Honeybee, *Apis mellifera*, round dance is influenced by trace components of floral nectar

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The round dance and mutual feeding (trophallaxis) enable honeybees to transfer information concerning a food source, including its profitability. For nectar, which consists mainly of sugars, profitability is usually defined by its energetic value. Nectars, however, also contain a wide range of trace components, some of which affect their attractiveness. Honeybees produce honey from nectar. We compared the round dance and trophallaxis behaviours of bees foraging on avocado and citrus honey solutions, as a substitute for nectars. These sources differ in their trace-elements composition, with avocado nectar and honey containing higher concentrations of minerals than citrus nectar and honey. In a second experiment, we compared the behaviour of bees foraging on sucrose solution and sucrose solution enriched with four major mineral components of avocado nectar. Subjects foraging on avocado honey had a significantly lower probability of dancing than those foraging on citrus honey, a rate of direction reversals that was almost one half, a lower total number of reversals, shorter dance duration and longer trophallaxis time. When avocado honey was supplied to bees that previously fed on citrus honey, most of them avoided it, indicating a strong context effect. When foraging on mineral-enriched sugar solution, dance variables tended to be lower compared with sucrose solution without minerals, but differences were smaller than the differences between the honey solutions. These results show that nectar trace components affect the estimation of nectar profitability by bees and consequently recruitment of new foragers to nectar sources.

Keywords: *Apis mellifera*; avocado; citrus; context-dependent evaluations; honey; honeybee; minerals; *Persea americana*

The round dance of the honeybee is a mean of communication among bees that enables a forager to inform nest mates about nearby food sources. The number of direction reversals in a dance, dance duration and rate of reversals correlate with food profitability (Frisch 1967; Waddington 1982; Seeley et al. 2000) and affect the number of new recruits (Seeley & Towne 1992). Honeybees also engage in trophallaxis, during which information about food source value is communicated by the number of contacts with surrounding nest mates and duration and rate of unloading (Farina & Nunez 1991; De Marco & Farina 2001). Profitability is usually defined by the energetic value of the nectar source. Profitability of a nectar source is affected by its sugar concentration (Waddington 1982; Tautz & Sandeman 2003; Seeley 1994), its distance from the hive (Seeley 1994), distance between flowers (Waddington 1982) and flow rate (Wainselboim & Farina 2003).

The effect of energetic value on dance and trophallaxis behaviour has been studied using sugar solutions as a substitute for floral nectar. Floral nectar consists mainly of sugars, but also contains a wide range of trace components, which although comprising a minor fraction of the nectar, may affect its attractiveness to bees (Adler 2000). Frisch (1942) and Lindauer (1948) found that adding aversive components such as sodium chloride, hydrochloric acid, and quinine to sucrose solution reduced the probability of dancing. However, it is not known whether the presence of naturally occurring trace components in nectar, at naturally occurring concentrations, affects dance variables and trophallaxis behaviour, and thereby recruitment to the nectar source.
Avocado, *Persea americana*, nectar is an example of the importance of trace components in affecting pollinator evaluation (Afik et al. 2006a, b). In fact, when alternative nectar sources become available, honeybees abandon the nectar-rich avocado bloom in favour of the alternative blooms (Ish-Am & Eisikowitch 1998). We hypothesize that the presence of repelling trace components in nectar affects dance and trophallaxis behaviour, and consequently forager recruitment.

It is difficult to obtain sufficient amounts of floral nectar necessary for behavioural studies. Honeybees, on the other hand, gather large amounts of nectar and store it in the hive in a concentrated form as honey. Honey consists of carbohydrates (about 79%), water (about 17%), and trace components (about 4%), which include minerals, vitamins, organic acids, proteins, amino acids, alkaloids, phenols and others (White 1992). Afik et al. (2006a) found that the minerals content of avocado honey is similar to that of avocado nectar, and that of citrus honey is similar to that of citrus nectar. Since avocado nectar and honey is much richer in minerals than citrus, we compared dance and trophallaxis behaviour of bees foraging on these two sources. In a follow-up experiment, we tested the effect of four major minerals of avocado nectar by comparing between bees foraging on pure sucrose solutions and sucrose solution enriched with minerals.

**METHODS**

**Subjects**

A honeybee, *Apis mellifera ligustica*, colony was placed in a two-frames observation hive inside a flight room measuring 3.2 × 3.5 × 2.5 m in Rehovot, Israel. The flight room was illuminated by 36 fluorescent lamps, every third lamp connected to a different phase of a tri-phasic electric current, thus reducing flicker and allowing high levels of bee activity inside the room. Bees were kept on a 12:12-h light:dark cycle, with constant room temperature of 25 ± 2°C. The bees were trained to visit a feeder (a Petri dish) 2 m away from the hive, containing 30% w/w sucrose solution. The colony was fed a pollen patty twice a week and had access to a water source. Honey stores were kept low to maintain high levels of motivation for collecting nectar (Richter & Waddington 1993).

We used two honeybee colonies, one for the experiment with avocado and citrus honey, and one for the experiment with sucrose solution and mineral-enriched sucrose solution. The first colony was introduced into the flight room in February, 2005, before the avocado and citrus started to bloom, to ensure that foragers were naive to these blooms. The second colony was introduced into the flight room in October, 2006.

**Honey and Sucrose Solutions**

Avocado honey is characterized by its dark colour, high minerals content and high pH value (Terrab & Heredia 2004; Dag et al. 2006), yet the most accurate method to identify this honey is by its perseitol concentration (Dvash et al. 2002; Dag et al. 2006). Perseitol is a unique avocado carbohydrate, which comprises up to 6% of the nectar sugars (Ish-Am 1994; Liu et al. 1995), but is not detected by honeybees (Afik et al. 2006a). The honey samples that were used in this study were extracted from colonies that were located in avocado or citrus orchards. We analysed perseitol concentrations in the honeys by high-performance liquid chromatography (Dag et al. 2003). Honey from the avocado orchard contained 2.5% perseitol of the total sugars, and thus was defined as predominantly of avocado origin (Dag et al. 2006), whereas no perseitol was detected in the honey from the citrus orchard, which was defined as predominantly of citrus origin.

Honey solutions were prepared by diluting honey with distilled water to a total dissolved solids (TDS) of 60% w/w, measured by a hand refractometer (brix units). Even though the tested honeys contain mainly glucose and fructose (Dag et al. 2006), their refractive index is similar to that of sucrose (Kearns & Inouye 1993).

In the second experiment, we used a 60% w/w sucrose solution. For the ‘minerals’ solution, we added four minerals that are found in high concentrations in avocado nectar (Afik et al. 2006a). We added K$_2$HPO$_4$ (J. T. Baker, CAS NO: 7758-11-4; Deventer, The Netherlands) and MgSO$_4$ (J. T. Baker, CAS NO: 10034-99-8; Japan). Their concentration in the solution was similar to that found in 60% w/w diluted avocado honey: potassium, 2826 mg/kg; phosphate, 1121 mg/kg; magnesium, 154 mg/kg; and sulphur, 203 mg/kg.

**Experimental Procedure**

In the morning of an experiment, a feeder was filled with 30% w/w sucrose solution, and bees soon started visiting it. The feeder was then replaced by a new, identical one, filled with one of the tested solutions and was covered by a Plexiglas cage. Only one bee at a time was allowed to enter the cage and to imbibe from the feeder. The bee was colour marked before leaving the cage and the experiment began at the next visit of the marked bee to the feeder. Each bee was allowed up to five successive visits to the feeder. During the experiment with honey solutions, subjects that had completed five visits to the first honey feeder, were allowed up to five more visits to a second feeder containing the other honey solution. Of a total of 47 bees tested in both experiments, 42 completed the first five visits to the first feeder (avocado honey: N = 10; citrus honey: N = 10; minerals solution: N = 11; sucrose solution: N = 11). Testing of each subject ended when the bee accomplished a total of 10 (honey experiment) or five (sucrose experiment) visits to the feeder or if the bee did not return to the feeder for 15 min.

We measured the time that a subject spent imbibing from the feeder during every visit and the time spent inside the hive between visits. We videotaped subjects inside the observation hive after each visit to the feeder. We later analysed the videos and quantified several behaviours following Waddington (1982). (1) Probability of dancing: the number of visits to the hive after visiting...
the feeder in which a subject danced. A dance was defined as circular running on the comb for longer than 5 s without interruptions. (2) Total dance duration: the sum of all the times a subject spent dancing during one visit to the hive. It was common to have more than one dance during a single visit to the hive. (3) Number of reversals: the total number of directional changes in dances during a single visit to the hive. (4) Rate of reversals: the number of reversals per total dance duration. (5) Total trophallaxis time: the sum of all the mutual feeding durations during a single visit to the hive. (6) Experiment-wise rate of reversals: the total number of reversals in all dances during all visits to a particular honey source per duration of experimental phase with each bee. Duration of experimental phase was from beginning of the experiment with one of the honey sources until either five visits were completed or 15 min with no further visits had elapsed. This last measurement provided an estimate of the overall recruitment effort that the colony experienced to the honey source.

Data Analysis

When criteria for parametric tests were met we used t tests to compare between two means. Means are presented with standard errors. In most cases the distributions were not amenable to parametric tests and we used the nonparametric Wilcoxon two-sample test. To avoid pseudoreplication, we used the average of five measurements for each bee for each parameter, except for the experiment-wise rate of reversals, for which there was only one measurement per bee. Statistical analyses were performed using JMP 5.0.1 software (SAS Institute, Inc., Cary, NC, U.S.A.). In cases of marginal statistical significance, we performed power analysis to calculate the sample size needed to reject the null hypothesis with power = 0.80 and alpha = 0.05.

RESULTS

Effect of Order of Reward: Honey Experiment

Most subjects completed five visits to the first honey that they encountered, regardless of its source. Only two of 12 subjects that first visited avocado honey stopped visiting the feeder after one or two visits. Only one of 10 subjects that first visited citrus honey stopped visiting the feeder (after two visits) for more than 15 min. This subject later reappeared and completed five additional visits. However, whereas all 10 subjects that first made five visits to avocado honey then completed five visits to citrus honey, only two of 10 subjects that first made five visits to citrus honey then completed five visits to avocado honey (Wilcoxon two-sample test: Z = 3.43, N_\text{avocado} = N_\text{citrus} = 10, P < 0.001; Fig. 1). Six subjects did not return to the feeder after their first encounter with avocado honey, and four of these sampled the solution for only short durations (less than 17 s) during that visit. Thus, avocado honey was acceptable to bees upon first encounter but unacceptable after they had experienced citrus honey. Since bees completely or partially avoided the avocado honey when it was presented after the citrus honey, we did not have a sufficient sample size to assess most dance parameters in this case. We could still calculate the experiment-wise rate of reversals, which is independent of the number of visits of each bee to the feeder. Experiment-wise rate of reversals of subjects visiting avocado honey was higher for those that experienced avocado honey first than for those that experienced avocado honey as a second reward (Wilcoxon two-sample test: Z = 2.97, N_\text{avocado} = N_\text{citrus} = 10, P = 0.003; Fig. 2). No such order effect was evident in dances of subjects visiting citrus honey (Wilcoxon two-sample test: Z = 0.04, N_\text{avocado} = N_\text{citrus} = 10, P = 0.97). Thus, overall recruitment effort to avocado honey was reduced if the forager had previously encountered citrus honey.

Evaluation of Reward on First Encounter: Honey Experiment

Because subjects tended to avoid the avocado honey when it was encountered second, to compare how subjects evaluated avocado and citrus honey we focus on the first phase of the experiment, in which subjects first encountered these honeys. Feeding time at the feeder was 67.8 ± 4.3 s and was not affected by honey source (t test: t_{18} = 0.28, P = 0.79). The total duration time spent in the hive after each visit to the feeder was 222.3 ± 18.7 s when feeding on avocado honey and 185.9 ± 13.3 s for citrus honey (t test: t_{18} = 1.59, P = 0.13).

Subjects were more likely to dance after visiting citrus than avocado honey (Wilcoxon two-sample test: Z = 1.87, N_\text{avocado} = N_\text{citrus} = 10, P = 0.06; Fig. 3); eight of 10 bees that foraged on citrus honey danced after all five visits to the feeder, whereas only three of 10 bees that foraged on avocado honey danced after all five visits. The experiment-wise rate of reversals of subjects visiting citrus honey was significantly higher than that of subjects visiting avocado honey (Wilcoxon two-sample test: Z = 2.0,
In comparing the various dance variables, we only included data from visits to the hive in which the subject performed a dance. Dances of subjects visiting citrus honey had higher rate of reversals than those visiting avocado (Wilcoxon two-sample test: $Z = 2.25$, $N_{\text{avocado}} = 9$, $N_{\text{citrus}} = 10$, $P = 0.045$; Fig. 2), and tended to contain more reversals (Wilcoxon two-sample test: $Z = 1.92$, $N_{\text{avocado}} = 9$, $N_{\text{citrus}} = 10$, $P = 0.055$; Fig. 4b). Power analysis showed that statistical significance would likely be achieved with $N = 16$ bees in each group. Dances of subjects visiting citrus honey also tended to be longer, but the difference was not statistically significant (Wilcoxon two-sample test: $Z = 1.14$, $N_{\text{avocado}} = 9$, $N_{\text{citrus}} = 10$, $P = 0.25$; Fig. 4c).

Total trophallaxis time was greater after feeding on avocado than on citrus honey (Wilcoxon two-sample test: $Z = 3.47$, $N_{\text{avocado}} = 10$, $N_{\text{citrus}} = 9$, $P < 0.001$; Fig. 4d).

**Evaluation of Reward: Sucrose Experiment**

When foraging on sucrose solution only one of 12 subjects stopped visiting the feeder after one visit and when foraging on the minerals solution two of 13 subjects stopped visiting the feeder after three or four visits. Feeding time at the feeder was $77.4 \pm 2.8$ s and was not affected by the presence of minerals in the sucrose solution ($t$ test: $t_{19} = 0.61$, $P = 0.55$, a single bee that stayed on the ‘minerals’ feeder for longer than 3 min was excluded from this analysis). The total duration time spent in the hive after each visit to the feeder was $211.9 \pm 16.6$ s when foraging on the minerals solution and $177.0 \pm 11.7$ s for the sucrose solution ($t$ test: $t_{20} = 1.72$, $P = 0.1$).

All of the subjects danced in the hive after each of five visits to sucrose solution, and nine of 11 subjects danced after each of five visits to the minerals solution (Wilcoxon two-sample test: $Z = 1.38$, $N_{\text{sucrose}} = N_{\text{minerals}} = 11$, $P = 0.17$). The differences in dance performance and trophallaxis of bees feeding on minerals and sucrose solutions showed a similar pattern to the differences between bees feeding on avocado and citrus honeys. The experiment-wise rate of reversals of subjects visiting the sucrose solution was higher than that of subjects visiting the minerals solution (Wilcoxon two-sample test: $N_{\text{sucrose}} = N_{\text{minerals}} = 11$; $Z = 1.71$, $P = 0.088$; Fig. 2); this is statistically significant if we consider that based on the findings from the honey experiment, we expected a higher rate for sucrose than for minerals ($P = 0.044$, one tail). Power analysis showed that statistical significance with a two-tailed hypothesis would likely be achieved with $N = 35$ bees in each group. Differences in the other variables measured were in the hypothesized direction, but were not

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**Figure 2.** Experiment-wise rate of reversals in the honey (left pane) and sucrose (right pane) experiments. This measure is the total number of reversals in all dances during all visits to a particular nectar source per duration of experimental phase with each bee. Results are presented according to nectar source and order of presentation (avocado honey presented first and citrus honey second or vice versa). Horizontal bars represent medians, box plots represent interquartiles and whiskers represent minimum and maximum values. Circles represent outliers that differ from the median by more than 1.5 interquartiles range. ***$P < 0.01$.

**Figure 3.** The number of visits to feeders that were followed by dancing in the hive, during the first phase of the honey experiment, where naive subjects first encountered avocado ($N = 10$) or citrus ($N = 10$) honey.
**DISCUSSION**

Several choice experiments have shown that preference between nectar sources is affected by the presence of trace components (Waller et al. 1972; Hagler & Buchmann 1993; London-Shafir et al. 2003; Singaravelan et al. 2005; Afik et al. in press). Crop load (Frisch 1950; Afik et al. 2006b), learning performance (Abramson et al. 2000; Kim & Smith 2000; Afik et al. in press) and longevity (Majak et al. 1980; Allsopp et al. 1998) are also affected by trace components. The present study provides the first evidence that information about trace components in nectar is communicated to the colony by the returning forager.

The experiment-wise rate of reversals is the best measure of overall recruitment effort. It is similar to the measure used by Seeley & Towne (1992) and Seeley (1995) to assess the overall recruitment effort of the waggle dance, by the total number of waggle runs observed in the hive per time.

This measure correlates well with the number of foragers recruited. The experiment-wise rate of reversals was greater for citrus than for avocado honey, which probably contributes to the greater number of bees foraging on citrus than avocado honey of the same concentration of TDS in choice experiments (Afik et al. 2006b). This behaviour may also contribute to the rapid abandonment of foragers from avocado to citrus when the citrus starts to bloom (Ish-Am & Eisinowitz 1998). Similarly, the tendency for greater experiment-wise rate of reversals to a sucrose solution than to a minerals-enriched sucrose solution, may contribute to the greater number of bees foraging on citrus honey than minerals-enriched citrus honey of the same TDS concentration in choice experiments (Afik et al. 2006a).

The low attractiveness of avocado nectar is believed to be partly because of its high minerals’ concentration (Afik et al. 2006a). Potassium concentration in particular is exceptional, as it is higher than the honeybee haemolymph concentration (Nicolson & Worswick 1990), and might be harmful for bees. Furthermore, minerals in the nectar may interfere with the sensory mechanism of the bee and reduce its perceived sweetness. Enriching sucrose solution with similar concentrations of the four most abundant minerals in avocado nectar reduced its perceived value as...
measured by the dance, but to a lesser extent than the difference between avocado and citrus honey. Evidently, additional trace components in avocado nectar further contribute to its aversive taste. In addition, there may be synergistic effects between these minerals and some honey components, so that their effect is more pronounced in honey than when in a sucrose solution.

We correlated between various measures of the round dance in relation to the bees’ evaluation of solutions that differed in composition of trace components. We found that the dance encodes information on perceived attractiveness of the trace components of nectar, similar to information on perceived energetic profitability (Waddington 1982) and perceived pollen quality (pure or diluted with alpha-cellulose; Waddington et al. 1998). The probability of dancing to the avocado honey in our study was reduced relative to the citrus honey, and similarly, the probability of dancing to energetically less profitable alternatives and lower-quality pollen is reduced relative to more profitable alternatives (Waddington 1982; Waddington et al. 1998). As in these studies, the measure of the round dance that correlated best with source quality was the rate of reversals, followed by the number of reversals and dance duration.

Trophallaxis is another way of communication between nest mates that provides information about nectar profitability. When a foraging bee evaluates the nectar source as of low profitability it reduces unloading rate (Farina & Nunez 1991; Wainselboim & Farina 2003). We did not measure unloading rate directly, but trophallaxis duration was longer after visits to the avocado honey feeder. Since bees collect smaller volumes of avocado than nonavocado honey (Afik et al. 2006b), unloading rate of avocado honey in our study was probably slower than that of citrus honey. This would reflect on the low subjective evaluation of the avocado honey.

It is now well established that evaluations are context dependent (Shafir 1994; Shafir et al. 2002, 2003; Wainselboim & Farina 2003). We found a strong background context effect in the bees’ subjective evaluation of the avocado honey. Whereas naïve subjects visited both food alternatives, after foraging on the citrus honey most subjects rejected the avocado honey. Similarly, Richter & Waddington (1993) found that whereas naïve subjects visited a feeder offering 20% sucrose solution, most subjects rejected this alternative after having foraged on 60% sucrose solution.

Furthermore, rate of reversals in the round dance after visiting a particular concentration of sucrose solution was lower for subjects that had previously encountered a higher sucrose concentration than for those that had first encountered a lower concentration (Richter & Waddington 1993). The lack of dances to the least attractive alternative in that study (20% sucrose concentration) and in ours (avocado honey), after having encountered an attractive alternative, made it difficult to analyse variables of individual dances in these cases. We calculated the experiment-wise rate of reversals, as a measure of the total number of reversals that potential recruits would experience while the foraging alternative was available. Recruitment effort to the less attractive avocado honey dropped to almost null if subjects had first foraged on the more attractive citrus honey. The experiment-wise rate of reversals to citrus honey was not affected by whether or not subjects had first foraged on avocado honey. Similarly, Richter & Waddington (1993) found a greater decrease in rate of reversals when switching from a high to low rewarding solution than an increase in rate of reversals when switching from a low to a high rewarding solution.

In conclusion, trace components in nectar seem to have a major effect on its subjective evaluation. This subjective evaluation is reflected in the communication between the returning forager and potential recruits in trophallaxis and dance performance. The response is graded. Initially, dance and trophallaxis behaviours are affected, followed by a reduction in the probability of dancing, and finally the abandonment of the nectar source. Thus, in addition to nectar volume and its sugars concentration, the composition of nectar trace components seems to be important in modulating floral visits by pollinators.

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References


