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How bees tune their dancing according to their colony's nectar influx: re-examining the role of the food-receivers' 'eagerness'

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Summary

***Apis mellifera* bees perform dances to communicate the presence of desirable nectar sources. The regulation of these dances does not depend exclusively on properties of the nectar sources, but also upon certain stimuli derived from the foraging status of the colony as a whole; i.e. bees exploiting a source of constant profitability are more likely to dance when the colony's nectar intake rate is low. Based on these stimuli, individual bees tune their dances according to their colony's nectar influx without visiting alternative nectar sources.**

Division of labour, in addition, is a common feature in honeybees. Upon returning to the nest, successful foragers transfer the content of their crops to food-receivers by means of a common behaviour in social insects called trophallaxis, i.e. the transfer of liquid food by mouth. Martin Lindauer stated that a returned forager may sense the foraging status of its colony on the basis of the food transfer process by computing how quickly and eagerly the food-receivers unload its crop.

This study focuses on the forager's experience during the food transfer process, its variability based on the colony's nectar influx, and the separate effects that the 'ease' and the 'eagerness' of the food-unloading have on the tuning of recruitment dances. Results indicate that foragers can rapidly sense variations in the colony's nectar influx, even when they experience no variation in the time interval between their return to the hive and the beginning of the food transfer. To accomplish this task they appear to use stimuli derived from the number of food-receivers, which enable them, in turn, to set their dance thresholds in relation to the nectar influx of their colony. The relevance of these findings is discussed in the context of communication and successful foraging.

Key words: *Apis mellifera*, dance behaviour, trophallaxis, colony's nectar influx.

Introduction

Forager honeybees (*Apis mellifera*) perform complex and highly controlled motor displays called 'dances' to communicate the presence of highly desirable resources (von Frisch, 1965). From the form, rhythm and orientation toward gravity of these dances, other bees present inside the hive (called dance followers) become aroused and search for the prospective spot in the field (Riley et al., 2005). Foragers perform round or waggle dances depending on the flown distance. They perform round dances when a food source is located near the hive and waggle dances when the source is far from the hive (von Frisch, 1965; Sen Sarma et al., 2004). Recently, comprehensive reviews of research on dance behaviour have described different aspects of its sensory basis, related social foraging strategies and evolutionary aspects (Dyer and Seeley, 1989; Seeley, 1995; Dyer, 2002; Michelsen, 2003).

In the case of nectar foraging, the key stimulus triggering dances is the presence of sugar solution at a given foraging spot. Hence, the current amount and sugar concentration of the nectar being collected must exceed a threshold previously defined by the foragers' central nervous system according to several properties of the nectar source (von Frisch, 1965; Núñez, 1970; Seeley, 1986; Seeley et al., 2000). Other non-source factors such as weather conditions (Lindauer, 1948; Boch, 1956) and the dancer's past foraging experience (Raveret-Richter and Waddingon, 1993; De Marco and Farina, 2001; De Marco et al., 2005) also influence intrinsic parameters controlling dancing displays. Most importantly, although honeybees do not directly compare alternative sources in the field (Keban and Baker, 1983; Chittka et al., 1999), and thus each foraging bee acquires information about the absolute profitability of its own source, both the probability and the strength of their dances depend (indirectly) upon the general nectar on offer. This means that the dance behaviour of a single forager is modulated by activities of other individuals, somehow encoded in certain stimuli associated with the colony's rate of nectar inflow (Lindauer, 1948; Núñez, 1970; Seeley, 1986, 1989, 1994, 1995; Seeley et al., 1991; Seeley and Towne, 1992; Seeley and Tovey, 1994; Thom et al., 2003), e.g. foragers exploiting a source of constant profitability are more likely to dance when the colony's nectar intake rate is low (Lindauer, 1948; Núñez, 1970; Seeley, 1995). This 'tuning' of dances based on the colony's nectar influx thus depends upon social feedback mechanisms providing returned foragers with information about the foraging status of the colony as a whole. At the group-level, it allows for adjustment to the rates of recruitment to and abandonment of different nectar sources, thus enhancing colony profits (Seeley, 1995).

Division of labour is a common feature in honeybees (Rösch, 1925; Lindauer, 1952). Upon entering the hive, successful foragers transfer the content of their crops to food-receivers (Doolittle, 1907). This food transfer occurs by means of a common behaviour in social insects (Wilson, 1971) called trophallaxis, i.e. the transfer of liquid food by mouth (Nixon and Ribbands, 1952; Free, 1956, 1957, 1959; Korst and Velthuis, 1982; Crailsheim, 1998). Throughout a set of pioneering experiments, Martin Lindauer (Lindauer, 1948) discovered that individual foragers are able to sense the foraging status of their colony, i.e. the rate of nectar flow into the colony (Seeley, 1986), on the basis of the process of food transfer. That is, by noting how 'quickly' and 'eagerly' they are unloaded by food-receivers inside the hive (see Seeley et al., 2002). Over the latest 20 years, Lindauer's early postulates on this subject (Lindauer, 1948) became not only supported but largely extended by both theoretical and empirical evidence (Seeley, 1995, 1998; Kühnholz and Seeley, 1997). Thus, for instance, it has been clearly demonstrated that the latency experienced by a successful returned forager while searching for a food-receiver constitutes both an accurate measure of the 'ease' of the food-transfer and a reliable time-based indicator of the colony's foraging status, thus influencing the forager's dance thresholds: the higher the colony's nectar intake rate the higher the experienced latency as well as the rise of individual dance thresholds (Seeley, 1995). The effect of the 'eagerness' of food-receivers on dance tuning has received less attention, however, so its separate effects and possible sensory bases remain unknown. This study focuses on the forager's experience during the process of food transfer, its variability based on the colony's nectar intake rate, and the separate effects of the 'ease' and the 'eagerness' of the food-unloading on the tuning of recruitment dances. The results are discussed in the context of communication and successful foraging.

Materials and methods

Bees

A colony of nearly 4000 *Apis mellifera ligustica* bees (with queen and workers) was placed in a two-frame observation hive (von Frisch, 1965). These bees had not been used for research before the experiments. The observation hive was connected to a 40 cm^3 chamber, in turn connected to a flight enclosure (i.e. a transparent polyethylene rectangular mesh of 6 m $\times$3 m$\times$2 m), arranged to control the totality of the nectar sources exploited by the colony during the experiments. By means of this chamber, the observation hive was also connected to a narrow corridor (6 cm$\times$1 cm high in cross-section and 1 m long) in which an artificial feeder was placed at its far end (see below). The complete structure was located outdoors and exposed to natural climatic conditions and natural light:darkness cycles. Experiments were conducted between March and May in the School of Exact and Natural Sciences of the University of Buenos Aires (34°32′S, 58°26′W).

Feeders

The behaviour of a forager bee is strongly influenced by the availability of nectar at a feeding place (Núñez, 1966, 1970, 1982). A forager tends to fill its crop upon finding unlimited sugar solution at an artificial feeder (Núñez, 1966; von Frisch, 1965). Moreover, under such conditions it forages regularly on the feeder and also tends to display intensive dances inside the hive (von Frisch, 1934, 1965). Such a reward situation, however, might conceal variations in the forager's dances derived from concurrent variations in the colony's nectar intake rate, i.e. the higher the dance strength the lower the probability of recording slight variations in its threshold, irrespective of the strength of those within-the-hive stimuli underlying dance tuning. Hence, instead of feeders offering unlimited sugar solution, I used artificial feeders that allowed me to control both the sugar concentration and the flow rate of the offered solution. The properties of these feeders have been described elsewhere (Núñez, 1966, 1970).

Experimental layout

Fig. 1 shows a schematic representation of the experimental layout employed to analyse dance tuning based on the colony's foraging status. Two different feeders were used during the experiments. These feeders always delivered unscented 1.8 mol l^{-1} sucrose solution. Hence, food-source profitability was exclusively defined on the basis of the flow rate of sugar solution. The first feeder (individual feeder, IF) was placed at the end of the corridor (see above) and offered sucrose solution at a constant flow rate of 5 μl min^{-1} throughout all the experimental series described below. During each experimental session, a single marked bee was trained to walk through the corridor in order to reach the feeder. Next it was allowed to forage individually on the IF throughout eight successive foraging cycles. Each foraging cycle involved the collection of sugar solution at the feeder and the transfer of the collected solution to food-receivers present inside the hive. It started when the animal left the hive toward the IF and finished at the time of its next departure. During each session, the behaviour of the single marked bee was video-recorded, both at the IF and inside the observation hive. Data were afterwards obtained from videotapes. The second feeder (group feeder,

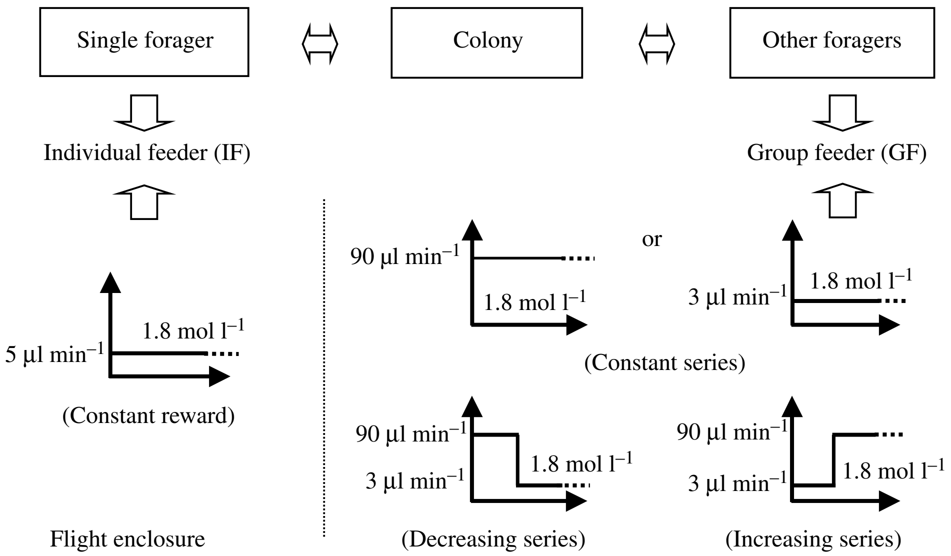

Fig. 1. Experimental layout to investigate how bees tune their dancing according to their colony's nectar influx. An observation hive was connected to a narrow corridor and to a flight enclosure. The flight enclosure was used to control the totality of the nectar sources exploited by the colony. Two different feeders were used, each delivering unscented 1.8 mol l^{-1} sucrose solution. The first one, individual feeder (IF), was placed at the end of the corridor and offered sucrose solution with a constant flow rate of 5 μl min^{-1}. During each recording session, marked bees were allowed to forage individually on the IF and their behaviours were video-recorded along eight successive foraging cycles. Simultaneously, the second feeder, group feeder (GF), was placed inside the flight enclosure and offered a constant solution flow rate of either 3 or 90 μl min^{-1} (according to three different experimental series: the constant, the decreasing and the increasing series, see Materials and methods for details). During each session, a group of foragers collected sucrose solution at the GF while a single marked bee foraged on the IF. Quantifiable variations in the colony's sugar solution intake rate were induced by modifying the solution flow rate offered at the GF.

GF) was placed inside the flight enclosure (see above). It offered a constant flow rate of solution at either 3 or 90 μl min^{-1}, according to the different experimental series described below. In each session, while the marked bee whose behaviour was being recorded foraged individually on the IF, a group of foragers was allowed to forage on the GF. Thus, the colony's sugar solution intake rate was manipulated by controlling the sugar solution flow rate offered at the GF.

Experimental series

Three different experimental series (Fig. 1) were defined based on the colony's nectar intake rate, i.e. the solution flow rate offered at the GF. In the first series (constant series), the colony was presented with a constant flow rate of either 3 or 90 μl min^{-1} throughout the totality of the foraging cycles made by the marked bee that foraged on the IF. In the remaining two series (transient series), the colony was presented with two different flow rates per session: 3 and 90 μl min^{-1}. In one of these series (decreasing series), the GF offered an initial flow rate of 90 μl min^{-1} throughout the foraging cycles 1–4 performed by the marked bee that foraged on the IF. Next it offered a flow rate of 3 μl min^{-1} during its foraging cycles 5–8. In the second transient series (increasing series), the GF offered an initial flow rate of 3 μl min^{-1} throughout the forager's cycles 1–4, which was then replaced by 90 μl min^{-1} up to the end of the session (foraging cycles 5–8). Different series were randomly assayed. Each experimental session lasted approximately 3 h. A maximum of two experimental sessions were assayed per day. The colony was allowed to exploit natural food sources daily after 17:30 h.

Behavioural recordings

The number of bees foraging concurrently on the GF was recorded at 5 min intervals throughout each experimental session. The number of simultaneous dances displayed by these foragers inside the hive was also recorded at 5 min intervals.

At the IF, I recorded the time that single marked bees took to collect the offered solution (visit time). Since the IF delivered solution only when the marked bee was present at the feeder, sugar solution did not accumulate inbetween successive visits. The crop load attained by marked bees was calculated by multiplying the visit time and the solution flow rate offered by the IF. I also recorded the time (in s) that single marked bees spent inside the hive in between two successive visits to the IF (hive time).

The following variables were also considered for the analysis:

(1) The number of dancing events, i.e. round circuits, displayed inside the hive. [Returned foragers execute round dances upon discovering a desirable source of nectar located near the hive (von Frisch, 1965). According to von Frisch's (1965) own descriptions of the round dance, a dancing bee runs in a circle of such small diameter that for the most part only a single cell lies within it. It runs about over the six adjacent cells, suddenly reversing direction and then turning again to its

original course. Between two reversals there are often one or two complete circles, but frequently only three-quarters or half of a circle. Each round dance involves a variable number of these circling runs. This study focuses exclusively on round dances because the experimental set-up employed during the experiment compelled bees to collect food at artificial feeders located close to the hive (see above). To facilitate comparison with the situation a bee may encounter at feeding places located at longer distances, in the present context I shall use the term 'dance' to refer to the round dance, since no information has been reported to suggest that rules controlling dance tuning according to colony-level attributes may differ depending on whether foragers perform round or waggle dances.]

(2) The number of trophallactic offering contacts lasting more than 4 s (food-unloading events), defined as the number of events in which the marked bee opened its mandibles during more than 4 s while one or more food-receivers contacted its prementum with their protruded tongues.

(3) The duration (in s) of each food-unloading event.

(4) The number of trophallactic offering contacts lasting up to 4 s (offering contacts), defined as the number of events in which the marked bee opened its mandibles during a time interval that lasted up to 4 s while one or more receivers contacted its prementum with their protruded tongues.

(5) The duration (in s) of each offering contact.

(6) The number of trophallactic begging contacts (begging contacts), defined as the number of events in which the marked bee protruded its own proboscis towards the mandibles of a nest-mate, also moving its antennae towards the nest-mate.

(7) The number of simultaneous food-receivers, defined as the maximum number of nest-mates that received sugar solution simultaneously during the forager's food-unloading.

(8) The total number of food-receivers, defined as the number of receiver nest-mates (being simultaneous or not) that contacted the mouthparts of the marked forager for more than 1 s during the food-unloading. Such short contacts do not necessarily allow the food-receivers to get sugar solution from their donors. Food-receivers, however, usually display intense antennal interactions with their donors prior to the beginning of trophallaxis (Free, 1956); these antennal interactions might convey stimuli that foragers use to tune their dancing.

(9) The duration (in s) of each of the trophallactic interactions performed by food-receivers during a single food-unloading event, as the time intervals between when they started contacting the forager's mouthparts and when they finished.

The following example illustrates differences between variables 7 and 8: a returned forager starts being unloaded by two simultaneous food-receivers, one of which soon stops receiving food. Next, while the forager is still being unloaded by the remaining food-receiver, two additional workers start receiving food until the end of the food-unloading. Thus, on the basis of the entire food transfer, the maximum number of simultaneous food-receivers is 3 (variable 7) while the total number of food-receivers is 4 (variable 8).

Dance probability

At the individual level, dance probability was measured as the proportion of foraging cycles in which a given marked bee performed round dances, calculated from the totality of the foraging cycles it made throughout the session under similar GF-reward rate conditions. Thus, each marked bee was considered as an experimental unit in the analysis. Individual dance probabilities were then averaged for the sake of comparisons among different series. In addition, dances that occurred before and after the food-unloading were separately analysed. Returning bees, however, may transfer the content of their crops through more than one trophallaxis. Indeed, bees performed an average of 1.4 food-unloading events per foraging cycle throughout the totality of the foraging cycles recorded in the different series. Each time a marked bee performed more than one trophallaxis, the longest food-unloading event was used to discriminate between dances occurring before and after the food-unloading.

Time-based cues

During the experiment, the totality of the nectar sources exploited by the colony was controlled by means of the flight enclosure. The relatively low sugar solution flow rates offered inside the enclosure reduced the number of bees foraging simultaneously on the GF. Thus, since the time a retuned forager takes to search for a food-receiver depends upon the ratio of employed foragers and food-receivers (Seeley and Tovey, 1994; Seeley, 1995), bees foraging on the IF usually experienced relatively short searches upon their return to the hive. Moreover, short and less variable searches inexorably reduce the availability of information encoded in time-based cues. In order to quantify variations in the time that returned marked foragers spent searching to find food-receivers, the following variables were included in the analysis: (1) the initial search time (in s), (2) the total search time (in s), (3) the delivery time (in s), (4) the time elapsed since the arrival of the forager until its first food-unloading (in s; TF_{FU}), (5) the time elapsed since the arrival of the forager until its longest food-unloading (in s; TL_{FU}).

Variables 1, 2 and 3 were defined according to Kühnholz and Seeley (1997). Each time a marked forager performed more than one trophallaxis, TF_{FU} was the time interval between when it entered the hive and when it began its first food-unloading, while TL_{FU} was the time interval between when it entered the hive and when it began its longest food-unloading (no difference appears between TF_{FU} and TL_{FU} whenever returning bees perform only one food-unloading).

Statistical analysis

Data from different foraging cycles recorded under each of the two different GF-reward rate conditions (3 and 90 μl min^{-1}) were averaged for the sake of comparisons. Data from different trophallaxes (i.e. the duration of the food-unloading, the number of simultaneous food-receivers and the total number of food-receivers) were averaged each time a marked forager performed more than one trophallaxis during a single foraging cycle. The behaviour of 17 and 15 marked bees was recorded in the constant series for each of the two different flow rates offered at the GF. In the decreasing and the increasing series, 7 and 6 bees were recorded, respectively. Comparisons were made by Mann–Whitney U-tests for independent samples, Wilcoxon matched-pair tests, G-tests, t-tests for both dependent and independent samples, Lilliefors tests and Shapiro–Wilk tests, and Pearson correlations (Zar, 1984).

Results

Foraging activity at the group feeder (GF): the colony's nectar influx

In all the series, the higher the sugar solution flow rate offered at the GF the higher the number of bees foraging simultaneously inside the enclosure [Table 1; constant series: U=36, N_1 (for 90 µl min^{-1}) =25, N_2 (for 3 µl min^{-1}) =23, P<0.0001, Mann–Whitney U-test; decreasing series: $t_{(6)}$=6.03, N=7, P<0.001, t-test; increasing series: Z=2.0, N=6, P=0.046, Wilcoxon matched pairs test]. Variations in the sugar solution flow rate offered at the GF thus corresponded to variations in the colony's solution intake rate. Further, the higher the flow rate at the GF the higher the number of simultaneous round dances displayed by these foragers (Table 1; constant series: U=0.5, N_1=25, N_2=23, P<0.0001, Mann–Whitney U-test; decreasing series: no comparison is possible due to the lack of dances observed for the lower solution flow rate; increasing series: Z=2.2, N=6, P=0.028, Wilcoxon matched-pairs test).

Crop load and hive time

When a trained bee forages regularly on an artificial feeder, the crop load it attains at the end of its single foraging visits depends upon the reward rate offered by the feeder (Núñez, 1966, 1970). Since the IF always offered a constant solution flow rate, no differences were found in the crop load attained by marked bees throughout the different series (Table 1; constant series: $t_{(28)}$=0.75, N_1=15, N_2=17, P=0.46; decreasing series: $t_{(6)}$=2.15, N=7, P=0.08; increasing series: $t_{(5)}$=–0.08, N=6, P=0.94, t-test). In addition, no changes were found in the hive time during both the constant and the decreasing series (Table 1; constant series: U=116, N_1=15, N_2=17, P=0.68, Mann–Whitney U-test; decreasing series: Z=1, N=7, P=0.31, Wilcoxon matched-pairs test). In the case of the increasing series, in contrast, the higher the colony's solution intake rate the lower the hive time (Table 1; $t_{(5)}$=4.9, N=6, P<0.005; t-test).

The number of food-unloading events

This number varied in the constant series. The higher the colony's nectar intake rate the higher the number of food-unloading events performed by marked bees (Table 1; $t_{(28)}$=2.31, N_1=15, N_2=17, P=0.028, t-test). No differences were found in the transient series (Table 1; decreasing series: $t_{(6)}$=–0.32, N=7, P=0.763, t-test; increasing series: Z=0.9, N=6, P=0.36, Wilcoxon matched-pairs test).

Mean duration of food-unloading events

The mean duration of the food-unloading increased in the constant series when the colony was presented with the lower sugar solution intake rate (Table 1; $t_{(28)}$=2.31, N_1=15, N_2=17, P=0.028, t-test). No differences were found throughout the transient series (Table 1; decreasing series: $t_{(6)}$=–0.78, N=7, P=0.463; increasing series: $t_{(5)}$=1.33, N=6, P=0.24, t-test).

Table 1. *Variables recorded in the different experimental series*

	Colony's nectar influx								
	Constant series			Decreasing series			Increasing series		
	Low	High	P	High	Low	P	Low	High	P
Bees foraging on the GF[a]	5.6±0.3	11.8±0.7	<0.01	14.2±0.9	6.7±0.9	<0.01	4.4±0.3	8.7±1.5	<0.05
Simultaneous dances[a]	0.02±0.01	1.31±0.1	<0.01	1.1±0.2	0		0.03±0.03	1.8±0.2	0.03
Crop load (µl)[b]	38.2±2.0	39.0±1.6	0.46	36.1±1.96	32.9±2.2	0.08	39.9±2.8	40.0±2.6	0.94
Hive time (s)[c]	179.3±14.1	196.7±32.4	0.68	152.2±23.3	201.2±50.5	0.31	159.2±24.0	124.7±26.0	<0.01
Food-unloading events[c]	1.3±0.06	1.5±0.08	0.03	1.4±0.12	1.4±0.13	0.76	1.3±0.12	1.2±0.10	0.36
Duration of the food unloading[c]	24.1±1.3	19.3±1.2	0.02	18.8±1.3	19.7±1.1	0.46	23.7±2.2	20.2±1.4	0.24
Offering contacts[c]	1.8±0.3	2.7±0.3	0.04	2.4±0.4	1.8±0.4	0.18	1.8±0.5	1.1±0.4	0.06
Begging contacts[c]	8.4±1.3	6.1±1.0	0.17	6.3±1.8	9.4±2.7	0.26	6.7±1.7	5.1±1.0	0.19
Initial search time (s)[c]	24.9±5.5	25.1±5.2	0.98	26.6±5.7	15.2±3.4	0.08	19.7±3.7	10.7±2.1	0.04
Total search time (s)[c]	35.8±5.5	50.3±8.8	0.22	46.5±10.8	27.3±4.0	0.09	29.3±1.6	16.4±4.4	0.03
Delivery time (s)[c]	60.7±5.4	70.0±8.4	0.60	65.7±10.8	46.7±5.3	0.07	53.9±2.0	37.9±4.3	<0.01
TF_{FU} (s)[c]	27.8±4.9	24.1±4.9	0.41	14.3±2.3	13.5±1.8	0.52	22.8±5.4	16.3±5.8	0.22
TL_{FU} (s)[c]	36.3±7.9	39.1±6.8	0.48	33.0±6.8	22.4±4.4	0.06	31.8±8.9	19.8±5.3	0.08

Results are presented for the two different sugar solution intake rates (Low, 3 µl min^{-1}; High, 90 µl min^{-1}) offered to the colony at the GF. Values are means ± s.e.m. for all variables (see Results for details).

[a] Recorded at 5 min intervals.

[b] Calculated as 'visit time × sugar solution flow rate offered at the IF (5 µl min^{-1})'.

[c] Recorded from bees that foraged individually on the IF.

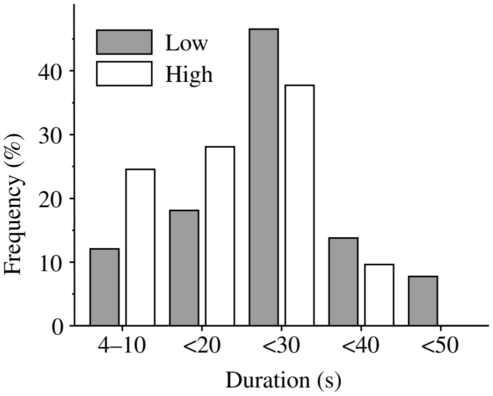

Fig. 2. Frequency distribution (%) of all the food-unloading events of different durations recorded in the constant series (each trophallaxis lasting more than 4 s was considered as an experimental unit). Data from 32 bees. Results are shown for each of the two different flow rates of sugar solution offered at the GF (grey bars, 3 μl min^{-1}; open bars, 90 μl min^{-1}).

Fig. 2 shows the frequency distribution of all the food-unloading events of different durations recorded in the constant series (each trophallaxis lasting more than 4 s was considered as an experimental unit). Data are shown separately for each of the two different solution flow rates offered at the GF (Fig. 2; 3 μl min^{-1}, grey bars: W=0.96, N=116, P<0.0015; 90 μl min^{-1}, open bars: W=0.96, N=114, P<0.0024; Shapiro–Wilk test). For both reward rates, results show a high proportion of food-unloading events lasting 20–30 s (Fig. 2, grey and open bars). Moreover, the proportion of events lasting 40–50 s increased for the lower reward rate (Fig. 2, grey bars), while the proportion of those lasting 4–20 s increased for the higher reward rate (Fig. 2, open bars).

A negative correlation thus appears in the constant series between the mean duration of single trophallaxes and the number of food-unloading events (Table 1): the lower the mean duration of single food-unloading events, the higher the number of events required to transfer the totality of the collected food.

Offering and begging contacts

In the constant series, the higher the colony's sugar solution intake rate the higher the number of offering contacts (Table 1; $t_{(28)}$=2.15, N_1=15, N_2=17, P=0.039, t-test), although the number of interactions lasting less than 2 s remained the same ($t_{(28)}$=–1.99, N_1=15, N_2=17, P=0.06, t-test). No differences were found in the transient series (Table 1; decreasing series: $t_{(6)}$=1.53, N=7, P=0.176; increasing series: $t_{(5)}$=2.39, N=6, P=0.062, t-test). Moreover, no significant variations were found in the number of begging contacts throughout all the series (Table 1; constant series: $t_{(28)}$=–1.40, N_1=15, N_2=17, P=0.172; decreasing series, $t_{(6)}$=–1.24, N=7, P=0.262; increasing series: $t_{(5)}$=1.52, N=6, P=0.19, t-test)

Time-based cues

Time-based cues varied neither in the constant nor in the decreasing series (Table 1; constant series, initial search time: U=127, N_1=15, N_2=17, P=0.98, total search time: U=94, N_1=15, N_2=17, P=0.22, delivery time: U=113, N_1=15, N_2=17, P=0.60, TF_{FU}: U=105, N_1=15, N_2=17, P=0.41, TL_{FU}: U=108, N_1=15, N_2=17, P=0.48, Mann–Whitney U-test; decreasing series, initial search time: $t_{(6)}$=2.09, N=7, P=0.08, total search time: $t_{(6)}$=2.05, N=7, P=0.09, delivery time: $t_{(6)}$=2.21, N=7, P=0.07, TF_{FU}: $t_{(6)}$=0.68, N=7, P=0.52, TL_{FU}: $t_{(6)}$=2.36, N=7, P=0.06, t-test). In the increasing series, in contrast, the lower the colony's sugar solution intake rate the higher the initial (Table 1; $t_{(5)}$=2.70, N=6, P=0.043, t-test) and the total search time (Table 1; Z=2.0, N=6, P=0.03, Wilcoxon matched-pairs test) as well as the delivery time (Table 1; $t_{(5)}$=5.55, N=6, P<0.003, t-test); although no variations were found for TF_{FU} and TL_{FU} (Table 1; TF_{FU}: $t_{(5)}$=1.40, N=6, P=0.22, TL_{FU}: $t_{(5)}$=2.16, N=6, P=0.08, t-test).

Dance probability

In all series, dance probability did not change before the foragers' food-unloading (Fig. 3, open bars; A, constant series: U=90, N_1=15, N_2=17, P=0.17, Mann–Whitney U-test; D, decreasing series: Z=0, N=7, P=1; G, increasing series: Z=1.6, N=6, P=0.11, Wilcoxon matched-pairs test). In contrast, in both the constant and the decreasing series it did change after the foragers' food-unloading (Fig. 3A,D, hatched bars). In both series, the lower the colony's sugar solution intake rate the higher the dance probability (Fig. 3, hatched bars; A, constant series: U=69, N_1=15, N_2=17, P=0.027, Mann–Whitney U-test; D, decreasing series: T=0.0, N=7, P=0.043, Wilcoxon matched-pairs test). Maximum values were recorded during the totality of the increasing series (Fig. 3G, hatched bars).

Data from the constant series also show that dance probability tends to be higher once the food transfer is finished (Fig. 3A; open *vs* hatched bars; 3 μl min^{-1} at the GF, Z=3.1, N=17, P<0.002; 90 μl min^{-1} at the GF, Z=2.5, N=15, P<0.02, Wilcoxon matched-pairs test).

Dance strength: the number of round circuits

Dancing bees may perform just a few or a hundred circuits, and the number of circuits strongly determines the rate of recruitment toward the indicated source (von Frisch, 1965). In the present context, I assume that the number of round circuits reflects the strength of the dancing display. Thus, before the food-unloading, dance strength did not change in the different series (Fig. 3, open bars; B, constant series: U=96, N_1=15, N_2=17, P=0.23, Mann–Whitney U-test; E, decreasing series: Z=0.4, N=7, P=0.72; H, increasing series: Z=1.8, N=6, P=0.08, Wilcoxon matched-pairs test). In contrast, it did change after the food-unloading in the decreasing series: the lower the colony's sugar solution intake rate the higher the dance strength (Fig. 3E, hatched bars; $t_{(6)}$=5.8, N=7, P<0.002, t-test). Differences were found in neither the constant (Fig. 3B, hatched bars; $t_{(28)}$=1.33, N_1=15, N_2=17, P<0.195, t-test) nor in the increasing series after the foragers' food-unloading (Fig. 3H, hatched bars; Z=0.5, N=6, P=0.60, Wilcoxon matched-pairs test).

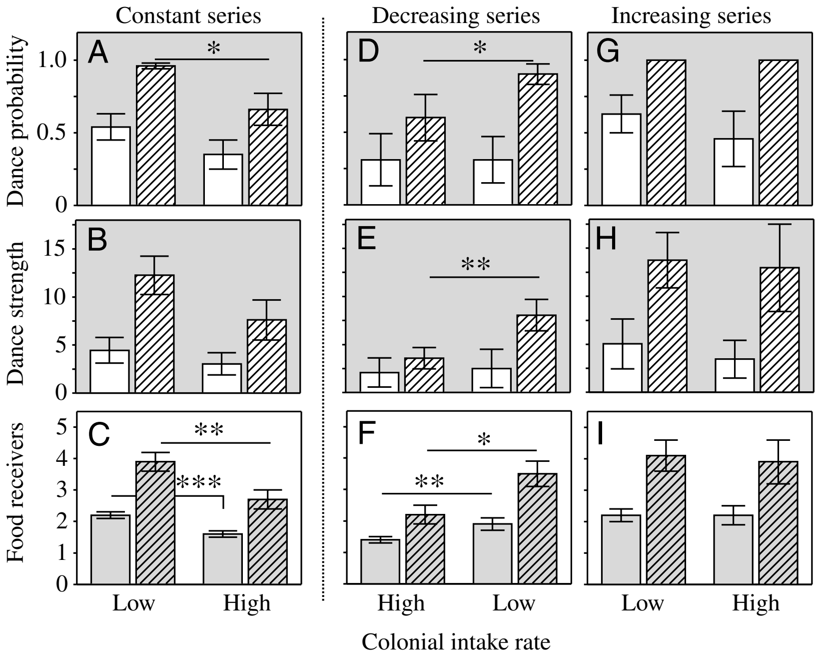

Fig. 3. (A,D,G) Dance probability before (open bars) and after (hatched bars) the foragers' food-unloading. (B,E,H) Dance strength, i.e. number of round circuits, before (open bars) and after (hatched bars) the food-unloading. (C,F,I) Maximum number of simultaneous food-receivers (grey open bars) and total number of food-receivers (grey hatched bars) involved in the foragers' food-unloading (see Materials and methods for details). Data correspond to marked bees that foraged individually on the IF. Results are shown for the two different reward conditions (Low: 3 μl min^{-1}, High: 90 μl min^{-1}) offered at the GF throughout the three different experimental series: A,B,C constant series (N_1=15, N_2=17), D,E,F decreasing series (N=7), and G,H,I increasing series (N=6). See Results for details on statistics. Values are means ± s.e.m.; *P<0.05, **P<0.01, ***P<0.001.

The number of food-receivers

Both the maximum number of simultaneous food-receivers (open bars) and the total number of food-receivers (hatched bars) that interacted with the returned marked bees during their single food-unloading events are shown in Fig. 3C,F,I, grey bars. In both the constant and the decreasing series, the lower the colony's sugar solution intake rate the higher the number of simultaneous food-receivers, as well as the total number of food-receivers (Fig. 3C, constant series, simultaneous food-receivers: $t_{(28)}$=3.84, N_1=15, N_2=17, P<0.0007, total number of food-receivers: $t_{(28)}$=2.75, N_1=15, N_2=17, P=0.01; F, decreasing series, simultaneous food-receivers: $t_{(6)}$=3.26, N=7, P=0.017, total number of food-receivers: $t_{(6)}$=4.65, N=7, P<0.004; t-test). No changes were recorded in these variables during the increasing series (Fig. 3I; simultaneous food-receivers: $t_{(5)}$=0.47, N=6, P=0.66, total number of food-receivers: $t_{(5)}$=0.33, N=6, P=0.75, t-test).

Throughout all the different series, both the probability and the strength of dances occurring after the foragers' food unloading appeared to be correlated to the mean number of food-receivers, being simultaneous or not (Fig. 4A, dance probability *vs* number of simultaneous food-receivers: before trophallaxis, filled circles, R^2=0.64, P=0.06; after trophallaxis, open circles: R^2=0.97, P<0.001; Fig. 4B, dance probability *vs* total number of food-receivers: before trophallaxis, filled circles, R^2=0.64, P=0.06; after trophallaxis, open circles, R^2=0.93, P<0.002; Fig. 4C, dance strength *vs* number of simultaneous food-receivers: before trophallaxis, filled circles, R^2=0.62, P=0.07; after trophallaxis, open circles, R^2=0.98, P<0.001; Fig. 4D, dance strength *vs* total number of food-receivers: before trophallaxis, filled circles, R^2=0.63, P=0.06; after trophallaxis, open circles, R^2=0.91, P<0.004; Pearson correlations).

Distribution of food-unloading events and dance circuits throughout the hive time

Next I used data collected during the decreasing series in order to evaluate the distribution of food-unloading events and dance circuits throughout the hive time (Fig. 5). To compare information from different bees and different foraging cycles, the hive time was divided into 20 periods of equal duration. An event (either a food-unloading or a dance circuit) was then included in a given period based on the time elapsed from the arrival of the marked bee until the occurrence of such event, and dividing this time lapse by the hive time. Finally, the frequency distribution of all the food-unloading events as well as all the dance circuits recorded in the series was analyzed separately for each of the two different colony's solution intake rates. Fig. 5 shows that food-unloading events occurred mainly during the first half of the hive time under both reward rate conditions (A and B, open and grey bars, respectively). The temporal distribution of the dance circuits slightly changed after the induced decrease in the colony's solution intake rate (Fig. 5C *vs* D, open and grey bars, respectively): a bimodal distribution of circuits was observed under both reward rate conditions, but an increase in the proportion of circuits occurring during the first half of the hive time was observed for the lower reward rate.

Duration of the receivers' trophallactic interactions

Data collected in the constant series were used to analyse the duration of trophallactic interactions performed by food-receivers interacting with marked bees. These interactions lasted from 1 s up to 50 s, and a higher proportion of brief interactions (lasting up to 2 s) was found for the lower colony's solution intake rate (Fig. 6, grey *vs* open bars: 50.7% *vs* 28.0%, G=34.8, d.f.=1, P<0.001, G-test).

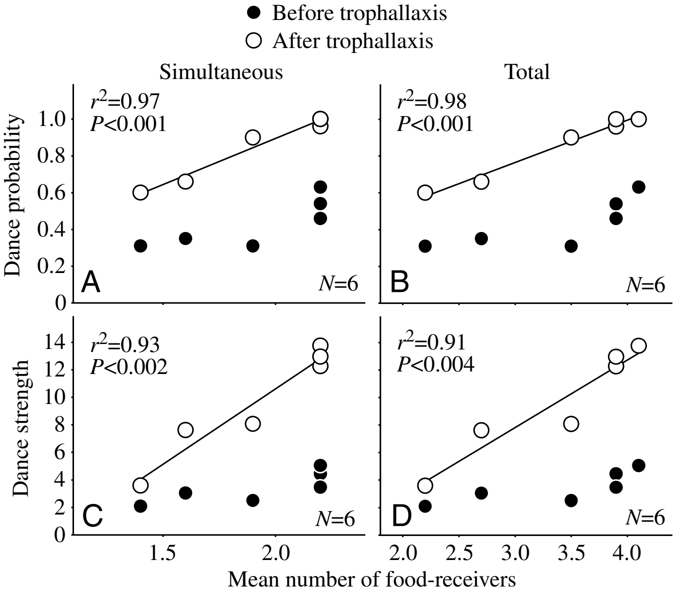

Fig. 4. Dance probability (A,B) and dance strength (C,D) plotted against the maximum number of simultaneous food-receivers (A,C) and the total number of food-receivers (B,D). Single records correspond to the mean values shown in Fig. 3. Data are shown for dances recorded before (filled circles) and after (open circles) the foragers' food-unloading. See Results for details on statistics.

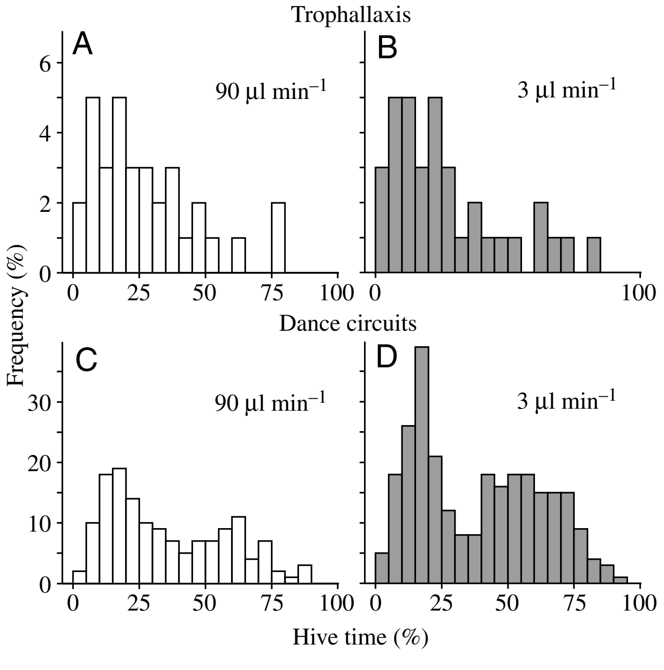

Fig. 5. Frequency distribution (%) of food-unloading events (A,B) and dance circuits (C,D) throughout the hive time. Data from the decreasing series. The hive time was divided into twenty periods of equal duration. Results are shown for each of the two different reward rates offered at the GF (open bars: 90 μl min^{-1}, grey bars: 3 μl min^{-1}). See Results for details.

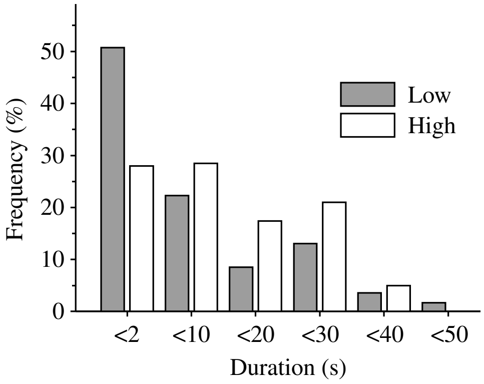

Fig. 6. Frequency distribution (%) of the duration of trophallactic interactions performed by food-receivers interacting with marked bees (see Materials and methods for details). Data from the constant series. Results are presented for each of the two different reward rates offered at the GF (grey bars: 3 μl min^{-1}, N=422, 105 food-unloading events, 17 marked bees; open bars: 90 μl min^{-1}, N=266, 101 food-unloading events, 15 marked bees).

Discussion

Shifts in dance thresholds

This study focused on how returned forager bees sense an attribute of the whole colony (i.e. its rate of nectar inflow) on the basis of its direct within-the-hive interactions with food-receivers, and the effects of the colony's nectar-foraging status on the tuning of individual dance thresholds. This means that dances were considered as a behavioural output capable of revealing the bee's perception of colony needs (Seeley, 1995). It also means that the colony's sugar solution intake rate was carefully manipulated during the experiments by controlling the totality of the nectar sources exploited by the colony. Results indicated that, upon being unloaded by food-receivers, returned bees modified their dances according to differences in the colony's nectar intake rate: dance probability was negatively correlated to the colony's solution intake rate in both the constant and the decreasing series (Fig. 3A,D, hatched bars). Dance strength, i.e. the number of round circuits, changed neither in the constant nor in the increasing series (Fig. 3B,H, hatched bars), but unloaded foragers danced more intensively during the decreasing series at times of low colony's nectar influx (Fig. 3E, hatched bars). Hence results also indicate that bees may rapidly change their dance thresholds after a sudden decrease in the colony's nectar intake rate. These shifts in dance thresholds can not be explained on the basis of the quality or the quantity of the offered reward (see Materials and methods and Results). In addition, neither the probability nor the strength of the foragers' dances changed in the increasing series (Fig. 3G, hatched bars). Since dance thresholds were already minimum at the beginning of this series (the probability of dancing was 1.00 when the colony's nectar influx was low), results indicate that changes in the values of those internal parameters regulating dance strength depends upon their current levels. Further, they also indicate that, during transient foraging conditions, loss and gain (with respect to the colony's nectar influx) might be not equally revealed through shifts in the foragers' dance behaviour. Since sucrose stimulation, the main stimulus triggering dances, remained constant during the experiments, the present results emphasize the flow of resource-related information that naturally occurs within honeybee colonies (Lindauer, 1948, 1954; Núñez, 1970; Seeley, 1995).

Time-based cues

The distribution of nectar inside the hive involves time costs (Seeley, 1995; Ratnieks and Anderson, 1999): i.e. the area where most trophallaxes occur is relatively large and contains other bees, so that foragers and receivers need to search for a partner, usually antennating several other bees before a partner is found (Seeley, 1995). It has been demonstrated that time-based cues derived from these searches encode information on colony needs, which foragers use to adjust their dance thresholds (Lindauer, 1948; Seeley, 1986, 1989, 1994, 1995, 1998; Seeley et al., 1991; Seeley and Tovey, 1994). In the present context, however, dance variations were found without recording simultaneous variations in the time-based cues that might have encoded variations in the colony's nectar influx (Table 1; Fig. 3A,D,E). The lack of variation in these cues must be interpreted as an artifact of the experimental layout. That is, although the colony's sugar solution intake rate was varied during the experiment, it was always maintained below $100\ \mu l\ min^{-1}$. This rate of reward is relatively low at the colony-level, and thus led to a small proportion of employed foragers (Table 1), according to the colony size (approximately 4000 individuals) and the expected proportion of foragers (25%, Seeley, 1995). Since time costs associated with the food transfer strongly depends upon the ratio of employed foragers and food-receivers (Seeley, 1995), a low proportion of employed foragers will reduce both the magnitude and variability of these costs whenever the number of employed food-receivers remains constant (Seeley and Tovey, 1994). In the present context, however, this does not imply that time-based cues were excluded from the store of reliable indicators of colony needs. Indeed, returned bees invested relatively short time-intervals while searching for food-receivers, which undoubtedly decreased their dance thresholds (Lindauer, 1948; Seeley, 1995). It means, instead, that the observed variations in the foragers' dance behaviour cannot be explained based on variations in the magnitude of the time-based cues they experienced upon returning to the hive (Table 1).

Duration of the food-unloading

A positive correlation has been reported between the duration of trophallaxis and those dances occurring upon the end of the food-unloading, suggesting that bees might tune their dances by computing the duration of the food-unloading (Farina, 2000). In this study, however, dance probability changed irrespective of the duration of the food-unloading (Table 1, Fig. 3D,E), indicating both that the duration of trophallaxis itself does not constitute a reliable indicator of the colony's nectar influx, and that foragers do not rely on the duration of the food-unloading to tune their dancing. Furthermore, the duration of trophallaxis appeared not to be correlated to the number of food-receivers involved in trophallaxis (Table 1, Fig. 3). The fact that sometimes it did increase together with the number of food-receivers (Table 1, constant series and Fig. 3C) indicates that the distribution of food to simultaneous food-receivers is not homogeneous. Since marked bees were always provided with a constant flow rate of sugar solution at the feeder (see Materials and methods), neither the crop load (Núñez, 1966) nor the rate of food transfer during trophallaxis (Farina and Núñez, 1991) can account for the differences in the mean duration of single food-unloading events found in the constant series (Table 1). These differences might be interpreted, at least partially, by considering additional factors that lead to simultaneous variations in the number of food-unloading events (Table 1). Indeed, the higher the number of trophallaxes the lower the mean duration of the food-unloading. Further, offering contacts (<4 s) also appeared to be inversely correlated to the duration of the food-unloading (Table 1), but no differences were found in the number of these short interactions when they lasted less than 2 s (see Results), a duration that probably does not allow an effective transfer of food.

Short trophallactic interactions

It has been reported that the number of short trophallactic interactions that returned foragers perform before initiating their actual food-unloading may constitute an indicator the colony's nectar intake rate (Seeley, 1986). In the constant series, differences were found in the number of these short interactions (Table 1), which matches previous results (Seeley, 1986). However, in both the decreasing and the increasing series, no differences were found in either the number of food-unloading events or the number of offering contacts (Table 1), which indicates that short trophallactic interactions do not necessarily encode the colony's nectar influx.

In addition, in all series, no changes were found in the number of begging contacts (Table 1). Previous evidence indicates that foragers might benefit from 'begging' to gather resource-related information within the colony (De Marco and Farina, 2001, 2003), although the foragers' begging behaviour seems to depend upon the level of resource uncertainty experienced in the field (De Marco and Farina, 2003), which remained constant throughout the present experiments (see Materials and methods).

Food-receivers

A returned forager usually transfers most of its crop to only one food-receiver, although trophallaxes involving several simultaneous food-receivers can also take place during the food transfer (Park, 1925; Lindauer, 1954; Seeley, 1986, 1989; Kirchner and Lindauer, 1994; Farina, 2000; De Marco and Farina, 2001; Huang and Seeley, 2003). Liquid food is likely to be successfully received by two simultaneous workers. However, when more simultaneous food-receivers are involved, those positioned more laterally with respect to the forager's mouthparts seem to receive food less efficiently (Farina and Wainselboim, 2001). It also happens that additional workers usually approach both foragers and food-receivers when they are already engaged in trophallaxes, protruding their probosces towards the foragers' mouthparts. These bees either interact with the foragers shortly or prolong their interactions so that they also become engaged in the forager's food-unloading until the end of trophallaxis. Further, whenever several food-receivers are simultaneously involved in trophallaxis, some of them may interrupt their interactions before the food-unloading is finished. In this study, I measured the maximum number of simultaneous food-receivers as well as the total number of food-receivers involved in a forager's food-unloading (see Materials and methods).

The higher the colony's nectar intake rate the lower the number of simultaneous as well as non-simultaneous food-receivers, in both the constant and the decreasing series (Fig. 3C,F, open and hatched bars); but not in the increasing series (Fig. 3I, open and hatched). In both the constant and the decreasing series, either one or two simultaneous food-receivers unloaded foragers at times of high colony nectar influx, and only one additional food-receiver contacted the foragers' mouthparts once trophallaxes were initiated, thus increasing the total number of food- receivers. When the colony's intake rate was low, however, two simultaneous food-receivers and one or two additional food-receivers were involved in the foragers' food-unloading (Fig. 3C,F, open and hatched bars). This means that when the colony's rate of nectar inflow remains stationary or diminishes abruptly, the crowd of food-receivers may be a reliable indicator of 'global' foraging conditions.

In addition, food-receivers executed trophallaxes that lasted between 1 and 50 s throughout the experiment and, among them, exhibited a high proportion of brief interactions (Fig. 6). Interestingly, these brief interactions appeared to be more frequent at times of low nectar influx (Fig. 6, grey and open bars). Video-recordings showed that additional food-receivers (those that approached the returned foragers when they were already engaged in trophallaxis), usually performed brief interactions (data not shown). When the colony's rate of nectar inflow was low, these additional food-receivers were frequently identified (based on individual marks) as bees that had previously foraged on the group feeder, suggesting that temporally unemployed foragers may be involved in trophallaxis with other employed foragers. This finding is in close agreement with previous evidence (De Marco and Farina, 2003).

The low proportion of employed foragers induced experimentally probably led to a high availability of food-receivers within the delivery area. But how did the number of food-receivers change together with the colony's rate of nectar inflow? Nectar is rapidly distributed within the colony (Nixon and Ribbands, 1952). Thus, the average crop load of the single food-receivers present within the delivery area might decrease at times of low nectar influx (Huang and Seeley, 2003; Gregson et al., 2003). If single food-receivers tend to 'achieve' a certain amount of solution before leaving the delivery area (in order to bring nectar into other areas of the comb), a decreased rate of nectar inflow may enhance the availability of food-receivers within this area, which may, in turn, enhance the number of food-receivers involved in trophallaxis with returning foragers. In order to test this hypothesis, however, both the crop contents of workers present within the delivery area and the colony's nectar intake rate must be simultaneously analyzed.

The number of food-receivers and the subsequent dances

Once the food-unloading is finished, dance probability appears to be positively correlated to the number of food-receivers that have been involved in trophallaxis (Fig. 4). A positive correlation between the number of food-receivers and the probability of the donor's subsequent dances has been reported elsewhere (Lindauer, 1948; Seeley, 1986; Farina, 2000), but the existence of separate effects on dance tuning of the crowd of food-receivers remained unclear since the foragers' dance thresholds also appeared to be correlated to a number of variables associated with the process of food transfer. I found no consistent correlation between the dance thresholds of unloaded foragers and the remaining variables considered in this study (Table 1, Figs 3 and 4). Results thus indicate that returned foragers compute stimuli derived from the number of food-receivers to tune their dances. These stimuli emerge during trophallaxis and seem to operate alongside time-based cues that foragers experience prior to trophallaxis (Seeley, 1995). Further, results also show that perception of even slight variations in the colony's nectar intake rate can be 'revealed' through the ensuing dance variations. In this study I considered two different variables to quantify Lindauer's 'eagerness' of food-receivers (Lindauer, 1948; see also Seeley et al., 2002): the number of simultaneous food-receivers and the total number of food-receivers (see Materials and methods). One might view the latter one, however, as a more reliable indicator of the colony's nectar influx because the maximum number of simultaneous food-receivers exhibits a lower scope (Fig. 3C,F,I).

Distribution of food-unloading events and dance circuits throughout the hive-time

If foragers tune their dances by computing stimuli derived from the number of food-receivers, it might be important to analyse the temporal distribution of both food-unloading events and dance circuits throughout the hive-time. I conducted this analysis with data from the decreasing series. Results show that foragers display a higher proportion of dance circuits during the first half of the hive-time when the colony's nectar intake rate suddenly decreases (Fig. 5C *vs* D). Food-unloading events occur mainly during this period (Fig. 5A,B), thus indicating that variations in the number of dance circuits, as related to recent variations in the colony's nectar intake rate, occur soon after trophallaxis, as expected if a direct relationship exists between stimuli available during the food-unloading and subsequent variations in the foragers' dances (von Holst and Mittelstaedt, 1950).

The effects of multiple food-receivers: previous evidence and a possible sensory modality

Throughout a set of pioneering descriptions of individual behaviours underlying homeostasis in honeybee colonies, Lindauer (1948, 1954) pointed out that returning foragers may benefit from certain stimuli derived from the process of food-transfer in order to adjust their recruitment behaviours according to the foraging status of the colony as a whole. According to Lindauer's early observations (Lindauer, 1948, 1954), this process involves cues derived from the time spent while searching to find a food-receiver and the time required to finish unloading the collected food (time-based cues), as well as the number of food-receivers involved in the forager's food-unloading.

The relationship between time-based cues and different aspects of the foragers' behaviour has been carefully described and is now well understood (Seeley, 1986, 1989, 1994, 1995, 1998). Significant changes in the maximum number of food-receivers with changes in a colony's nectar influx have also been consistently reported (Seeley, 1986, 1989; Kühnholz and Seeley, 1997). However, the separate effects of the number of food-receivers on dance tuning has received less attention. This may be because the crowd of food-receivers usually varies together with a number of additional variables associated with the food transfer process. Thus the sensory bases of these separate effects (as related to their effects on dance tuning) remain unknown. A reasonable hypothesis to account for the effects of the number of food-receivers might rely on the intense antennal and mouth-to-mouth interactions that occur between donor and food-receivers (Montagner and Galliot, 1982; Goyret and Farina, 2003). Indeed, the integrity of the food-receivers' antenna are essential for the food transfer to occur (Free, 1956). Returned foragers might thus integrate repetitive stimulation encoded in some form of mechanical stimuli derived from antennal and mouth-to-mouth interactions.

Acquiring information during trophallaxis

Lindauer (1948, 1954, 1961) pointed out that mutual begging and the transfer of liquid food by mouth are directly involve in the regulation of task priorities and individual foraging behaviours within honeybee colonies. Present results indicate that the number of food-receivers involved in trophallaxis correlates to both the probability and the strength of subsequent dances, irrespective of variations in time-based cues experienced by foragers upon their return to the hive. Since the number of food-receivers appeared to be negatively correlated to the colony's rate of nectar inflow, it must be concluded that stimuli perceived during trophallaxis enhance the flow of informational cues allowing foragers to tune their recruitment behaviours according to colony needs.

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