

CHANGES IN THE THORACIC TEMPERATURE OF HONEYBEES WHILE RECEIVING NECTAR FROM FORAGERS COLLECTING AT DIFFERENT REWARD RATES

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Summary

Mouth-to-mouth food exchange in eusocial insects (trophallaxis) contributes to the organization of complex social activities. In the case of honeybees, foragers returning from a nectar source transfer the food collected to receiver colony-mates through oral contact. Previous studies have shown that the speed of nectar transfer within each contact (unloading rate) increases when foragers return from feeding sites with higher profitability, i.e. with more concentrated sugar solutions or higher solution flow rates. However, there is no evidence that the nectar unloading rate is actually evaluated by hive-mates during food exchange. To investigate this, trophallaxis between donor bees returning from a feeder with different flow rates of sucrose solution (range 1.0–8.2 $\mu\text{l min}^{-1}$ of 50% w/w sucrose solution) and receiver hive-mates was studied by combining behavioural and infrared thermal analysis.

The results show that when foraging bees returned from a feeder delivering a higher flow rate they initiated unloading at higher thoracic temperatures and transferred the solution at higher speed. During these food exchanges, the thoraces of receiver bees warmed up faster in proportion to increasing forager temperature and unloading rate. Therefore, whatever the variable actually evaluated by receivers (mostly nectar processors, i.e. bees that handle nectar in the hive) during trophallaxis (unloading rate and/or donor thoracic temperature), they raised their activity level in proportion to that of the foragers. In this way, receiver bees will intensify their nectar processing when nectar foragers return from more profitable sites.

Key words: honeybee, *Apis mellifera carnica*, foraging, profitability, trophallaxis, information transfer, thermography.

Introduction

Trophallaxis in honeybees involves a donor that opens its mandibles to expose a drop of regurgitated nectar and one or more receivers that receive the liquid food offered by the donor (von Frisch, 1967). If hive bees have gathered similar nectar previously in the field, they may be alerted or recruited by the familiar odours transmitted by unloading contact. Nectar odours thus play a key role in recruitment, since the transfer of scented food seems to stimulate unemployed foragers to resume foraging activity even in the absence of dance manoeuvres (von Frisch, 1923).

Trophallaxis performed by returning foragers has been shown to vary according to different nectar properties that define its profitability, such as sugar concentration and delivery rate (flow). Quantitative changes in trophallaxis have been measured in response to changes in the volume and concentration of sucrose solution carried by bees and the flow rate offered at the feeding site (Farina, 1996; Farina and Núñez, 1991; Tezze and Farina, 1999; Wainseboim and Farina, 2000a; Wainseboim and Farina, 2000b). Such changes were found both in experimental arenas containing loaded and

unloaded honeybee foragers (Farina and Núñez, 1991; Tezze and Farina, 1999; Wainseboim and Farina, 2000a; Wainseboim and Farina, 2000b) and in the hive (Farina, 1996). For instance, the unloading rate between partners (i.e. the speed at which the solution is transferred from donor to receiver) increases with the volume of solution carried by the donor bee, a variable that also depends on the flow rate at the food source (Núñez, 1966). Specifically, previous studies (Farina, 1996; Farina and Núñez, 1991; Wainseboim and Farina, 2000a; Wainseboim and Farina, 2000b) demonstrated that foragers modulate the unloading rate according to the flow of solution previously exploited at the food source. If receiver bees could evaluate this modulation in rate of nectar unloading, they could acquire information that might be used to adjust their behaviour inside the hive. Therefore, a critical issue regarding this hypothesis is whether, during trophallaxis, the modulation found in the unloading rate of the returning forager is actually evaluated by receiver bees.

Previous observations suggest that bees collecting food regulate their thoracic temperature while ingesting solutions

of different sugar concentration at a feeding place (Schmaranzer and Stabentheiner, 1988), so a similar active thermoregulation of thoracic muscles from hive bees while receiving solution at different unloading rates from foraging bees is to be expected, since both variables are related to the profitability perceived during the ingestion process. This result would imply that, through trophallaxis, receiver bees could acquire information about the activity level of the returning forager or respond to the quantity of nectar they receive from the returning forager. To test this hypothesis, the thermal behaviour of hive bees involved in nectar unloading was analysed with respect to changes in the flow of solution offered to food-donor foragers by means of infrared thermography, since this has proved a useful non-invasive technique for monitoring body temperature (Schmaranzer and Stabentheiner, 1988; Stabentheiner, 1996; Stabentheiner and Hagmüller, 1991; Stabentheiner et al., 1995).

Materials and methods

This study was undertaken during the summer of 1998 at the Bee Station of the University of Würzburg, Germany. A two-frame observation hive of *Apis mellifera carnica* (Pollm.) was placed inside a laboratory that shielded the measurement site from solar heat and sunlight. To obtain infrared thermographic recordings, the glass front of the observation hive was removed during experiments and replaced with infrared-transmitting plastic foil.

Procedure

Single individually marked bees (marked with a spot of paint) were trained to collect 50% (w/w) scented sucrose solution ($80\mu\text{l l}^{-1}$ vanilla essence) from a feeder 180 m from the colony. At the feeder, sucrose solution was provided at different flow rates (1.0, 2.4 and $8.2\mu\text{l min}^{-1}$) via a pump driven by a synchomotor (Núñez, 1970). The synchomotor was switched on when a bee arrived at the feeder and off immediately after it returned to the hive. In this way, no solution accumulated at the feeder between foraging bouts.

To analyse the foraging behaviour of individual bees, all recruited bees were captured during experiments. The flow rate at the feeder was kept constant for 3–4 successive visits. The behaviour of individual bees was recorded both at the feeder and inside the hive over 9–19 foraging cycles, during which three flow rates were offered sequentially and randomly. This experimental arrangement allowed us to compensate for transient responses to changes in flow and to control for possible effects of daily activity rhythms.

Behavioural measurements

At the feeder, we measured the total feeding time (in min), defined as the time spent by a bee at the feeder from its arrival until it left to return to the hive. By multiplying the feeding time by the sucrose solution flow rate, the crop load carried in the foraging trip was obtained. Núñez (Núñez, 1966; Núñez,

1974) found no difference between forager crop loads calculated by weighing the animal before and after feeding and the method used here. However, this method is valid only when the flow rate offered at the feeder is lower than the bee's maximal intake rate (approximately $60\mu\text{l min}^{-1}$ for a 50% w/w sucrose solution; see Núñez, 1966) and when only one bee at a time visits the feeder.

At the observation hive, we recorded (i) hive time (in min), defined as the time foragers remained in the hive and (ii) unloading time (in s), defined as the time a forager spent with its mandibles open while one or more receiver bees contacted its prementum. From data on the forager's crop load (in μl) and unloading time (in s), the rate at which the forager unloaded solution to the receiver hive-mates (unloading rate, in $\mu\text{l s}^{-1}$) was calculated. It was assumed that bees transferred the entire collected load before returning to forage, thus arriving at the feeder with little or no crop load (Scholze et al., 1964).

Thermographic recordings

Thermographic recordings at the observation hive were obtained using a Radiance PM-Amber thermo-camera. In the centre of the image produced, a cross allowed the surface temperature of a particular point to be recorded continuously; this was used to measure the thoracic temperature of the experimental bees (see Fig. 1). The thermal behaviour of bees involved in food transfer was recorded on videotape for later analysis of the following variables: (i) honeycomb surface temperature, measured within a 5 cm area of the partners involved in trophallaxis; and (ii) the instantaneous thoracic temperature of the receiver bee positioned in front of the donor. A linear increase in temperature of the receivers during trophallaxis was observed (see Results) and the slope, defined as the thoracic heating rate (in $^{\circ}\text{C s}^{-1}$), was obtained for each receiver from the instantaneous temperature (which was recorded continuously throughout the contact) and the thermographic recording time data. (iii) The thoracic temperatures of the donor bee at the beginning and at the end of the contact (in $^{\circ}\text{C}$) were also recorded. The difference between these two values was divided by the total contact time to estimate a heating rate for each donor bee. The infrared emissivity used was 0.95, a value that allowed absolute body surface temperature to be calculated.

Statistical analyses

Data were analysed using one-way analysis of variance (ANOVA), linear regressions, correlation analysis and analysis of covariance (ANCOVA) (Sokal and Rohlf, 1981). Since interindividual variability in honeybee behaviour is well known (Farina, 1996; Waddington and Kirchner, 1992), an ANCOVA was performed to factor out such effects among bees. To avoid pseudoreplication (Hurlbert, 1984), measurements taken at a constant feeder flow rate were pooled to give a sample mean before statistical analysis. In total, 128 foraging cycles performed by nine bees during nine observation days were analysed.

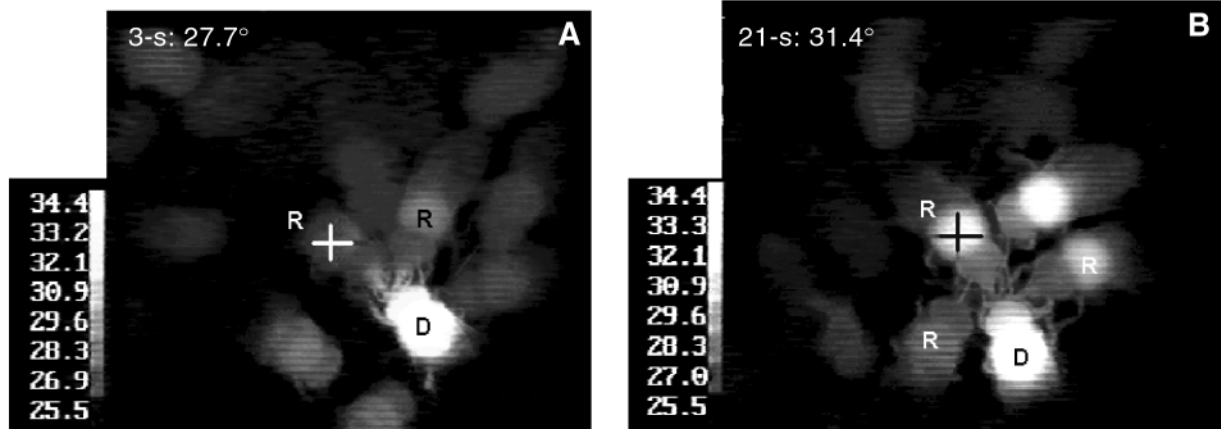


Fig. 1. Thermograms showing the surface temperatures of a food-donor forager (D) and receiver hive-mates (R) at two different times during one bout of trophallaxis. The cross at the centre of each picture is used to measure the instantaneous thoracic surface temperature of the same receiver bee: 27.7 °C in A 3 s after the beginning of the contact, and 31.4 °C in B after 21 s. The donor bee had returned from a feeder offering a 50% w/w sucrose solution at a flow of 8.2 $\mu\text{l min}^{-1}$ located 180 m from the hive.

Results

At higher feeder flow rates, trained bees imbibed solution for shorter periods, but achieved a higher final crop load (Table 1). When these bees arrived at the hive, they invested similar times in unloading food (Table 1), although the unloading rate at which the liquid was transferred increased with solution flow rate at the feeder. Thus, as predicted from previous studies (Farina, 1996; Farina and Núñez, 1991; Núñez, 1966; Núñez, 1970; Wainelboim and Farina, 2000a; Wainelboim and Farina, 2000b), the flow rate of sucrose solution at the feeder influenced not only the behaviour at the feeding site but also food unloading inside the hive.

The thoracic surface temperatures of bees involved in food transfer were also monitored. An example of infrared thermal images at two time points during one trophallaxis episode is shown in Fig. 1. The contact in this case involves one bee receiving food from a forager that had returned from the feeder delivering a flow rate of 8.2 $\mu\text{l min}^{-1}$. The thermograms show a large thoracic temperature increase (3.7 °C) in the receiver bee during 18 s of food unloading; its abdomen and head temperatures changed to a much smaller extent. Donor head and thorax temperatures remained relatively unchanged during the food-transfer process (Fig. 1).

The thoracic temperature of receiver bees increased linearly with contact time with donor foragers for all feeder flow rates (Fig. 2). The thoraces of receivers warmed up in 73 out of 74 recorded contacts (in one case, the receiver's thorax cooled down while interacting with a donor that had fed at 1.0 $\mu\text{l min}^{-1}$; see Fig. 2A). All data were fitted well by linear regressions (Fig. 2). Donors had higher initial thoracic temperatures at the beginning of trophallaxis when they returned from feeders with higher flow rates (Fig. 3A, open circles). The initial thoracic temperature of receivers, although showing a positive relationship with honeycomb surface temperature ($r=0.43$, $P<0.0001$, $N=69$), did not correlate with the flow experienced by donors (Fig. 3A, filled circles). In

Table 1. Behavioural variables recorded at the food source and inside the hive as a function of the solution flow rate exploited by trained foragers

	Solution flow rate ($\mu\text{l min}^{-1}$)			Σ
	1.0 ($N=5$)	2.4 ($N=7$)	8.2 ($N=8$)	
At the food source				
Feeding time (min) ^a	24.1 \pm 5.08	15.8 \pm 1.14	6.0 \pm 0.42	50
Crop load (μl)	24.6	37.8	49.4	
T_a (°C) ^b	19.7 \pm 1.09	20.25 \pm 0.72	19.6 \pm 0.77	53
At the hive				
Unloading time (s) ^c	23.4 \pm 4.08	22.8 \pm 1.29	26.4 \pm 3.46	50
Unloading rate ($\mu\text{l s}^{-1}$)	1.1	1.8	2.1	
T_h (°C) ^d	25.7 \pm 0.68	25.2 \pm 0.37	25.1 \pm 0.31	53

Values are means \pm S.E.M., except for mean crop loads and unloading rates, which were estimated as described in Materials and methods.

Values measured at the same flow rate were pooled to give a sample mean before performing statistical analyses.

N , number of bees; Σ , sum of replicates; T_a , ambient temperature; T_h , hive temperature.

^aResults from ANCOVA: $\log(\text{flow rate}) F_{1,10}=19.38$, $P<0.005$; individual $F_{6,10}=1.10$, not significant; interaction term $F_{6,4}=0.56$, not significant.

^bResults from one-way ANOVA: $F_{2,50}=0.21$, not significant.

^cResults from ANCOVA: $\log(\text{flow rate}) F_{1,10}=2.92$, not significant; individual $F_{6,10}=0.89$, not significant; interaction term $F_{6,4}=2.09$, not significant.

^dResults from one-way ANOVA: $F_{2,50}=0.47$, not significant.

addition, while no thermal change was observed after trophallaxis for the thoraces of donors experiencing different flow rates (Fig. 3B, open circles), the thoraces of the receivers warmed up to a greater extent with increasing flow rates of solution previously experienced by the donor bees (Fig. 3B,

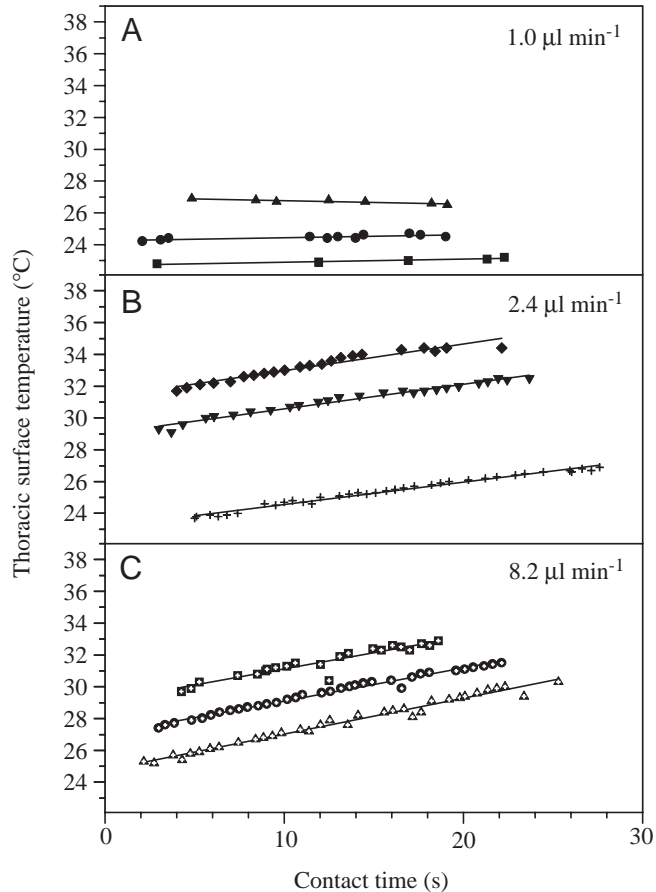


Fig. 2. Examples of instantaneous thoracic surface temperature T_{th} ($^{\circ}\text{C}$) of different receiver bees at different times t during trophallaxis with foragers returning from a feeder supplying different flow rates. (A) $1.0 \mu\text{l min}^{-1}$; the regression lines are: $T_{th}=27.0-0.02t$, $r^2=0.82$, $P<0.005$, $N=7$ (triangles); $T_{th}=24.3+0.02t$, $r^2=0.66$, $P<0.005$, $N=11$ (circles); $T_{th}=22.7+0.02t$, $r^2=0.92$, $P<0.02$, $N=5$ (squares). (B) $2.4 \mu\text{l min}^{-1}$: $T_{th}=29.0+0.15t$, $r^2=0.97$, $P<0.0001$, $N=26$ (triangles); $T_{th}=23.2+0.14t$, $r^2=0.98$, $P<0.0001$, $N=26$ (plus signs); $T_{th}=31.3+0.17t$, $r^2=0.94$, $P<0.0001$, $N=22$ (diamonds). (C) $8.2 \mu\text{l min}^{-1}$: $T_{th}=24.8+0.23t$, $r^2=0.98$, $P<0.0001$, $N=34$ (triangles); $T_{th}=27.0+0.21t$, $r^2=0.985$, $P<0.0001$, $N=34$ (circles); $T_{th}=29.1+0.20t$, $r^2=0.89$, $P<0.0001$, $N=22$ (squares).

filled circles). These heating rates were independent of the thoracic temperature at which receivers initiated the contact ($r=-0.027$, not significant, $N=74$) and of the number of bees feeding simultaneously from the donor forager (for $1 \mu\text{l min}^{-1}$, $r=0.547$, not significant, $N=7$; for $2.4 \mu\text{l min}^{-1}$, $r=-0.037$, not significant, $N=34$; for $8.2 \mu\text{l min}^{-1}$, $r=0.196$, not significant, $N=28$), although they did depend on the orientation of the receiver to the donor, i.e. receivers positioned in front of the donor foragers warmed up faster than those positioned laterally.

Discussion

Previous results have provided evidence that honeybees collecting food at a feeder not only elevate their thoracic

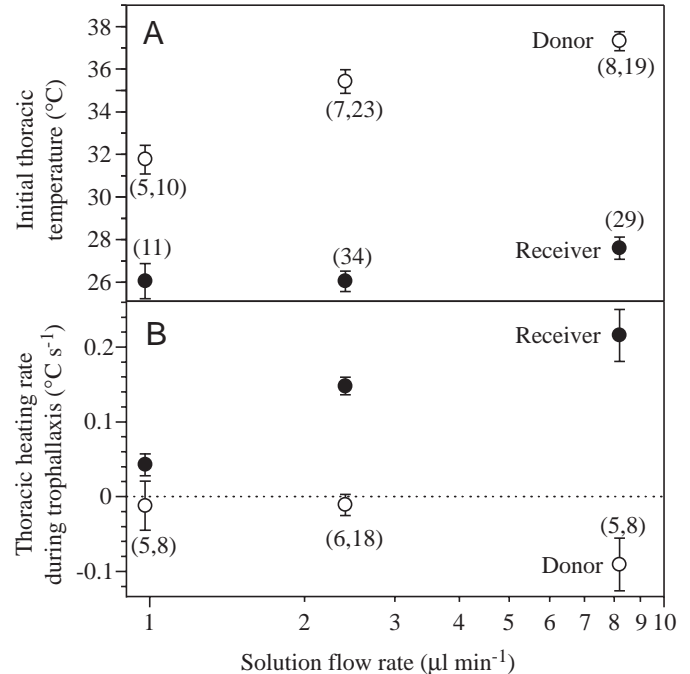


Fig. 3. Initial thoracic temperature and heating rate (means \pm S.E.M.) of the donor and receiver bees during food unloading as a function of the feeder solution flow rate. (A) Results of ANCOVA. Thoracic temperatures for donors: effect of $\log(\text{flow rate})$ $F_{1,10}=10.99$, $P<0.01$; effect of individual $F_{6,10}=0.35$, not significant; interaction term $F_{6,4}=0.39$, not significant; results of ANOVA for receivers $F_{2,71}=2.74$, not significant. (B) Thoracic heating rates: results of ANOVA (because of the low number of values for donors, ANCOVA could not be performed) for donors $F_{2,13}=2.63$, not significant; results of ANOVA for receptors $F_{2,71}=7.62$, $P<0.002$. The dotted line represents no heating of the thoraces. For donors, the number of animals and the number of measurements are given for each animal in parentheses; for receivers, the number of measurements is given in parentheses in A; this value also applies to B.

temperature to a level necessary for flight, but also regulate it when collecting sugar solutions of different concentrations (Schmaranzer and Stabentheiner, 1988). Bees can also regulate their thermal behaviour upon return to the hive during dance displaying, walking and unloading food with respect to the sugar concentration and the distance to the recently exploited food source (Stabentheiner, 1996; Stabentheiner and Hagmüller, 1991; Stabentheiner et al., 1995). In the present study, bees returning from feeders supplying higher flow rates initiated food transfer at higher thoracic temperatures than did those that had experienced lower flow rates, so the temperature of the exchanged solution might have been higher for higher flow rates experienced by the donors. The gradual thermal changes observed in receivers, at first glance, could potentially be explained as a passive result of the heat transferred in the nectar. However, a simple calculation shows that the heat contribution from the transferred solution is probably small. If no heat loss during food transfer is assumed, the heat energy released by the

solution would be equal to the heat energy absorbed by the receiver:

$$m_{\text{S}}c_{\text{S}}dT_{\text{S}} = m_{\text{R}}c_{\text{R}}dT_{\text{R}}, \quad (1)$$

where the subscript S indicates variables pertaining to the sugar solution, the subscript R indicates those pertaining to the receiver, m is mass, c is specific heat capacity and dT is the temperature change. If we assume that the specific heat capacities of the sucrose solution and bee tissues are approximately equal to each other and to that of water, then:

$$m_{\text{S}}/m_{\text{R}} = dT_{\text{R}}/dT_{\text{S}}. \quad (2)$$

For contacts that attained the highest trophallactic unloading rates, the result would be as follows. Taking a value of 80 mg for the mass of an empty receiver, with a maximum load to be received of 49.4 μl (Table 1) of 50% w/w sucrose solution (60.7 mg of solution), a donor thoracic temperature of 37.3 °C (Fig. 3A) (and assuming that the unloaded nectar had the same temperature as the donor's thorax) and a receiver temperature of 27.6 °C (Fig. 3A), the receiver should warm passively to a maximum thoracic temperature of 31.7 °C. This estimate will decrease if there is any heat loss from the nectar or if the worker is heavier than 80 mg. It will increase if the heat capacity of nectar is higher than that of bee tissue, as is likely. However, the mean thoracic temperature of receiver bees unloaded at the highest rates would increase to 33.4 °C at the end of food-exchange contact, assuming an unloading time of 26.4 s and a heating rate of 0.22 °C s⁻¹ (Table 1; Fig. 3B). This simple calculation therefore suggests that other factors, in addition to the passive warm-up of the receiver during food unloading, must be involved.

If the heating were passive, the head should warm up faster than the thorax, since (i) the solution passes through the head before it reaches the thorax and, thus, is at a higher temperature in the head than when it reaches the thorax; (ii) the mass of the head is considerably lower than that of the thorax and, consequently, the head can exchange heat three times faster than the thorax (Heinrich, 1993). Nevertheless, the present results show that the receiver's head temperature increased considerably less than that of the thorax during trophallaxis (Fig. 1). Another argument against the passive warm-up of the receivers is that receiver thorax temperature increased linearly during food transfer in 73 of the 74 cases (Fig. 2). For passive warming of a body, temperature increases asymptotically, and this pattern should therefore have been observed in at least a proportion of our recordings. The present observations agree with previous studies reporting that active warm-up in honeybees is highly linear over small intervals of thoracic temperature change (Heinrich, 1993). Thus, in the same way that previous results have suggested that the thoracic temperature of bees could be used to estimate their motivation to forage (Schmaranzer and Stabentheiner, 1988; Stabentheiner, 1996; Stabentheiner and Haggmüller, 1991; Stabentheiner et al., 1995), the active thermoregulation of bees while receiving food suggests the stimulation of receivers through trophallaxis.

The example presented in Fig. 1 showed an increase of 3.7 °C over 18 s in the receiver bee, which is equivalent to 12.3 °C min⁻¹. This warm-up rate, although high, is lower than values recorded in honeybees ingesting sucrose solution at an *ad libitum* feeder: 3.7 °C over 6 s for a honeybee following food ingestion and preparing to take off (Schmaranzer and Stabentheiner, 1988). Since a higher thoracic temperature facilitates energy turnover in muscles that arise in the thorax, such as flight and walking muscles (Crailsheim et al., 1999; Goller and Esch, 1991), bees involved in receiving nectar may also match their activity level to the activity level of the donor bees which, in turn, is influenced by the profitability of the food sources exploited by these foragers. Thus, receiver bees could intensify the behaviours involved in nectar processing when nectar foragers return from more profitable sites.

Which cue or signal provides receivers with information about the profitability of the food sources exploited by the arriving nectar foragers? Receivers may be able to monitor the nectar and/or head temperature of the donor using thermal receptors located on the antennae (Stabentheiner and Haggmüller, 1991). However, this sensory capacity does not necessarily mean that receivers do use temperature as the relevant cue (Stabentheiner et al., 1995). The presence of sucrose chemoreceptors in the honeybee's proboscis (Whitehead, 1978; Whitehead and Larsen, 1976) and behavioural evidence suggesting that the sucrose intake rate is monitored during ingestion by foragers (Farina and Núñez, 1991; Wainseboim and Farina, 2000a; Varjú and Núñez, 1991) indicate that this variable could also be evaluated by receivers through the unloading rate.

At first glance, the donor's head temperature would be the simplest variable to measure, since trophallaxis involving more than one receiver would require receivers to multiply their own reward rate by the number of bees feeding from the forager to assess the actual unloading rate of the donor. However, the unloaded nectar may not be equally partitioned among all the receivers involved. Indeed, we observed throughout the present experiments that the receiver bee positioned in front of the forager (always our recorded bee) heated up considerably faster than did other receivers. This should not have been the case if receivers were evaluating forager temperature as the relevant cue or were receiving equal proportions of the unloaded solution. Bees positioned in front of the donor forager may receive more nectar than those in a more lateral position because of their more favourable place during trophallaxis. As mentioned above, the observed heating rates show a dependency on the receiver's position that could be explained if the heating rate were to depend on the quantity of nectar received per unit time by the receiver bee.

Whatever the variable actually evaluated by the receivers (mostly nectar processors) during trophallaxis (donor thoracic temperature and/or unloading rate), the thoracic temperature of receivers is raised through trophallaxis, presumably resulting in an increased activity level. Thus, while dance followers acquire information about nectar-source location (von Frisch, 1967) and nectar-specific odour (von Frisch, 1923), nectar receivers may

acquire quantitative information about the nectar source exploited by returning foragers, which may allow the receiver bees to adjust their nectar processing in accordance with nectar site profitability. This would enable the members of a colony involved in nectar collection and nectar processing to adjust their behaviour to distribute the colony's work efficiently (Seeley, 1995).

Finally, it is worth mentioning that, although the bees that unload nectar foragers mostly remain inside the hive processing nectar (Seeley, 1995), some unemployed foragers also receive nectar from the arriving bees. As von Frisch (von Frisch, 1968) pointed out, unemployed foragers could be induced to begin foraging again when food becomes re-available at their food source by contact with a successful returning forager even in the absence of dancing. Active warm-up of an unemployed forager during food reception from a recently arrived forager could enable the former to resume exploitation of such a site more swiftly. Assessment of the information contained in the unloading contact could allow an unemployed forager to estimate the current value of the foraging site. However, no direct evidence yet exists to support this hypothesis (von Frisch, 1968).

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