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Original article

Trophallaxis within the dancing context: a behavioral and thermographic analysis in honeybees (*Apis mellifera*)[1]

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Abstract – Honeybee workers that follow a dancing bee within the hive may contact its mouthparts to receive a food sample. It is currently unknown which proportion of begging bees actually receive food samples via trophallaxis from a dancer. We combined behavioral and thermographic recordings to analyze trophallactic behavior considering the informational context in which these interactions occurred. Dance followers engaged in shorter oral contacts and achieved a lower proportion of effective receptions (26%) than non-followers (58%). These results show that oral contacts often occur between dancers and followers, but their brief duration suggests that followers may just probe the incoming nectar. However, short contacts might allow unemployed nectar foragers either to taste or smell (or both) the solution exposed between the dancer's mandibles, which may contribute to the acquisition of information involved in the decision to visit that food source.

***Apis mellifera* / honeybee / trophallaxis / dance / thermography**

1. INTRODUCTION

When a successful nectar forager honeybee arrives at the hive she often performs dance maneuvers while followed by a group of hive mates (i.e., dance followers). During this display some of the bees contact with their proboscis the dancer's mandibles even during the performance of these runs. These begging bees can follow the dance, stand near or even face the dancer (Ribbands, 1955; von Frisch, 1967). Once a forager stops her dance and begins to unload the nectar collected, it is often observed that some of the bees that have followed her from behind or laterally can contact the dancer's mouthparts resulting in trophallaxis (von Frisch, 1967). Within this recruiting context, oral contacts may involve both dance followers as well as hive bees that have not followed the dancing runs (Bozic and Valentincic, 1991). Bees that both follow dances and contact the forager's mouthparts would obtain more diverse information related to the exploited food source than dance followers only. It is currently unknown, however, what proportion of begging bees actually receive food samples from the returning forager while following dances.

By considering these facts our aim is to quantitatively analyze the trophallactic behavior between returning foragers and hive mates according to the informational context in which these oral contacts occur, i.e. involving or not involving the following of dances. Under these two situations we measured the durations of the contacts. Furthermore, we complemented this analysis by using infrared

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thermography as a non-invasive method for determining if an incoming nectar forager transferred food to a hive mate (Farina and Wainselboim, 2001). In this way it was possible to combine behavioral and thermographic recordings to analyze trophallactic behavior considering the informational context in which these interactions occurred.

2. MATERIALS AND METHODS

The 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* L. was placed inside a laboratory that shielded the measurement site from solar heat and sunlight. In order to obtain infrared thermographic recordings the pane was removed during experiments, and replaced by an infrared transmissive plastic foil.

Bees, one at a time and marked with a spot of paint, were trained to collect 50% w/w scented sucrose solution (80 µl vanilla essence/L) from a rate-feeder placed at a station 180-m away from the colony. At the rate-feeder, sucrose solution was provided at a constant flow rate of 8.2 µl/min via a pump driven by a synchromotor. The synchromotor was switched on when a bee arrived at the rate-feeder, and off immediately after it returned to the hive. In this way no amount of solution accumulated between foraging bouts.

Thermographic recordings at the observation hive were obtained by using a Radiance PM-Amber thermo-camera. In the center of the image produced, a cross allowed the absolute temperature of a particular point to be recorded continuously; this was used to measure the instantaneous temperature of the experimental bees. At the edge of the image a color scale indicated the measured temperature range. The thermal behavior of bees involved in oral contacts was recorded on videotape for later analysis of the following variables: (i) the contact time, in s, defined as the total time that a begging bee's proboscis contacted the mandibles of the trained forager; and (ii) whether the proboscis temperature of the begging bee increased during the contact time with a trained forager. An effective passage of liquid was considered when the proboscis temperature of the begging bee increased at least 0.85 °C (range: 0.85–3.5 °C), which corresponded to the minimum distinguishable color change according to the above mentioned color scale (for details see Farina and Wainselboim, 2001). The infrared emissivity used was 0.95, a value that allowed measurement of absolute body surface temperature (i.e., 0.95 is the lower limit of the band emissivity for the honeybee thoracic cuticle; see Schmaranzer and Stabentheiner, 1988).

The measured variables were categorized into two behavioral groups depending on whether the begging bee had or had not followed dancing displayed by the returning forager before the oral contact. In the first case the begging bees had indeed followed the dancing movements of the returning forager (henceforth: dance followers), being positioned either behind or laterally of the dancing forager before contacting her mouthparts. In the second case the begging bee was only standing near or facing the dancing forager, without actually following the dancer in her waggle runs (henceforth: non-followers). Additionally, this second group also included oral contacts in the absence of dancing displays by the trained forager.

Data were obtained from 353 trophallactic interactions recorded during 128 foraging cycles performed by nine forager bees, and were analyzed by means of Mann-Whitney test.

3. RESULTS

When an oral contact involves transfer of food, a rapid advance of a heat front proceeding from the tip to the base of the receiver's proboscis is observed by thermography with a brief time-lapse (Farina and Wainselboim, 2001). From this observation we determined that these changes (increases) of the begging bee's proboscis temperature during an oral contact corresponded to an effective passage of solution.

Oral contacts with dance followers showed shorter durations than did contacts with non-followers of waggle dances (Mann-Whitney test: $z = -7.12$, $P < 0.00001$, $n = 353$). Of the total contacts observed under both situations, 48.7% presented increases of proboscis temperature. From these effective food transfers, 84.3% took place outside of the dancing context (i.e., either involved non-dancers as donors or begging bees that did not follow the dance maneuvers) and 15.7% involved followers of dance as receivers of food.

Contact durations between dancers and followers were highly variable (Fig. 1 top; range: 0–26 s). Nevertheless, the majority of these oral contacts was very brief and did not show a temperature increase of the begging bee's proboscis (e.g., approximately 70% of these interactions lasted less than 1 s; median value

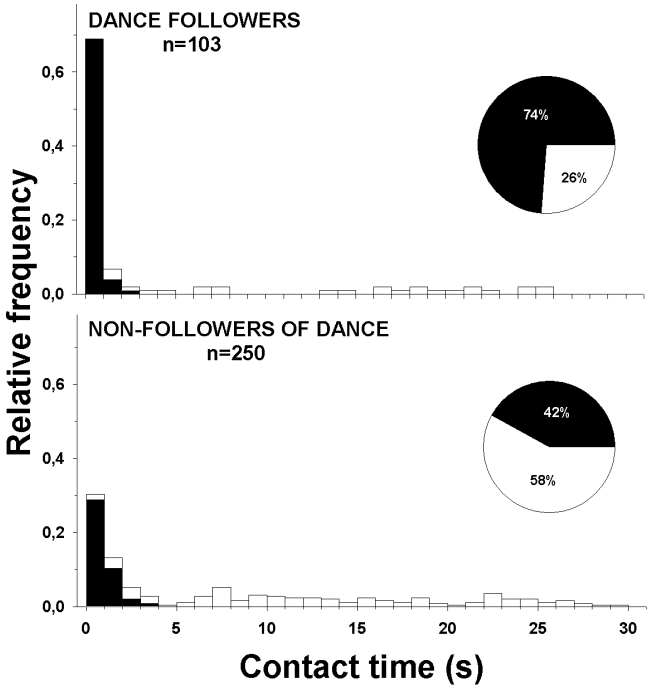

Figure 1. Relative frequency distribution of trophallactic contacts, in s, performed between returning foragers and begging hive-mates. Top: Contacts involving dance followers as begging bees; bottom: contacts involving non-followers of dance as begging bees (see Methods for description). Black part of bars: contacts without increase in the begging bee's proboscis temperature; white part of bars: contacts with an increase in proboscis temperature. Pie plots represent the fraction of contacts (in percentage) without/ with increase of proboscis temperature under each condition.

of the total data population was 0.5 s, N = 103). Approximately 50% of contacts lasting 1–2 s showed a temperature increase in the begging bee's proboscis of at least 0.85 °C. Contacts lasting longer than 3 s always presented an increase of proboscis temperature. Considering all of the contacts observed under the situation in which dance followers behaved as begging bees only 26% involved effective transfer of food (i.e., the begging bee's proboscis temperature increased at least 0.85 °C).

In the case of contacts in which begging bees had not followed dances ("non-followers of dance"), contact times were also highly variable (Fig. 1 bottom; range: 0–30 s). Compared with the dancing context, brief contacts were less frequently observed (approximately 30% of the interactions lasted less than 1 s achieving a median value for the total data population of 3.5 s, N = 250). Contacts longer than 3 s also presented an increase in the begging bee's proboscis temperature by at least 0.85 °C. In this context, a higher proportion of the total contacts involved an increase of proboscis temperature (58% of the total cases, Fig. 1 bottom).

4. DISCUSSION

Our results suggest that the majority of the oral contacts that involved returning foragers did not involve effective transfers of food (Farina and Wainselboim, 2001; this study). This tendency was much more noticeable within the dancing context, since followers mainly performed short oral contacts. Begging followers, as expected, did not preferably perform long-lasting contacts, which are more likely to be observed in the class of food-storer bees (Seeley, 1995). Begging dance followers seem to be more interested in gaining information about the nectar exposed at the dancer's mandibles than in food reception. In this sense, short contacts may indeed transfer small samples of food (which were not detected because of the 0.85 °C threshold of temperature change) or may allow probing the solution exposed between the donor's mandibles. The acquisition of chemosensory information about the nectar exploited (i.e., the odor and the sugar concentration of the nectar) does not require transfer of food. The sense organs involved are on the tongue and the antennae, and food intake is not necessary to stimulate them. Therefore,

very brief begging contacts by dance followers would suffice to transmit information about a food source.

Besides the role of the honeybee dance of transferring information about the location and profitability of a food source (von Frisch, 1967; Seeley et al., 2000), it was proposed that dances provide odor cues impregnated onto the dancers' surface within the hive (von Frisch, 1968; Wenner and Wells, 1990; Kirchner and Grasser, 1998). Nevertheless, other odor cues produced by the flowers could be also relevant to be transferred among hive mates, particularly the nectar fragrances that could be highly conservative preserved into the foragers´ honey sacs until the food unloading inside the hive (von Frisch, 1946). The evaluation of this kind of feature while following dances could thus be relevant during the decision-making process occurring within the hive, changing potential foragers' thresholds to initiate or reinitiate the foraging mode (Johnson, 1967; von Frisch, 1968).

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Résumé – La trophallaxie dans le contexte de la danse : analyse comportementale et thermographique chez l'Abeille domestique (*Apis mellifera*). Lorsqu'une ouvrière butineuse revient à la ruche chargée de nectar, elle effectue souvent des manœuvres de danse et est alors suivie par un groupe d'ouvrières de la colonie. Au cours de la danse, certaines abeilles établissent à l'aide de leur proboscis un contact avec les mandibules de la danseuse. Si les suiveuses entrent en contact avec la nourriture sur les pièces buccales de la danseuse, elles devraient obtenir plus d'informations sur la source de nectar exploitée que les suiveuses qui n'ont pas établi de tels contacts. Plusieurs évènements trophallactiques de différentes durées ont généralement lieu entre les butineuses de nectar qui entrent et leurs consoeurs, mais on ne sait pas à l'heure actuelle quelle proportion d'abeilles demandeuses reçoit réellement des échantillons de nourriture de la danseuse, ni combien durent ces contacts dans le contexte comportemental. Nous avons combiné des enregistrements de comportement et de thermographie pour analyser le comportement trophallactique en prenant en compte le contexte d'échanges d'informations dans lequel ont lieu ces échanges, à savoir s'ils se manifestent ou non lorsque la danseuse est suivie. Grâce à des enregistrements thermographiques non invasifs il a été possible d'observer que le nectar tiède régurgité par les butineuses qui rentrent réchauffait brutalement le proboscis de la receveuse. Nous avons donc utilisé l'augmentation de la température du proboscis de la receveuse comme indicateur d'un transfert effectif de nourriture au cours du contact. Dans la majorité des cas observés les contacts sont brefs et sans passage de nourriture (51,3 %). Les suiveuses se sont engagées dans des contacts oraux courts et ont présenté une plus faible proportion de réception effective de nourriture (26 %) que les abeilles qui n'avaient pas suivi la danse (Fig. 1). Ces résultats montrent que des contacts oraux entre danseuses et suiveuses ont souvent lieu, mais leur courte durée suggère que les suiveuses n'obtiennent que rarement des échantillons de nectar. Pourtant des contacts brefs pourraient permettre à des butineuses de nectar « au chômage » de goûter et/ou de sentir l'échantillon de nectar présent entre les mandibules de la danseuse. Ceci est très important pour des butineuses au chômage afin qu'elles obtiennent l'information pour décider de visiter une source donnée de nourriture.

***Apis mellifera* / trophallaxie / danse / thermographie**

Zusammenfassung – Trophallaxis im Kontext des Bienentanzes: eine Verhaltens- und thermographische Analyse an Honigbienen (*Apis mellifera*). Wenn eine erfolgreiche Nektarsammlerin im Stock ankommt, vollführt sie häufig Tanzmanöver, die von anderen Bienen gefolgt werden. Während dieser Darbietung nehmen einige Bienen selbst während der Tanzläufe mit ihre Saugrüssel Kontakt zu den Mandibeln der Tänzerin auf. Falls die Folgebienen dabei mit ihrem Proboscis in Kontakt mit Nahrung auf den Mundwerkzeugen der Tänzerin kommen, sollten sie mehr Information über die ausgebeutete Futterquelle erhalten als Folgebienen, die keine solchen Kontakte aufgenommen haben. Zwischen rückkehrenden Nektarsammlerinnen und ihren Geschwistern im Stock kommt es dabei gewöhnlich zu Futteraustauschereignissen unterschiedlicher Dauer. Bisher ist allerdings unbekannt, welches der Anteil bettelnder Bienen ist, die von der Tänzerin tatsächlich Futterproben erhalten, und wie lange diese Kontakte im Verhaltenskontext dauern. Wir kombinierten Verhaltensuntersuchungen mit thermographischen Aufnahmen, um das Trophallaxisverhalten im Kontext des Informationsaustausches zu untersuchen, in dem diese Interaktionen zu beobachten waren, d.h.ob sie beim Folgen der Tänzerin auftraten oder nicht. Mittels nichtinvasiver Thermographie war es möglich zu beobachten, wie der von der rückkehrenden Sammlerin regurgitierte warme Nektar die Proboscis der empfangenden

Biene abrupt aufheizte. Wir nutzten deshalb die Temperatur des Saugrüssels der Empfängerbiene als Indikator für einen effektiven Futtertransfer während des Kontakts. Die Mehrzahl der beobachteten Kontakte (51,3 %) war kurz und zeigte keinen Hinweis auf einen Futteraustausch. Tanzfolgebienen zeigten vergleichsweise kürzere orale Kontakte mit einem geringeren Anteil an effektivem Empfang (26 %) als Bienen, die dem Tanz nicht gefolgt waren (58 %) (Abb. 1). Diese Ergebnisse zeigen, dass es zwischen der Tänzerin und Folgebienen oft zu oralen Kontakten kommt. Deren kurze Dauer weist darauf hin, dass die Folgebienen dabei aber offensichtlich nur kleine Proben des hereinkommenden Nektars erhalten. Diese kurzen Kontakte sollten es jedoch erlauben, dass „arbeitslose" Nektarsammlerinnen die Nektarprobe zwischen den Mandibeln der Tänzerin zu riechen oder schmecken bekommen, oder beides. Dies kann für eine arbeitslose Sammlerin von Bedeutung sein, da sie darin Information gewinnen könnte für die Entscheidung, eine bestimmte Futterquelle aufzusuchen.

Honigbiene / Trophallaxis / Tanz / Thermographie

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