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Trophallaxis in forager honeybees (*Apis mellifera*): resource uncertainty enhances begging contacts?

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Abstract Trophallaxis among adult worker honeybees is the transfer of liquid food by mouth from one individual to another. Within the colony, nectar foragers perform offering contacts (as food-donors) to transfer the contents of their crops to recipient nest-mates and, in addition, they also perform begging contacts (as food-receivers). The biological relevance of these last interactions remains unknown. Previous evidence suggests that begging may be involved in the exchange of information on food resources that occurs naturally between employed foragers and nest-mates. This work was aimed to reveal possible connections between the information obtained while foraging and the begging behavior displayed inside the nest. Experiments were intended to (1) analyze whether chemosensory information obtained while foraging, i.e., odors and sucrose concentrations, affects begging behavior, and (2) determine whether resource uncertainty enhances begging contacts. Results showed that: (1) most begging contacts lasted less than 1 s, a duration which only allows receiving food samples from nest-mates; (2) the diversity of odors and sucrose concentrations at the feeding place enhances the occurrence of begging contacts; and (3) an increased resource uncertainty enhances the forager begging behavior. In addition, results suggest that foragers may direct their begging contacts frequently to other employed nectar foragers.

Keywords *Apis mellifera* · Foraging · Information exchange · Trophallaxis

Dedicated to Prof. Dr. Josué A. Núñez on the occasion of his 78th birthday.

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Abbreviations RF rate feeder · T treatment

Introduction

Trophallaxis in social insects is the exchange of liquid food among individuals of the same colony (Wilson 1971). Among adult worker honeybees, it takes place inside the nest through direct mouth-to-mouth contacts that also involve intense antennal interactions (Free 1956, 1957, 1959; Istomina-Tsvetkova 1960; Crailsheim 1998). During a single trophallactic contact, a worker may display two different behaviors. As a food-donor, she may open her mandibles keeping her antennae downward and close to the head while a variable number of recipient nest-mates start contacting her prementum with their protruded proboscis. This behavior is called 'offering contact'. On the other hand, as a food-receiver she may protrude her own proboscis towards the mandibles of a donor nest-mate also moving her antennae towards the donor. This behavior is called 'begging contact' (Free 1957, 1959).

Employed nectar foragers perform offering contacts to transfer the content of their crops to recipient nest-mates (Doolittle 1907; Rösch 1925; Nixon and Ribbands 1952; Michener 1974) and, in addition, they perform begging contacts (Istomina-Tsvetkova 1960; von Frisch 1967; Núñez 1970; Farina 1996; De Marco and Farina 2001). The biological relevance of begging in foragers remains unknown and two main hypotheses were proposed to explain its function. On the one hand, begging contacts were considered acts of refueling; i.e., it was assumed that foragers supply foraging costs starting from the sugar solutions they obtained through begging (Beutler 1950). Alongside this, it was reported a positive correlation between the amounts of sugar solution carried by foragers arriving at a known feeding place and the distance they recently traveled from the hive (Istomina-Tsvetkova 1960). Begging was then considered as a mean through which fuel is acquired (von Frisch 1967; Brandstetter et al. 1988) although no evidence showed a direct relationship between the crop contents of the arriving foragers and their previous begging behavior.

On the other hand, Núñez (1970) found that foragers changed their begging behavior even when they exploited artificial feeders placed at the same distance from the hive: the occurrence of begging increased as the rate of reward offered at the feeding place decreased. Similar results were reported by Farina (1996). In addition, begging also was negatively correlated to the offered sugar concentration (De Marco and Farina 2001). Hence, several reports signaled a negative relationship between begging and food source profitability. Starting from this, since food source profitability affects resource-related communicating behaviors (von Frisch 1967), it was proposed that begging might be involved in the exchange of information that occurs at each moment between employed foragers and nest-mates.

Mouth-to-mouth interactions allow honeybees to perceive the odor and taste of the collected nectar (von Frisch 1967). These cues serve the foragers to search and recognize their foraging targets (Butler 1951; von Frisch 1967). In addition, the recognition of a given food delivered by employed foragers may stimulate unemployed foragers to leave the hive and inspect the prospective food source (Ribbands 1952, 1954; von Frisch 1967). Accordingly, whenever the foraging trips become energetically deficient, begging may serve the foragers to perceive nectar-related chemosensory cues that would be useful to make sound foraging decisions.

While addressing whether the forager begging behavior is involved in the exchange of information on food resources, the goal of this work was to reveal connections between the information acquired during foraging and the begging behavior displayed inside the nest. To address this we considered two distinctive features of honeybee foraging: (1) foragers exhibit floral constancy and use floral odors in searching and recognizing their foraging targets (von Frisch 1967), and (2) that choice behavior is not random, but guided by specific memories (Greggers and Menzel 1993; Menzel 1999) that result in reduced foraging time, risk and cost, usually leading to an optimization of relative profits (Lindauer 1961; Heinrich 1975; Pyke 1978; Waddington and Holden 1979; Menzel 1985). According to these features, the uncertainty of food resources that foragers may experience increases as the number of possible foraging choices increases. Thus, when the quality as well as the diversity of food sources changes at an artificial feeding place, i.e., by changing the number of odor-reward combinations, an increased exchange of information is required to ensure efficient foraging decisions. We changed resource uncertainty experimentally to determine (1) whether chemosensory information acquired during foraging, i.e. odors and sucrose concentration present at the feeding place, affects begging behavior, and (2) whether an increased uncertainty enhances begging contacts.

Materials and methods

Experimental schedule

Apis mellifera honeybees placed inside one or two frames observation hives (von Frisch 1967) were used. In all experiments we connected a single observation hive to a flight enclosure located outdoors. The flight enclosure was used to control the food sources exploited by the colony, which were those offered within the enclosure. It consisted of a transparent polyethylene rectangular mesh of 6 m×3 m×2 m. The observation hive was connected to a 40-cm^3 chamber in turn connected to the inner flight enclosure. In some of the experiments, this chamber was also connected to a narrow corridor of 6 cm×1 cm high in cross-section and 1 m long, leading to a feeding place where marked bees were trained to forage individually. The complete structure was exposed to natural climatic conditions and a natural light-dark cycle. Instead of ad libitum feeders (i.e., unlimited sources of sugar solution), we used artificial feeders (henceforth: rate-feeders) that allowed the sucrose concentration as well as the solution flow rate offered at the feeding place to be controlled, i.e., the parameters that modulate honeybee foraging behavior (Núñez 1966, 1970; von Frisch 1967). The characteristics and properties of the rate-feeders have been described previously (see Núñez 1966, 1970). We conducted the experiments during the autumn in the Experimental Field of the University of Buenos Aires (34° 32’ S, 58° 26’ W).

Duration of the forager begging contacts

The duration of begging contacts was evaluated under controlled foraging conditions for both the foragers whose begging behavior was recorded and the colony as a whole. To address this, a two-frame observation hive with a colony of nearly 4,000 bees was connected to the chamber that was connected in turn to both the flight enclosure and the corridor. Two different feeding places were established, each presenting a rate-feeder that always delivered unscented 1.8 mol l^{-1} sucrose solution. The first rate-feeder was located at the end of the corridor (corridor-RF) and offered a constant solution flow rate of 5 $\mu l\ min^{-1}$. Marked bees were trained to walk through the corridor in order to reach the feeding place. Afterwards, they were allowed to forage individually on the corridor-RF and their behavior was video-recorded (both at the feeding place and inside the observation hive) along eight successive foraging cycles for each recording session. The second rate-feeder (flight enclosure-RF) was located inside the flight enclosure and always offered a constant solution flow rate of 90 $\mu l\ min^{-1}$ throughout the different recording sessions. While marked foragers exploited individually the corridor-RF, the remaining foragers were allowed to forage on the flight enclosure-RF. The colony sugar solution intake rate was thus controlled during measurements. For each marked forager collecting sugar solution at the corridor-RF, we recorded: (1) the number of begging contacts per stay in the hive, defined as the number of events in which the forager touched with her protruded proboscis the prementum of a nest-mate; and (2) the duration of each of these contacts (in seconds).

Effects of changing odor identity and sucrose concentration at the feeding place

A colony was placed in a one-frame observation hive that was connected to the flight enclosure. Foragers were trained to exploit an artificial patch of three rate-feeders (henceforth: 3-feeder patch) located inside the flight enclosure 3 m away from the hive entrance. At the patch, rate-feeders delivered sugar solutions that could present different odors and sucrose concentrations, although the sum of the offered sucrose concentrations as well as the whole solution flow rate of the feeding place was kept constant. However, the 3-feeder patch presented an abnormal foraging situation. It compelled the foragers to randomly collect solution among its rate-feeders. Thus, though foragers were able to perceive the odor as well as the sucrose concentration of the offered solutions, they were unable to collect solution uninterruptedly or even select a given rate-feeder among the others. Throughout the treatments described below, the behavior of the foragers was video-recorded both at the 3-feeder patch and within the observation hive. Details on behavioral measurements are described below.

Rationale

To optimize their foraging choices nectar foragers use visual cues as well as odors and sucrose concentrations of the sources they exploit. In addition, they exhibit floral constancy, i.e., they tend to remain foraging on a single floral species that is recognized according to its odor. Therefore, if sugar solutions of different sucrose concentration are differentially scented and offered simultaneously at an artificial patch of feeders (which does not present other signals allowing the foragers to recognize a particular feeder), foragers will use the odors to distinguish the more profitable one, thus optimizing their choices. Nevertheless, if the same patch now compels the foragers to collect solution randomly among its feeders, a trade-off will arise between the optimizing behavior of the foragers (with its well-documented flower constancy) and the impossibility of choosing the more profitable feeder. Under these exceptional foraging circumstances, foragers should search for information to compensate the increased uncertainty about the foraging target, and they should modify their begging behavior whenever begging contacts are used to request information about floral cues.

The 3-feeder patch

Figure 1 shows a schematic representation of the 3-feeder patch. It consisted of two superposed acrylic-plastic discs located in a hole of a wooden structure (25 cm×15 cm×1 cm). The upper disc was 79 mm in diameter and 7.5 mm thick, while the lower disc was 58 mm and 10 mm, respectively. A distance of 2 mm separated both surfaces. The upper disc presented six bowed slots (width: 2 mm, length: 13 mm) allowing the foragers to reach the sugar solutions that were continuously delivered by the three rate-feeders placed 31 mm from each other and located symmetrically on the lower disc. Each rate-feeder presented a flow rate of 11 $\mu\mathrm{l\ min}^{-1}$ of sugar solution. Thus, the total flow rate offered by the patch was 33 $\mu\mathrm{l\ min}^{-1}$. The upper disc was rotated by a syncromotor using a bronze axis-section in order to avoid use of positional information to distinguish between feeders. Rotations were established such that a bee feeding on a bowed slot could access the sucrose solution for an average of 3.5 s. In addition, a cylindrical wall of white filter paper (10 cm high and 6 cm in diameter) was located around the upper disc in order to diminish the visual input of the foragers staying on its surface. Through the rotations of the upper disc, foragers were forced to divide their feeding time equally between the three rate-feeders despite the fact that they offered different sucrose concentrations. To confirm this, prior to the experiments we quantified individually the feeding time on each of the three rate-feeders, and the total feeding time attained at the end of the visit. Measurements were performed for all the foraging conditions described below (T1–T8). For each condition, we compared the mean percentage feeding times that marked foragers spent on each of the rate-feeders with respect to the total feeding time they spent at the patch. Results confirmed that the 3-feeder patch compels the foragers to randomly distribute their feeding times between the rate-feeders (T1: $H=0.5$, $n=18$, NS; T2: $H=2.5$, $n=18$, NS; T3: $H=1.1$, $n=18$, NS; T4: $H=3.5$, $n=18$, NS; T5: $H=0.8$, $n=18$, NS; T6: $H=1.3$, $n=18$, NS; T7: $H=5.1$, $n=18$, NS; T8: $H=2.3$, $n=18$, NS; Kruskal-Wallis test; Zar 1996).

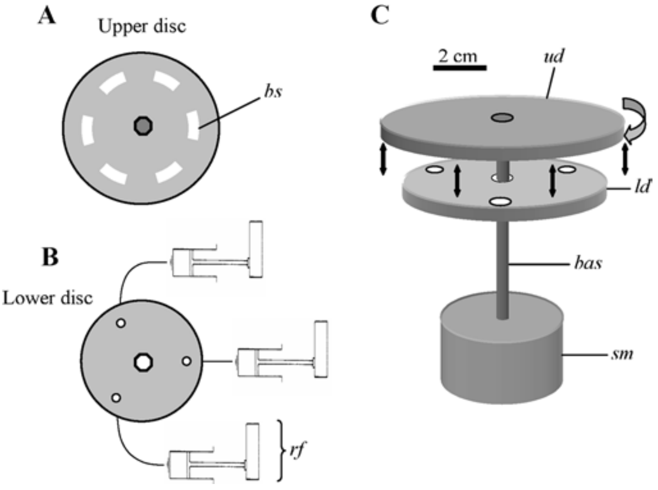

Fig. 1A–C Schematic representation of the 3-feeder patch. It consisted of two superposed acrylic plastic discs placed in a wooden structure. The upper disc (**A**) presented six bowed slots (*bs*) allowing the trained foragers to reach the sugar solutions symmetrically offered on the surface of the lower disc (**B**). Sucrose solutions were delivered by three rate-feeders (*rf*), each offering a constant flow rate of 11 $\mu\mathrm{l\ min}^{-1}$. **C** The upper disc (*ud*) was rotated by a syncromotor (*sm*) using a bronze axis-section (*bas*). Through the compulsive rotations of the upper disc, foragers were thus faced to distribute equally their feeding time between the three rate-feeders (see Materials and methods for details). The sum of sucrose concentrations as well as the whole flow rate of sugar solution offered by the patch was kept constant along treatments summarized in Table 1

Treatments

Eight treatments were defined based on the odor identity and sucrose concentrations offered at the 3-feeder patch (Table 1). Odors were mint, rose and vanilla (Pfeiffer-Gerhards Töpferhof Laboratory), and were always presented with the same dilution of 50 µl of essence per liter of sugar solution. Sucrose concentrations were 0.5, 1, and 1.5 mol l^{-1}, which lay within the range of concentrations that can be found in floral nectar (Beutler and Schöntag 1940; von Frisch 1934; Maurizio 1960) and, in addition, honeybees can perceive its differences (von Frisch 1934).

The first four treatments (T1–T4) offered the same 1 mol l^{-1} sucrose concentration in each of the three rate-feeders (equal concentration treatments). In T1, the sugar solutions present in the rate-feeders were unscented. In T2, the solutions had the same odor (being a pure essence). In T3, each solution had a different odor. Finally, in T4 each solution had the same compound odor that resulted from mixing the three pure substances in a unique volume of sucrose solution. In doing this, we maintained the same final dilution of 50 µl of odor per liter of solution. In the fourth remaining treatments (T5–T8) each of the three rate-feeders presented a different sucrose concentration (0.5, 1, and 1.5 mol l^{-1}; different concentration treatments). Otherwise, odor presentation was as in treatments T1–T4, respectively. Despite the three different concentrations used in T5–T8, the average concentration (1 mol l^{-1}) was the same as in T1–T4.

Measurements

The individual behavior of the foragers was video recorded both at the 3-feeder patch and the observation hive. Data were afterwards obtained from the videotapes. Each experiment lasted approximately 30 min.

Table 1 Treatments assayed to analyze effects of changing odor identity and sucrose concentrations at the 3-feeder patch. They were defined based on to the diversity of odors and sucrose concentrations offered by the three feeders of the patch (Fig. 1).*Circles* indicate sucrose concentration: *white circles*, 0.5 mol l^{-1}; *gray circles*, 1 mol l^{-1}; *blackcircles*, 1.5 mol l^{-1}. *Squares* indicate odors (50 μl l^{-1}): *white squares*, mint; *gray squares*, rose; *black squares*, vanilla. Squares in T4and T8 represent an odor compound resulting from mixing the three onesemployed. In doing this, the final dilution of 50 μl l^{-1} was maintained. T1 and T5 offered unscented solutions. Each rate-feeder delivered sugar solution at a flow rate of 11 μl min^{-1}. [a]Treatments T5–T8 were repeated in the control experiment (see Materials and methods). In thisexperiment, squares in T6 and T7 do not represent particular odors but the same or three different odors. Circles indicate concentrations as in the second series

Equal concentration treatments				Different concentration treatments [a]			
◍◍◍	◍◍◍ ▨▨▨	◍◍◍ □▨■	◍◍◍ ▥▥▥	○◍●	○◍● □□□	○◍● □▨■	○◍● ▥▥▥
T1	T2	T3	T4	T5	T6	T7	T8

Recordings at the 3-feeder patch

The number of bees foraging at the 3-feeder patch was recorded at 15-s intervals. Six marked bees per treatment were randomly selected among the totality of the foragers and the time they spent collecting solution from the patch was recorded.

Recordings at the observation hive

For each returning marked forager we recorded: (1) the number of begging contacts (defined as previously) per stay in the hive; (2) the number begging contacts specifically performed with any other marked forager; (3) the number of marked foragers present each time a marked forager entered the nest; (4) the hive time (in seconds), defined as the time spent by a forager inside the hive between two consecutive foraging trips; (5) the number of offering contacts per stay in the hive, defined as the number of events in which the returning forager opened her mandibles while one or more recipient bees contacted her prementum with their protruded proboscis; and (6) the duration of each offering contact (in seconds).

Controls for other sources of variability

We studied the following possible sources of variations for the number of begging contacts recorded along the treatments: (1) inter-individual variations; (2) odor-specific effects; and (3) the number of foragers collecting solution simultaneously at the 3-feeder patch. The experiment was similar to that described in the previous section except for the treatments presenting different sucrose concentrations (Table 1). While different individuals were involved in the treatments of the previous experiment, the same group of foragers was now exposed to the different treatments randomly presented. Inter-individual variations were thus included in the analysis. In addition, throughout the previous experiment only one of the three odors was employed in the treatments that offered equally scented sugar solutions (T2 and T6). Furthermore, in the treatment that offered different odors and concentrations (T7), a given odor was assigned only to a given sucrose concentration. Therefore, throughout the previous experiment we could not discard odor-specific effects on the number of begging contacts recorded in T6 and T7. Throughout this experiment, on the contrary, we repeated these treatments in order to test each of the three odors as the single odor offered at the patch in T6, as well as all the possible combinations between the different odors and sucrose concentrations in T7. Finally, we controlled the number of foragers exploiting the patch along the treatments. As previously, trained foragers were individualized and their behaviors were video recorded both at the 3-feeder patch and the observation hive. All the variables mentioned in the previous section were analyzed.

Data analysis

Regression analysis was used to determine the function describing the frequency distribution of begging times. While analyzing effects of changing odor identity and sucrose concentration at the feeding place, each forager was considered as an experimental unit and data from the first and the second foraging cycle (therefore averaged) were considered. The same criterion was employed for results from the control experiment. Data were analyzed through ANOVAs, Tukey-Kramer and Newman-Keuls comparisons, Kruskal-Wallis tests, and Spearman Rank Order tests (Zar 1996).

Results

Duration of the forager begging contacts

The frequency distribution of the duration of the forager begging contacts (Fig. 2) fits an exponential decay function $Y=99.6e^{(-1.4x)}$ ($r^2=0.99$, $n=1222$, $P<0.0001$), where Y is the proportion of begging contacts and x represents the contact duration. Accordingly, begging contacts mainly lasted less than 0.5 s and 77.2% of these

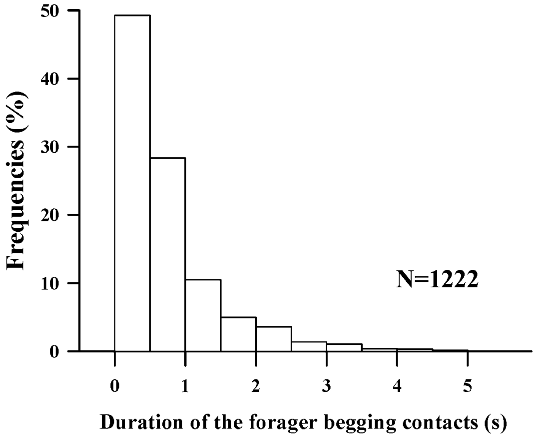

Fig. 2 Relative frequency distribution (in percentage) of the forager begging contacts categorized according to its durations. Data correspond to 1,222 begging contacts performed within the hive by 32 returning foragers along 165 foraging cycles. Foragers collected 1.8 mol l^{-1} sucrose solution at a rate-feeder which presented a flow rate of 5 μl min^{-1}

interactions lasted less than 1 s. The maximum duration recorded for a single interaction was 5 s.

Effects of changing odor identity and sucrose concentration at the feeding place

The mean number of begging contacts performed by the foragers per stay in the hive changed throughout the treatments (Fig. 3, interaction term $F_{(3,92)} = 9.2$, $P < 0.0001$, two-way ANOVA, after log transformation). In the treatments in which a unique concentration was used (black bars), the higher number of begging contacts was found when each feeder of the patch presented a different odor (T3). On the contrary, the lower number of interactions was found in the absence of odors (T1). No difference was found between the treatments in which the feeders had a single odor (pure essence or compound, T2 and T4, respectively). These treatments showed values that were intermediate between those obtained for the treatments T1 and T3 ($P < 0.05$; Tukey-Kramer comparisons).

In the treatments in which different concentrations were used (Fig. 3, white bars), no statistical differences were found in the number of begging contacts between treatments in which solutions were unscented (T5), had the same odor (T6), and three different odors were offered (T7). Nevertheless, a tendency to perform more interactions when a single odor is offered could be observed (T6). In addition, when the rate-feeders presented the same odor compound (T8), foragers performed more begging contacts than in the treatments T5 and T7 (unscented sugar solutions and three different odors, respectively; $P < 0.05$, Tukey-Kramer comparisons).

Finally, while comparing treatments presenting a similar arrangement of the olfactory stimulation but equal or different sucrose concentrations (Fig. 3, black versus white bars), more begging contacts were recorded whenever different concentrations were presented at the patch (asterisks; without odor: $F_{(1,92)} = 15.2$, $P = 0.0002$; with the same odor: $F_{(1,92)} = 12.9$, $P = 0.0005$; with the same mixed odor: $F_{(1,92)} = 18.6$, $P < 0.0001$; one-way ANOVA), except when sugar solutions had different odors (T7, $F_{(1,92)} = 3.5$, $P = 0.06$; one-way ANOVA).

Table 2 summarizes the results obtained for the other variables recorded at the patch and within the observation hive. We found differences in the number of bees foraging at the patch along the treatments (see Table 2 for statistics). Furthermore, the duration of the foraging

Fig. 3 Mean number (±SE) of begging contacts performed by returning foragers per stay in the hive. Eight treatments were defined based on the odor identity and sucrose concentrations offered at the 3-feeder patch (see Table 1). *Black bars* indicate results from the treatments *T1–T4*, when the patch offered the same sucrose concentration in each of its rate-feeders. White bars indicate results from the treatments *T5–T8*, when three different sucrose concentrations were offered in the rate-feeders. The number of individuals recorded in each of the treatments is shown in *parentheses*. The forager begging behavior changed along the treatments ($F_{(3,92)} = 9.2$, $P < 0.0001$; two-way and main effects ANOVA and Tukey-Kramer comparisons, after log transformation). *Letters* indicate differences between treatments when a single concentration was offered at the patch as well as when different concentrations were offered. *Asterisks* indicate differences between treatments offering a similar arrangement of the olfactory stimulation at the patch but a single concentration or three different concentrations in its feeders

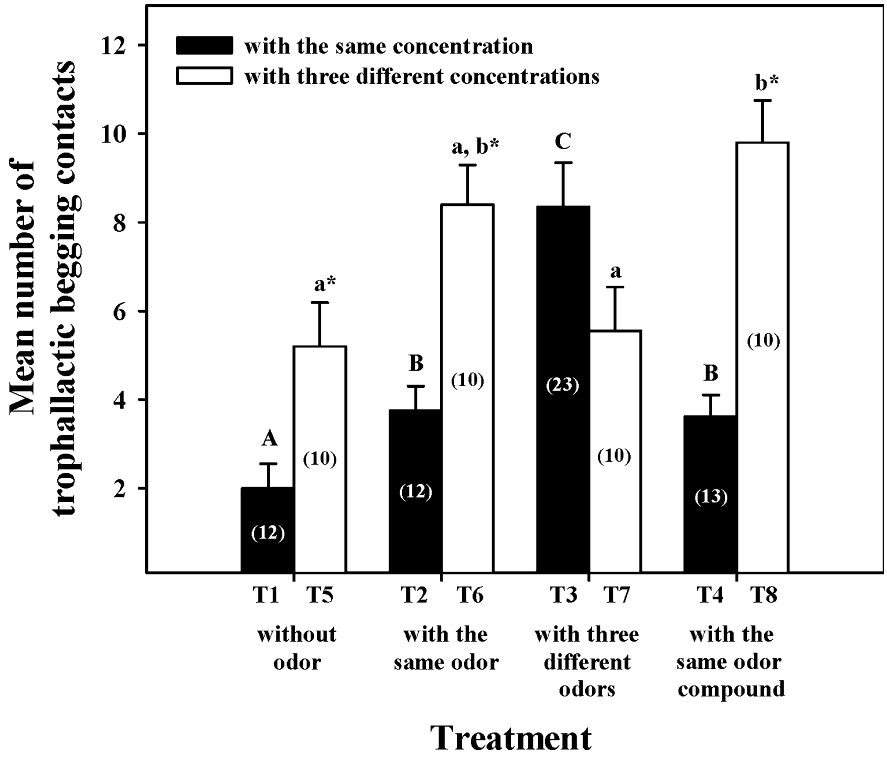

Table 2 Mean values (±SE) of individual foraging parameters recorded both at the 3-feeder patch and the observation hive (see Materials and methods for details). Superscript letters indicate results of statistical analyses: [a]results from two-wayANOVA: odor $F_{(3,40)} = 1.6$, $P = 0.21$; concentration$F_{(1,40)} = 8.9$, $P = 0.005$; interaction term $F_{(3,\ 40)} = 8.9$, $P = 0.05$. [b]Results from Kruskal-Wallis test: $H = 173.0$, $n = 341$, $P < 0.0001$. [c]Results from two-way ANOVA, after log transformation: odor$F_{(3,92)} = 1.6$, $P = 0.20$; concentration $F_{(1,92)} = 4.0$, $P = 0.05$; interaction term $F_{(3,92)} = 8.9$, $P = 0.65$. [d]Results from two-way ANOVA: odor $F_{(3,92)} = 0.6$,$P = 0.65$; concentration $F_{(1,92)} = 1.6$, $P = 0.21$; interaction term $F_{(3,92)} = 1.8$, $P = 0.16$. [e]Results from two-way ANOVA: odor $F_{(3,92)} = 0.5$, $P = 0.69$; concentration $F_{(1,92)} = 1.8$, $P = 0.18$; interaction term $F_{(3,92)} = 0.4$, $P = 0.77$

	Equal concentration treatments				Different concentration treatments			
	◍◍◍	◍◍◍ ▨▨▨	◍◍◍ □▨■	◍◍◍ ▥▥▥	○◍●	○◍● □□□	○◍● □▨■	○◍● ▥▥▥
	T1	T2	T3	T4	T5	T6	T7	T8
At the 3-feeder patch								
Visit time (s) [a]	695.0 ± 113.6	723.8 ± 152.3	705.6 ± 90.7	796.4 ± 140.1	717.7 ± 131.4	302.5 ± 47.9	666.8 ± 145.1	255.1 ± 52.6
Number of bees [b]	10.8 ± 1.0	11.8 ± 0.39	10.8 ± 0.5	11.5 ± 0.6	11.3 ± 0.5	6.8 ± 0.3	9.2 ± 0.2	4.3 ±0.2
At the observation hive								
Hive time (s) [c]	116.8 ± 12.8	160.7 ± 28.8	122.9 ± 10.9	159.9 ± 27.7	111.3 ± 9.7	108.2 ± 6.0	103.8 ± 9.7	125.3 ± 15.0
Number of offering contacts [d]	2.8 ± 0.4	3.6 ± 0.5	3.9 ± 0.4	3.2 ± 0.5	4.8 ± 0.4	3.8 ± 0.4	3.6 ± 0.8	3.2 ± 0.6
Total offering time (s) [e]	30.9 ± 3.2	26.2 ± 3.0	25.7 ± 2.1	25.9 ± 2.5	30.0 ± 3.0	31.3 ± 3.7	28.4 ± 3.2	30.2 ± 3.0

visits also changed along the treatments, showing lower values for the treatments offering different sucrose concentration. Therefore, after a one-way ANOVA ($F_{(7,40)} = 3.2$, $P = 0.009$), post-hoc comparisons were used to analyze variations in the visit time. The visit time was lower in T8 than in T1, T4 and T7 ($P = 0.049$, $P = 0.038$, and $P = 0.042$, respectively; Newman-Keuls comparisons). There were also differences between T6 and T7 ($P = 0.032$; Newman-Keuls comparisons). In spite of this, a correlation was found between the visit time and the number of bees that simultaneously foraged at the patch ($r = 0.95$, $n = 8$, $P = 0.0003$; Spearman rank-order correlation). This result was expected and, in addition, it can explain the variations observed in both the visit time and the number of simultaneous foragers. That is, the visit time strongly depends on the flow rate of solution offered at the feeding place (Núñez 1966). Thus, since the flow rate offered at the patch was constant throughout the experiment, the availability of sugar solution per bee decreases as the number of simultaneous foragers at the feeding place increases. Finally, we found no difference in the hive time, the number of offering contacts and the total offering time recorded throughout the treatments.

Controls for other sources of variability

The same group of foragers was exposed to the different concentration treatments randomly presented (Table 1, T5–T8). We found no inter-individual variations in the forager begging behavior (individual term $F_{(17,51)} = 1.2$, $P = 0.28$; randomized-block design ANOVA, after log transformation). As odors and sucrose concentrations were paired in all possible combinations, we compared the number of begging contacts recorded throughout repetitions of the treatments T6 and T7, with a single odor and three different odors, respectively. Throughout these repetitions, each of the three odors was alternately assigned either to a single feeder in T7 or to the whole patch in T6. Results showed that the number of begging contacts did not change throughout these repetitions (T6: $H = 3.4$, $n = 25$, $P = 0.18$; T7: $H = 0.003$, $n = 28$, $P = 0.998$; Kruskal-Wallis test). Thus, we found no odor-specific effects on the forager begging behavior recorded under these treatments.

As in the previous experiment, however, statistical differences were found in the number of begging contacts while comparing results among the treatments, ($F_{(3,68)} = 5.1$, $P = 0.003$; one-way ANOVA after log transformation). When unscented sugar solutions were offered (T5), a lower number of begging contacts was recorded regarding treatments in which the same odor (pure essence or compound) was offered at the patch (T6 and T8, respectively, $P < 0.05$; Newman-Keuls comparisons). In addition, when the feeders offered different odors (T7), foragers performed less begging contacts in comparison to the number recorded when they offered the same odor compound (T8, $P = 0.009$; Newman-Keuls comparisons). No difference was found between T6 and T7 (when one or three odors were offered, respectively; $P = 0.058$); or between T6 and T8 (when a pure essence and the odor compound were offered, respectively; $P = 0.345$; Newman-Keuls comparisons). However, as previously, in comparison to the treatment in which three odors were offered (T7), a tendency to perform more begging contacts when a single odor was offered (T6) was observed.

The mean number of bees foraging at the patch did not change throughout these treatments ($H = 2.8$, $n = 262$, $P = 0.43$, Kruskal-Wallis test; mean = 7.4, SD = 2.6). We also found no difference in the mean visit time ($F_{(3,32)} = 0.434$, $P = 0.73$, one-way ANOVA; mean = 428.2, SD = 165.3). As previously, no difference was found along the treatments in the hive time, the number of offering contacts and the total offering time ($F_{(3,68)} = 1.02$, $P = 0.39$; $F_{(3,68)} = 0.56$, $P = 0.64$; and $F_{(3,68)} = 0.11$, $P = 0.96$, respectively; one-way ANOVAs).

Receivers of the forager begging contacts

We next evaluated whether foragers performed their begging contacts randomly with any worker within the colony or whether they often directed these interactions to a particular group of individuals. Explicitly, we analyzed how frequently the marked foragers begged for food to other marked foragers. Data were obtained from the control experiment. For each entire experimental session, we considered the following measurements: (1) the number of begging contacts that each of the marked foragers performed per stay in the hive, (2) the number of foraging cycles they performed, (3) the total number of marked (employed) foragers whose behaviors were recorded, (4) the number of begging contacts occurring between marked foragers, and finally (5) the number of marked foragers that were inside the hive each time a single marked forager returned from the patch. Table 3 shows mean values of these variables for each treatment and, in addition, the percentage of begging contacts between marked foragers related to the whole number of begging contacts recorded. This percentage ranged from 4.8% up to 6.6% for all treatments.

Video-recordings showed that marked foragers performed their begging contacts within the first half of the comb, where several hundreds of workers were always present during the trials. For the sake of calculating, we will assume presence of at least 100 individuals in the first half of the comb. According to present results, in addition, each time a marked forager entered the colony during the experiments approximately two other marked foragers were already present inside the hive (means from different treatments ranged from 1.4 up to 1.9). Therefore, according to these results as well as the previous assumption, whenever begging contacts are randomly performed 2/100 should be the expected proportion of begging contacts that single marked foragers could perform with other marked forager per foraging cycle. Thus, for single trials, the expected percentage of begging contacts between marked foragers can be easily calculated by multiplying: (1) 2/100, (2) the mean number of begging contacts performed by marked foragers per foraging cycle, (3) the mean number of foraging cycles performed by marked foragers during the trial, and finally (4) the mean number of recorded marked foragers. This calculation yields values that ranged from 0.5% up to 1% depending on the different treatments (Table 3). These values contrast with the percentages actually observed, which were significantly higher (see above). Results thus indicate that returning foragers directed their begging contacts frequently to other employed foragers.

Discussion

Forager honeybees perform offering contacts as well as begging contacts within the colony (Istomina-Tsvetkova 1960; von Frisch 1967; Núñez 1970; Farina 1996; De Marco and Farina 2001). Earlier works suggested that begging might serve the foragers to exchange information on food resources. While addressing this hypothesis, our goal was to reveal possible connections between the information acquired during foraging and the begging behavior displayed inside the nest.

Duration of the forager begging contacts

Results showed that begging contacts, which occur mainly after the nectar collected was transferred to nestmates (De Marco and Farina 2001), are shorter than the offering ones by which returned foragers discharge their crops. During the experiments presented here, begging contacts mainly lasted less than 0.5 s (Fig. 2) while an offering contact often lasts more than 10 s (Korst and Velthuis 1982; De Marco and Farina 2001). Non-invasive thermographic recordings have shown that most trophallactic contacts lasting up to 1 s do not allow an effective transfer of food between bees (Farina and Wainselboim 2001). Hence, throughout a single begging contact returning foragers usually obtain just a sample of food, which, however, allowing perception of the odor and taste of the nectar presented in the mouthparts of their trophallactic-mates (von Frisch 1946, 1967; Free 1959).

Chemosensory information obtained during foraging affects the forager begging behavior

We presented changes in odor identity and sucrose concentration at an artificial feeding place. Under these

Table 3 Data from the control experiment employed to analyze how frequently the marked foragers begged for food to other marked foragers (see Results for details). *Superscript letters*: $e = (d/(a \times b \times c)) \times 100$

	Different concentration treatments			
	○◍●	○◍● □□□	○◍● □▩■	○◍● ▥▥▥
	T5	T6	T7	T8
Begging contacts performed by single marked foragers per stay in the hive [a]	2.1 ± 0.3	3.0 ± 0.3	2.3 ± 0.5	4.2 ± 0.4
Foraging cycles performed by single marked foragers [b]	1.8 ± 0.1	2.4 ± 0.2	2.1 ± 0.1	2.0 ± 0.1
Marked foragers recorded [c]	6.0 ± 0.9	6.1 ± 0.8	6.6 ± 0.7	5.7 ± 0.9
Begging contacts between marked foragers [d]	1.5 ± 0.7	2.8 ± 1.2	1.8 ± 1.0	2.3 ± 1.3
Marked foragers present inside the hive each time a single marked forager returned from the 3-feeder patch.	1.4 ± 0.1	1.4 ± 0.2	1.9 ± 0.3	1.9 ± 0.1
Percentage of begging contacts between marked foragers [e]	6.6%	6.4%	5.6%	4.8%

circumstances, we found variations in the forager begging behavior while the overall profitability of the feeding place remained constant, i.e., the feeding place location, the time spent collecting food, and the sugar content as well as the crop size attained by foragers remained without changes (Fig. 3 and Results). Additionally, we found no differences for variables such as the hive time, the number of offering contacts, and the total offering time, which are strongly affected by food source profitability (Núñez 1970; Farina 1996; De Marco and Farina 2001). The results thus indicate that begging is affected by chemosensory information received during foraging.

We showed that begging was affected by the diversity of odors as well as the diversity of sucrose concentrations offered at the patch (Fig. 3). If begging allows the foragers to obtain information about the odors and/or sucrose concentrations available inside the colony, these findings could be explained according to the following hypotheses. First, when odor diversity was increased at the patch (T3 versus T2 and T4) foragers begged more often to distinguish the odor to be searched for preferentially during their next trips, since they naturally exhibit floral constancy (von Frisch 1967). Second, when concentration diversity was increased (T1 versus T5, T2 versus T6, and T4 versus T8), begging served the foragers to distinguish the sucrose concentration to be searched at the patch, since they tend to optimize foraging relative profits (Heinrich 1975). Third, the uncertainty on the feeder that would be preferred at the patch, however, depended on both the diversity of odors and sucrose concentrations. Thus, whenever a given odor was unambiguously associated to a given sucrose concentration (T3 versus T7), the task 'to obtain information' decreased because it was reduced to use odors as a guide to find the best sucrose concentration at the patch. Accordingly, the number of begging contacts in turn was reduced. In the control experiment we found results similar than those described in Fig. 3 that, in addition, were independent of inter-individual variations. This suggests that heritable components may underlie the observed begging behavior.

Additionally, we found no differences in the forager begging behavior when a pure essence or an odor compound was presented at the patch (Fig. 3; T2 versus T4 and T6 versus T8). A similar result was obtained in the control experiment (see Materials and methods and Results). In this case, however, treatments were presented randomly. Thus, results indicate that a mixture of three different odors was perceived as a single odor in T8 in spite of recent chemosensory experiences that involved perceiving each of the single odors differentially rewarded, as in T7.

Receivers of the forager begging contacts

We also examined whether foragers performed their begging contacts randomly with any worker present within the colony (see Results and Table 3). Under our experimental conditions, begging contacts were frequently aimed towards other employed foragers, thus revealing heterogeneity among nest-mates as receivers of the forager begging contacts. As mouth-to-mouth interactions may be involved in communication about resources (von Frisch 1946; Ribbands 1954, 1955; Free 1959; Lindauer 1961; Wilson 1971), begging contacts between employed nectar foragers may be also related to the transfer of information on food sources. If begging serves the foragers to distinguish the odor and sugar concentration of the nectar being handled within the colony, foragers may optimize their search for information by selectively asking for food to other foragers since they normally carry freshly nectar in their crops. Earlier results support the idea of frequent mouth-to-mouth interactions among foragers. Nixon and Ribbands (1952) showed that the distribution of signaled sugar solution offered at an artificial feeder was greater among foragers than among other workers of the colony. Furthermore, although Free (1957) observed that workers of all ages fed and were fed by workers of a wide range of ages, he reported a tendency for food to be transferred between workers of the same age. Further research is necessary to analyze this issue.

Are olfactory memories retrieved through begging contacts?

Foragers invested similar times collecting sugar solution from each feeder of the patch, thus attaining mixed crops. Therefore, odors and sucrose concentrations present at the feeding place were always mixed before being transferred from foragers to nest-mates. Taking into account that an increased number of odors at the patch enhanced begging contacts (Fig. 3, T2, T3, and T4 versus T1), the possibility arises that known odors present in the mouthparts of nest-mates might act as a triggering-stimuli in the elicitation of the forager begging behavior.

Regarding this, for instance, it is well known that honeybees extend their proboscis reflexively when the sucrose receptors at the antennae and mouthparts are stimulated. This proboscis extension reflex can be conditioned to olfactory stimuli if the bees are allowed to taste sucrose solution immediately after the presentation of odors (Takeda 1961). Olfactory conditioning of the proboscis extension reflex has been largely employed to analyze different aspects of appetitive learning and memory in honeybees (Bitterman et al. 1983; Bitterman 1988; Menzel 1990; Hammer and Menzel 1995; Menzel and Müller 1996). Thus, it was demonstrated that appetitive olfactory memories established during flower visits are retrieved during the proboscis extension reflex conditioning performed under laboratory conditions (Gerber et al. 1996). Memory retrieval of floral odors thus appeared to be independent of contextual parameters. Under our experimental conditions, an increased uncertainty on the preferred feeder enhanced the forager begging behavior (Fig. 3, T3, T6, and T8). If known odors present in the mouthparts of nest-mates act as triggering stimuli in the elicitation of begging, results may be also indicating that appetitive olfactory memories established during foraging would be afterwards retrieved through begging contacts.

Begging as a self-ruling behavior employed in gathering resource-related information

Observations on dance communication in honeybees emphasized the nectar samples that followers may obtain from dancers and by which they can receive specific chemosensory cues about the nectar being exploited (von Frisch 1967). Since dance followers obtain nectar samples through begging, these observations must be interpreted as initial reports on begging being involved in gathering information on food resources. Unfortunately, the forager begging behavior and the possibility that it may represent a self-ruling behavior employed in gathering information have received little attention. The reason for this can be found in the fact that most studies on honeybee foraging are performed with artificial feeders presenting unlimited sources of sugar solutions, thus establishing foraging conditions that evidently conceal the forager begging behavior because its occurrence shows a negative correlation with the availability of nectar at the feeding place.

According to the present results, begging might be involved in contrasting chemosensory cues perceived during flower visits with the same kind of stimuli available within the colony. Under this point of view, an increased begging could be interpreted as a behavioral output that tends to reduce an increased uncertainty related to the current foraging target; which could be elicited, for instance, when the profitability of the forager own source is marginal. Thus, when the feeding place under exploitation becomes energetically deficient, begging may serve a forager to request information that, perhaps working alongside memories about nectar odors, would be useful to assess whether the nectar that she is currently collecting is being highly or poorly exploited by the colony as a whole. This information could in turn affect her subsequent foraging activity in terms of her foraging tempo or even her decision about to continue or to stop collecting food.

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