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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.

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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 $3.2 \times 3.5 \times 2.5$ 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 naïve 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_2HPO_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