model via body grooming that occurs after reward ingestion.

Materials and Methods

I observed the foraging of individual bumblebee workers collected from a single colony or at random near Berkeley, California. Bees were trained to forage in a foraging apparatus in the laboratory over a period of 1–2 days before they were used in experiments. The foraging apparatus consisted of a plexiglas flight box with rewarding flower wells that were refillable with microliter syringes and syringe dispensers. A drawing of this apparatus is shown in Fig. 1. I manipulated the quantity and concentration of reward

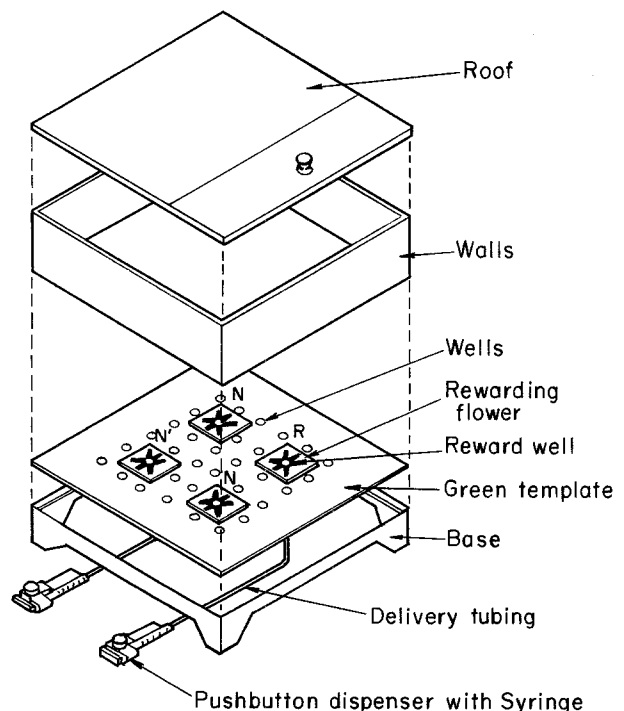


Fig. 1. Fully assembled foraging box

at artificial flowers that could be moved about the foraging 'field' in this apparatus as desired. Bees flew normally back and forth among four blue flowers spaced 22 cm apart at the corners of a square (31 cm diagonal distance), which were used for all experiments. The flour-flower configuration presented the foragers with a plausible foraging situation; in the field a bee probably does not choose from more than a small number of flowers at a time when it is moving from one flower to the next within a close range (Pyke 1978).

Results

Under symmetrical conditions (flowers equidistant and physically identical, rewarding 1 microliter of 50% honey/water solution with each visit) the bees treated all flowers approximately equally; the distribution of 799 visits in 4 experimental series using 17 bumblebees yielded 24.0%, 24.9%, 23.6%, and 24.9% visits to each flower, which does not significantly differ from equal treatment (χ^2 -test). The behavior is consistent with a random, or uniform foraging pattern. Given these results, would the bees treat the flowers differentially if some flowers were rewarding, others not?

I investigated this question, again using four equidistant flowers. One flower rewarded 1 microliter of 50% honey at each visit, while the other three gave no rewards but were scented by placing filter paper with 4–5 drops of honey around the well beneath the flower (out of sight and reach of the bees). The total number of visits was found to be very small; the bees visited the rewarding flower only 3–4% more often than the non-rewarding flowers ($28.6 \pm 0.01\%$ to rewarding flower; $P < 0.001$) (Fig. 2).

On the other hand, during these experiments the bees behaved differently on rewarding flowers; sometimes they turned clockwise, then counter-clockwise ('danced') on the rewarding flowers. They appeared to probe rewarding but not non-rewarding flowers. Therefore, I repeated the experiment, recording whether or not a bee probed when it landed on a flower. The results were strikingly different; they probed at rewarding flowers with a frequency of 98.9% of 48 visits and in none of 59 visits to non-rewarding flowers ($P < 0.001$). The bees were in fact strongly discriminating the rewarding from the non-rewarding flowers, not by preferential visits but by preferential probing. This indicates that the discrimination was occurring at close range. Similar experiments were conducted with a reward of 50% sucrose, a substance considered to be odorless to bees (Schwarz 1955; von Frisch 1967). The results were almost identical to those conducted with honey: bees probed the rewarding flowers 94% of 158 visits and the non-rewarding about 4% of 259 visits ($P < 0.001$). The bees thus appeared to be probing at a flower

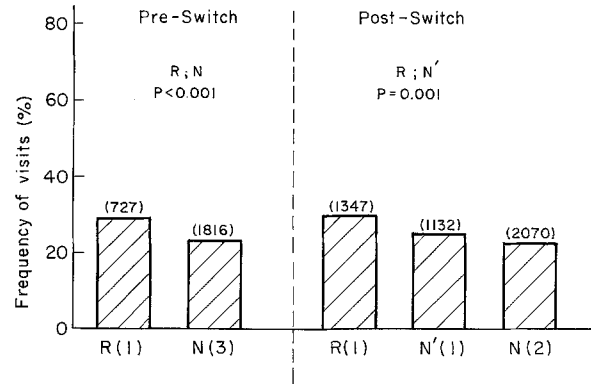


Fig. 2. Distribution of visits to rewarding (*R*), non-rewarding (*N*), and previously rewarding (*N'*) flowers during pre- and post-switch foraging, with scent pads at flowers. The rewarding flower contained 1 microliter 50% honey. Numerals in parentheses next to letters *R*, *N* and *N'* indicate number of flowers of each type that were available to the bees. Numerals on top of each bar indicate total number of visits made to respective flowers. Frequency of visits to *N* flowers represents the average of all visits made to the three non-rewarding flowers. Letters separated by semicolon, with their respective *P*-values (e.g., *R*; *N*, $P = 0.001$) indicate the significance of the results when one population (e.g., *R*) is compared with another (e.g., *N*). 20 bees were tested over 13 days

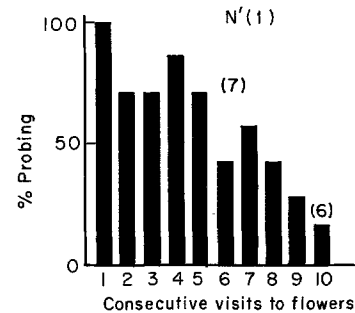


Fig. 3. Frequency of visits to *N'* that resulted in probing at the first 10 consecutive flowers visited, when sucrose was rewarding at the rewarding flower. Numerals indicate number of bees making consecutive probing visits

independent of the odor (or lack of odor) of the rewarding substance.

Was probing a response to a chemical stimulus (e.g. reward contamination, secretion by bee) left on the rewarding flower during previous visits? Were this the case, exchanging the rewarding flower (after being visited by the forager) with only one of the three non-rewarding flowers would be expected to cause the forager to probe this flower (the 'previously rewarding flower') in expectation of a reward. This experiment showed that bees in fact probed the previously rewarding flower 67% (of 46 visits) when honey had been the rewarding substance ($P < 0.001$) and 57% (of 153 visits) when sucrose had been rewarding ($P < 0.001$). In each case the never rewarding flowers were probed only 0–4%, while the rewarding ones

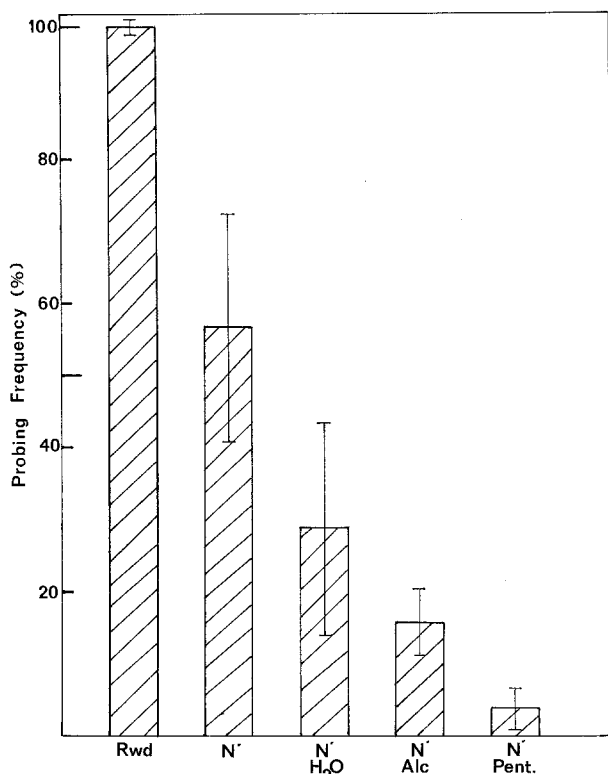


Fig. 4. Frequency of probing by bumblebees on differently treated flowers: rewarding (Rwd=98.0 ± 1.1%), previously rewarding (N' = 54.6 ± 15.5%), previously rewarding flower washed in water (N' H₂O=28.8% ± 14.6%), previously rewarding washed in ethyl alcohol (N' Alc=15.5 ± 4.8%), and previously rewarding washed in pentane (N' Pent=4.1 ± 2.8%)

received about 95% probing ($P < 0.001$). Furthermore, all bees probed on their first visits to the previously rewarding flower (N') after it was moved from the rewarding position (Fig. 3 shows the results for sucrose). Probing frequency decreased on subsequent visits and after 5 visits was 50% or less. By the tenth consecutive visit only about 20% of the bees continued to probe. Other experiments demonstrated that evaporation was not the cause of probing cessation.

The possibility that probing at the previously rewarding flower was the result of honey contamination left on the flower by the forager was tested by washing the rewarding flower in water before transferring it to a non-rewarding position. When the flower was washed in a large volume of deionized water the bees continued to probe it with significantly greater frequency than the non-rewarding flowers, 30% vs 0%, respectively ($P < 0.001$) although there was about a 20% decrease from that of the unwashed flower (Fig. 4). The same experiment was conducted with ethyl alcohol as the solvent. The results were similar: after the wash the bees continued to probe the flower with a frequency of 15.5%; this was significantly greater than probing of the non-rewarding flowers

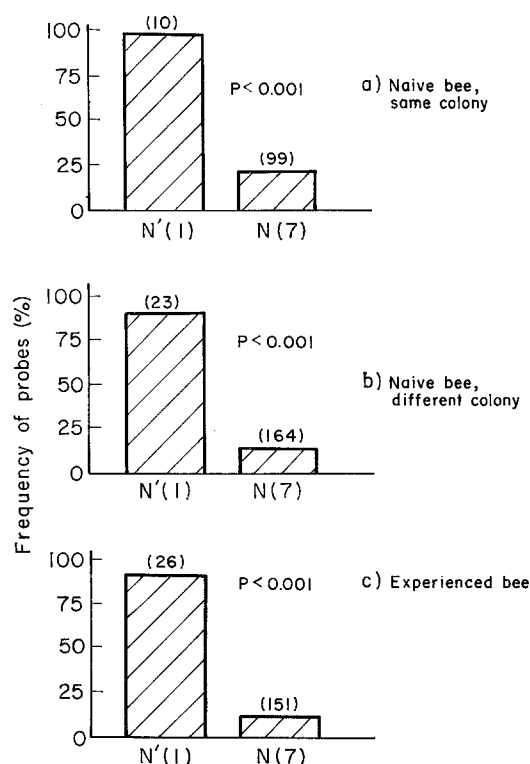


Fig. 5a-c. Frequency of probing previously rewarding flower (N') and non-rewarding flowers (N) by a naive bee, from same colony (4 bees; 2 days); b naive bee, from different colony (7 bees; 4 days); c experienced bee (4 bees; 2 days). Numbers of visits in parentheses above column

($P < 0.001$). Lastly, the rewarding flower was washed with pentane, a solvent for lipid-type compounds. Now, workers no longer probed preferentially at the pentane-washed flower ($P < 0.001$) (Fig. 4). Hexane also erases probing; this was the case when *B. terricola* and *B. impatiens* were tested in the same fashion as the previous experiments with *B. vosnesenskii*.

The foregoing evidence establishes that the deposit left on the rewarding flower is probably not reward contamination alone. Another experiment demonstrated that the bees did not orient by visual memory of the rewarding flower. New bees ($n > 10$), trained to forage but without previous exposure to the test system, were introduced immediately after the flower was exchanged; these bees probed it 90–100%, approximately the same as the initial forager (Fig. 5).

The experiments in which pentane was used as a solvent indicate that the reward cue may be a compound similar to pheromones secreted by *Bombus* (Svensson and Bergström 1977). A possible mechanism for deposition of a scent is grooming. A strong correlation was observed between grooming and probing on flowers. Workers groomed 52% on rewarding flowers as compared to 1.2% on non-reward-

ing flowers. Wax (and other) glands covering the surface of bumblebees could provide the source compounds which, during combing of the exterior portion of the body, could be transferred from bee to flower (Cederberg 1977).

Discussion

This study indicates that foraging bumblebees deposit an organic substance on rewarding flowers which assists in discrimination between rewarding and non-rewarding flowers. Thus bumblebees *can* use scent-marking, which affects subsequent foraging behavior. However, it is not known to what extent a chemical deposit is actually used by bumblebees in nature. These observations seem counter-intuitive when considered in the light of adaptive behavior: reprobating empty flowers would not be selectively advantageous. However, many species of flowers secrete nectar either continuously or cyclically, so that marking could be adaptive. The odor of the flower type may be passively communicated within the nest (Free 1970; Brian 1951; Cameron, experiments in progress) and marking could be confirmatory to 'recruiting' of foragers. It could thus reduce the time spent searching by naive foragers. Possibly the deposit is a signal whose message depends on the context; for example, to signal to nestmates areas of high-reward flowers (either whole plants or patches of plants) or to communicate the non-rewarding aspect of just visited flowers.

The fact that bumblebees deposit odors on a substrate has been demonstrated by Cederberg (1978). He found that bumblebees leave a pheromone in their nest tunnel, which they deposit as they exit. This same behavior has been demonstrated for honeybees and wasps by Butler (1969), who postulates a 'foot-print' pheromone. Flower marking may be viewed

as one of a number of different possibilities that allow bumblebees to maintain a high degree of flexibility in an ever-changing environment.

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