Social learning of food odours and the implications for collective foraging in honeybees (*Apis mellifera*)

Inauguraldissertation
der Philosophisch-naturwissenschaftlichen Fakultät
der Universität Bern

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Photo:	Honeybees collecting pollen on *Hypericum sp.*
Cover, layout & photo:	Christoph Grüter
Printed by:	Bookstation GmbH

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Von der Philosophisch-naturwissenschaftlichen Fakultät
angenommen.

Der Dekan

Bern, den 27. Februar 2008

Prof. Dr. P. Messerli

Those who have treated of the sciences have been either empirics or dogmatical. The former like ants only heap up and use their store, the latter like spiders spin out their webs. The bee, a mean between both, extracts matter from the flowers of the garden and the field, but works and fashions it by its own efforts. The true labour of philosophy resembles hers, for it neither relies entirely nor principally on the powers of the mind, nor yet lays up in the memory the matter afforded by the experiments of natural history and mechanics in its raw state, but changes and works in the understanding.

Sir Francis Bacon

Contents

1

General Introduction

Taking decisions is an important part of animal life, for example when choosing a mate, a nest site or a food source. Gaining information about relevant ecological parameters is crucial for an animal in order to behave adaptively (Danchin et al. 2004; Dall et al. 2005). Important sources of information are other individuals and their products. In social insects, acquiring this kind of information about the needs of the brood, nest quality, the optimal allocation of insects among different tasks or foraging opportunities is vital for colony success (Wilson 1971; Robinson 1992; Seeley 1995; Gordon 1996,1999; Jeanne 1999; Pankiw 2004; Pratt 2005).

1.1 Types of social information

Social information can be provided in the form of signals or inadvertent social information cues (ISI). A signal is an action or trait that has been shaped by natural selection specifically to convey information (Lloyd 1983; Seeley 1995; Bradbury & Vehrencamp 1998; Maynard-Smith 2000). The term ”communication” has often been restricted to cases where information is transferred via signals (Wilson 1971; Lloyd 1983; Seeley 1995; Bradbury & Vehrencamp 1998;

Jablonka 2002). Examples of signals are pheromones (Wilson 1971) or the honeybee waggle dance (von Frisch 1967).

ISI is a form of information, which is provided as a by-product of the actions or traits of animals. It can be divided into public information (Valone 1989; Danchin et al. 2004; Dall et al. 2005) and social cues (Dall et al. 2005). Public information conveys continuous, graded information about the quality of a feature, e.g. a mating partner. Social cues convey discrete information about the presence or absence of a feature, e.g. a food patch.

It has been suggested, that cues are more important than signals in colony organisation (Seeley 1989). Information transfer via cues should evolve more readily than through signals, because the evolution of cuing involves only the formation of an adaptive response to a pre-existing stimulus, whereas the evolution of signalling involves the adaptive modification of both the stimulus and a response. For example, in honeybees about 17 signals and at least twice as many cues have been proposed to help organizing the information flow of colonies (Seeley 1998).

1.2 The honeybee as a model system

Of all social insects, the use of social information has most thoroughly been studied in the context of honeybee (*Apis mellifera*) foraging. There are several reasons why the honeybee is an excellent and popular study animal since many decades. The most obvious ones are their importance for pollination, honey and wax production. Honeybees can now be found on all continents except Antarctica and they occupy a wide range of habitats (Michener 2007). Honeybee colonies are easy and cheap to keep and are resistant to experimental manipulation. The most important reason, however, is that honeybees have a fascinating recruitment and communication system (reviewed in Ribbands 1953; von Frisch 1967; Seeley 1995; Dyer 2002). Our knowledge about honeybees allows educated guesses about the biology of other social insect species, particularly other social bee species.

1.3 Foraging in honeybees

1.3.1 Resources collected by honeybees

Pollen and nectar are the main food sources for honeybees. Nectar is the most important source of carbohydrates, which is the principal energy source. In some temperate habitats, non-floral resources such as sap of woody vegetation (honeydew) and fruits are the predominant source of energy (Roubik 1989). Pollen is the principal protein source, but it also contains lipids, vitamins and minerals (Winston 1987).

Water is collected mainly for temperature control of the nest and the nutrition of larvae (Lindauer 1954; Seeley 1995). Furthermore, honeybees collect resin from sticky tree buds (Michener 1974; Seeley 1995) and waxes from other unoccupied or even occupied nests (Roubik 1989) as building material for their own nest.

1.3.2 Foraging specialisation: Nectar, pollen and water foraging

Foraging is performed by the oldest individuals of a colony. After performing in-hive duties such as cell cleaning, caring for brood, receiving and processing nectar for about 2-3 weeks, bees leave the hive and start foraging (Rösch 1925; Lindauer 1952; Seeley 1982). However, foragers do not collect resources randomly, instead they show preferences to collect either nectar or pollen or water. The different forager types are characterised by different behavioural syndromes, i.e. different suits of behaviours that correlate through time and across contexts (Sih et al. 2004; Bell 2007). A central factor of the behavioural syndrome is the bees' responsiveness to sucrose (reviewed in Page & Erber 2002; Scheiner et al. 2004). Bees with a high sensitivity to sucrose start foraging earlier in life (Pankiw 2003), they show higher locomotion immediately after emergence (Rueppell et al. 2006), better learning performance (Scheiner et al. 1999; Scheiner et al. 2001a,b; Scheiner et al. 2003), have higher ovariole numbers (Amdam et al. 2006) and prefer to collect pollen or water (Pankiw & Page 1999; Pankiw & Page 2000; Scheiner et al. 2003). Bees with a low responsiveness for sucrose collect predominantly nectar. These differences among individuals depend on age, experience, and genotype (Page & Erber 2002; Scheiner et al. 2004).

While some bees collect either nectar or pollen throughout their lives, others collect both (Ribbands 1952; Free 1960). Sometimes, bees can be observed collecting one type of food in the morning and the other type in the afternoon (Free 1963). However, there is a further step in specialisation. Foragers often specialize on a particular floral species, a phenomenon called flower constancy (Free 1963) and even on a particular patch of a flower species. If foraging conditions are favourable, foragers continue visiting these "fixation areas" for consecutive days up to three weeks (Butler 1945; Ribbands 1949).

In such an area, single nectar foragers have to visit from several dozen to several hundred (up to 1400) blossoms per foraging trip, including revisits of the same blossoms (Ribbands 1949; Free 1960). Pollen foragers return after fewer visits (up to 120). It is no surprise then that foraging trips can last from a few minutes up to 100 minutes (Park 1926; Butler et al. 1943). These values strongly depend on the characteristics and profitability of a food patch.

1.3.3 Foraging range

Different studies found different average flight ranges for honeybee colonies. The average foraging distance of foragers of a colony was found to be between 745 m to 6100 m, depending on colony, the location of plant species and the season (Visscher & Seeley 1982; Waddington et al. 1994; Beekman & Ratnieks 2000; Steffan-Dewenter & Kuhn 2003). Often foragers go as far as 10 km from the hive, which means that the potential foraging area of a single colony is more than 300 km^2.

1.4 Foraging communication

Honeybee colonies must have the ability to direct their foraging force to the most profitable food sources in a sometimes rapidly changing environment in order to acquire enough food for feeding and heating the brood and maintaining the colony in winter. This requires that foragers acquire information about characteristics of good food sources from their nest mates. The place where most of this information is available is the hive (von Frisch 1967; Seeley 1995).

1.4.1 Trophallaxis

Trophallaxis is the transfer of a liquid between individuals (Wheeler 1918; Wilson 1971). In honeybees and many other social bees species, foragers transfer the collected liquid food to hive mates for further processing and storing (Park 1925; von Frisch 1967; Hart & Ratnieks 2002). The donor bee opens her mandibles to expose a drop of regurgitated nectar and one or more receivers contact the donor's prementum with their protruded proboscis. Foragers perform 1-2 long unloading contacts of about 20 s, mainly with middle aged food processor bees and a few shorter offering contacts mainly with other foragers (von Frisch 1967; De Marco & Farina 2001). Aspects of forager behaviour such as the transfer rate during single trophallaxes or chains of trophallaxes (Farina & Núñez 1991; Farina & Wainselboim 2001; Goyret & Farina 2005; Tezze & Farina 1999) and the frequency of short trophallaxes (Farina 1996; De Marco & Farina 2001)

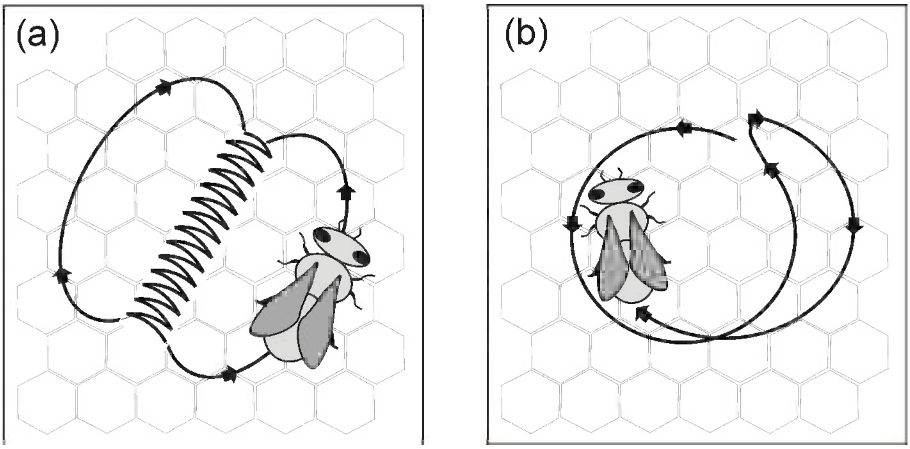

Figure 1.1: Waggle dance (a) and round dance (b) of honey bees. The direction to the food source is encoded in the orientation of the waggle run relative to gravity. The distance to the food source is encoded in the duration of the waggle run.

correlate with the food source profitability and may convey information about food source profitability (Pírez & Farina 2004). The role of trophallaxis as a mechanism of information transfer will be discussed in more detail in chapter 11.

1.4.2 The waggle dance

When a honeybee forager returns from a very profitable food source, she scrambles inside the hive and starts running through a figure-eight pattern near the hive entrance. She performs a straight waggle run followed by a turn to one side to circle back to the starting point of the waggle run, she starts another waggle run followed by a turn to the other side to circle back to the starting point, and so on in a regular alternation between right and left turns after waggle runs (von Frisch 1967; Seeley 1995, Fig. 1.1a). While she is doing this, she is surrounded by other bees that face towards her, some follow for a few waggle runs (von Frisch 1967; Božič & Valentinčič 1991; Michelsen 2003). In the case of nectar foragers, the dancer interrupts dancing from time to time and distributes small samples of nectar to the surrounding bees (von Frisch 1923; Park 1925; Farina 1996; De Marco & Farina 2001).

The waggle dance is one of the most intriguing communication signals in the animal world and was already described by Aristotle in his Historia Animalium (330 B.C.). However, it was Karl von Frisch who first suggested that the waggle dance conveys information about the distance and direction to the visited food source to surrounding bees (von Frisch 1946). He discovered that the orientation of the waggle run and its duration correlate with the direction and the distance of the flight from the hive to the food source (reviewed in von Frisch 1967; Seeley 1995; Dyer 2002; Michelsen 2003). von Frisch also showed that foragers perform a different kind of dance if food sources are close to the colony, i.e. less than about 100 m, a dance called the round dance (von Frisch 1923; Fig. 1.1b). In this case, the bee runs in a small circle and suddenly reverses the running direction before she again turns around to her original course and so on. The round dance does not provide information about the direction to a food source, but only about its proximity (von Frisch 1967).

As mentioned, only high quality food sources are advertised by dancers, i.e. about 10% of all foragers of a colony perform recruitment dances after returning with food (Seeley 1995; Weidenmuller & Tautz 2002). There are many factors that affect the likelihood and the duration of dancing, such as the sugar concentration of the liquid food (von Frisch 1923; Lindauer 1948), the nectar flow rate (Núñez 1970) and the distance to the food source (Boch 1956). These factors determine the energetic profitability of a food source (Seeley et al. 2000). However, the motivation to dance does not only depend on the profitability of the food source, but also on the availability of other food sources to the colony (Lindauer 1948,1954; Boch 1956). If the environment offers many good food sources, most of the foragers of a colony are active and return with food. As a consequence, bees responsible for the unloading of foragers are busy. In such a situation, finding an unloading partner becomes more difficult (Lindauer 1948,1954). Foragers respond to an increase of waiting time and a decrease of the number of food receivers during unloading with a reduced motivation to dance (Lindauer 1948,1954; Seeley et al. 1991; Seeley & Tovey 1994; Farina 2000; De Marco 2006). In this way, foragers do not recruit more foragers if the colony's maximum processing capacity has been reached (Seeley 1995). Hence, foragers integrate private information acquired at the food source and social information acquired in the hive to adjust the level of dancing.

The dance is famous for the location information it provides, however, it also provides other kinds of information, which might be even more important from an ecological perspective. First, it signals the presence of an attractive food source (von Frisch 1923). Many bees surrounding the dancer are immediately attracted to the dancing bee and show an increased propensity to leave the hive and search for food after following a dance (von Frisch 1923; Božič & Valentinčič 1991; Thom et al. 2007). Hydrocarbons produced by the dancer and released onto the cuticular surface and into the air seem to be an important cause of this reaction (Thom et al. 2007).

The third type of information specifies the exploited plant species (von Frisch 1923; von Frisch & Rösch 1926). When foragers return with nectar or pollen, food odour molecules are brought back on the body and in the collected food source (von Frisch & Rösch 1926; von Frisch 1967; Raguso 2004). Odour molecules cling especially tenaciously to the body of honeybees (Steinhoff 1948).

Von Frisch showed that bees that have been alarmed and recruited by dances show a preference during their search outside the hive for the odour of the food the dancer collected (von Frisch 1923; Wenner & Wells 1990). He showed that this effect is especially strong if the odour is present in the collected solution and proposed that recruits learn this scent when they receive samples of food via trophallaxis from the dancing bee (von Frisch 1967). The role of this olfactory information for foraging decisions of individual honeybees seemed so important and obvious that a fierce controversy arose about whether bees that follow dances are able to decode the location information, or instead rely exclusively on odour (Wenner et al. 1969; Gould 1974,1976; Lindauer 1971; Wells & Wenner 1971,1973; Wenner & Wells 1990; Wenner 2002). By now, the honeybees' ability to decode the location information provided by dancing bees has been demonstrated beyond any doubt (Michelsen et al. 1989; Esch et al. 2001; Sherman & Visscher 2002; Riley et al. 2005).

However, despite the importance of socially acquired olfactory information for honeybee foragers, many basic questions remained unanswered for a long time, e.g. do bees learn odours associatively inside the hive? What kind of bees of a colony learn the odours and in which behavioural situation? What are the properties of the established memories? What are the consequences of socially acquired olfactory information for collective foraging? The aim of this project was to find answers to these questions and to identify new questions for further study.

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2

Nectar distribution and its relation to food quality in honeybee colonies

Grüter, C. and Farina, W.M. 2007. *Insectes Sociaux* 54: 87-94

2.1 Abstract

In honeybees (*Apis mellifera*), the process of nectar collection is considered a straightforward example of task partitioning with two subtasks or two intersecting cycles of activity: (1) foraging and (2) storing of nectar, linked via its transfer between foragers and food processors. Many observations suggest, however, that nectar collection and processing in honeybees is a complex process, involving workers of other sub-castes and depending on variables such as resource profitability or the amount of stored honey. It has been observed that food processor bees often distribute food to other hive bees after receiving it from incoming foragers, instead of storing it immediately in honey cells. While there is little information about the sub-caste affiliation and the behaviour of these second-order receivers, this stage may be important for the rapid distribution of nutrients and related information. To investigate the identity of these second-order receivers, we quantified behaviours following nectar transfer and compared these behaviours with the behaviour of average worker hive-bees. Furthermore, we tested whether food quality (sugar concentration) affects the behaviour of the second-order receivers. Of all identified second-order receivers, 59.3% performed nurse duties, 18.5% performed food-processor duties and 22.2% performed forager duties. After food intake, these bees were more active, had more trophallaxes (especially offering contacts) compared to average workers and they were found mainly in the brood area, independent of food quality. Our results show that the liquid food can be distributed rapidly among many bees of the three main worker sub-castes, without being stored in honey cells first. Furthermore, the results suggest that the rapid distribution of food partly depends on the high activity of second-order receivers.

2.2 Introduction

Task partitioning, the division of a piece of work among two or more colony mates, is likely to enhance the performance of the individual and the colony (Ratnieks & Anderson 1999a). Nectar collection in honeybees have been described as a straightforward process of two intersecting cycles of activity, a forage cycle and a storage cycle (Seeley 1989; Ratnieks & Anderson 1999a). Successful foragers transfer the gathered liquid to hive mates, the food processors (also called receivers or food storers), through trophallaxis (mouth-to-mouth contacts), which then initiate the processing of the fresh nectar to honey and store the food in honey cells (Park 1925).

Observational and experimental evidence suggests, however, that nectar collection and processing is a more complex process, i.e. the nectar flows into various directions before or even without being stored in honey cells. First, observations on the behaviour of food processors show that bees often feed other bees (second- order receivers) on their way to the honey cells, sometimes large nectar samples (von Frisch 1923; Rösch 1925; Seeley 1989; Pírez & Farina 2004). Aspects of this feeding behaviour of the processor bees have been shown to depend on variables such as food source profitability (Pírez & Farina 2004) and the nutritional state of the colony (Seeley 1989). The proportion of food processors donating food to second-order receivers is between about 50% (Pírez & Farina 2004) and 95% (Seeley 1989). Secondly, it has been shown that small quantities of food collected by foragers can become widely distributed among the members of different worker castes of a colony within a few hours (Nixon & Ribbands 1952; see also DeGrandi-Hoffman & Hagler 2000), which suggests that the view of a primarily unidirectional nectar flow from foragers to food processors to honey cells misses important aspects of nectar collection.

Information about nectar flow, about the behaviour and the caste of bees that receive food from food processors is not only important from a nutritional perspective, but also from an informational one. The major disadvantage of task partitioning with direct transfer of material is the time costs caused by queuing, searching and transfer delays. However, these delays may in fact offer opportunities for information acquisition about environmental conditions and colony work allocation as shown by empirical (Lindauer 1948; Seeley 1995; Hart & Ratnieks 2001) and theoretical studies (Ratnieks & Anderson 1999b). Furthermore, information cues and signals present in the transferred nectar such as the sucrose concentration (Pankiw et al. 2004), food scents (von Frisch 1967; Farina et al. 2005,2007; Gil & DeMarco 2005, 2006; Grüter et al. 2006) or pheromones (Wilson 1971; Naumann et al. 1991; Crailsheim 1998) may spread rapidly within the entire colony if nectar flow is multidirectional, involving many bees performing different tasks. Finally, aspects of trophallactic behaviour such as the transfer rate during single trophallaxes or chains of trophallaxes (Farina & Núñez 1991; Farina & Wainselboim 2001a; Goyret & Farina 2005; Tezze & Farina 1999) and the frequency of trophallaxes (Farina 1996; DeMarco & Farina 2001) correlate with food source characteristics and may convey information about food source profitability. The ability to respond to these different information cues which are available as a consequence of nectar transfer from bee to bee might allow for a more accurate colony response to the current environmental conditions (Seeley 1995; Dall et al. 2005).

For a better understanding of nectar flow within the honeybee hive, information about the caste affiliation and behaviours related to food processing after food reception of second-order receivers is needed. Therefore, we did an experiment in order to find out what kind of tasks the second-order receivers mainly perform after nectar reception. Furthermore, we quantified various behaviours of second-order receivers after they received liquid food and compared their behaviour with the behaviour of average worker bees of unknown age and caste. We were interested in behaviours relevant for nectar and information flow like trophallactic and locomotion activity. As forager and food processor behaviours are affected by food source profitability (Núñez 1966, 1970; von Frisch 1967; Seeley 1986; Farina 1996; De Marco & Farina 2001; Farina & Wainselboim 2001a; Pírez & Farina 2004), we also tested whether the sucrose concentration of the liquid food affects the behaviour of second-order receivers.

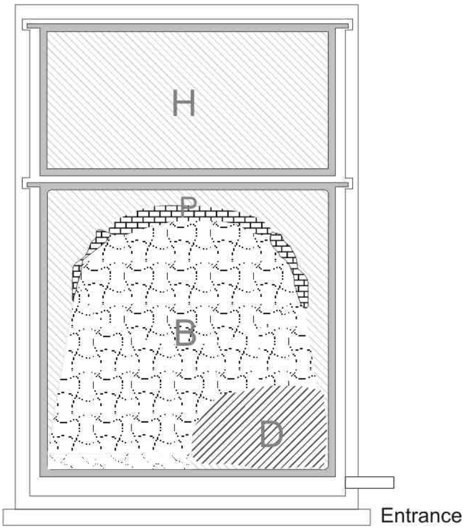

Figure 2.1: The observation hive with two frames of unequal size. The indicated areas are the honey area (H), the brood area (B), the delivery area (D) and the pollen area (P).

2.3 Methods

2.3.1 Study site and animals

The experiment was conducted at the ethological field station near Bern, Switzerland. We used two two-frame observation hives (Schneider measure, brood comb 30 x 30 cm, Fig. 2.1) containing a colony of about 2500 Buckfast honeybees (a cross between *Apis mellifera ligustica* and *A. m. mellifera*) each. Colonies had a queen, brood and honey reserves. A group of bees was trained to collect unscented sucrose solution that was of 15% w/w sucrose concentration at an ad libitum-feeder located 85 m from the hive. Bees were marked individually and a number of about 5 to 15 foragers was maintained throughout the experiment.

2.3.2 Experimental procedure

4 to 6 numbered foragers collected unscented sucrose solution that was either of 56% w/w sucrose concentration (high quality food; H treatment) or 13% w/w sucrose concentration (low quality food; L treatment) for about 60 minutes once

or twice a day between 10:00 and 17:00 hours. Newly arriving bees were captured to maintain a constant number of foragers in both treatments. Bees that received solution from the numbered bees for at least 5 sec were considered food processor bees (first-order receiver). These first-order receivers were observed until they transferred solution to another bee (second-order receiver) for at least 1.5 sec. It has been shown that shorter trophallactic contacts often do not guarantee successful food transfer (Farina & Wainselboim 2001b). Since we were also interested in the flow of information in the colonies, our threshold accounted for that fact that learning of food odours can happen during contacts as short as 1.2 seconds (Gil & De Marco 2005). These second-order receivers were then filmed with a digital camera for a maximum of 20 min (561 ± 49.3 s, N = 49, mean ± SE). Often it was not possible to observe the bee for the 20 min because other bees covered them or they were simply lost. For the behavioural analysis we analysed the behaviour of the filmed bees (only bees that had been filmed for at least 3 min were used). Furthermore, we filmed worker bees randomly chosen throughout the hive to compare their behaviour with the behaviour of the secondorder receiver bees. Therefore we divided the entire area on one side of the observation hive into 35 (5x7) equally sized rectangles. The rectangles were numbered and a sequence of random numbers was generated to randomly choose rectangles and therefore randomise the position of the chosen bee. The bee that was closest to the right upper corner of a rectangle was chosen and filmed (579.2 ± 10.6 s, N = 35, mean ± SE). Bee densities were similar in the different hive areas (2500 bees approach the carrying capacity of our hives).

2.3.3 Behavioural observations

To identify a second-order receiver as forager, food processor or nurse bee, we looked for behaviours that are typical for bees working in a particular caste. A bee was considered a

i. forager if the bee was foraging, dancing or following dances (Rösch 1925; Lindauer 1952; von Frisch 1967; Seeley 1995).

ii. food processor if the bee unloaded food (> 5 sec) from a returning forager (Rösch 1925; Lindauer 1952; von Frisch 1967; Seeley 1995). These unloading contacts had to occur after bees received food from first-order receivers.

iii. nurse bee if the bee was observed entering (> 4 sec) brood or pollen cells (Rösch 1925; Lindauer 1952; von Frisch 1967) or fanning on brood comb (Winston 1987).

Even though in particular cases one can not be sure, that a bee performing one of these behaviours belongs to the corresponding group, an analysis based on these criteria's provides a good general representation of the distribution of bees among the three main worker castes.

To compare the behaviour of bees that received high quality food, low quality food and average workers, we analysed the following behaviours or states:

(a) States (% of total duration): walking, standing, entering a honey cell, entering a brood cell, entering a pollen cell, dancing.

(b) Frequency of behaviours (per 10 min): trophallactic contacts, offering contacts, begging contacts, honey cell inspections, brood cell inspections, pollen cell inspections.

(c) Duration of behaviours (in seconds): offering contacts, begging contacts.

(d) Position (% of total duration): delivery area, brood area, honey area, position of the 1st and the 2nd trophallaxes (Fig. 2.1).

To get a general estimation of the activity of second-order receivers and average bees, we calculated an activity index, which is the time walking divided by the total time the bee was walking and standing. All filmed observations were recorded with the OBSERVER 3.0 program (Noldus, Wageningen, the Netherlands).

2.3.4 Estimation of trophallactic activity

To get a general estimation of the trophallactic activity of hive bees under the two reward conditions, we filmed six 8 x 10 cm rectangles for 1 min with a digital camera. Of these six rectangles, three were filmed on each side of the hives, one in the lower part of the hive (delivery area and brood area), one in the middle part of the hive (brood area) and one in the upper part of the hive (honey area). The filming of all 6 rectangles for 1 minute constituted one sampling period. The area filmed during one sampling period represents 16% of the total comb area. The recordings were later watched once at normal speed by one observer and all trophallactic contacts were counted.

2.3.5 Statistical analysis

For most analyses, we used general linear models (GLM) in SPSS 12.0. We mostly used both treatment and colony (hive 1 and 2) as explanatory variables to test for effects on the dependent variable. The treatments were (1) H treatment, (2) L treatment and (3) average worker. Cases were only H and L treatments were compared are indicated.We pooled the data of average workers observed under both reward conditions because we found no significant differences between the two groups of workers (not shown). When we tested data sets several times, we corrected for multiple testing and adjusted the significance level using the sequential Bonferroni method (Sokal and Rohlf 1995). Values of $p < 0.05$ after correction are indicated with one asterisk, results no longer significant are indicated with two asterisks. To test for differences in the position data and the cell inspection data between the 3 bee groups, we used non-parametric statistics because the assumptions for parametric statistics were not met. Descriptive statistics are given as mean ± SE (standard error) or median [quartiles]. All tests were two tailed.

2.4 Results

We observed 54 second-order receivers and found caste specific behaviours in 27 bees (13 of 30 in the L treatment; 14 of 24 in the H treatment). 16 (59.3%) of the identified bees performed nurse duties (mean time in brood or pollen cell: 204 ± 57.8 sec, N = 16; mean time fanning: 83 sec, N = 2), 5 (18.5%) performed food processor duties (duration of unloading contacts: 18.4 ± 3.8 sec), 6 (22.2%) performed forager duties (3 bees followed between 2 and 5 dances; 2 bees danced; 3 bees left the hive at least once during recording (2 were numbered foragers)). These proportions were almost identical in the H and the L treatment (Fig. 2.2).

2.4.1 Behavioural observations

We found a significant positive relation between the duration of the 1st (23.3 ± 1.04 sec, N = 53) and the 2nd (11.8 ± 1.37 sec, N = 58) trophallactic contact but no effect of treatment (L and H) or colony (GLM: 1st troph. x 2nd troph.: $F_{1,53} = 5.25$, p = 0.026; treatment x 2nd troph.: $F_{1,53} = 0.58$, p = 0.45; colony x 2nd troph.: $F_{1,53} = 0.51$, p = 0.481) was found. All 1st trophallaxes took place in the delivery area, but only 40% (H treatment) to 37.9% (L treatment) of the 2nd trophallaxes took place in this area. A substantial proportion of 2nd trophallaxes, between 55% (H treatment) and 48.3% (L treatment), took place in the brood area. Between 5% (H treatment) and 13.8% (L treatment) took place in the honey area (Fig. 2.3).

When we compared the activity of bees of the H treatment, the L treatment and the average workers, we found that both H treatment bees and L treatment

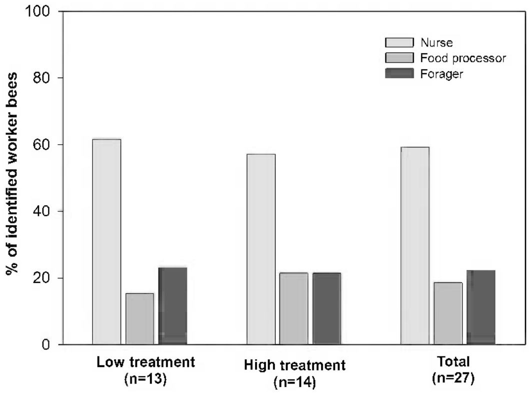

Figure 2.2: The percentage of second-order receivers identified as nurse bees, food processors or foragers of all identified bees, L treatment bees and the H treatment bees.

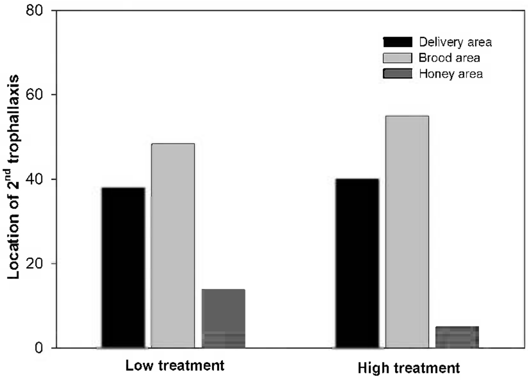

Figure 2.3: The area in which the trophallaxis between first-order receivers (food processor bees) and second-order receivers took place (2nd trophallactic food transfer).

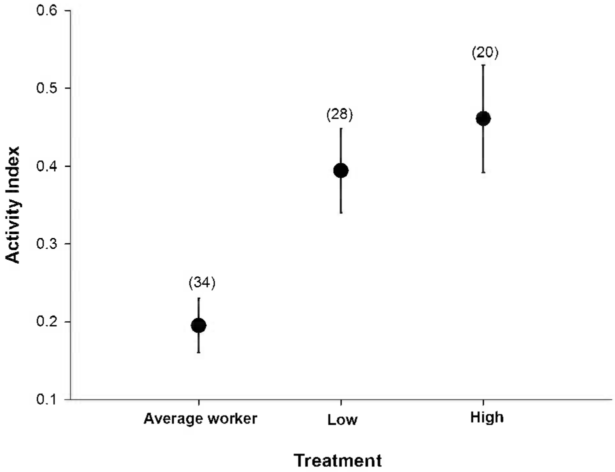

Figure 2.4: The activity index (mean ± SE) of bees from the L treatment, the H treatment and average bees. Numbers above bars represent the number of bees.

bees were more active than the average worker, while there was no difference between H treatment bees and L treatment bees (GLM: treatment x activity: $F_{2,82} = 8.57$, $p < 0.001$*; colony x activity: $F_{1,82} = 1.4$, $p = 0.24$; Fig. 2.4. Post-hoc Tukey-Kramer comparison between groups: H vs. average worker: $p = 0.001$; L vs. average worker: $p = 0.006$; H vs. L: $p = 0.6$). When we tested for the effect of treatment and colony on the number of trophallactic contacts/10 min, we again found that H and L treatment bees had significantly more trophallactic contacts than average workers, while there was no difference between the H and L treatment groups and the two colonies (GLM: treatment x troph./10 min: $F_{2,84} = 18.4$, $p < 0.001$*; colony x troph./10 min: $F_{1,84} = 1.1$, $p = 0.3$. Post-hoc Tukey- Kramer comparisons between treatments: H vs. average worker: $p < 0.001$; L vs. average worker: $p < 0.001$; H vs. L: $p = 0.87$).

We then tested whether this difference in the number of trophallactic contacts between groups was due to differences in the number of offering contacts or begging contacts or both. When we compared the number of offering contacts between groups we found that H and L treatment bees had more offering contacts than average workers and a significant positive relation with the number

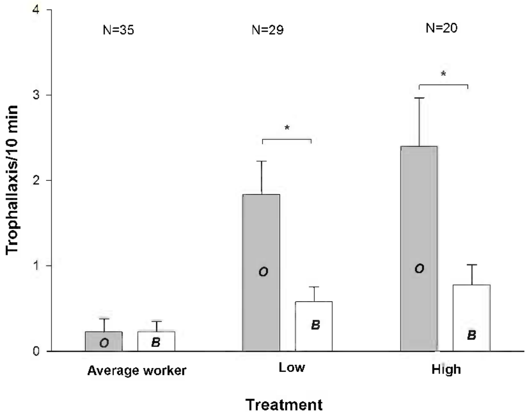

Figure 2.5: The number of offering contacts (G; grey) and begging contacts (R; white) per 10 minutes (mean ± SE) of bees from the H treatment, the L treatment and average bees.

of begging contacts (bees having more offering contacts also had more begging contacts), but no effect of colony (GLM: treatment x troph. offering/10 min: $F_{2,84} = 14.9$, $p < 0.001$*; troph. begging/10 min x troph. offering/10 min: $F_{1,84} = 6.74$, $p = 0.011$*; colony x troph. offering/10 min: $F_{1,84} = 1.07$, $p = 0.3$; Fig. 2.5. Tukey-Kramer comparisons: H vs. average worker: $p < 0.001$; L vs. average worker: $p < 0.001$; H vs. L: $p = 0.72$). The number of begging contacts, on the other side, did not differ neither between bee groups nor between colonies (GLM: treatment x troph. Begging/10 min: $F_{2,84} = 2.89$, $p = 0.061$; colony x troph. begging/10 min: $F_{1,84} = 0.32$, $p = 0.57$; Fig. 2.5). In both the H treatment and the L treatment bees had more offering contacts than begging contacts (GLM: H treatment: type of troph. x number of troph.: $F_{1,40} = 7.5$, $p = 0.01$*; colony x number of troph.: $F_{1,40} = 3$, $p = 0.091$; L treatment: type of troph. x number of troph.: $F_{1,52} = 7.15$, $p = 0.01$*; colony x number of troph.: $F_{1,52} = 0.54$, $p = 0.46$; Fig. 2.5).

We also tested whether the mean duration of trophallactic contacts differed between the H and L treatment bees. We found no differences between both groups in the mean duration of offering contacts and begging contacts that the second-order receivers had during the filming (GLM: offering contacts: treatment x duration offering troph.: $F_{1,36} = 0.4$, $p = 0.53$; H treatment: 4.4 ±

Table 2.1: Dunn's test for multiple comparisons of frequencies. Frequencies of brood and honey cell inspections and percentages of observation time in delivery area, brood area and pollen area between bees of the L group, H group and average workers.

Comparison	*N*	*rank difference*	*critical value*	*p*
Brood cell inspections				
L vs. H	20/29	3.45	14.87	n.s.
L vs. average worker	29/35	11.85	12.85	n.s.
H vs. average worker	20/35	15.14	14.34	$p < 0.05$
Honey cell inspections				
L vs. H	20/29	6.37	12.61	n.s.
L vs. average worker	29/35	14.53	10.90	$p < 0.05$
H vs. average worker	20/35	8.16	12.17	n.s.
Delivery area				
L vs. H	20/29	4.22	16.59	n.s.
L vs. average worker	29/35	25.69	15.99	$p < 0.05$
H vs. average worker	20/35	21.57	14.33	$p < 0.05$
Brood area				
L vs. H	20/29	1.71	16.59	n.s.
L vs. average worker	29/35	19.8	15.99	$p < 0.05$
H vs. average worker	20/35	18.09	14.33	$p < 0.05$
Honey area				
L vs. H	20/29	3.46	16.59	n.s.
L vs. average worker	29/35	26.7	15.99	$p < 0.05$
H vs. average worker	20/35	3.2	14.33	$p < 0.05$

1.02 sec, L treatment: 3.5 ± 0.51 sec; colony x duration offering troph.: $F_{1,36}$ = 0.88, p = 0.354; begging contacts: treatment x duration begging troph.: $F_{1,21}$ = 1.48, p = 0.24; H treatment: 12.2 ± 5.97 sec, L treatment: 4.6 ± 1.95 sec; colony x duration begging troph.: $F_{1,21}$ = 0.002, p = 0.97). Only 2 of 84 filmed bees inspected pollen cells during the observation period. Brood cell inspections (35 of 84 bees) and honey cell inspections were more frequent (21 of 84 bees). We found significant differences between the 3 bee groups in both brood cell inspection frequencies (Kruskal-Wallis ANOVA: χ^2 = 7.64, df = 2, p = 0.022) and honey cell inspection frequencies (Kruskal-Wallis ANOVA: χ^2 = 9.84, df = 2, p = 0.007). Post-hoc Dunn tests showed that average workers inspected fewer brood cells than H treatment bees, but more honey cells than L treatment bees (Table 2.1).

2.4.2 Comparison between identified and unidentified bees

The distribution of bees among the three worker castes found in identified bees is probably not representative for all second-order receivers. To find evidence for behavioural differences between identified and unidentified bees, we compared activity (GLM: group x activity: $F_{1,48}$ = 1.873, p = 0.178; treatment x activity: $F_{1,48}$ = 0.177, p = 0.68; colony x activity: $F_{1,48}$ = 4.87, p = 0.033**), the total number of trophallaxes per 10 min (GLM: group x troph/10 min: $F_{1,49}$ = 0.71, p = 0.41; treatment x troph/ 10 min: $F_{1,49}$ = 0.431, p = 0.52; colony x troph/10 min: $F_{1,49}$ = 2.73, p = 0.105) and the time spent in the brood area (GLM: group x brood: $F_{1,49}$ = 1.41, p = 0.24; treatment x brood: $F_{1,49}$ = 2.25, p = 0.14; colony x brood: $F_{1,49}$ = 15.5, p < 0.001*; the colony effect is caused by a smaller brood area in hive 2) between these two groups of bees and found no differences.

2.4.3 Position of bees

Behavioural differences between the different bee groups may also concern the position of the second-order receiver during the observations. Therefore we compared the percentage of the observation time bees spent in the three most important hive areas, the delivery area, the brood area and the honey area (Table 2.1). We found that bees of the H and the L group spent significantly more time in the delivery area than average workers (Kruskal- Wallis ANOVA, χ^2 = 24.4, N = 84, p < 0.001; H group: 20.17% [0, 59.5], L group: 3.86% [0, 61.6], average worker: 0% [0, 0]; Table 2.1). They also spent more time in the brood area compared to average workers (Kruskal- Wallis ANOVA, χ^2 = 13.11, N = 84, p = 0.001*; H group: 56.24% [25.4, 81.9], L group: 90.74% [5.4, 99.48], average worker: 0% [0, 63.27]; Table 2.1). But H and L treatment bees spent less time in the honey area than average bees (Kruskal-Wallis ANOVA, $\chi^2 = 25.3$, N = 84, $p < 0.001$; H group: 0% [0, 9.18], L group: 0% [0, 1.99], average worker: 49.22% [2.2, 99.1]; Table 2.1). Table 2.1 shows the results of the pair-wise Dunn tests for multiple comparisons.

2.4.4 General trophallactic activity

We recorded 11 sampling periods (6 x 1 min per sampling period), 4 under high quality food conditions and 7 under low quality food conditions. There were no effects of food condition and colony on the total number of trophallaxes observed during a sampling period (GLM: treatment x troph.: $F_{1,11} = 0.074$, p = 0.792; colony x troph.: $F_{1,11} = 0.0$, p = 0.99; in total 10.1 ± 0.99 trophallaxis per sampling period, range: 6 – 16). Using these values to estimate the average number of trophallaxes/bee in 10 min provides a value of about 0.25 trophallaxes per average worker in our colonies. Average workers recorded individually with video had 0.5 ± 0.15 trophallaxes in 10 minutes. One reason for the difference may be that the sampling of many bees at the same time (scan sampling) made it more difficult to see all short contacts than when filming one single bee for a longer period.

2.5 Discussion

On the way from the delivery area to the honey cells, food processors feed various bees which indicates that nectar is distributed rapidly amongst hive bees (von Frisch 1923; Rösch 1925; Seeley 1989). However, it was not yet known what kind of bees receive food from food processors. In our study, we identified bees performing foraging duties (22.2%), food-processing duties (18.5%) and mainly nurse duties (59.3%) as second-order receivers. This shows, that hive bees of the three major worker castes receive food samples within a few minutes after the nectar has been carried to the hive. These results help to explain how small quantities of food collected by foragers can become widely distributed among the members of different worker castes of a colony within a few hours (Nixon & Ribbands 1952). Second-order receivers of unknown subcaste showed similar trophallactic behaviours and locomotion activity as the identified bees. The conclusion that nurse bees are the main recipients of food from food processors is also supported by the findings that most trophallaxes between food processors and second-order receivers took place in the brood area and that second-order receivers subsequently spent most of their time in the brood area. It also accords with observations showing a general tendency for food to pass from older bees to younger bees within colonies (Free 1957; Crailsheim 1998). The young nurse bees, normally 3-11 days old, are responsible for preparing nutrients from pollen and distribute the nutritionally valuable proteins produced by their hypopharyngeal glands, nectar and honey mainly to larvae but also to other hive bees (Winston 1987; Crailsheim 1998). 22.2% of the identified second-order receivers performed foraging duties. This shows that foragers can obtain information cues about the current foraging opportunities (such as floral scents) not only from foragers, but also via food processors.

The second-order receivers were much more active than the average worker bees during the observation period. The low activity of our average workers accords with the high rate of inactivity found in other studies (Rösch 1925; Lindauer 1952; Seeley 1995) and may be more pronounced in our study, because the study has been performed at the end of the flowering season. The increased activity of the second-order receivers was found to correlate with a high trophallactic activity. While the average number of trophallaxes/10 min was between 0.25 - 0.5 in average bees, second-order receivers had almost 4. This difference between second-order receivers and average worker bees was mainly due to a high number of trophallactic-offering contacts (Fig. 2.5). In other words, the liquid food received from the food processors is rapidly distributed to other hive workers. Seeley (1989) found that active food processors (first-order receivers) had between 4.3 and 10.5 offering contacts per 10 minutes, depending on the nutritional state of the colony (calculated from his Table 3). These result and our own suggest that the rapid distribution of food within a colony may be the result of a very high trophallactic-offering activity of a relatively small proportion of bees in a colony. However, since we filmed on average only about 10 min per bee, it is not possible to say for what time period second-order receivers show this increased behavioural activity.

Results further show, that the 1st trophallaxes (between foragers and food processors) are about twice as long as 2nd trophallaxes (between food processors and second-order receivers), while 2nd trophallaxes are about twice as long as 3rd trophallaxes (offering contacts of second-order receivers). Bees retain food for themselves or for additional offerings. This coincides with results obtained in a laboratory study (Goyret & Farina 2005), which found a similar reduction in transfer time from the 1st to the 2nd trophallaxis and a reduction in the transfer rate of about $35 - 40\%$. The transfer rates of the 1st and the 2nd trophallaxis correlated positively. As there is a positive relation between transfer rate and food source profitability (Farina & Núñez 1991; Tezze & Farina 1999), bees momentarily not involved in foraging potentially obtain quantitative information about the profitability of the exploited food sources from hive-bees. This may affect the decision to start or resume foraging activities or it may cause adjustments of in-hive activities related to nectar processing.

It has often been shown, that the food source profitability, either expressed in terms of nectar flow rate or sucrose concentration, affects in-hive behaviours such as dancing (Lindauer 1948; von Frisch 1967; Seeley 1995) or trophallactic behaviour of foragers (Farina 1996; De Marco & Farina 2001) and of food processors (Pírez & Farina 2004). The changes in forager and food processor behaviours according to food source profitability can be seen as part of a colony level response to the current environmental conditions (Seeley 1995). In our study, we found no significant effect of sucrose concentration on the second-order receiver behaviours that were analysed. Our ad libitum-feeders did not allow the adjustment of the nectar flow rate. The nectar flow rate has been shown to affect food processor behaviour previously (Pírez & Farina 2004). It is possible that our bees evaluated the low sucrose concentration food as a high quality food source.We observed that a substantial number of foragers showed recruitment dances even when foraging at the food source with 13% sucrose concentration. This indicates low thresholds for dancing, caused by a lack of alternative food sources at this late stage of the flower season (Seeley 1995). In summary, food source profitability has an effect on the nectar flow pathways, but probably not at all stages. Fig. 2.6 graphically shows the flow of nectar inside the honeybee colony in late summer and its relation to food source profitability. (1) The width of the arrows coming from the returning foragers reflects the positive dependence of the crop load on food source profitability (Núñez 1966,1970). (2) Foragers exploiting high profitability food sources perform more offering trophallaxes upon arrival at the hive and interact with other foragers more frequently (Farina 1996; De Marco & Farina 2001). (3) Subsequently, food processors receiving food from foragers exploiting high profitability food sources are more likely to go directly to the storing area and less likely to engage in offering contacts only compared to food processors under low reward conditions (Pírez & Farina 2004). With the exception of the time spent in the brood area (where we found a difference between the two colonies due to a smaller brood area in H2), we found no effect of colony with respect to the main results.

Food collection by honeybee colonies is a rather complex process, which involves bees of different subcastes and, as a consequence of the numerous interactions, creates a food network. It is important to mention that aspects of food sharing potentially depend on many more factors such as nutritional state of a colony, amount of brood, nectar influx, season and colony size (Free 1959;

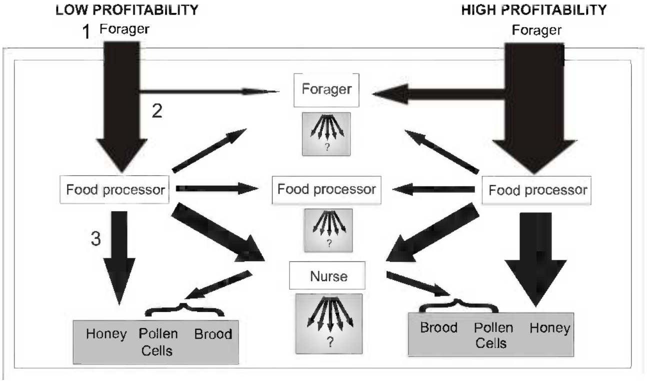

Figure 2.6: The schematic flow of nectar of a single forager load in a honeybee colony in late summer, coming either from a food source of high or low profitability (either in terms of nectar flow or sugar concentration). The width of the arrows reflects roughly the estimated amount of nectar flowing along its route. 1) From Núñez 1966; 2) Farina 1996, De Marco & Farina 2001; 3) from Pírez & Farina 2004.

Istomina-Tsvetkova 1960; Howard & Tschinkel 1980; Seeley 1989). The relative amount of nectar that is transported either to honey cells or is fed directly to other bees, for example, may be very variable, depending on these factors.

Food sharing seems much more extensive than would be required merely to prevent individuals from starving when food is available (Ribbands 1953). Hence, it has been suggested that the majority of trophallactic contacts serve communicational purposes rather than being food transfer attempts (Korst & Velthuis 1982). The numerous social interactions have a potential benefit in the spread of information through the colony (Ribbands 1953; Crailsheim 1998; Grüter et al. 2006). Information cues and signals present in the processed and shared nectar, such as food scents, sucrose concentration or pheromones can affect the behaviour of potentially all hive bees (Wilson 1971; Pankiw et al. 2004; Grüter et al. 2006). In our study, 49.5% of all trophallaxes of second-order receivers and 44% of all trophallaxes in average workers were shorter than 1.5 seconds. These contacts are normally too short for effective food transfer (Farina & Wainselboim 2001b). But even when trophallactic interactions are too short to guarantee food transfer, they may have important informational implications. These contacts (i.e. search time for a receiver bee) cause time delays which in turn offer opportunities for information acquisition about environmental conditions and colony work allocation (Lindauer 1948; Seeley 1995). Natural selection may favour a high trophallactic activity and extensive circulation of liquid food, if this leads to better informed hive individuals which in turn allow for a more adequate colony level response to present internal and external conditions.

Acknowledgments

We are deeply indebted to Peter Stettler for all his help with the experiments. Without his support, this experiment could not have been performed. We are also very grateful to Michael Taborsky for logistic support. We thank Sabina Joos, Severine Loosli, Rebecca Schreier, Andrea Woodtli, Adrian Britschitsch and Tomi Reichlin for help with data collection. This study was supported by funds from the VDRB (Verein deutschsprachiger und rätoromanischer Bienenfreunde), Dr. De Giacomi Stiftung, Basler Stiftung für biologische Forschung and Berner Hochschulstiftung. WMF is supported by funds from ANPCYT (0112319), and University of Buenos Aires (X 036). When we carried out our experiments, we adhered to the legal requirements of Switzerland.

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3

Social learning of floral odours inside the honeybee hive

Farina, W.M., Grüter, C.* and Díaz, P.C. 2005. *Proceedings of the Royal Society London B* 272: 1923-1928 (*co-first author)

3.1 Abstract

A honeybee hive serves as an information centre in which communication among bees allows the colony to exploit the most profitable resources in a continuously changing environment. The best-studied communication behaviour in this context is the waggle dance performed by returning foragers, which encodes information about the distance and direction to the food source. It has been suggested that another information cue, floral scents transferred within the hive, is also important for recruitment to food sources, as bee recruits aremore strongly attracted to odours previously brought back by foragers in both honeybees and bumble-bees. These observations suggested that honeybees learn the odour from successful foragers before leaving the hive. However, this has never been shown directly and the mechanisms and properties of the learning process

remain obscure.We tested the learning andmemory of recruited bees in the laboratory using the proboscis extension response (PER) paradigm, and show that recruits indeed learn the nectar odours brought back by foragers by associative learning and retrieve this memory in the PER paradigm. The associative nature of this learning reveals that information was gained duringmouth-to-mouth contacts among bees (trophallaxis). Results further suggest that the information is transferred to long-term memory. Associative learning of food odours in a social context may help recruits to find a particular food source faster.

3.2 Introduction

Recruitment dynamics to scented food sources strongly suggest that information transfer about floral odours inside the hive is an important component of honeybee (*Apis mellifera*) recruitment (von Frisch 1967; Wenner et al. 1969). The role of this information transfer seemed so important that controversy has arisen about whether bees that follow dances decode the vector information, or instead rely exclusively on odour (von Frisch 1967; Wenner et al. 1969; Gould 1974). This controversy is now considered to be resolved (Gould 1974; Esch et al. 2001; Sherman & Visscher 2002) and the new findings strongly indicate that the dance as well as the olfactory information transferred inside the hive are used to find a particular food source (von Frisch 1967; Wenner et al. 1969; Seeley 1995; Kirchner & Grasser 1998; Esch et al. 2001; Sherman & Visscher 2002). Despite the importance of this olfactory information transfer for recruitment to food sources, questions remain unanswered with respect to whether or not recruits do indeed learn the association between odour and food, when they learn it and what kind of properties the established memory has.

Gerber et al. (1996) showed that olfactory memories of free flying bees established during flower visits in an operant context can be transferred to the proboscis extension response (PER) paradigm in the laboratory in which harnessed bees may extend their proboscis when presented with odorants, depending on their previous experiences with this odour. When the antennae of bees are touched with sucrose solution (unconditioned stimulus; US), they will reflexively extend their proboscis to drink the solution. If an odour (conditioned stimulus; CS) is presented shortly before it becomes associated with the US and subsequently elicits the response (Bitterman et al. 1983). This associative learning paradigm offers a convenient method to quantify retention for an odour in single bees, by testing whether associations between the nectar reward and odours have been acquired during flower visits or within the hive (Gerber et al.

1996; Menzel & Giurfa 2001).

Foraging bees that return from nectar sources transfer the gathered liquid to hive mates through several trophallactic contacts (von Frisch 1967; De Marco & Farina 2001; Farina & Wainselboim 2001a). It has been suggested that recruits may learn the odour of nectar brought back in the honey stomach during these contacts and most honeybees recruited to a source containing an artificial dye were observed to have received a sample from the forager inside the hive (von Frisch 1967). Even during short contacts (less than 4 s long) the regurgitated food may be transferred or just probed and thereby allow receivers to taste the incoming nectar (Farina & Wainselboim 2001b).

Using the PER paradigm, we tested whether bees recruited to a scented food source extended their proboscis on the first presentation of the corresponding odour (spontaneous response) and, therefore, had learned the association between food and odour inside the hive during trophallactic contacts.We further analysed the development of retention for the learned odour during 3 consecutive days. Bees may have experienced the combination of odour and solution even if they did not respond spontaneously to the odour (Menzel 1999). In such a case, these bees should learn the odour faster as a consequence of a previous experience. Therefore, we tested their learning performance for the solution odour in a differential PER conditioning (Bitterman et al. 1983): one odour (the odorant diluted in the sugar solution) is paired with sucrose (CS+, CS) and the other odour (the odorant presented at the hive entrance) is presented unpaired (CS–) between CS+ trials. The bees learn to respond to the CS+ and not to the CS–.

3.3 Methods

3.3.1 Study site and animals

The experiment was performed at the end of the nectar flow season (March-April 2004) at the experimental field of the University of Buenos Aires. We used two two-frame observation hives containing a colony of about 4000 European honeybees (hybrid descendants of *A. mellifera ligustica*) each. Hive bees were marked with coloured paint on the thorax. A group of bees was trained to collect 0.5 M (5 μl min^{-1} flow rate) unscented sucrose solution at an artificial feeder located 160 m from the hive. These bees were given individual marks and the group was renewed every 3-6 days to maintain a number of about 5 to 10 foragers.

3.3.2 Hive and solution odour

The hive odour served two purposes. First, we wanted to reduce the effect of odour molecules clinging onto the foragers body, which may be perceived during trophallaxis as well. Our aim was that bees would associate the odour covering the forager with a non-appetitive hive context. Second, we were interested to see whether bees also would respond to the hive odour. The hive and its entrance were scented by putting absorbent paper (diameter 3 cm) soaked with 50 μl pure odorant inside a box connected to the entrance by a wire mesh. The paper was renewed once a week. Returning foragers passed the box when entering the hive. In two parts of the experiments we used two different sets of odorants. In part 1 (beginning on day 1), we used phenylacetaldehyde as the hive odour and linalool (LIO) as the odorant diluted in the reward (henceforth, solution odour). In part 2 (beginning on day 19) we used LIO as the hive odour and 2-nonanone as the solution odour (Fig. 3.1b,e). We avoided using Phenylacetaldehyde as the solution odour in part 2 because its use as hive odour in part 1 could affect olfactory conditioning of this odour, for instance by latent inhibition (Chandra et al. 2000). The three odours are natural flower odours (Knudsen et al. 1993). We used the same odour combinations for the differential conditioning. In this way, we repeated the experimental situation, where bees experienced one odour in an appetitive context (solution odour) and one in a non-appetitive context (hive odour) in the laboratory. Odours were obtained from Sigma-Aldrich, Steinheim, Germany.

3.3.3 Bee capture

Bees were captured during 5 periods. Part 1 of the experiment consisted of period 1 (days 3–5), period 2 (days 12–14) and period 3 (days 16–18). Part 2 consisted of period 4 (days 38–40) and period 5 (days 43, 44). Experimental hive: during sampling periods of 3 h, a group of 5–8 marked bees from the experimental hive collected a 2.0 Msucrose solution (40 μl min^{-1} flow rate) at a feeder and recruited hive-mates. The solutions were scented with 50 μl pure odour per litre. Several days prior to periods 2 and 4, trained bees were already foraging small amounts (6 ml before period 2 and 4, respectively) of solution scented with the same odour as was used afterwards. After periods with scented solution, the feeders were replaced by clean feeders. Arriving recruits were captured with plastic tubes before they touched the solution; otherwise they were killed with alcohol. Captured bees were fed a drop of a 1.8 M unscented sucrose solution. The interval between capture and feeding was 30-60 min. Control hive: changes in spontaneous response probabilities to odours and learning of odours could reflect changes in the availability of natural food source. To exclude this possibility we used a control hive placed about 5 m from the experimental hive. During the periods 2–5, bees leaving the control hive were captured and fed after the same interval mentioned above with a drop of 1.8 M sucrose solution.

3.3.4 Harnessing

After 1-3 h in captivity, bees were harnessed in plastic tubes so that they could move their mouthparts and antennae freely (Bitterman et al. 1983). They were fed 1.8 M sucrose solution for about 3 s and kept in an incubator (25°C, 55% relative humidity, darkness) for at least 3 h.

3.3.5 Differential PER conditioning

We subjected the harnessed bees to standard differential PER conditioning (Bittermanet al. 1983), in which two pure odours are presented, one rewarded (CS+) with 1.8 M sucrose solution (US) and the other unrewarded (CS–), four times each, in a pseudo-randomized order. The inter-trial interval lasted 10-15 min. Only bees that showed the unconditioned response (the reflexive extension of the proboscis after applying a 1.8 M sucrose solution to the antennae) and that did not respond to the mechanical air flow stimulus were used. A device that delivered a continuous airflow was used for odorant application. Trials lasted for 46 s and consisted of 20 s of air flow, 6 s of odour (CS) and 20 s of air flow. During rewarded trials, the reward (US) was delivered upon the last 3 s of CS. Bees that responded to the first presentation of the CS (spontaneous response) were not used in the PER conditioning. In part 1, the CS+ was LIO (solution odour) and the CS– was phenylacetaldehyde (hive odour). In part 2, the CS+ was 2-nonanone (NON; solution odour), and the CS– was LIO (hive odour). In this way, the experimental situation, where bees experienced one odour in an appetitive context (solution odour) and one in a non-appetitive context (hive odour) was repeated in the laboratory.

3.3.6 Statistical analysis

G-tests were used to compare proboscis extension frequencies between groups. We corrected G-values for multiple comparisons within hive and indicated corresponding p-values with (*). Performance during conditioning was analysed using a discrimination index (Pelz et al. 1997), that was calculated as the cumulative sum of a bees responses to the CS+ minus the cumulative sum of that bees responses to the CS–. This index was then used in Kruskal-Wallis

ANOVAs. A Dunns test was used for multiple comparisons between groups.

3.4 Results

3.4.1 Spontaneous response

The percentage of recruits that extended the proboscis on the first presentation of the odour differed among days in the experimental hive over the experiment, but not in the control hive (experimental hive: G-test, $G_{adj} = 88.7$, df = 13, $p < 0.001$; control hive: G-test, $G_{adj} = 6.25$, df = 10, p = 0.5). During the scented period with LIO (period 2) the spontaneous response increased from day 1 to 3 (day 12 versus day 14 of the experimental period: G-tests, LIO,G_{adj} = 8.45, df = 1, *$p < 0.05$; Fig. 3.1a). On day 3 of the LIO period (day 14), the spontaneous response shown by recruits was significantly higher than that of the foragers coming from a control hive captured the same day (G-test, G_{adj} = 15.7, df = 1, $p < 0.001$, Fig. 3.1a,c), which shows that bees learned the scents from their hive companions. Additionally, spontaneous responses on day 3 of the LIO period were higher than on day 3 (day 40) of the NON period (G-test, $G_{adj} = 8.31$, df = 1, *$p < 0.05$, Fig. 3.1d). After the LIO period, we captured bees recruited to unscented solution for 3 days (period 3) to analyse whether bees recruited several days later also remember the odour. We found a high spontaneous response on the first day (day 16), which decreased by the third day (day 18, G-test, $G_{adj} = 7.78$, df = 1, *$p < 0.05$; Fig. 3.1a).

During the period when solution was scented with NON (period 4), the spontaneous response probability did not differ between days 1 and 3, in either the experimental hive or the control hive (Table 3.1). However, more recruits from the experimental hive than the control hive responded to NON during this period (G-test, $G_{adj} = 16.85$, df = 1, $p < 0.001$; Fig. 3.1d,f). This suggests that recruits also learned the odour of NON from their companions. After the NON period, we also captured bees recruited to unscented solution for 2 days (period 5, Fig. 3.1d). We found a decreasing spontaneous response, attaining a null spontaneous response after 4 days with no NON in the solution (day 44; Fig. 3.1d).

3.4.2 Differential PER conditioning

The bees that showed no spontaneous response (above) were used in a differential PER conditioning. There were strong differences in acquisition performance between the bees captured during the two periods when the solution was scented

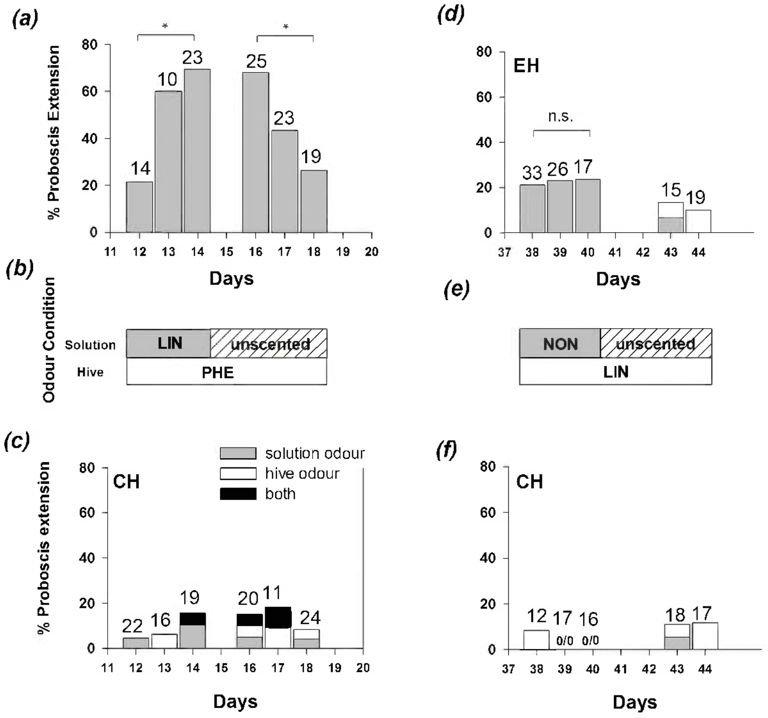

Figure 3.1: Percentage of bees that extended the proboscis on the first presentation of the odour. (a) Responses from the experimental hive (EH) during part 1. (b) Its corresponding odour condition: linalool in solution and phenylacetaldehyde in the EH during days 12, 13 and 14; unscented solution and phenylacetaldehyde in the EH between 15 and 18 days. (c) Responses from the control hive (CH) during part 1. (d) Responses from the EH during part 2. (e) Its corresponding odour condition: 2-nonanone in solution and linalool in the EH during days 38, 39 and 40, unscented solution and linalool in the EH between 41 and 44 days. (f) Responses from the CH during part 2. Responses for solution odour (grey), hive odour (white) and for both odours (black) for the EH and the CH. The CH was untreated in both situations. Asterisks indicate statistical differences (G-test, *$p < 0.05$, n.s., not significant; see 3 for details). Number of tested bees above bars.

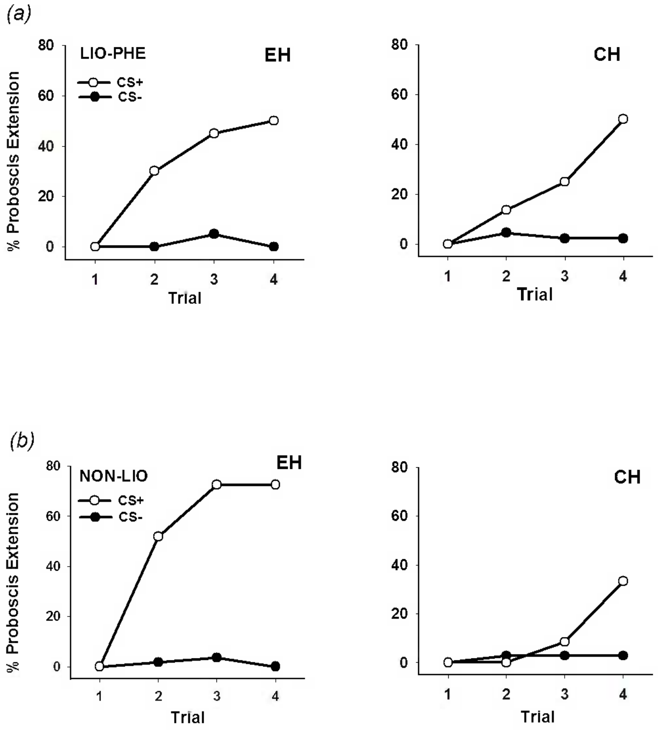

Figure 3.2: Percentage of bees that extended the proboscis during a given trial. (a) Acquisition of bees from the experimental hive (EH) and control hive (CH) during the course of the differential PER conditioning. LIO was the CS+ and PHE was the CS–. (b) For conditioning with NON as CS+ and LIO as CS–.

Table 3.1: Comparison of PER frequencies between days 1 and 3 of a given period. (EH, experimental hive; CH, control hive; NON, 2-nonanone.)

analysis	*N*	G_{adj}	*p*
spontaneous response			
NON treatment EH	45	0.044	0.98
NON treatment CH	76	0.0	1.0

(periods 2 and 4), and the bees from the control hive captured during the corresponding periods (acquisition: Kruskal-Wallis ANOVA for the four groups of bees, N = 158, df = 3, H = 38.5, $p < 0.0001$; comparison between days 1 and 3 for LIO period: experimental hive, N = 20, df = 2, H = 1.58, p = 0.45; control hive, N = 44, df = 2, H = 0.93, p = 0.63; pooled data for day 13 for NON period: experimental hive, N = 58, df = 2, H = 4.05, p = 0.13; control hive, N = 36, df = 2, H = 0.22, p = 0.89; Fig. 3.2a,b). Bees recruited by foragers collecting solution with NON showed better learning of the solution odour than bees from the two control groups (Fig. 3.2, Table 3.2) but did not differ from recruits in the LIO treatment (Table 3.2). On day 44, 4 days after the end of the NON period, learning performance in the experimental hive was still higher than in the control hive bees (Mann-Whitney U-test, U = 44, z = 3.1, $p < 0.005$).

Table 3.2: Multiple comparisons of acquisition performance between days 1 and 3 of a given period. (EH, experimental hive; CH, control hive; DI, discrimination index; NON, 2-nonanone; LIO, linalool.)

analysis	*N*	*Q*	*p*
acquisition (DI)			
Dunn's test for multiple-comparisons			
LIO EH versus LIO CH	20/44	1.18	n.s.
LIO EH versus NON EH	20/58	2	n.s.
LIO CH versus NON CH	44/36	1.5	n.s.
NON EH versus NON CH	58/36	5.53	< 0.001

3.5 Discussion

A honeybee colony must rapidly deploy its foragers among many different flower patches in the surrounding environment and therefore needs to acquire information about the different foraging opportunities. Previous studies suggested that the floral odour brought back to the hive by successful foragers is an important information cue for information flow in the control of a colonys foraging operation (von Frisch 1967; Wenner et al. 1969). However, despite the importance of olfactory information transfer inside the hive for recruitment to food sources, themechanisms underlying this information transfer are poorly understood.

We used two procedures to document that recruited bees learned odours that had been brought back by other foragers. Applying the PER paradigm, we also show that recruits transfer the odour learned in a social context to the classical conditioning situation of the PER test. The associative nature of the PER paradigm (Bitterman et al. 1983) reveals that the learned association took place during trophallactic interactions where the transferred solution functioned as a US and the odour (in the nectar or clinging onto the bees body) as the CS. We cannot exclude the possibility that recruits perceived odour molecules clinging onto the body of the donor, but our experimental design, as well as that used in earlier studies by von Frisch (1967), suggest that the odour present in the solution was perceived during food transfer.

The spontaneous response levels in the PER test (Fig. 3.1) differed between the two odours used. Although the same volumes of LIO and NON solution were collected by the recruiting bees from the experimental hive, recruits to NON solution showed a lower spontaneous response probability than recruits to LIO. On the other hand, recruits showed faster acquisition functions during differential conditioning as compared to bees from the control hive. The difference between the spontaneous response frequencies of recruits to LIO and NON may result from prior learning in the natural context (Bitterman et al. 1983; Menzel & Giurfa 2001) or from innate differences reflecting the biological relevance of the odours used (Knudsen et al. 1993). It may also be that the use of LIO in both parts of the experiments (as solution odour in part 1 and as hive odour in part 2) impaired the learning abilities of the bees in the experimental hive in the second part of the experiment. This, however, seems unlikely because during the 24 days between parts 1 and 2 the colony was almost completely renewed. That bees did not confuse the two contexts in which LIO was used is also supported by the observation that bees did not respond to LIO in part 2.

The potential role of mouth-to-mouth contacts as an information channel for food source characteristics and as a mechanism to efficiently direct the foraging activity of the colony has been suggested in earlier studies, which showed that aspects of trophallaxis correlate with food source profitability, such as nectar unloading rate (Farina & Núñez 1991), frequency and duration of contacts (Farina 1996; De Marco & Farina 2001) or thoracic temperature of food donors (Farina & Wainselboim 2001a). While information on distance and direction transferred during dancing is perceived only by bees following the dancers, information about food source characteristics, such as its odour, may be transferred to most members of the colony through a rapid (within a few hours) distribution of small quantities of food inside the hive (Nixon & Ribbands 1952). This could explain the high response frequency (68%) of foragers that were recruited to unscented solution 2 days after the end of the LIO period. Since the total amount of scented solution carried into the hive during each scented period is very small (approx. 28 ml of sugar solution), the high spontaneous response 2 and 3 days after the LIO period and the higher acquisition rate in the experimental hive than in the control hive 4 days after the end of the NON period may be interpreted in two ways. This olfactory information could be transferred to an early long-term memory, even after a single trophallaxis as it was recently reported (Gil & De Marco 2005). This memory trace is stable over 1-2 days but needs updating on a regular basis for transfer into late long-term memory, a form of memory that controls behaviour 3 days after learning (Menzel 1999). Or the receiving bee may be exposed to multiple experiences within a short period of time, e.g. attending several recruitment dances in a row. In that case, memory consolidation would undergo a sequential transfer from early to late long-term memory (Menzel 1999). Because our bees were killed after the differential PER conditioning, we probably underestimated the stability of the established memories if the bees exposed to the PER test represent a large proportion of the nest mates that learned the odour.

Learning floral odours in a social context such as a hive leads to long-lasting preferences for communicated odours and may affect a larger proportion of foragers and thereby influencing flower choice in the field for several days. Social learning of nectar scents in bees is thus remarkably similar to social transmission of information regarding food odours in some mammals such as the Norway rats, *Rattus norvegicus.* Rats learn food odours on the breath of co-specifics that have recently eaten and will show a preference for this food, even after weeks (Galef & Giraldeau 2001).

Acknowledgments

We are deeply indebted to M. Spivak, M. Giurfa, I.M. Hamilton and D.

Heg for suggestions and valuable comments on the original manuscript. We also thank M. Giurfa for the donation of the PER set-up, and A. Arenas, A. Martínez and H. Verna for technical assistance. This study was supported by funds from ANPCyT (01-12319), CONICET (02049), UBACyT (X 036) and Fundacion Antorchas to W.M.F. C.G. was supported by the Dr. De Giacomi Stiftung and the Janggen-Pöhn Stiftung.

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4

Honeybees learn floral odours while receiving nectar from foragers within the hive

Farina, W.M., Grüter, C., Acosta, L. and Mc Cabe, S. 2007. *Naturwissenschaften* 94: 55-60

4.1 Abstract

Recent studies showed that nectar odours brought back by honeybee foragers can be learned associatively inside the hive. In the present study, we focused on the learning abilities of bees, which directly interact via trophallaxis with the incoming nectar foragers: the workers that perform nectar-receiving tasks inside the hive. Workers that have received food directly from foragers coming back from a feeder offering either unscented or scented sugar solution [phenylacetaldehyde (PHE) or nonanal diluted] were captured from two observational hives, and their olfactory memories were tested using the proboscis extension response paradigm. Bees that have received scented solution from incoming for-

agers showed significantly increased response frequencies for the corresponding solution odour in comparison with those that have received unscented solution. No differences in the response frequencies were found between food odours and colonies. The results indicate that first-order receivers learn via trophallaxis the association between the scent and the sugar solution transferred by incoming foragers. The implications of these results should be considered at three levels: the operational cohesion of bees involved in foraging-related tasks, the information propagation inside the hive related to the floral type exploited, and the putative effect of these memories on future preferences for resources.

4.2 Introduction

Nectivorous insects, such as honeybees, use floral odorants to search for and identify food sources (von Frisch 1919). Odour cues present in nectar and pollen can be learned during the first foraging trips and help bees to return to the recently discovered feeding places (von Frisch 1967). These olfactory memories can be retained during several days (Beekman 2005), being retrieved either when bees fly in the close range of a known floral patch or inside the hive when scents of known food sources reactivate experienced foragers to resume collecting tasks (Ribbands 1954; Johnson & Wenner 1966).

Olfactory learning can also happen inside the colonies while the incoming scented nectar is shared among hive mates and through the food odour clinging on the returning foragers body (von Frisch 1967; Wenner et al. 1969; Farina et al. 2005; Grüter et al. 2006). Recent studies demonstrated that floral scents present in the nectar brought back by foragers can be learned by hive mates that later will be recruited to the advertised flower type (Farina et al. 2005; Grüter et al. 2006). In this social context, trophallaxis would be the most plausible mechanism by which the liquid food and its odours are associated. In fact, it is already known that associative learning occurs among caged honeybees through single mouth-to-mouth trophallactic contacts (Gil & De Marco 2005). Olfactory conditioning could not only be relevant for the recruitment to specific floral species but also for the organization of foraging-related tasks within the hive. Accordingly, a recent study reported that the occurrence of a transfer of food with a given scent between nectar foragers and hive mates is not random but is affected by olfactory experiences made during previous food exchanges (Goyret & Farina 2005).

As nectar receivers initiate nectar distribution within the colony (Seeley 1995), the analysis of their learning abilities is crucial for understanding how chemosensory information related to the incoming nectar can be propagated at the social level. With this in mind, first-order nectar receivers that interacted with a group of trained foragers were captured, and their proboscis extension responses (PERs) to odours diluted in sugar solution were assessed in the laboratory. We also tested whether nectar receivers showed differences in their odour responses depending on their odour experience. Among the odours tested, we presented those diluted in the nectar previously brought back by foragers and which were experienced by receivers during trophallactic contacts.

4.3 Methods

The experiment was performed at the end of the nectar flow season (February-April) at the experimental field of the University of Buenos Aires. We used two two-frame observation hives (henceforth: H1 and H2) containing a colony of about 3,200 European honeybees (*Apis mellifera ligustica*) each. Colonies had a queen, brood, and reserves.

4.3.1 Experimental procedure

A group of foragers was trained to collect a 2.0-M unscented sugar solution at a small plate feeder (about 8 cm diameter), placed at a distance of about 30 cm from the hive entrance for about 30 min. During the training period, foragers were marked with a colored spot onto the thorax. After this period, we offered at the feeder a solution having the same sucrose concentration and that was either unscented (day 1) or scented (days 2 and 9) for 60-90 min. During this period, we marked (with a new color) the hive bees that received the solution via trophallactic contacts for at least 5 s from the color-marked foragers (Fig. 4.1). This duration guarantees an effective passage of food during trophallaxis (Farina & Wainselboim 2001). To mark the receiver bees, we used a sliding acrylic wall that was partly covered with a mosquito screen, which allowed us to paint the receivers thorax while they interacted with the marked incoming foragers. This device could be moved horizontally, from side to side, allowing us to scan the whole area of the exposed face of the hive. Afterward, the mosquitoscreen piece of the sliding wall was replaced by an acrylic one (3 x 27 cm) with an opening in the center (2.5-cm diameter) that allowed the insertion of a suction tube to capture the marked receiver bees (Fig. 4.1c). This new sliding wall allowed us to move the opening in two dimensions. The capture of the marked hive bees, i.e., those that received food from marked foragers, lasted 30-45 min. After the capture, the bees were anesthetized with CO^2 and harnessed in plastic tubes (Fig. 4.1d), allowing the antennae and the proboscis to move freely (Bitterman et al. 1983). The bees were then kept in the dark (25°C, 55% relative humidity) for 1 h.

4.3.2 Odours used

Each colony was exposed to a solution-odour sequence: in H1, the unscented solution was presented on day 1, phenylacetaldehyde (PHE) in solution (all scented solutions contained 50 μl of pure odour per liter of solution) on day 2, and nonanal in solution on day 9; in H2, it was unscented solution on day 1, nonanal in solution on day 2, and PHE in solution on day 9. Three odours were presented in the PER paradigm: PHE, nonanal, and 2-octanol. The odours tested presented a similar carbon-chain length (eight or nine carbons) and relative low vapor pressures (Table 4.1). With this combination, we compared PER values to: (1) the same test odours for the different conditions and (2) the different solution odours. All odours used were natural flower compounds (Knudsen et al. 1993) and were obtained from Sigma-Aldrich, Steinheim, Germany.

4.3.3 PER testing

We tested the receiver PERs to the test odours. Bees that showed the unconditioned response (UR, the reflexive extension of the proboscis after applying a 1.0-M sucrose solution to the antennae) and did not respond to the mechanical airflow stimulus were used. For both colonies, the bees were allocated in equal numbers to the six possible odour sequences, e.g., one-sixth of the bees captured were tested in the sequence PHE–nonanal–2-octanol and the remaining five groups in the rest of the possible sequences. The PER of the bees that had received unscented sugar solution gave us a general picture of spontaneous response frequencies for the test odours. Hive bees receiving scented solution were exposed to the solution odour collected by the forager mates (nonanal or PHE) and to the test odours (2-octanol or the alternative solution odour, either nonanal or PHE). After odour presentations, the bees were tested again for the UR, and bees not responding (less than 5%) were excluded from the analysis. The interval between the odour presentations lasted about 15 min. A device that delivered a continuous airflow was used for odorant application (for details of this setup, see the work of Guerrieri et al. 2005). Test trials lasted for 46 s. During the first 20 s, the bees received clean air followed by 6 s of odour stimulation and then 20 s of clean air again.

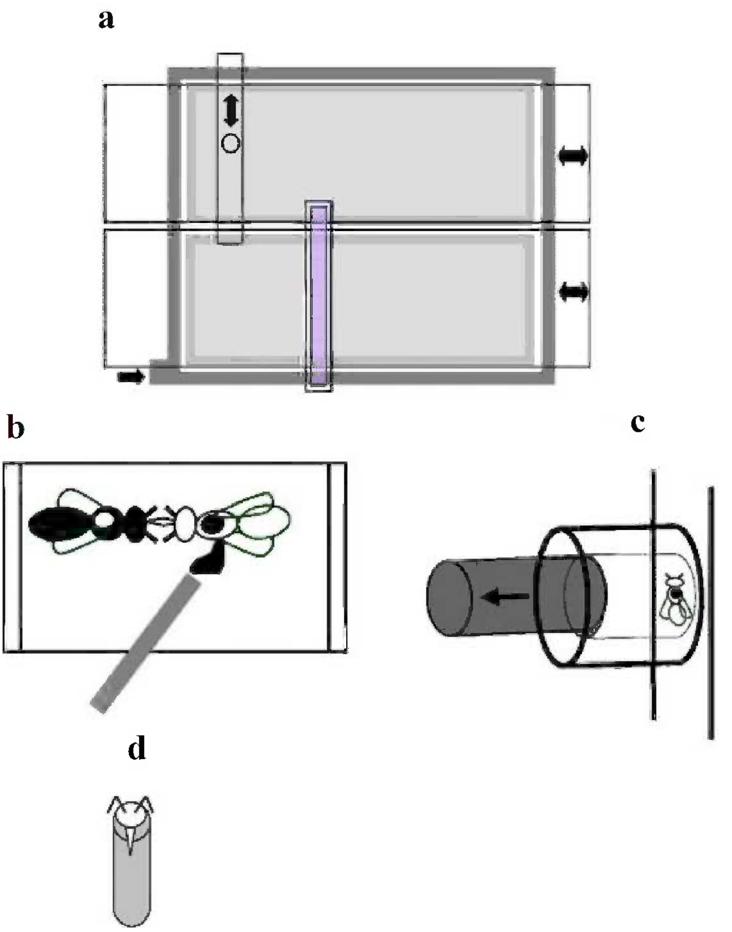


Figure 4.1: Experimental device and procedure to capture nectar-receiver bees inside the hive to test their olfactory memories in a PER assay. a) The experimental hive with its sliding acrylic walls. One of them (the lower one) was partly covered with a mosquito screen. This allowed us to paint the receivers thorax (white bee) during the trophallaxis with a marked forager (black bee). b) Afterward, the mosquito-screen piece was replaced by an acrylic one with an opening in the center [see the upper comb in a)] that allowed us the insertion of a suction tube to capture the marked receivers c). The captured receiver was then anesthetized and harnessed in plastic tubes, allowing the antennae and the proboscis to move freely d).

4.3.4 Statistical analysis

We compared PER frequencies using log-linear models (Zar 1999). Interactions between PER frequencies, presence or absence of solution odours, and colonies were tested. We ran three models, one for each solution odour separately and one to compare the two solution odours. We adjusted alpha levels of the mutual independence tests for multiple comparisons using the Dunn-Sidak correction (Sokal & Rohlf 1981). All significant p-values remained significant after the correction.

4.4 Results

The percentages of responses for the three test odours in H1 are shown in Fig. 4.2a. From 49 bees receiving unscented solution in the hive, 4% responded only to PHE, 2% responded only to nonanal, and about 8% responded only to 2-octanol. From 22 bees receiving PHE solution in the hive, 32% responded only to PHE, and 5% responded only to nonanal. From 30 bees receiving nonanal solution in the hive, 33% responded only to nonanal, while 3% responded to the three test odours. Fig. 4.2b shows the percentage of responses in H2 to the same three odours tested in H1. From 37 bees receiving unscented solution in the hive, 8% of the bees responded only to PHE, and about 3% responded to nonanal. From 39 bees receiving nonanal solution in the hive, 26% responded only to nonanal, 3% responded only to PHE, and about 10% responded to more than one test odour. Seven days later, we used PHE in the solution. From 33 bees receiving PHE solution in the hive, 24% responded only to PHE, 3% responded only to 2-octanol, and 6% responded to more than one test odour. We tested interactions between PER frequencies (PER, variable 1) for PHE, the presence or absence of PHE in the previously collected solution (odour, variable 2), and the two colonies (hive, variable 3). A global test of mutual independence among the three variables using log-linear models revealed significant mutual dependence ($G = 17.42$, $df = 4$, $N = 141$, $p = 0.002$). Partial independence tests suggested that there is a significant interaction between the presence of odour in solution and the PER frequencies (odour vs. hive and PER: $G = 17.14$, $df = 3$, $p < 0.001$; PER vs. hive and odour: $G = 13.53$, $df = 3$, $p = 0.004$) but no effect of hive (hive vs. odour and PER: $G = 4.88$, $df = 3$, $p = 0.18$). This was tested using a two-dimensional contingency table (G-test: $G = 12.53$, $df = 1$, $p < 0.001$).

Similar results were found in the case of nonanal. After finding mutual

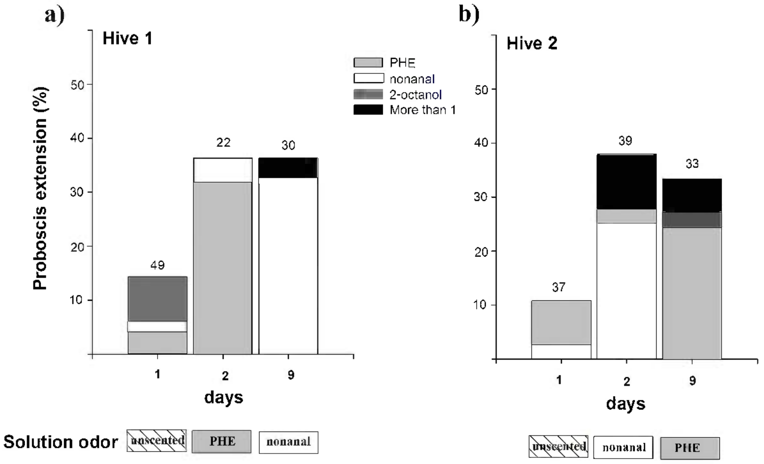
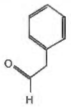


Figure 4.2: Proboscis extension response (PER) percentages for food-receiver bees that extended the proboscis on the first presentation of an odour in two observation hives. a) Responses from the hive 1 during the experimental period. Its corresponding odour condition was: unscented solution, PHE (phenylacetaldehyde) in solution, and nonanal in solution were collected by a group of trained forager mates at a feeder offered with a 2.0-M sucrose solution during days 1, 2, and 9, respectively. b) Responses from the hive 2 during the experimental period. Its corresponding odour condition was: unscented solution, nonanal in solution, and PHE in solution were collected by a group of trained forager mates at a feeder offered with a 2.0-M sucrose solution during days 1, 2, and 9, respectively. A random presentation of test odours had been performed during the PER test (for details, see Materials and methods). Responses for PHE (gray), nonanal (white), 2-octanol (dark gray), and for more than one test odour (black). Number of tested bees above bars

Table 4.1: Functional groups, chemical structures, carbonchain lengths, and vapor pressures of the odours used in the experiment. The compounds are general floral odorants (after Knudsen et al. 1993).

Functional group	Compound	Structure	Carbon-chain length	Vapour Pressure (mm Hg; 25°C)
Aldehyde	Nonanal		9	0.37
Aldehyde	PHE		8	0.392
Secondary alcohol	2-Octanol		8	0.24

dependence between the variables (G = 28.87, df = 4, N = 155, $p < 0.001$), we tested for partial independence. Again, the results suggested a significant interaction between the odour presence in solution and PER frequencies (odour vs. hive and PER: G = 27.87, df = 3, $p < 0.001$; PER vs. hive and odour: G = 25.07, df = 3, $p < 0.001$) but no effect of hive (hive vs. odour and PER: G = 3.32, df = 3, p = 0.35). A subsequent G-test showed a significant effect of odour presence on PER frequencies (G-test: G = 24.55, df = 1, $p < 0.001$). These results show that both odours present in the solution had a significant effect on PER frequencies, but that there was no difference in PER frequencies for the odours between the two hives. We then tested if the PER frequencies for PHE and nonanal were different when they were in the solution and if there were colony effects, but found no significant mutual dependence (G = 1.06, df = 4, N = 124, p = 0.90).

4.5 Discussion

Hive bees that received scented solution from incoming foragers showed a significant increase in PER for the corresponding solution odour compared to those that received unscented solution. These differences were found when foragers collected a scented solution, irrespective of the identity of the odorant used. The lack of differences found between colonies also suggests that the order of food odour presentation did not affect the olfactory learning abilities for the different odour compounds diluted in the solution.

These results suggest that first-order receiver honeybees can learn via trophallaxis the nectar odour brought back by foragers inside the hive. The fact that this odour triggers the appetitive response (PER) of nectar receivers clearly shows an odour reward association. It has already been reported that olfactory learning occurs within honeybee colonies (von Frisch 1967; Wenner et al. 1969; Farina et al. 2005); however, there was, until now, no direct evidence that effective food receivers learn the association between reward and its odour. Our nectar receivers most likely received solution during one trophallactic contact (even though trophallaxes involving already marked receivers were observed in a few instances) which would be the equivalent of a single learning trial and tested 2 h later (corresponding to the temporal window of a medium-term memory, Menzel 1999). Although the PER values were significant for the odours received via trophallaxis, the responses found were lower than expected for single learning trials in laboratory studies (Menzel 1999; Gil & De Marco 2005). This could be explained by the effect of the dramatic change of context suffered by the hive bees after the capture (from the hive to the harnessing tubes in the laboratory; see Bouton & Moody 2004 for a review). A recent study showed that honeybees captured inside the hive presented increasing PERs with increasing foraging time at a scented food source (Grüter et al. 2006). Therefore, it is possible that the rather low response frequencies found in this study are due to the small amount of collected solution and/or the short time in which the hive bees were exposed to the scented food. Furthermore, the complex in-hive environment could lead to an unpredictable olfactory training, which is difficult to control experimentally.

The acquired olfactory information may be especially important at two different stages during adult life. First, because nectar receivers perform tasks inside the nest exclusively (Seeley 1995), the capability to learn the scent of the incoming nectar might affect decision-making of receivers once they return to the delivery area of the hive to unload new samples of fresh nectar. In this sense, it was recently reported that the probability of trophallactic interactions among incoming foragers and receivers is affected by olfactory experiences established during previous interactions inside the hive (Goyret & Farina 2005). Therefore, it is likely that the olfactory memories formed by receivers will affect the occurrence of subsequent trophallaxes with nectar foragers.

On the other hand, these memories may affect the behaviour of bees once they become foragers. Foraging follows nectar receiving and processing tasks (i.e., receivers are normally younger than foragers; Seeley 1995). Because long-term olfactory memories can be established inside the hive (Farina et al. 2005), the olfactory information acquired by receivers is likely to cause preferences for food sources once these bees initiated foraging tasks.

In summary, hive bees can learn the contingency between odour and reward during unloading contacts with nectar foragers. This fact will be crucial for the olfactory information management at the social level. In the shortterm, this capability will affect the operational cohesion of bees involved in foraging-related tasks and the propagation of olfactory information within the hive. In the long-term, it could affect putative preferences for resources once hive bees initiate foraging flights.

Acknowledgments

We are indebted to A. Arenas and H. Verna for their technical assistance and to one anonymous referee for the helpful comments on the earlier versions of the manuscript. This study was supported by funds from ANPCYT (01-12319) and the University of Buenos Aires (X 036) to WMF. CG is supported by the VDRB, commission for travel grants of the SANW, Dr. De Giacomi Stiftung, Basler Stiftung für biologische Forschung and Berner Hochschulstiftung. The present study complies with the current laws of the state country in which experiments were performed.

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5

Propagation of olfactory information within the honeybee hive

Grüter, C., Acosta, L. and Farina, W.M. 2006. *Behavioral Ecology and Sociobiology* 60: 707-715

5.1 Abstract

Transfer of information about food source characteristics within insect societies is essential to colony-foraging success. The food odour communicated within honeybee hives has been shown to be important for food source exploitation. When successful foragers return to the nest and transfer the collected nectar to hive mates through mouth-to-mouth contacts (trophallaxis), potential recruits receiving these samples learn the food odour by associative learning. The food then becomes rapidly distributed among colony members, which is mainly a consequence of the numerous trophallaxes between hive-mates of all ages during food processing. We tested whether the distribution of food among hive mates causes a propagation of olfactory information within the hive. Using the proboscis extension response paradigm, we show that large proportions of

bees of the age groups representing the main worker castes, 4 to 9-day-old bees (nurse-aged bees), 12 to 16-day-old bees (food processor-aged bees), and actual foragers (about 17+ day old bees) associatively learn the food odour in the course of processing food that has been collected by only a few foragers. Results further suggest that the information is shared more or less equally between bees of the three age groups. This shows that olfactory information about the flower species exploited by foragers is distributed within the entire colony and is acquired by bees of all age groups, which may influence many behaviours inside and outside the hive.

5.2 Introduction

Information acquisition and transfer among individuals in an insect society is crucial for adaptive colony-level responses to relevant ecological parameters in a variable world; the better informed a colony, the better it can adjust its behaviour to meet the demands of its environment (Seeley 1995; Dall et al. 2005). Olfactory information transferred within the hive can help experienced foragers exploit known food sources (von Frisch 1923; Johnson 1967; Reinhard et al. 2004) and new recruits locate food sources advertised by dances (von Frisch 1923,1967; Wenner et al. 1969). Recruits can perceive both the food odour clinging on the returning foragers bodies (von Frisch 1923,1967) and that contained in the nectar transferred during mouth-to-mouth contacts (trophallaxis) (von Frisch 1923,1967; Farina et al. 2005). The latter has been shown to be more important for recruitment (von Frisch 1923,1967). Successful incoming foragers unload their nectar through several trophallactic contacts of different durations to their hive mates (von Frisch 1923; Farina & Wainselboim 2001). Receivers are either food processors (main unloading contacts) (Seeley et al. 1996, Pírez & Farina 2004) or other foragers (short contacts) (De Marco & Farina 2003). During short contacts, small samples of food can be transferred, thereby allowing the receivers to taste and/or smell the nectar (Farina & Wainselboim 2001,2005). However, only recently, it has been shown that receiving foragers learn the odour/food association during trophallaxis within the hive by directly testing recruits in the laboratory using the proboscis extension response (PER) assay (Farina et al. 2005).

Trophallaxis plays a key role in the organization of food processing of many species of social insects as it links the various subtasks in a partitioned task, i.e., the sequential stages in the handling and processing of material (Wilson 1971; Ratnieks & Anderson 1999). Food processing involves honeybees belonging to the three main worker castes: nurses, food processors, and foragers (Nixon & Ribbands 1952; Seeley 1995), which are bees of three different age groups (Lindauer 1952; Seeley 1982). While foraging is normally performed by bees older than about 20 days, food receiving and processing (distribution and storage) is performed by middle-aged bees (between 11 20 days old) (Rösch 1925; Seeley 1982). Nurse bees are young bees, normally 3-11 days old, that are largely responsible for preparing nutrients from pollen and distribute the nutritionally valuable proteins produced by their hypopharyngeal glands, nectar, and honey to larvae and practically all hive mates (Crailsheim 1998; DeGrandi-Hoffman & Hagler 2000). The distribution of food within the hive has been shown to be rapid and extensive, i.e., a large proportion of all hive bees comes into contact with the nectar within a few hours (Nixon & Ribbands 1952; DeGrandi-Hoffman & Hagler 2000). The circulation of food within the hive via trophallaxis not only serves nutritional purposes, but is also considered to have informational importance, even though there exists little direct evidence (Crailsheim 1998). It is not yet known, for example, whether young hive bees, e.g., bees performing nurse tasks, also learn the odour of the incoming nectar. However, information cues present in the circulating nectar may provide the colony with global information, which means that the information leads to a response in most colony members and provides them with information about the current foraging opportunities (Pankiw et al. 2004).

In a recent attempt to test olfactory experiences made within the hive, it was shown that the PER assay offers a powerful method to test associations established between odour and sugar present in the liquid solution transferred amongst colony members (Farina et al. 2005). Bees reflexively extend their proboscis to drink solution when the antennae are touched with sucrose solution (unconditioned stimulus; US). In classical conditioning in the laboratory, an odour as a conditioned stimulus (CS) is pairedwith the US,which causes the odour itself to become capable of eliciting proboscis extension as a conditioned response (Kuwabara 1957; Bitterman et al. 1983). During trophallaxis, the solution transferred functions as unconditioned stimulus (US), while the food odour functions as CS (Gil & De Marco 2005).

We hypothesised that the distribution of food bearing a floral scent also distributes olfactory information among workers of different age groups performing different tasks within their caste. The nectar distribution amongst workers during the course of food processing could result in many olfactory conditioning events within the hive. To test our hypothesis, we fed foragers with scented solution and then captured four groups of bees belonging to three different age groups to test their proboscis extension response on the first presentation of the solution odour and a novel odour in the laboratory (proboscis extension response; PER). The four groups were (1) 4-9 days old bees, normally performing nurse tasks at this age, (2) 12-16 days old bees, normally performing food-processor tasks at this age, (3) a group of randomly captured foragers, and (4) foragers recruited to the scented food source.

In this study, the PER assay allowed us for the first time to (1) measure the propagation of information about a food source characteristic (floral scent) within the different age groups, (2) measure the speed of information acquisition day by day during our experimental periods, and (3) compare the proportions of bees that learned the food odour between the age groups.

5.3 Methods

5.3.1 Study site and animals

The experiment was performed at the end of the nectar flow season (March-April 2005) at the experimental field of the University of Buenos Aires. We used two two-frame observation hives (H1 and H2) containing a colony of about 3,800 European honeybees (*Apis mellifera ligustica*) each (Table 5.1). Colonies had a queen, brood, and reserves. A group of bees was trained to collect 1 M unscented sucrose solution at an artificial feeder located 160 m from the hive. Bees were marked individually and a number of about 5 to 10 foragers was maintained throughout the experiment by training new foragers whenever necessary.

5.3.2 Experimental procedure

At the beginning of the experiment, combs with preemerging brood from several hives housed in the apiary of the University of Buenos Aires were obtained and maintained in an incubator (temperature: 32°C, relative humidity 55%). On the day of emergence, bees were color-marked and introduced to H1 and H2. Honeybee colonies readily accept newly emerged bees (Breed et al. 2004). Using a different color every 2 days made it possible to determine the age of the marked bees in H1 and H2. The experiment consisted of two parts. The trained and marked foragers (that were trained to the 1 M unscented sucrose solution) of the experimental hive (EH) collected scented solution during 6 days (part 1) and 7 days (part 2) for 3 h each day at a feeder offering 2 M sucrose solution (about 70 μl/min flow rate). The solutions were scented with 50 μl pure odour per liter. The control hive (CH) had no access to solution offered at an artificial feeder. All the nectar that entered the CH was collected by foragers foraging at natural food sources. In part 1, H1 was used as experimental hive (EH) and H2 as control hive (CH). In part 2, the situation was reversed. Information about the odours used, population sizes of both colonies, number of collecting bees per day and amount of solution collected per day can be found in Table 5.1.

Table 5.1: Experimental conditions in part 1 and part 2 in the experimental hive (EH) and the control hive (CH).

	Part 1		Part 2	
Hive 1	EH		CH	
Hive 2	CH		EH	
Solution odour	Linalool		Phenylacetaldehyde	
No. of trained bees/day	7.2		7.9	
Solution fed/day	11.8 ml		12.6 ml	
	Start	*End*	*Start*	*End*
Population hive 1*	3'740	3'900	3'900	3'270
Population hive 2*	3'700	3'800	3'800	3'180

*Estimation following Seeley 1995

5.3.3 Bee capture

In the experiment, three groups of bees (foragers, 4-9 days old bees and 12-16 days old bees) were captured each from EH and CH. An additional group of bees (recruits) was captured from the EH (bees recruited to the experimental feeder are very likely to have interacted with our numbered foragers, von Frisch 1923,1967). During part 1, bees were captured on days 0, 2, 4, and 6 from the EH and on days 3 and 5 from the CH. During part 2, bees were captured on days 0, 1, 3, 5, and 7 in the EH and on days 2, 4, and 6 in the CH. This sampling schedule was used to reduce time effects on learning performance that could cause differences in learning performance between EH bees and CH bees. The interval between part 1 and part 2 was 6 days. During the 3 h when the numbered bees collected scented solution, recruits arriving at the feeder

(EH only) were captured with plastic tubes on the feeding platform before they touched the scented solution, otherwise they were killed with alcohol. Captured bees were fed a drop of 2 M unscented sucrose solution.

About 60 min after the 3-h sampling period, we started to capture bees of the following three groups.

- Foragers. A group of random foragers was captured with plastic tubes while feeding from a small plate (about 8 cm in diameter), filled with 2 M unscented sucrose solution at a distance of some centimeter from the entrance. Foragers leaving the hive and passing the plate will normally accept the offered solution at this time of the year. If they had color marks, their age was determined (about 10-20% of the captured bees had marks). There was no difference in forager age between the two colonies (ANOVA: $F_{1,69} = 0.49$, P = 0.826, mean: 23.9 ± 5.97 and 24.2 ± 5.03 days old, H1 and H2, respectively).

- Four to nine days old bees (nurse aged bees) and 12-16 days old bees (food processor aged bees). Bees were identified by their color mark and captured from the hive. The windows of the observation hive were movable acrylic walls (movable horizontally). In the middle of these walls were rectangular holes (the height of the holes almost equaled the height of the walls). The rectangles contained acrylic slides that were movable vertically (up and down). The acrylic slides contained an aperture (3.5 cm width) that allowed to capture the bees with a plastic tube. This device allowed us to scan the whole comb area.

None of the captured bees had contact with the scented solution at the feeding station and associations could therefore only have been established within the hive. Captured bees of all groups were harnessed in plastic tubes so that they could move freely their mouthparts and antennae (Bitterman et al. 1983). They were fed 1.8 M unscented sucrose solution ad libitum and kept overnight in an incubator (25°C, 55% relative humidity, darkness). This guaranteed that bees had similar motivation levels when they were tested the next morning.

5.3.4 PER testing

The morning after harnessing, we tested the bees once for their responses to the solution odour (LIO in part 1, PHE in part 2) and once for their spontaneous responses to a novel odour, which was 2-octanol (2-OCT) in both parts. The interval between the two presentations lasted about 15 min. A device that delivered a continuous air flow was used for odorant application. We used 4 μl of pure odour impregnated on a filter paper (about 30 x 3 mm), which was placed inside a syringe. When odour was presented, the air flow produced by our setup passed through this syringe. Only bees that showed the unconditioned response (the reflexive extension of the proboscis after applying a 1.8 M sucrose solution to the antennae) and that did not respond to the mechanical air flow stimulus were used (this was tested when the harnessed bees were put in the continuous air flow before presenting the odours. Less than 1% of all tested bees did respond to the air flow). Test trials lasted for 46 s and consisted of 20 s of air flow, 6 s of odour (CS), and 20 s of air flow. The three odours are natural components of flower odours (Knudsen et al. 1993) and were obtained from Sigma-Aldrich, Steinheim, Germany.

5.3.5 Statistical analysis

G-tests were used to compare PER frequencies between groups. We used the Williams correction for adjustment of G-values, except in cases were we had 0 responses. In these cases, the more conservative Yates correction was used (see Sokal & Rohlf 1981 for discussion of the two corrections). For statistical analysis, we pooled the data of control hive (CH) bees for all four groups in part 1 and separately in part 2. Forager age comparison and the relation between percentage of PER and amount of scented solution (general linear model, GLM, with odour as a fixed factor and amount of scented solution as a covariate to explain the percentage of PER) that entered the colony, were done using parametric statistics as assumptions were met (Grafen & Hails 2002). The descriptive statistics are given in mean ± SE.

5.4 Results

Proboscis extension response frequencies for the solution odour (bees responding only to the solution odour) increased over experimental time and reached values between 30% for 4-9 days old bees (nurse-aged bees) and 56% for recruits on the last day of part 1 (day 6 using linalool; Fig. 5.1a, Table 5.1), and between 30% for foragers and 43% for 12-16 days old bees (processor-aged bees) on the last day of part 2 (day 7 using phenylacetaldehyde; Fig. 5.2a, Table 5.1). PER frequencies of control bees for the solution odour were low during both parts (Figs. 5.1b and 5.2b). Neither in part 1 (comparison between day 3 and 5: 4-9 days old bees: G-test, $G_{adj} < 0.001$, df = 1, p = 0.99; 12-16 days old bees:

G-test, $G_{adj} = 0.027$, df = 1, p = 0.87; foragers: G-test, $G_{adj} = 1.61$, df = 1, p = 0.20; Fig. 5.1b) nor in part 2 (comparison between days 2, 4, and 6: 49 days old bees: G-test, $G_{adj} = 0.34$, df = 2, p = 0.84; 1216 days old bees: G-test, $G_{adj} = 0.58$, df = 2, p = 0.75; foragers: G-test, $G_{adj} = 1.15$, df = 2, P = 0.56; Fig. 5.2b), did we find significant differences in PER frequencies for the solution odour (bees responding to the solution odour only).

Table 5.2: Comparison of PER frequencies between experimental hive (EH) bees on day 6 (part 1) or day 7 (part 2) and control hive (CH) bees.

		N	*G-value*	*P*
Part 1				
4-9 days old	EH vs. CH	10/30	2.81	0.09
12-16 days old	EH vs. CH	17/30	9.41*	< 0.005
Foragers	EH vs. CH	14/41	13.75*	< 0.001
Part 2				
4-9 days old	EH vs. CH	20/38	13.04	< 0.001
12-16 days old	EH vs. CH	14/55	11.90	< 0.001
Foragers	EH vs. CH	10/53	4.78	< 0.05

N refers to the number of bees in both hives. *G-values adjusted using the Yates correction.

There is also no reason why the response frequencies for the solution odour should change during the course of the experiment in CH bees, as these bees never had access to the offered scented solution. For further statistical analysis, we pooled the data obtained on days 3 and 5 in part 1 and the data obtained on days 2, 4, and 6 in part 2 for the three groups of bees (4-9 days old bees, 12-16 days old bees, and foragers) separately. The differences between PER frequencies of experimental bees at the end of the experimental periods of part 1 and 2 and control bees were wide (between 23.3% for 4-9 day old bees (nurse-aged bees) and 56% for recruits (compared to foragers of the control group) in part 1 and between 26.2% for foragers and 39.3% for 12-16 days old bees (food processor-aged bees) in part 2; Figs. 5.1a and 5.2a). Therefore, we compared the PER frequencies of bees captured in the experimental hive on day 6 (i.e. at

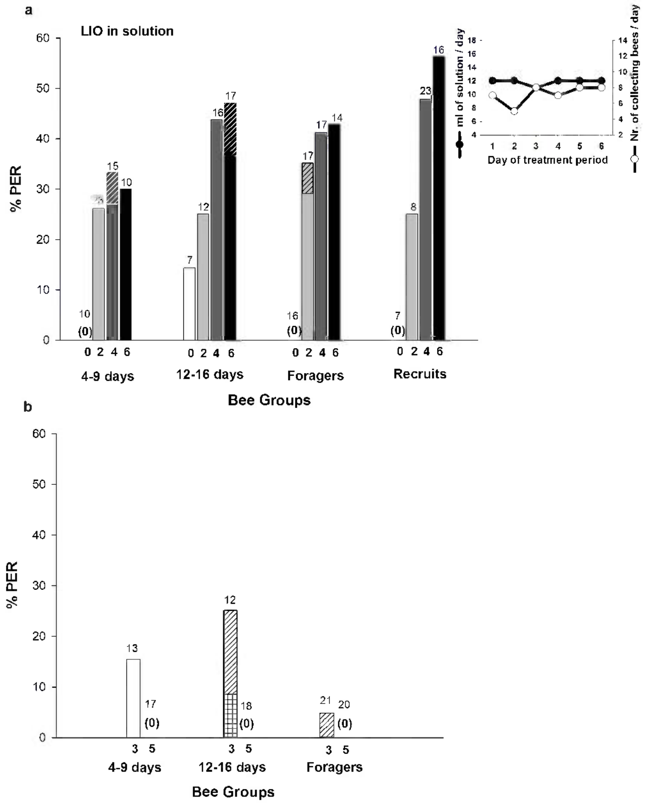

Figure 5.1: Proboscis extension response (PER) frequencies for 4-9 days old bees, 12-16 days old bees, foragers, and recruits (EH) (the percentage of bees that extended the proboscis on the first presentation of the odour in the laboratory) for the solution odour (filled bars), the novel odour (crossed bars), or both odours (hatched bars). a) EH: the PER frequencies measured on days 0, 2, 4, and 6 after starting to feed with scented solution (hive 1, H1, was used as experimental hive, EH). Linalool, LIO, was used as solution odour. b) CH: the PER frequencies measured on days 3 and 5 (H2 was used as CH). Linalool, LIO, was used as solution odour. Number of tested bees above bars. Insert figure shows the quantity of scented sucrose solution, in milliliter (emptied circles), that entered the EH every day and the number of trained foragers (filled circles).

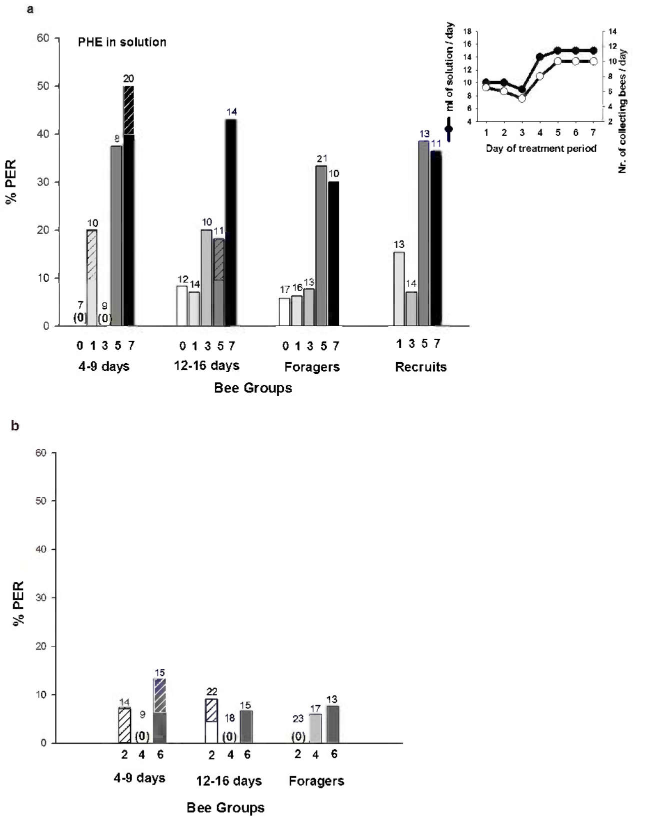

Figure 5.2: PER frequencies for 4-9 days old bees, 12-16 days old bees, foragers, and recruits (EH). a) EH: the PER frequencies measured on days 0, 1, 3, 5, and 7 after starting to feed with scented solution (H2 was used as EH). Phenylacetaldehyde, PHE, was used as solution odour. b) CH: the PER frequencies measured on days 2, 4, and 6 after starting to feed with scented solution (H1 was used as CH).

Table 5.3: Comparison of PER frequencies between the different bee groups (4-9 days old bees, 12-16 days old bees, foragers, and recruits).

	df	*N*	*G-value*	*P*
Part 1				
Day 0	3	7/16/7/10	3.61	0.31
Day 2	3	8/17/12/23	0.1	0.99
Day 4	3	23/17/16/15	1.76	0.62
Day 6	3	10/17/14/16	2.15	0.54
Part 2				
Day 0	2*	12/17/7	0.14*	0.93
Day 1	3	13/16/14/10	0.66	0.88
Day 3	3	14/13/10/9	2.91	0.41
Day 5	3	13/21/11/8	3.35	0.35
Day 7	3	20/14/10/11	0.44	0.93

N refers to the number of bees per group. *No data about recruits collected.

the end of conditioning) in part 1 with the PER frequencies of bees captured from the CH (days 3 and 5) in part 1 and on day 7 in part 2 with the PER frequencies of bees captured from the CH in part 2 (days 2, 4, and 6). We found significant differences in all cases, except for 4-9 days old bees (nurse-aged bees) during part 1 (Figs. 5.1a and 5.2a; Table 5.2). Unfortunately, the number of bees belonging to this category was very low, making the detection of significant differences difficult. (Table 5.2). Neither in part 1, nor in part 2, did we find significant differences in PER frequencies between the four groups (Table 5.3). To visualize the increase in PER frequencies in the entire colony and its relation to the amount of scented solution entering the hives, we summed the data of the different bee groups in Fig. 5.3a,b. There is a significant positive relationship between the amount of scented solution that entered the hive and the percentage of PER, but no difference between the odours (GLM: amount solution x percentage of PER: $F_{1,9} = 42.7$, p = 0.001; odour x percentage of PER: $F_{1,9} = 5.36$, p = 0.06).

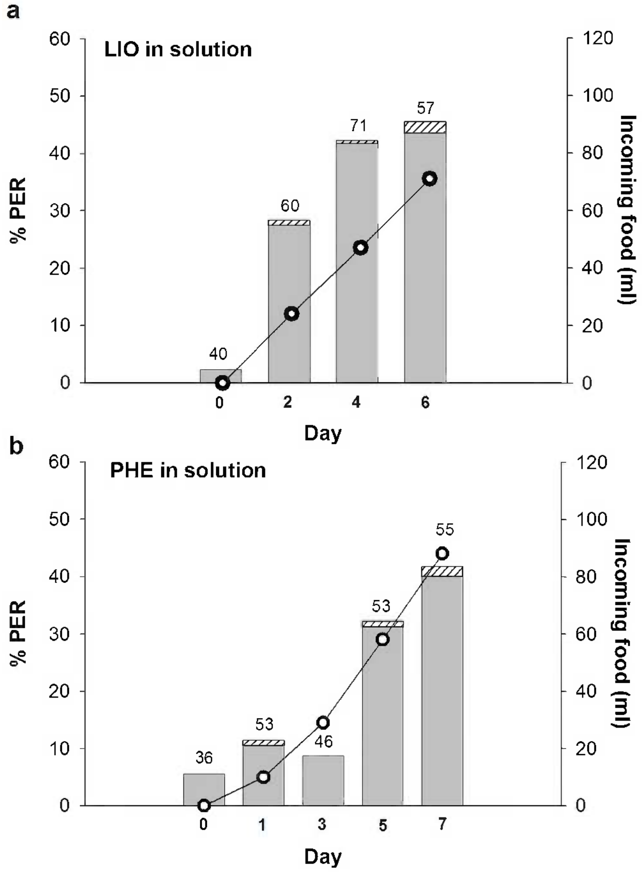

Figure 5.3: PER frequencies for bees of all four bee groups at different days (bars) and the amount of scented solution (ml) that entered the colonies until the given day (curve). a) the PER frequencies measured on days 0, 2, 4, and 6 during part 1; Linalool, LIO, was used as solution odour. b) the PER frequencies measured on days 0, 1, 3, 5, and 7 during part 2; phenylacetaldehyde, PHE, was used as solution odour.

5.5 Discussion

Within a honeybee hive, transfer of information between bees allows the colony to adaptively respond to its changing environment. Previous studies suggested that the food odour transferred within the hive is an important information cue for foraging decisions of honeybees (von Frisch 1923,1967; Wenner et al. 1969). However, extensive olfactory information propagation within honeybee colonies in the course of the processing food has never been reported. Our experiment allowed measuring the patterns of information propagation within the colony over time and comparing information acquisition between the different worker castes. The high PER frequencies for the solution odour, but not for the novel odour, observed in all tested groups and the low PER frequencies for the solution odour for CH bees demonstrates that propagation of olfactory information within the hive occurs. Results, therefore, clearly show that also very young hive bees (4-9 days old) having the age of nurse bees learn the odour of the incoming solution. The pattern of information propagation was different in the two colonies. While in part 1, PER frequencies on day 2 were above 25% for all groups, PER frequencies on day 3 in part 2 remained below 20%. The propagation pattern will be modified by many variables, e.g., hive population, the number of employed foragers, and the amount of solution entering the hive. A decrease of about 20% in colony size during part 2, combined with the low amount of solution that entered the EH in part 2 until day 3 and the increased nectar influx from day 4 on may explain to some extent the different pattern found in the second part and it shows a correlation between the speed of information propagation and the amount of solution entering the hive, and consequently the number of collecting foragers. The social structure of a colony (i.e. the age and the number of bees performing tasks) is also likely to influence the pattern of propagation of olfactory information. However, we found no difference in forager age between the two colonies and have no evidence of a difference in the social structure between the two colonies. Variables such as food odour characteristics (Smith 1991), previous olfactory experience of bees (Sandoz et al. 2000), sugar response thresholds (Scheiner et al. 2004), time of year (Ray & Ferneyhough 1997), and genotype (Bhagavan et al. 1994) are related to learning performance of honeybees and are therefore likely to influence the pattern of information propagation in yet unknown ways.

It is interesting that our results do not show consistent differences in PER frequencies between the four groups of bees. In neither part 1 nor part 2 did we find significant differences in PER frequencies between the different age groups. It seems that the information is shared more or less equally between bees of the three age groups. Differences in olfactory learning performances between bees of different age are also likely to influence the propagation of information within the hive. Earlier studies suggest that the acquisition process is similar in bees older than 8 days (Bhagavan et al. 1994; Laloi et al. 2001; Ichikawa & Sasaki 2003). For bees younger than 8 days, previous studies provided contradictory results (Ray & Ferneyhough 1997, Laloi et al. 2001; Ichikawa & Sasaki 2003). While some studies showed agedependent learning abilities (Ray & Ferneyhough 1997; Ichikawa & Sasaki 2003), others did not (Bhagavan et al. 1994; Laloi et al. 2001).

Olfactory associative learning relies on the discrete pairing of odour and reward and cannot be accomplished by passive exposure to the scent in the hive, which has been shown to have inhibitory effects on associative learning (Menzel et al. 1993; Gerber et al. 1996; Sandoz et al. 2000). It is likely that the information propagation relied mostly on trophallaxis, rather than on individual feeding from the honey cells because the season and the small amount of scented food gathered by the foragers prevented an accumulation of scented solution within the colony. Food offerings via trophallaxis is a common behaviour of returning foragers (von Frisch 1923, 1967), and it is often seen in nectar processors after receiving the incoming food (Pírez & Farina 2004). However, stored honey may be an important source of olfactory information, functioning like an odour library of previous and present food sources (Free 1969).

Solution gathered by only 5-10 bees (about 1% of all foragers of our colonies, estimation after Seeley 1995) is likely to become mixed with other nectar samples during the course of food processing, which would cause different US and CS intensities experienced by bees of the different age groups involved at different stages of food processing. This affects the strength of the association made between odour and sucrose solution (Pelz et al. 1997; Gil & De Marco 2005) and therefore makes a simple relationship between number of trophallactic contacts and PER frequencies found in the three age groups unlikely. More studies are needed to unravel the food transmission pathways and functional identity of bees involved at the different stages of food processing.

It has recently been shown that the rate at which a receiver unloads nectar to another bee is positively correlated with the rate at which she received it from a food donor (Goyret & Farina 2005a). Therefore it could be that also quantitative aspects of trophallaxis are propagated during food distribution among honeybees. Propagation of chemosensory (olfactory and gustatory) information during the course of food processing is a highly economic information transfer that could influence many within-hive behaviours of young bees. It has been shown, for example, that experience with olfactory cues present in solution during trophallaxis would lead to the observed nonrandom occurrence of trophallaxis between foragers and receivers (Goyret & Farina 2005b). Transfer of information about floral scents also provides bees not involved in foraging with information about the food sources currently and recently exploited. It establishes associations in preforagers between nectar and floral odours they are likely to encounter some days later when they become foragers. In other words, bees leaving a hive for a foraging trip (independently of whether the bee is an experienced forager or a novice forager leaving for the first foraging trip) acquired information about foraging opportunities many times in the past during social interactions. This challenges the traditional scout-recruit concept, an important concept in honeybee foraging (Rösch 1925; Lindauer 1952; Seeley 1995; Anderson 2001; Biesmeijer & de Vries 2001; Dechaume-Moncharmont et al. 2005) in which foragers are divided into two groups: scouts that set out independently and recruits that use information provided by returning foragers (see also Biesmeijer & de Vries 2001 for a criticism).

Even if foragers leave the hive without following dances that provide location information, they possess memorized information about food source characteristics acquired inside the nest. Therefore, the question seems not to be whether a bee should use information regarding foraging opportunities provided by other foragers (Dechaume-Moncharmont et al. 2005), but what kind of information she should use at any given moment. Foraging honeybees are capable of using more than one sensory cue to enhance their foraging efficiency in the field. While learned odours may help foragers to identify rewarding flowers when they come near to the food source, visual cues that are not learned inside the hive may attract their attention when they are still some distance away from the source (Giurfa et al. 1994, Galizia et al. 2004). Then, the notion of scout might be still valid when referred, for instance, to visual guidance. Adaptive decisions will then depend on the costs and benefits of using the different forms of information that are available (Dall et al. 2005).

Even if treated bees did not show a PER towards the solution odour, they may have experienced the combination of odour and solution previously (Menzel 1999) and as a consequence, learn the odour faster than bees without this experience (Farina et al. 2005). Olfactory learning by means of trophallaxis leads to long-term memory (Farina et al. 2005; Gil & De Marco 2005) and the behaviour of bees and therefore the colony-foraging pattern could be affected for several days.

Acknowledgments

We are deeply indebted to A. Arenas for much thoughtful advice and support during the experiment and I. Hamilton and two anonymous referees for helpful comments on earlier versions of the manuscript. We also thank A. Arenas, and H. Verna for technical assistance. This study was supported by funds from ANPCYT (01-12319), CONICET (02049) and University of Buenos Aires (X 036) to WMF. CG is supported by the VDRB, Commission for travelgrants of the SANW, Dr. De Giacomi Stiftung, Basler Stiftung für biologische Forschung. When we carried out our experiments, we adhered to the legal requirements of the country.

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6

Does food quality affect the propagation of floral scent information in honeybee colonies?

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6.1 Abstract

In many social insect species, foraging success depends crucially on the colonies' ability to discover new profitable food sources. Yet, little is known about whether mechanisms exist, which allow insect colonies to discover new high quality food sources more efficiently than low quality food sources. In honeybees (*Apis mellifera*), as in many other social insect species, floral food odours are important information cues in foraging. By means of associative learning, food sharing inside the colonies causes a propagation of this olfactory information among hive bees. Here we tested, whether olfactory information present in high quality food is propagated more extensively than olfactory information present in low quality food. Using the proboscis extension response (PER) test,

we found a positive effect of food quality (sugar concentration) on the proportion of colony bees that respond to the food odour after feeding the hives with relatively small amounts of scented food: 36.6% of all tested bees after feeding high quality food vs. 19.8% after feeding low quality food. This result may be explained by the positive effects of stronger unconditioned stimulus (US, sugar solution) on learning performance in associative learning and the amplification of olfactory information transfer by means of dancing. Since olfactory learning helps foragers to find a food source of a particular scent, we propose that quality dependent information propagation helps colonies to discover food patches of plant species that offer highly concentrated nectar more frequently than food patches of plant species offering nectar with low sugar concentrations.

6.2 Introduction

Colonies of honeybees (*Apis mellifera*), as colonies of other social insect species, are able to selectively exploit more profitable food sources while avoiding food sources of low profitability (Seeley 1995). In order to do so, foragers can adjust their recruitment behaviour according to the profitability of the exploited food source (von Frisch 1967; Seeley 1995). However, insect colonies not only have to exploit known food sources efficiently, they also have to constantly discover new profitable food sources. Little is known about whether mechanisms exist that allow social insect colonies to discover high quality food sources with higher probability than low quality food sources. Such a mechanism could, for example, cause colonies to show general preferences for particular plant species - e.g. based on plant species-specific odours (Dobson 1994) - which are highly rewarding in the present or have been so in the past.

Food odours are important information cues for foraging honeybees (von Frisch 1967). Olfactory learning inside the hive leads to a strong preference for the learned food odour in the field (von Frisch 1923,1967; Wenner & Wells 1990; Arenas et al. 2007) and it helps to discover new food sources of the same scent (von Frisch 1923,1967; Wenner & Wells 1990). Potential recruits can associatively learn the food scent while interacting with successful foragers. Foragers distribute their load to various bees via mouth-to-mouth feeding (trophallaxis) (von Frisch 1923) and bees receiving food can learn to associate the nectar, which functions as an unconditioned stimulus (US; a stimulus that naturally and automatically triggers a response) with the food odour, which functions as conditioned stimulus (CS; a previously neutral stimulus) (Farina et al. 2005,2007; Gil & De Marco 2005; Grüter et al. 2006). This kind of learning can be tested with the proboscis extension response (PER) method (Giurfa 2007). If the bee has learnt the association reward-scent, then the presentation of the scent to the antennae causes the bee to extend its proboscis (conditioned response; CR). Subsequently, the food becomes rapidly distributed amongst hive bees of all castes (Nixon & Ribbands 1952; Grüter & Farina 2007), which causes a propagation of the food scent information within the entire colony (Grüter et al. 2006).

In associative learning, the strength of the reward usually affects the learning performance (Rescorla & Wagner 1972). Correspondingly, stronger US (higher sucrose concentrations) have been shown to positively affect learning in bees (Bitterman et al. 1983; Gil & De Marco 2005). Sugar concentration is one key determinant of nectar quality for honeybees (von Frisch 1967; Seeley 1995). Therefore, it seemed straightforward to hypothesise that nectar quality in terms of sucrose concentration per se affects the propagation of olfactory information within the colony. Given the differences between plant species in average sugar concentrations of the nectar they offer (Butler 1945), a higher proportion of bees in a colony would acquire a memory for plant species that offer high quality food. If subsequently more new food source patches with food odour A are approached than patches with odour B because more foragers learnt the odour A, then food quality dependent learning would provide a simple mechanism to establish higher food patch discovery rates for certain plant species. To test our hypothesis, we fed a few foragers of different colonies equal amounts (70 ml in total) of either scented high quality food (2 M sucrose solution) or low quality food (0.5 M sucrose solution) and measured information propagation with the PER method.

6.3 Methods

Six colonies of European honeybees (*Apis mellifera ligustica*) were housed in two-frame observation hives (H1-H4) at the experimental field of the University of Buenos Aires. Four hives (H1-H4) were used as experimental hives; two additional full sized hives (MH1 and MH2) were used to monitor PER frequencies of foragers in non-treated hives at particular moments. H1 and H2 started with about 3'200 bees per colony; H3 and H4 started with about 3'900 bees per colony. Colonies had a queen, brood and reserves.

6.3.1 Experimental procedure

Before data collection started, combs with pre-emerging brood from various hives housed in the apiary of the University of Buenos Aires were obtained and maintained in an incubator (temperature: 36° C, relative humidity 55%). On the day of emergence, about 100 bees (per hive) were color-marked and introduced to H1-H4. By using a different color every two days, we could determine the age of the marked bees in H1-H4 during the entire experiment.

To compare the effect of the two different food qualities, we fed colonies either with 2 M or 0.5 M sucrose solution containing the same scent for 5 days. In 2005 (part 1), H1 was treated with high quality food (H treatment), H2 with low quality food (L treatment; started one day later). In 2006 (part 2a,b), H3 first received the H treatment and H4 the L treatment (part 2a; the L treatment started four days after the H treatment); subsequently the treatments were reversed for both colonies (part 2b; the H treatment started five days after the L treatment). In 2005, part 1 was performed at the very end of the season. Environmental conditions did not allow the reversal of treatment conditions for H1 and H2. In part 1 and 2b, phenylacetaldehyde (PHE) was used as solution odour; in part 2a we used Linalool (LIO) as solution odour. We tried to collect the data of two colonies (one receiving the high quality food, the other receiving the low quality food) as synchronously as possible to reduce time effects. Hence, while we trained a group of individually marked foragers of one colony to an artificial feeder A, a group of individually marked foragers of a second colony was trained to a feeder B. The two feeders were placed about 80 m from the hives. The angle between the directions from the hives to feeder A and feeder B was about 90°. During experimental periods, we maintained a number of 8 foragers collecting a total amount of 14 ml scented sucrose solution per day for 5 days (70 ml). A previous study showed that this amount causes a substantial proportion of all bees to learn the food odour (Grüter et al. 2006). New recruits arriving at the feeder were captured with plastic tubes before they could touch the solution; otherwise they were killed with alcohol. Captured recruits were released at the end of the day (Overall: 115 ± 52.1 recruits/per day were captured when offering 2 M solution; 35 ± 32.4 recruits were captured when offering 0.5 M solution, means ± SD). The solutions were scented with 50-μl pure odour per liter of sugar solution (i.e. the lowest odour concentration which still leads to efficient learning; Gil & De Marco 2005).

We were interested in the PER of bees belonging to three different bee groups: (i) foragers, (ii) 4-9 days old bees and (iii) 12-16 days old bees, all belonging to H1-4. We measured the PER of these bees at three time points:

Before offering scented solution for the first time (day 0), on day two of the treatment period (day 2) and on the last day of the treatment period (day 5)(Fig.7.1). At the end of each part, the PER of foragers belonging to MH1 (part 1) and MH2 (parts 2 and 2b) was tested in order to gain additional information about natural PER levels for the odours used during our experiment. However, we know from previous experiments performed at the same site in the same season, that PER frequencies for the odours used are normally not higher than 10% (Farina et al. 2005, Grüter et al. 2006).

6.3.2 Bee capturing

Bees of the following 3 groups were captured between 15:00-18:00 hrs (after offering scented food).

- Foragers: A group of random foragers was captured with plastic tubes while feeding from a small plate (about 8 cm diameter), filled with 2 M unscented sucrose solution at a distance of some cm from the entrance.
- 4-9 days old bees (nurse-aged bees); and 12-16 days old bees (food processor-aged bees) (Seeley 1982): bees were identified by their color mark and captured from the hive (capturing method as in Farina et al. 2007).

Our capture method guarantees that captured bees of all groups could only have experienced the scented solution within the hive. Captured bees of all groups were harnessed in plastic tubes so that they could move freely their mouthparts and antennae (Bitterman et al. 1983). They were fed 1.8 M unscented sucrose solution ad libitum and kept overnight in an incubator (25° C, 55% relative humidity, darkness).

6.3.3 PER testing

The morning following harnessing, we tested the bees once for their responses to the solution odour and once for their spontaneous responses to a novel odour (i.e. an odour which was never used to scent solution throughout the experiment), which was 2-Octanol (2-OCT). In part 2, bees were tested with both LIO and PHE, which allowed measuring acquisition of PHE and the retention of LIO simultaneously. Odour presentations alternated between bees, so that equal numbers of bees were tested in all possible odour sequences to avoid order effects. The interval between the presentations lasted about 15 min. A device that delivered a continuous airflow was used for odorant application. We used 4 μl of pure odour impregnated on a filter paper (about 30 x 3 mm), which was placed inside a syringe. When odour was presented, the airflow produced by our set-up passed through this syringe. Only bees that showed the unconditioned response (the reflexive extension of the proboscis after applying a 1.8 M sucrose solution to the antennae) and that did not respond to the mechanical airflow stimulus were used (less than 1% of all tested bees did respond to the air flow). Test trials lasted for 46 s and consisted of 20 s of airflow, 6 s of odour (CS) and 20 s of airflow. All used odours are natural component of flower odours (Knudsen et al. 1993). Odours were obtained from Sigma-Aldrich, Steinheim, Germany.

6.3.4 Statistical analyses

Log-linear models were used to analyse the PER frequencies with SPSS 12.0. The Log-linear analysis procedure allows testing several hypotheses in one model (Zar 1999). SPSS uses the Logit Loglinear Analysis procedure to analyse interactions among categorical variables by analyzing cell counts of the cross-tabulation table formed by the cross-classification of dependent variable and the predictor variables. We used PER as dependent variable and treatment and age group as predictor variables. In part 2, the effect of odour was included as an additional factor. SPSS performed goodness-of-fit tests to test whether our unsaturated models adequately fitted the data. All goodness-of-fit tests had values of $p > 0.1$.

6.4 Results

Our main hypothesis predicted that more bees of a colony learn the food odour if the sugar solution collected has a higher sugar concentration compared to low concentration food. Therefore, we compared the number of bees showing the PER towards the solution odour (either PHE or LIO) in colonies fed with high-quality food and colonies fed with low-quality food.

Additionally, we measured the PER towards a novel odour (2-Oct). While PER percentages for the solution odours increased from day 0 to day 5 to up to 50% (Fig. 6.1), PER percentages for 2-Oct remained low. During the entire experiment, only 5.6% of all tested bees responded towards 2-Oct only ($N = 1121$). The highest value was 13.9% and was recorded on day 2 of the L-treatment in H2. Therefore, responses towards 2-Oct were not further analysed. In part 1, 4.7% of all bees responded towards both odours ($N = 327$). 4% of all bees responded towards both solution odours and 1.8% responded towards

Table 6.1: Effect of treatment, age group and odour on PER frequencies. Age groups are 4-9 days old bees (N), 12-16 days old bees (P) and foragers (F).

		N	*Chi-value*	*P*
Part 1, day 0				
PER vs. Treatment		90	0.00	0.99
PER vs. Age group	F vs. N	35/35	0.00	0.99
	P vs. N	20/35	0.00	0.99
	F vs. P	35/20	0.00	0.99
Part 2, day 0				
PER vs. Treatment		212	0.19	0.66
PER vs. Odour		212	4.83	*0.028*
PER vs. Age group	F vs. N	91/66	1.67	0.2
	P vs. N	55/66	0.24	0.64
	F vs. P	91/55	2.95	0.086
Part 1, day 2				
PER vs. Treatment		101	0.07	0.79
PER vs. Age group	F vs. N	46/34	1.74	0.19
	P vs. N	21/34	0.36	0.55
	F vs. P	46/21	0.29	0.57
Part 2, day 2				
PER vs. Treatment		246	3.37	0.07
PER vs. Odour		246	9.31	*0.002*
PER vs. Age group	F vs. N	93/86	3.46	0.06
	P vs. N	67/86	0.69	0.41
	F vs. P	93/67	0.77	0.38
Part 1, day 5				
PER vs. Treatment		136	7.53	*0.006*
PER vs. Age group	F vs. N	45/54	4.47	*0.034*
	P vs. N	37/54	0.12	0.73
	F vs. P	45/37	1.99	0.16
Part 2, day 5				
PER vs. Treatment		336	4.38	*0.036*
PER vs. Odour		336	18.1	*<0.001*
PER vs. Age group	F vs. N	131/111	4.45	*0.035*
	P vs. N	94/111	3.42	0.064
	F vs. P	131/94	13.32	*<0.001*

all three odours (N = 794). In MH1 and MH2, 7.3% of the captured foragers responded only to PHE (N = 55), 7.3% to 2-Oct (N = 55) and 5.55% to LIO only (N = 36; LIO was not presented during part 1).

For day 0, we found no significant relationship between the predictor variable treatment (sugar concentration) and the dependent variable PER (Table 6.1; Fig. 6.1a). The same was true for day 2 (Fig. 6.1b). On day 5, at the end of the treatment period, we found a significant positive relationship between the sugar concentration and the PER frequency in both part 1 and 2 (Table 6.1, Fig. 6.1c). In part 2, there were more PERs towards the solution odour in part 2a when LIO was used compared to part 2b when PHE was used. This was true for all test days (Table 6.1). Furthermore, the model estimated higher PER percentages for foragers than for nurse-aged bees on day 5 (Table 6.1).

6.5 Discussion

We found that the food odour of high quality food was learned and retrieved by more bees of a colony than the food odour of a low quality food source in both parts of the experiment (Fig. 6.1c). This suggests that the food quality directly affected the social propagation of olfactory information within the colony. While there were no differences between colonies before the start of the treatment and after two days of feeding colonies with scented food, on day 5 more bees showed the PER for the solution odour in colonies treated with high quality food (36.6%, N = 224) compared to colonies fed with low quality food (19.8%, N = 248). This treatment effect may be explained by the commonly found positive relationship between US strength and learning performance in associative learning (Rescorla & Wagner 1972; Bitterman et al. 1983; Gil & De Marco 2005). However, food quality could also positively affect the propagation of olfactory information by increasing the probability of dancing (von Frisch 1967, Seeley 1995) and the number of trophallactic-offering contacts (De Marco & Farina 2001). Even though we did not quantify these in-hive behaviours, it is likely that foragers performed more dances and more trophallaxes when collecting the high quality food in our experiment since we captured many more recruits when offering high quality food. Dancing bees attract both other foragers and food processor bees. As a consequence, foragers that dance before unloading their food find more unloading partners (Farina 2000). In this way, the dance facilitates the distribution of olfactory information (see also Díaz et al. 2007). However, a look at Figure 7.1 shows that nurse-aged bees, which normally have little contact with dancing bees (Seeley 1995) but often receive food from food processors (Grüter

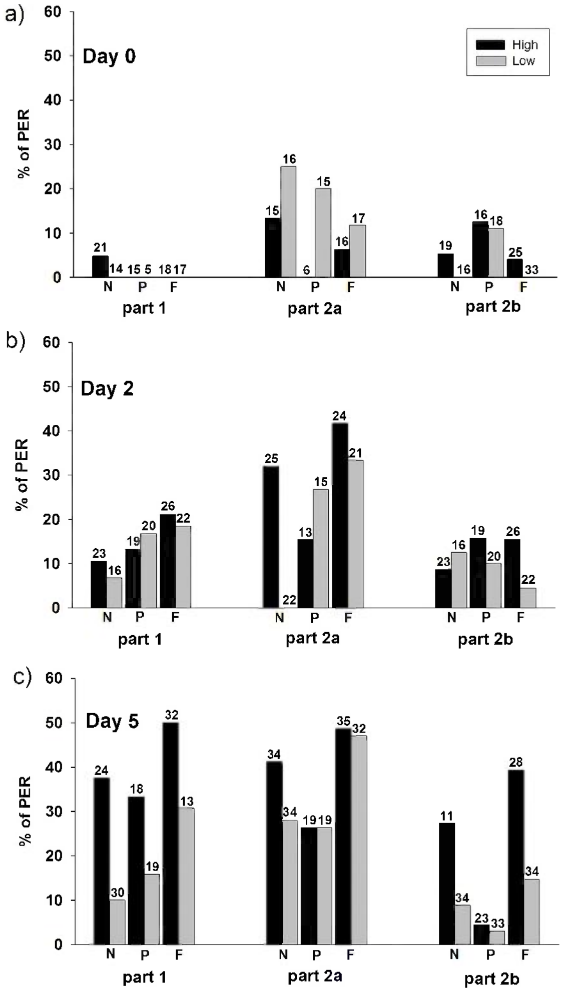

Figure 6.1: Proboscis extension response (PER) percentages for the solution odour for bees of 4-9 days (nurse ages; N), 12-16 days (food-processor ages; P) and foragers (F) at day 0, 2 and 5 for colonies treated with high quality food (black bars) and low quality food (light gray bars) during part 1 and 2. Number of tested bees above each bar.

& Farina 2007) responded similarly to the treatment. It seems, therefore, that the positive effect of US strength on associative learning is the primary reason for the observed treatment effect.

In part 1, treatment conditions were not reversed to control for general colony differences in learning performance between H1 and H2. However, the higher PER percentages in H1 are unlikely to be caused by learning differences between colonies, because learning performance of bees of H1 and H2 were not different (unpublished data).

Olfactory learning inside the hive leads to a strong preference for the learned food odour in the field (von Frisch 1923,1943,1967; Wenner & Wells 1990; Arenas et al. 2007) and it helps to discover new food sources of the same scent (von Frisch 1923,1943; Wenner & Wells 1990). Since plant species differ considerably in both sugar concentration of nectars (Butler 1945) and odour composition (Dobson 1994), we propose that quality dependent information propagation leads to an increased number of discoveries of high quality food patches of particular plant species compared to low quality food patches of other plant species. In part 2, we found a strong odour effect on the percentage of PER in both colonies. This could be the consequence of differences in the salience of the two odours used (Smith 1991) or seasonal effects on learning (Ray & Ferneyhough 1997) or both.

While the waggle dance is known only in honeybees, learning of food odours by recruits within insect nests is taxonomically widespread. It has been shown in stingless bees (Lindauer & Kerr 1958), bumblebees (Dornhaus & Chittka 1999), wasps (Jandt & Jeanne 2005) and ants (Roces 1990). It is not known, whether individuals learn by means of associative learning during social interactions and if food functions as a US in these species. If this turns out to be the case, then food quality dependent information transfer could be a more common mechanism, which helps social insect colonies to enhance their foraging efficiency.

Acknowledgments

We are deeply indebted to A. Arenas for much thoughtful advices and support during the experiment and H. Verna for technical assistance. This study was supported by funds from ANPCYT (01-12319), CONICET and University of Buenos Aires (X 036) to WMF. CG is supported by the VDRB, Commission for travelgrants of the SANW, Dr. De Giacomi Stiftung, Basler Stiftung für biologische Forschung and the Janggen-Pöhn Stiftung. When we carried out our experiments, we adhered to the legal requirements of the country.

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Floral scents affect the distribution of hive bees around dancers

Díaz, P.C., Grüter, C. and Farina, W.M. 2007. *Behavioral Ecology and Sociobiology* 61: 1589-1597. (From P.C. Díaz' diploma thesis)

7.1 Abstract

Floral scents are important information cues used to organize foraging-related tasks in honeybees. The waggle dance, apart from encoding spatial information about food sources, might facilitate the transfer of olfactory information by increasing the dissipation of volatiles brought back by successful foragers. By assuming that food scents are more intensive on specific body parts of returning foragers, i.e., the posterior legs of pollen foragers and mouthparts of nectar foragers, we quantified the interactions between hive mates and foragers during dances advertising different types of food sources. For natural sources, a higher proportion of hive mates contacted the hind legs of pollen dancers (where the pollen loads were located) with their heads compared to nonpollen dancers. On the other hand, the proportion of head-tohead contacts was higher

for non-pollen foragers during the waggle runs. When the food scent was manipulated, dancers collecting scented sugar solution had a higher proportion of head-to-head contacts and a lower proportion around their hind legs compared to dancers collecting unscented solution. The presence of food odours did not affect in-hive behaviours of dancers, but it increased the number of trophallaxes inbetween waggle runs (i.e., during circle phases). These results suggest that the honeybee dance facilitates the olfactory information transfer between incoming foragers and hive mates, and we propose that excitatory displays in other social insect species serve the same purpose. While recent empirical and theoretical findings suggested that the colony level foraging benefits of the spatial information encoded in the waggle dance vary seasonally and with habitats, the role of the dance as a compound signal not only indicating the presence of a profitable resource but also amplifying the information transfer regarding floral odours may be important under any ecological circumstances.

7.2 Introduction

Communication is indispensable for the survival of animal societies. Within the insect taxa, honeybee (*Apis mellifera*) colonies are good models to study communication because they have efficient channels for transferring information about food source characteristics. The different communication mechanisms allow bees to exploit selectively the most profitable food sources in an often unstable environment (von Frisch 1967; Seeley 1995). The most studied and conspicuous behaviour that involves signal transmission in honeybees is the waggle dance (von Frisch 1967). When foragers find highly profitable food sources nearby, they perform round dances, while waggle dances are displayed when food sources are far from the hive (von Frisch 1967). The waggle dance has been shown to encode spatial information about food sources (von Frisch 1967; Gould 1974; Esch et al. 2001; Riley et al. 2005). The duration and the rate of waggle-run production are tuned to the resource profitability allowing for an adaptive distribution of recruits among the various food sources (von Frisch 1967; Seeley et al. 1991,2000). However, the benefits to hive foraging efficiency of the spatial information encoded in the waggle dance seem to depend on the particular environmental circumstances, e.g. the spatial and temporal distribution of food sources (Sherman & Visscher 2002; Dornhaus & Chittka 2004).

It has often been suggested that the dance serves at least two other informational purposes. Firstly, it increases the attention and activity of bees in the vicinity thereby communicating the presence of an attractive food source (von Frisch 1923, 1967; Božič & Valentinčič 1991). If a forager is performing a waggle dance, the increased attention of unemployed foragers will facilitate their perception of the acousticvibratory signals emitted by the intensive movements of the wings that conforms the acoustic near field of the dancer (Michelsen et al. 1987; Michelsen 2003). However, the dance display not only attracts potential foragers but also food processor bees that initiate trophallactic interactions with the incoming foragers, whereby the latter would receive information about the nutritional state of the colony (Farina 2000). Secondly, the dance seems to be relevant to transfer food odours (von Frisch 1923,1967; Johnson 1967; Wenner et al. 1969; Wells & Wenner 1973). The particles of floral odours impregnated onto the foragers body as well as the pollen loads carried on hind legs of the incoming foragers are enough to reactivate unemployed experienced foragers to resume collecting at known food sources (von Frisch 1923). While odours carried on the foragers body could be dissipated, the nectar odour preserved in the honey sac is maintained intact regardless of flight distance (von Frisch 1967). As a consequence, recruits can learn the nectar odour brought back by foragers (Farina et al. 2005; Gil & De Marco 2006; Grüter et al. 2006) via mouth-to-mouth trophallactic contacts (in lab experiments: Gil & De Marco 2005, in hive experiments: Farina et al. 2007). Thus, a dancing bee can provide different kinds of information during the same behaviour, and the dance, therefore, functions as a compound signal (Bradbury & Vehrencamp 1998).

It is known that information cues can be transmitted in parallel or complementary to signal transmission. Contrary to the evolved signals, cues are provided inadvertently (Danchin et al. 2004; Dall et al. 2005). In this sense, the honeybee is an excellent model to study the role of these incidental cues during the performance of a conspicuous behaviour that involves signal transmission such as the waggle dance.

Until now, follower behaviour and distribution of hive bees around dancers have been explained in the context of spatial information acquisition (Michelsen et al. 1987; Rohrseitz & Tautz 1999). The fact that followers are primarily positioned around the abdomen of a dancer, where the acoustic near field is most intense, has been explained by improved acquisition of information about the transmitted signal (Michelsen et al. 1987; Michelsen 2003). However, given the importance of odour cues and assuming that dancers provide olfactory information during this motor display, we hypothesised that hive bees located around the dancer may present behavioural patterns which particularly facilitate the acquisition of olfactory information.

A quantitative study analyzing the distribution of hive bees around the dancer and its dependence on the presence of food odours was performed to address this hypothesis. By using video recording, we analysed the distribution of hive mates around dancers foraging under natural situations (dancers returning from natural pollen and nectar sources) and in a controlled situation (dancers collecting scented or unscented sugar solution). Dancer behaviour and trophallactic behaviour during dancing were also measured for the controlled situation.

7.3 Methods

The experiments were performed at the end of the nectar flow season (March-April of 2004) at the experimental field of University of Buenos Aires. We used a two-frame observation hive containing a colony of about 4,000 European honeybees (*A. mellifera ligustica*) with a queen, brood, and food reserves.

7.3.1 In-hive behavioural recordings

Dances of foragers coming from natural food sources, (natural situation) or coming from an artificial feeder of regulated rate (controlled situation) were video-recorded. In the natural situation, dances were divided in two groups, depending on the type of food source exploited: (a) Pollen foragers were distinguished from (b) non-pollen foragers by the pollen loads carried on the posterior legs (primarily nectar foragers).

In the controlled situation, we experimentally manipulated olfactory cues of a sucrose solution that was offered at an artificial feeder to a group of foragers. This group of bees from the experimental hive was previously trained to collect 0.5 M unscented sucrose solution at a feeder that was located 160 m from the hive and provided solution at a constant flow rate of 5 μl/min during a period of 8 h daily. A new group of five to eight trained foragers at the experimental feeder were individually marked each experimental day. The sucrose solution concentration and the flow rate were increased only for 3 h per day for data recording sessions. During a first period of 3 days, the groups of foragers collected 2.0 M unscented sucrose solution with a constant flow rate of 40 μl/min at the feeder.

This period was followed by another 3 days, during which the trained foragers collected 2.0 M sucrose solution at the feeder scented with 50 μl of Linalool (LIO) per liter of sugar solution, with the same flow rate of 40 μl/min. These conditions of flow rate ensured the highest probability of dance to arrive to the hive (Cogorno et al. 1998). Complete times foragers spent inside the hive from her arrival to the hive until her departure to the food source (hive stays) were recorded by using a Sony DCR-TRV520 video camera. Video recordings of the dances were analysed with a resolution of 30 frames per second by using the Ulead Video Capture for Windows program (6.0 version).

7.3.2 Measurements

For both the natural and controlled situations, we recorded the position of hive bees around the body of the dancers during all the waggle phases of their hive stays. As the movements of the antennae (the insects olfactory organs) could not be distinguished due to the temporal resolution of the video, the head contacts were quantified because their recordings guaranteed that the hive bees antennae indeed contacted the dancer body. All the head contacts of hive bees with the dancers body were recorded by using transparent acetate sheets, the medium position of the frontal part of the heads of the hive bees that contacted the dancer body were marked as points around a diagram of the dancer. Trophallactic interactions were not counted as head contacts. In this way, more than one contact per hive bee could have been recorded, as these bees were not individually marked. Each contact was recorded only once, although some lasted for more than one frame during the analysis.

The space around the diagram (360°) was subdivided in 10° intervals (Fig. 7.1). The numbers of contacts in each of the intervals were registered. The contacts of the two corresponding intervals of both sides (e.g. 0-10° and 350-360°) were added up for the analysis because we assumed that bees around dancers have no general preference for a particular dancer side. Henceforth, all angles are between 0° and 180°.

In the controlled situation, additionally, we measured (1) the duration of hive stays of experimental foragers, (2) the latency until the first waggle run began, (3) the number of waggle runs per hive stay, (4) the total time of trophallaxis, (5) the number of receivers per trophallaxis, and (6) the number of trophallactic contacts. For this last variable, we separated the trophallactic contacts that occurred during the dancing period (during the circle phases) from those that occurred in the absence of dancing (i.e., before and after the dancing period). If foragers stopped dancing for a period longer than 4 s, it was considered a period of absence of dancing (see Waddington 1982). Each contact was considered a mouth-to-mouth contact (trophallaxis) between the forager and the receiver when the receivers head looked like a triangle, and its proboscis was extended towards the opened mandibles of the forager that offered the sucrose solution.

7.3.3 Hive odour

During the controlled situation, the entrance of the observation hive was scented with a different odour by putting an absorptive paper (diameter 3 cm) soaked with 50 μl of pure odorant, phenylacetaldehyde, inside a box connected to the entrance by a wire mesh. Returning foragers passed the box when entering the hive. This device was used to minimize the effect of food odours clinging on the forager bodies (von Frisch 1967; Farina et al. 2005). Hive and solution odours are pure natural flower components, and they were obtained from Sigma-Aldrich, Steinheim, Germany.

7.3.4 Statistical analysis

Because the assumptions of normality and homogeneity of variances were not met, nonparametric analyses were used. Mann-Whitney U-tests were used to compare frequencies of hive bee contacts, and behavioural parameters of the experimental foragers such as the hive time, the latency, the number of waggle runs, the total trophallactic time, the total offering contacts, and the food receivers involved. Wilcoxon matched-pairs tests were used to compare the number of trophallaxis between stages of hive stays, i.e. during dancing period or in the absence of dancing (Zar 1999). Descriptive statistics are given as medians and quartiles (in brackets). For the descriptive part, 24 dancers were analysed; for the experimental part, 31. Only 28 of these 31 bees could be recorded completely during their whole hive stays.

7.4 Results

7.4.1 Natural situation

The highest proportion of head contacts of hive bees around the dancers during the waggle-run phases was observed at the total area of abdomen of the dancers (more than 60% of all cases, Fig. 7.2). However, the distribution of head contacts around dancers foraging at natural food sources differed between the types of resources exploited (Fig. 7.2a). Head contacts around the hind legs were more frequent when hive bees followed pollen dancers (with pollen loads in their hind legs) than when they followed non-pollen dancers (abdomen including posterior legs without pollen vs. abdomen including posterior legs with pollen: $U = 18$, $p = 0.0018$, $N = 24$, Mann-Whitney U-test; Fig. 7.2b). Hive bees around nonpollen dancers presented a higher proportion of contacts with the

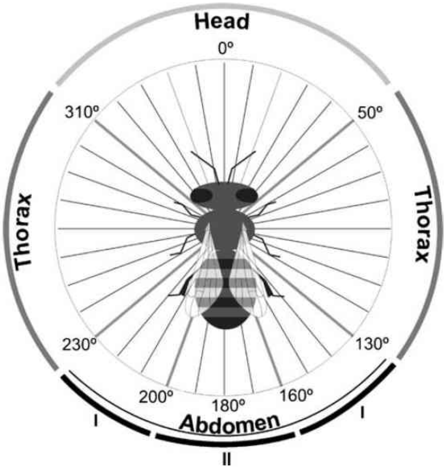

Figure 7.1: Sketch of a bee with the intervals, in degree, representing the different body parts. Interval I represents the abdomen including the posterior legs; II the abdomen excluding the posterior legs.

anterior part of the dancer compared to hive bees around the pollen dancers (head without pollen vs. head with pollen: U = 18, p = 0.0018, N = 24; and thorax without pollen vs thorax with pollen: U = 33, p = 0.024, N = 24, Mann-Whitney U-test; Fig. 7.2b). When we analysed the part of the abdomen that did not include posterior legs (range 160-200°), no differences were found between proportions of head contacts by hive bees comparing non-pollen dancers to pollen dancers (abdomen not including posterior legs without pollen vs abdomen not including posterior legs with pollen: U = 61, p = 0.53, N = 24, Mann-Whitney U-test; Fig. 7.2b).

7.4.2 Controlled situation

When the dancers collected scented sucrose solution from the controlled rate feeder, a higher proportion of hive bee contacts was observed during the waggle-run phase with the anterior part of the dancers body compared to the situation in which dancers collected unscented solution (Fig. 7.3a). When contacts were grouped according to the same dancer body parts analysed in natural situation, the differences were clear. For the head, the proportion of contacts was higher for the situation with odour (head without odour vs. head with odour: U = 0.5,

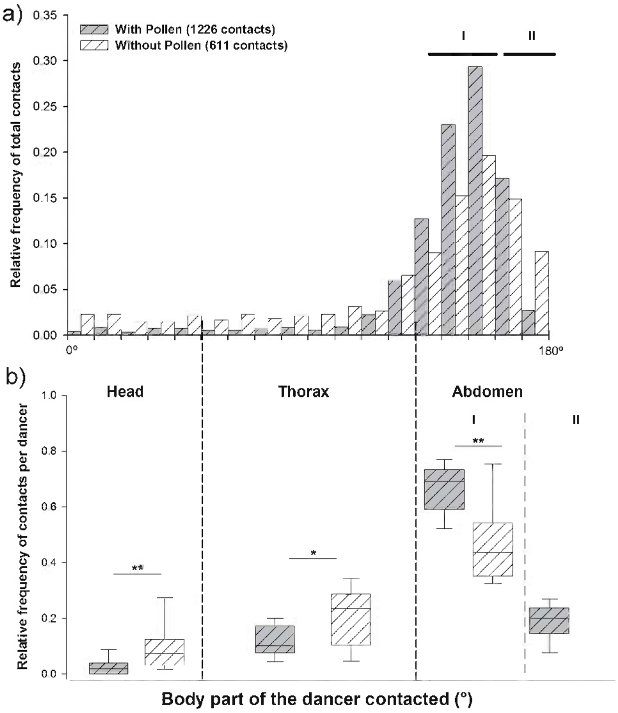

Figure 7.2: Distribution of the head contacts of hive bees around the incoming foragers during the display of the waggle-run phase. a) Total head contacts (in relative frequencies) of hive mates performed around the body of 24 dancers returning from natural food sources. Dancers without pollen loads in the posterior legs were considered to be non-pollen foragers (white bordered bars, without pollen, N = 12). Dancers with pollen loads in the posterior legs were considered to be pollen foragers (gray bordered bars, with pollen, N = 12). 0° corresponds to the frontal part of the dancers head and 180° to the posterior extreme of its abdomen. b) Total head contacts relative to each dancer and grouped according to the part of the dancers body that was contacted: head; thorax; posterior legs (I); the rest of dancers abdomen, (II). Medians, quartiles, and the 5th and 95th percentiles are represented. Asterisks indicate statistical differences (**$p < 0.01$; *$p < 0.05$; see Results for details).

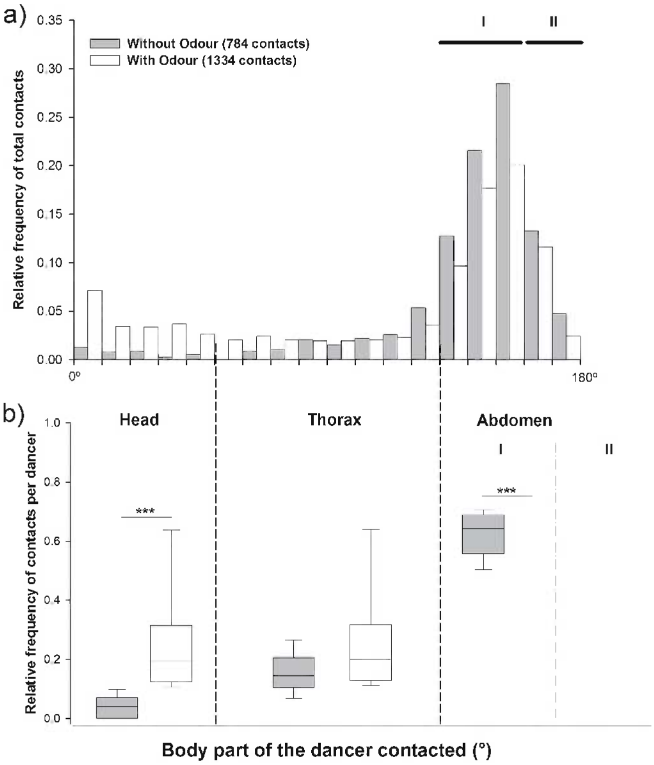

Figure 7.3: Distribution of the head contacts of hive bees around the incoming foragers during the display of the waggle-run phases. a) Total head contacts (in relative frequencies) of hive mates performed around the body of 28 dancers returning from a rate-feeder located 160 m from the hive. Dancers that collected unscented 1.8 M sucrose solution (gray bars, without odour, N = 14) were compared with dances of another group foraging at a 1.8 M sucrose solution scented with LIO (white bars, with odour, N = 14). 0° corresponds to the frontal part of the dancers head and 180° to the posterior extreme of its abdomen. b) Total head contacts relative to each dancer and grouped according to the part of the dancers body that was contacted: head; thorax; posterior legs, I; the rest of dancers abdomen, II. Medians, quartiles, and the 5th and 95th percentiles are shown. Asterisks indicate statistical differences (***$p < 0.001$; see Results for details).

Table 7.1: In-hive behavioural parameters of the trained forager bees.

	No odour	*With odour*	*U*	*P*	*N*
Hive time (s)	102 (67, 139)	129 (87, 191)	84.5	0.16	31
Latency (s)	27 (11, 38)	23 (12, 34)	94.5	0.65	29
Number of waggle runs	24 (19, 34)	41.5 (14, 74)	101.5	0.46	31
Total troph. time (s)	36 (28, 42)	37 (25, 41)	93	0.82	28
Total offering contacts	5 (3, 8)	7.5 (5, 12)	82.5	0.14	28
Food receivers involved	7.5 (4, 9)	9 (7, 12)	69.5	0.19	28

Behaviours of dancers collecting unscented sucrose solution were compared with dancers collecting scented sucrose solution. Medians and quartiles (in parentheses) and values for Mann-Whitney U-test are shown.

$p < 0.0001$, N = 28, Mann-Whitney U-test; Fig. 7.3b). At the abdomen, we also found differences but only for the part that included the posterior legs (abdomen including posterior legs without odour vs. abdomen including posterior legs with odour: U = 11.5, $p < 0.0001$, N = 28; abdomen not including posterior legs without odour vs. abdomen not including posterior legs with odour: U = 72.5, p = 0.241, N = 28, Mann-Whitney U test; Fig. 7.3b). In this case, the higher proportion was found in the treatment without odour. Around the thorax, there were no differences between treatments (thorax without odour vs. thorax with odour: U = 73, p = 0.75, N = 28; Mann- Whitney U-test, Fig. 7.3b).

Neither foragers coming from a scented sucrose solution nor those coming from unscented sucrose solution differed in their hive stay duration, the latency before dance, the number of waggle runs per hive stay, the total time of trophallaxis, the number of receivers per trophallaxis, and the total number of trophallaxis (see Table 7.1 for details). However, if we consider the trophallactic contacts according to the stage at which they occur (during dances, specifically during circle phases, or in the absence of it), the number of trophallactic-offering contacts was significantly higher during the dancing period only for the situation with odour (for the unscented situation: T = 31.5, p = 0.105, N = 15, Wilcoxon-test, Fig. 7.3a; for the scented situation: T = 2, p = 0.0009, N = 16, Wilcoxon-test, Fig. 7.4b).

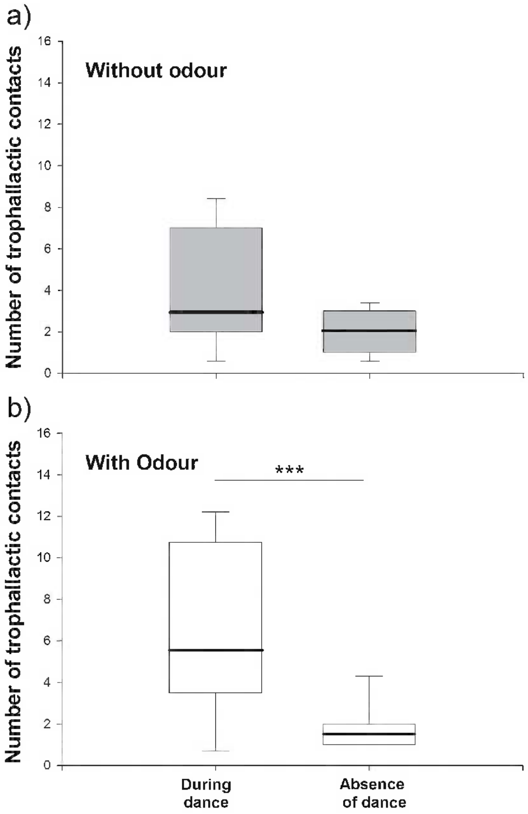

Figure 7.4: Number of trophallactic-offering contacts performed by the trained foragers inside the hive. a) Unscented sucrose solution (without odour, N = 15), b) scented sucrose solution (with odour, N = 16) with LIO. The trophallactic contacts were grouped according to the period during which they occurred: during the dancing display (in-between waggle-run phases, i.e. during the circle phases) or before the first waggle-run and/or after the last waggle-run phase observed (absence of dancing). Medians, quartiles, and the 5th and 95th percentiles are shown. Asterisks indicate statistical differences (***$p < 0.001$; see Results for details).

7.5 Discussion

The results show that odour cues of the food brought back by dancing foragers affect the distribution of hive mates around the dancers. We observed that these differences were not caused by conspicuous changes in the in-hive behaviours of the dancers, as all of the behavioural variables recorded in the trained foragers did not show differences between treatments. Therefore, the crop scent per se, specifically the odour concentrated on the mouthparts of the dancers, affected not only the head-to-head contacts between the dancer and hive mates but also the occurrence of trophallaxis amongst them.

7.5.1 Intensive interactions during scented dances

As expected, the highest proportion of hive bee contacts was observed around the hind legs of the dancers in all of the analysed cases. This could be the result of bees trying to improve the acquisition of the transmitted signal (Michelsen et al. 1987), which supposedly is strongest at the rear part of the dancer or due to the body oscillation during the waggle runs, which presents the highest amplification at the abdomen. This could, incidentally, increase the probability of contacts with bees around her. Nevertheless, the general pattern of contacts also depended on the type of food source and the presence of odour in the food bees collected. We found a higher proportion of head-to-head contacts when dancers came from natural non-pollen sites and when foragers collected scented sugar solution at an artificial feeder. Food odour effects seem to be stronger when odours are located on the dancer mouthparts, while the fragrances of the pollen loads are probably less effectively transferred to other colony members (von Frisch 1943). Moreover, if we compare the proportion of head-to-head contacts between non-pollen dances and scented solution dances, more contacts are observed for the dancers collecting scented solutions at the artificial feeder, which suggests a more salient response for this food odour compared with the natural odour. This could be a consequence of differences in odour concentration, odour identities, or both. Another reason may be that the hive entrance was scented only during the controlled situation, which reduces the putative effect of the food odours clinging on the forager bodies to the controlled situation. Therefore, while food odours brought back by the trained foragers were mainly concentrated onto their mouthparts, the dancers analysed during the natural situation could have had food odours more equally distributed on the body surface. Oral contacts during dance performance have already been reported (von Frisch 1923,1967; Park 1925). The occurrence of dance performance positively correlates with the number of nectar receivers, once the dancer stops its motor display (Farina 2000), which suggests that both behaviours, dance and trophallaxis, are coupled. Present results go beyond this, as we show that the presence of odour did not modify the number of trophallaxis during each foragers hive stay but increased the proportion of these trophallaxes while the active forager danced. Thus, honeybee dances, apart from encoding spatial information, function as a congregating mechanism, while the crop scent concentrated on the mouthparts would orientate the hive bees to obtain samples of the food recently collected. The higher proportion of the head-to-head contacts between scented nectar dancers and hive bees compared to the unscented situation could lead to a higher number of mouth-to-mouth contacts during the return phases of the waggle dance. During the trophallactic contacts, the food receivers can obtain information related to the odour and the profitability of the discovered nectar source (von Frisch 1967; Farina 1996; Crailsheim 1998; Gil & De Marco 2005; Farina et al. 2007). Thus, the performance of trophallactic contacts during dances might indicate that this display would facilitate the transfer of chemical cues about the new floral type found, while it encodes more complex information such as the food location.

Transfer of food odour information is an important mechanism of recruitment that has been observed also in other social insects (stingless bees: Lindauer & Kerr 1958; bumblebees: Dornhaus & Chittka 1999; wasps: Jandt & Jeanne 2005; and ants: Roces 1990). Additionally, many social bee species also show excitatory recruitment displays (Lindauer 1961; Hölldobler 1977; Nieh 1998; Dornhaus & Chittka 2001). It has been suggested that excitatory motor displays and the transfer of olfactory information during these behaviours represent more primitive recruitment mechanisms from which the waggle dance seems to have originated (Ribbands 1954; Hölldobler 1977; Nieh 2004). Therefore, the presence of odours might affect behavioural patterns of potential recruits in similar ways in other social insect species that use socially transmitted food odour information.

7.5.2 Putative appetitive learning within the dancing context

Bee dances could be a suitable context for the acquisition of olfactory information through olfactory conditioning within the hive. This motor display may arouse the following bees and lead to better learning of the contingency between odour and reward. Indeed, honeybee recruits can be conditioned to the floral odour before arriving at a particular food source, which shows that olfactory memories for a specific odour can be formed during the recruitment displays inside the hive (von Frisch 1967; Farina et al. 2005; Grüter et al. 2006). This strongly suggests that recruits learn the contingency reward odour through mouth-to-mouth contacts while following dances. In this sense, Dirschedl (1960) showed that 96% of all recruits arriving at the food source received food samples from the recruiting foragers (i.e. carried dyed syrup collected by trained foragers).

The crop scent cannot only be learned within the hive, but it can also cause a conditioned response depending on the knowledge of the inactive forager about this floral odour. In fact, most dance followers are experienced foragers (Biesmeijer & Seeley 2005), and the dance is the most frequent social interaction during the reactivation process to a profitable food source (Gil & Farina 2002). Thus, inactive foragers can confirm the reappearance of their food source by only perceiving the odour of the floral type (von Frisch 1923,1967; Ribbands 1954).

Although we have not identified the follower bees, it is known that the bees attending dancers often are recruits (Riley et al. 2005; Biesmeijer & Seeley 2005), reactivated foragers (Gil & Farina 2002; Biesmeijer & Seeley 2005), and also nectar processors (Farina 2000). The differences found in followers behaviour, depending on food source type, will not only affect the process of foraging activation (and reactivation) but also foragingrelated tasks inside the nest such as nectar processing and storing. Floral odours are learnt inside the hive (Gil & De Marco 2006; Farina et al. 2007), they affect the occurrence of trophallaxes between foragers and nectar processors (Goyret & Farina 2005), and this food scent information is rapidly propagated amongst nestmates of all ages (Grüter et al. 2006).

Recent empirical and theoretical findings suggest that the benefits of the spatial information encoded in the waggle dance to foraging vary seasonally and with habitats (Sherman & Visscher 2002; Dornhaus & Chittka 2004; Dechaume-Moncharmont et al. 2005; Dornhaus et al. 2006). However, to understand the costs and benefits of dancing or of following dances and, therefore, the selection pressures that act on this extraordinary behaviour, we need to consider the fact that the waggle dance is a compound signal, providing different kinds of information. The role of the dance (waggle or round dance) as a mechanism for the amplification of olfactory information within the hive is likely to be relevant under any ecological circumstances of the honeybee hive.

Acknowledgments

We are indebted with A. Arenas and H. Verna for technical assistance. This study was supported by funds from ANPCYT (01-12310), University of Buenos Aires (X 036), and CONICET to WMF. PCD is supported by a CONICET fellowship, and CG is supported by the Verein deutschschweizerischer und rätoromanischer Bienenfreunde, commission for travel grants of the SANW, Dr. De Giacomi Stiftung, Janggen-Pöhn Stiftung, Basler Stiftung für biologische Forschung. The present study complies with the current laws of the state country in which experiments were performed.

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8

Informational conflicts created by the waggle dance

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8.1 Abstract

The honeybee waggle dance is one of the most intriguing animal communication signals. A dancing bee communicates the location of a profitable food source and its odour. Followers may often experience situations in which dancers indicate an unfamiliar location but carry the scent of a flower species the followers experienced previously at different locations. Food scents often reactivate bees to resume food collection at previously visited food patches. This double function of the dance creates a conflict between the social vector information and the private navigational information. We investigated, which kind of information followers use in this situation and found that followers usually ignored the spatial information encoded by the waggle dance even if they followed a dance

thoroughly (= 5 waggle runs). They relied on private information about food source locations instead (in 93% of all cases). Furthermore, foragers preferred to follow dancers carrying food odours they knew from previous field trips, independently of the spatial information encoded in the dance. Surprisingly, neither odour identity nor the location indicated by the dancer was an important factor for the reactivation success of a dance type. Our results contrast with the assumption that (1) followers usually try to decode the vector information and (2) that dances indicating an unfamiliar location are of little interest to experienced foragers.

8.2 Introduction

The waggle dance of the honeybee (*Apis mellifera*) is probably the best-known communication signal in the insect world. A dancing bee provides its followers with at least three types of information, which are important for the organization of collective foraging in honeybees: (1) the distance and direction to the exploited food source (vector-information, the "dance language"; von Frisch 1967; Riley et al. 2005) (2) the odour of the food source (von Frisch 1967; Wenner & Wells 1990; Díaz et al. 2007) and (3) the presence of a profitable food source (von Frisch 1923; Wells & Wenner 1973; Thom et al. 2007). While the vector information is unique to the waggle dance, the other two types of information are involved in recruitment in many other social insects (Lindauer & Kerr 1960; Dornhaus & Chittka 1999; Hrncir et al. 2007) Most of the bees interacting with dancers are foragers with field experience and many of them follow dances after being temporally inactive (Biesmeijer & Seeley 2005). These followers can use the vector-information in order to find the location of the food source (von Frisch 1967; Riley et al. 2005). In addition, they can learn floral cues such as the odour of the flower species, which are carried on the body and in the collected food itself (von Frisch 1967; Farina et al. 2005; Grüter et al. 2006). Both kinds of information help foragers to locate the indicated food source (von Frisch 1967).

On the other hand, temporarily inactive foragers, which possess self-acquired (private) information about the location of food sources from previous field trips, can be reactivated to resume foraging at known food sources (e.g. after nightfall, bad weather or the end of nectar or pollen production periods of particular plant species) by encountering the scent of a previously visited food source in the hive (via following round dances (von Frisch 1923) or simply by encountering the scent (Ribbands 1954; Wenner & Johnson 1966)). In such a case, the familiar scent triggers navigational and visual memories (Reinhard et al. 2004). Hence, dances provide followers both social information for the discovery of a food source and the social context for the activation of private navigational information about a previously profitable food source.

It is largely unknown which kind of information foragers with field experience use after following waggle dances in natural situations. In other words, which strategy do followers choose when the dancer carries the odour of a familiar flower species, but indicates an unknown location? In such a situation the waggle dance creates a conflict and a bee could either (i) use her self-acquired information and fly to memorized food source locations or (ii) use the social vector information and fly to the place indicated by the dancer. There is preliminary support for both strategies. On one hand, von Frisch repeatedly reported that dances with "wrong" vector information are of little interest to experienced foragers (von Frisch & Rösch 1925; von Frisch 1946,1967). He suggested that these dances have very low reactivation success and that consequently, the vector information is the primary source of information used by experienced foragers (von Frisch 1967). Since then it has often been assumed that the vector information provided by the dance is used to discover the indicated food patch when bees follow dances (Seeley 1983; Seeley & Visscher 1988). On the other hand, Johnson (Johnson 1967) observed experienced foragers following waggle dances indicating an unknown location but carrying a known odour and he reported that these foragers subsequently used the private navigational information to fly to the food location where this odour had been learnt. He concluded that bees with field experience normally ignore the vector information and rely on their olfactory memories.

Until now, experiments with quantitative and qualitative analyses of different kinds of in-hive interactions like dance following or other social interactions (e.g. trophallactic contacts) are lacking and it is, therefore, still unclear what kind of strategy foragers pursue. Resolving this contradiction is obviously important to understand how honeybees use the waggle dance and how this signal affects collective foraging patterns at the colony level. In this experiment, we exposed inactive foragers to dancers, which indicate an unknown location but carry an odour they previously learnt at a different location and compared this situation with an alternative one in which there is no conflict between private and social information. We analysed in-hive interactions between active foragers and inactive experienced foragers in order to quantify the attractiveness and the reactivation success of the different dance types.

8.3 Methods

Four colonies with about 3,000 honeybees each, housed in two-frame observation hives (H1-H4) were used. Two *Apis mellifera ligustica* colonies (H1 and H2) were held at the experimental field of the University of Buenos Aires and two Buckfast colonies (H3 and H4; a cross between *A. m. ligustica* and *A. m. mellifera*) were held at the ethological field station of the University of Bern. Colonies had a queen, brood and reserves.

8.3.1 Experimental procedure

The experiment was done in 2006 (H1) and 2007 (H2-H4). We used one hive at a time to perform the experiment. Two groups of 15-30 bees coming from one hive were trained to collect a 2 M unscented sucrose solution at two different feeders with unrestricted flow 110 m from the hive. This feeder-hive distance guaranteed that our foragers showed waggle dances from which the observer could easily see which of the two feeders was advertised. The distance between the two feeders was 170 m, and the angle between the two directions from the hive to the feeders was 100° (see Fig. 8.1). The bees trained to the feeders were numbered with thoracic plastic tags (Opalithplättchen) for individual identification. One day after the two groups of foragers had been established, both feeders offered 2 M differently scented sucrose solutions (50 μl essential oil per liter sucrose solution) for 60 min from 10:00 until 11:00 in the morning (henceforth: training session). We used rose, vanilla (H1), jasmine, peppermint (H2), eucalyptus, anise (H3), lavender and lemon (H4) as scents for the two different feeders. The numbers of all foragers that collected scented food during this time were recorded. During these 60 min foragers of both groups learnt the link between the location and the scent. Then the feeders were removed, cleaned (with water) and placed in their original position 110 m from the hive. From 11:00 to about 15:00 treated foragers could freely inspect the now empty feeders. From 15:00 to 15:40 we again offered 2 M scented sucrose solutions. During twenty minutes immediately before offering food again, we noted all treated foragers that inspected the feeders to get an idea about the inspection rates in absence of food. One or two of the inspecting bees were captured and released at the beginning of the feeding period to start recruitment. During the feeding period, two numbered foragers per feeder were allowed to collect food and recruit other bees (henceforth: testing session). The high concentration of the offered sucrose solution guaranteed a high probability of dancing by the collecting foragers. All other foragers arriving at the two feeders after the start of this reactivation period were captured with plastic tubes after they started drinking. The time of arrival and their numbers were noted. At the same time, the hive interactions between the four employed foragers (2 per feeder) and the other numbered bees were filmed using a Sony DCR-TRV520 video camera. Training of bees in the morning and reactivation in the afternoon was considered one trial.

We did two trials with each colony, a "same-situation" trial and a "changed-situation" trial. The "same-situation" trial was planned to create a situation in which employed foragers showed dances, which created a situation of matching private and social location information. A feeder offered the same scent in the solution during the testing session as during the training session. The "changed-situation" trial created a situation in which unemployed dance followers were confronted with conflicting social and private information. Therefore, wee exchanged the two scents for the testing session, i.e. during the testing session feeder A offered the scent that was offered by feeder B during the training session and vice versa. Hence, dance followers with olfactory experience established at a particular feeder could thus experience four types of dance (Fig. 8.1):

- OV-dance: dancers collect the familiar odour, and indicate the location of the known feeder ("same-situation")
- NN-dance: dancers collect the unfamiliar odour and indicate the location of the unknown feeder ("same-situation")
- O-dance: dancers collect the familiar odour, but indicate the location of the unknown feeder ("changed-situation")
- V-dance: dancers collect the unfamiliar odour, but indicate the known feeder ("changed-situation")

In the cases of OV-dances and the NN-dances, a mismatch between private location information and social location information occurs. In the case of NN-dances, however, the unfamiliar odour has not been learnt at the known feeder location. Every bee was used only once.

8.3.2 Behavioural observations

We quantified the time and the type of interaction between the employed foragers and the individually marked and treated inactive foragers inside the hive. The types of interactions were:

- Dance following: bees that are located around the dancing bee within one bee-length of the dancer, facing the dancer and moving so that her head stayed facing the dancer during dance circuits (Biesmeijer & Seeley 2005). The number of waggle runs followed was also recorded.

- Trophallaxis: mouth-to-mouth contacts between active (incoming) foragers and treated inactive foragers. The active forager opens her mandibles and regurgitates a drop of solution between her mouthparts; the receiver protrudes her tongue towards the mandibles of the donor and tries to drink the solution.

8.3.3 Statistical Analyses

For data analysis we used generalized linear mixed-effects models (GLMM) in R 2.5.1 (R Development Core Team 2006). R fitted the models using the lme4 package (Bates 2007). We used hive and trial as random effects and dance type or odour situation as fixed effects. All dependent variables had a Poisson distribution. In order to test for the significance of a fixed effect, we compared the model containing the fixed effect with the model without fixed effect. A likelihood ratio test then compared the two models (Faraway 2006). When the fixed effect had more than two levels, pair wise comparisons between levels were performed if a significant overall effect was found. We corrected for multiple testing of a data set and adjusted the significance level by using the sequential Bonferroni method (Sokal & Rohlf 1995). Descriptive statistics are given as mean ± SE (standard error).

8.4 Results

8.4.1 Private vs. social information

In both the "same-situation" and the "changed-situation" most experienced foragers were reactivated to visit the feeder location they knew from previous field trips (Fig. 8.2). Only in H3 in the "same-situation" three foragers arrived at the unknown feeder location. These three foragers followed dances of the opposite forager group (NN-dances) for 12, 19, 22 waggle runs respectively and are the only bees for which we have clear evidence that the vector information provided by the waggle dance was used. 30 bees followed dancers that were collecting the known odour but indicated the unknown feeder location (O-dances). Of those, 26 bees landed at the feeder location where they previously experienced the food odour. 0 were captured at the feeder indicated by the vector information of the dance (Table 8.1). 18 bees followed dancers carrying the unknown odour and indicating the known feeder (V-dance). All of them were afterwards captured at the known feeder location. The same was true for bees following OV-dances. In summary, 43 bees followed dances indicating the unknown feeder location (17 NN-dances, 26 O-dances). Only 3 (7%) used this information.

Table 8.1: Number of bees following the different types of waggle dances (Dance), the number of bees captured (Captured), the number of waggle runs followed (Waggles) and the reactivation delay (Delay).

					Feeder location	
Dance	*N*	*Captured*	*Waggles*	*Delay*	*known*	*unknown*
OV	41	36	4.68 ± 2.7	3.73 ± 3.3	36	0
NN	20	17	7.7 ± 6.8	4.29 ± 2.87	14	3
O	30	26	5.07 ± 3.9	3.93 ± 3.6	26	0
V	18	18	2.53 ± 2.3	4.29 ± 4.22	18	0

Overall difference between the different dance types (OV, O, NN and V) with respect to the number of waggle runs followed per forager ($\chi^2 = 26.6$, df = 3, $p < 0.001$).

8.4.2 Dance choice

Figure 8.3 shows the number of experienced foragers following the different dance types in both situations. In all 8 trials, more bees followed dances with familiar odours (54% of all cases) than dances with unfamiliar odours (30.2% of all cases). 15.8% of bees followed dances of both kinds (B-group). A GLMM showed that bees preferred to follow a dancer that collected food with a familiar food odour compared to dances with an unfamiliar odour (GLMM, $\chi^2 = 10.2$, df = 1, p = 0.001; Fig. 8.3). There was no difference between dances indicating the known feeder location and those indicating the unknown feeder location ($\chi^2 = 0.74$, df = 1, p = 0.39), i.e. we found no indication of an effect of the vector information on the dance choice.

8.4.3 Waggle runs followed

Reactivated bees, which have been filmed following dances, followed 4.6 ± 3.38 waggle runs (N = 106, range: 1-17). 62 bees (58.5%) followed less than 5 waggle

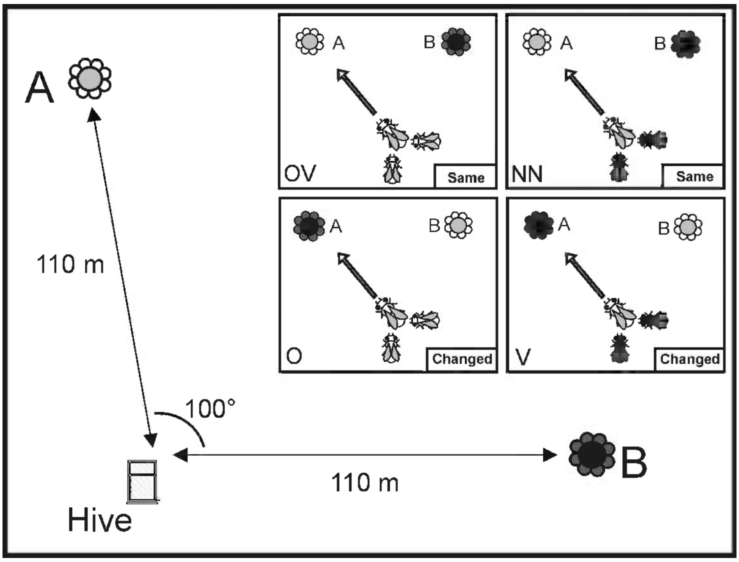

Figure 8.1: Arrangement of the feeding locations and the observation hive in both experimental fields in Argentina and Switzerland. Letters (A and B) represent locations while dark and bright flower dummies different odours. The four insert figures represent the four different kinds of dance types experienced by inactive forages in the "same-situation" and the "changed-situation". OV: experienced foragers (small bees) follow dancers (big bees) that collect the familiar odour (both dancer and follower are bright) and indicate the location of the known feeder (bright flower). NN: experienced foragers follow dancers that collect the unfamiliar odour (bright dancer vs. dark followers) and indicate the location of the unknown feeder ("same-situation"). O: experienced foragers follow dancers that collect the familiar odour, but indicate the location of the unknown feeder. V: experienced foragers follow dancers that collect the unfamiliar odour but indicate the known feeder.

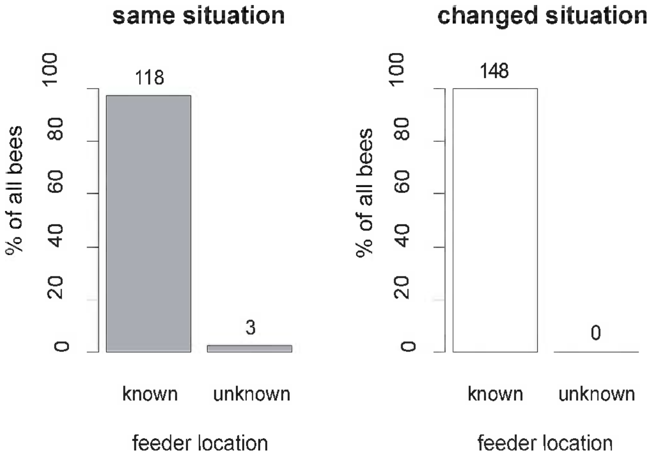

Figure 8.2: The percentage of bees being captured at the known and the unknown feeder location in both situations. Numbers above bars represent the number of bees.

runs, 44 bees (41.5%) followed at least 5 waggle runs. From this last category, 11 bees (10.4%) followed at least 10 waggle runs and 2 bees (1.9%) followed more than 15 waggle runs. The attractiveness of a dance type may be apparent also on a second level, the total number of waggle-runs followed per experienced forager for the different dance types (Table 8.1). First we tested for an overall effect of dance type (OV, O, NN and V) and found a significant effect ($\chi^2 = 26.6$, df $= 3$, $p < 0.001$). Table 8.2 shows the comparisons between the different dance types. When looking at dances promoting the known odour, there was no difference in the number of waggle-runs followed by experienced foragers between those that indicated the known location (OV-dance) and those indicating the unknown location (O-dance). On the other side, dancers carrying both unknown vector and unknown odour (NN-dance) were followed for more waggle runs than those carrying the known odour and indicating the known vector (OV-dance). In the inverted situation, dancers carrying the familiar odour (O-dance) were followed longer than those indicating the known vector (V-dance).

8.4.4 Reactivation delay

It might be argued that more bees used the vector information of the dance and tried unsuccessfully to find the indicated feeder location. After failing to

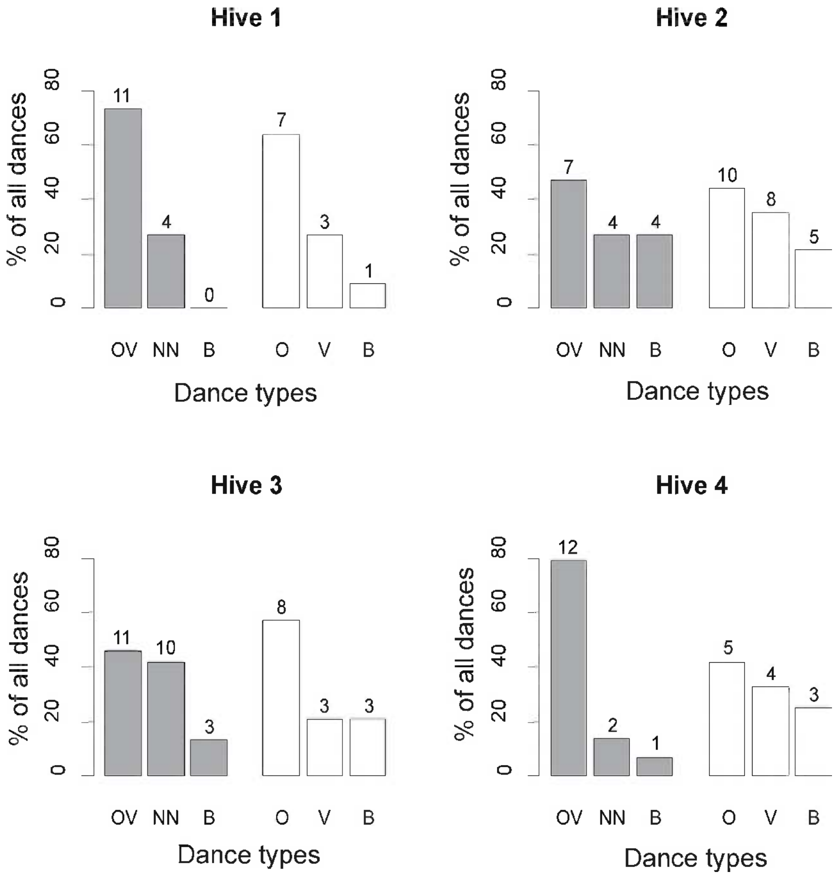

Figure 8.3: The percentage of bees following dances of a particular type in both situations (gray for "same-situation"; white for "changed-situation"). OV, NN, O and V are defined in epigraph of Fig. 8.1; B = experienced foragers follow both types of dances of a given situation.

Table 8.2: Number of bees following the different types of waggle dances and the number of bees captured at the two feeders.

Comparison	*Total N*	χ^2*-value*	*p-value*
OV vs. NN	38*/20	9.47	0.002**
OV vs. O	38*/30	0.63	0.43
OV vs. V	38*/17*	3.47	0.062
NN vs. O	20/30	0.04	0.84
NN vs. V	20/17*	1.97	0.16
O vs. V	30/17*	15.5	< 0.001**

*Lower numbers compared to Table 1 because in a few cases it was not possible to record the exact numbers of waggle runs followed. **Significant after sequential Bonferroni correction.

find the correct feeding site, these foragers would have then flown to the known feeder location. If this were true, one would expect that the time delay between dance following and capture at the feeder would be longer in the case of dancers providing conflicting information compared to those providing no conflict (OV-dance vs. O-dance). However, we found no significant differences between foragers following different dance types ($\chi^2 = 1.67$, df = 3, p = 0.64).

8.4.5 Trophallactic interactions amongst foragers

We tested whether field experiences with a particular food odour affect the occurrence of trophallaxis between inactive foragers and active foragers. Overall, we recorded 74 trophallactic-begging contacts of inactive foragers with active foragers returning with a familiar-scent and 39 trophallactic-begging contacts with the foragers returning with the unfamiliar-food scent. Thus, inactive foragers were more likely to receive food from a forager offering an odour, which the inactive forager had previously learnt in the field ($\chi^2 = 11.0$, df = 1, $p < 0.001$).

8.4.6 Reactivation success

von Frisch stated that the location information provided by a dancing bee is important for the reactivation success of the dancer (von Frisch 1946,1967).

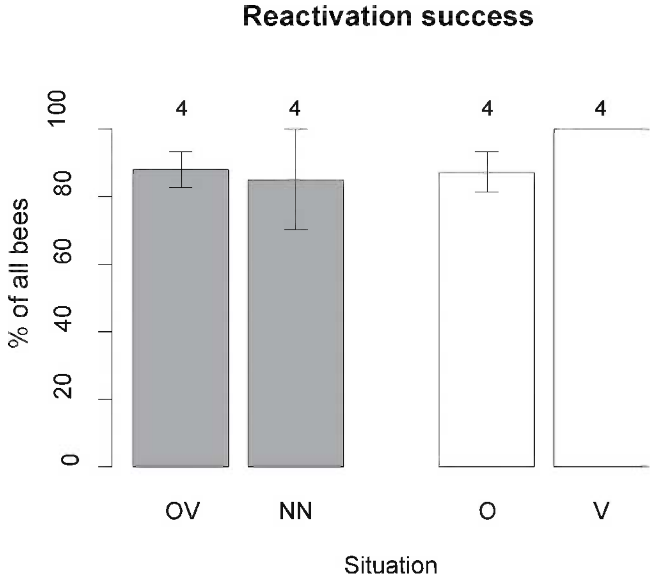

Figure 8.4: Reactivation success for the different dance types. Bars represent the percentage of bees (mean ± standard error) that were captured at the familiar feeder after following a dance of a certain type in the "same-situation" (gray) and the "changed-situation" (white). The numbers above the bars represent the four hives.

Therefore, we measured the reactivation success of different dance types, i.e. the proportion of foragers flying back to the known feeder after interacting with a dancing bee in the hive. Irrespective of whether followers knew the odour carried or the vector indicated by a dancer, reactivation success was above 80% for all types of dances in both situations (Fig. 8.4) and no significant difference between the dance types with respect to the reactivation success (in percent) was found ($\chi^2 = 4.69$, df = 3, p = 0.20).

8.5 Discussion

In 93% of all cases when bees were following dances providing spatial information that diverged from their private navigational information, followers subsequently relied on self-acquired information. These results suggest that foragers with self-acquired (private) information about the location of profitable food sources are usually not interested in the vector information transmitted by the dancers, even if they follow dances thoroughly. 41.5% of all reactivated bees followed more than 5 waggle runs, 10.4% followed more than 10 waggle runs. One bee was captured at the known feeder after following 17 waggle runs of a dancer that indicated the unknown location. This is remarkable given that followers can decode the vector information already after following 5 waggle runs (von Frisch & Jander 1957).

None of 26 foragers followed the instructions of the dancers when dancers collected a scent, which the foragers learnt at a different location (O-dance). Surprisingly, the food scent does not seem to be essential either. 82% of all bees following dancers indicating an unknown location and collecting an unfamiliar scent (NN-dance) also were reactivated and flew to the previously visited feeding site. The simple presence of a dancing bee motivates some foragers to fly to previously visited food patches, irrespective of vector and food odour information. Behaviourally active chemicals produced and released onto the cuticular surface of the dancer and into the air might alert experienced inactive foragers to generally good foraging conditions (Thom et al. 2007). However, since bees show a preference for following dancers, which collected food odours they had previously learnt in the field, food odours seem to speed up the reactivation process.

It seems puzzling that dance followers ignore the spatial information of the dance because the dancer obviously advertises a high quality food source (von Frisch 1967; Seeley 1995) while there is no guarantee that reactivated foragers will find food of similar quality at previously visited sites. On the other hand, many flower species offer nectar at particular periods during the day (von Frisch 1967; Vogel 1983) and there is a high probability that flowers of a given species offer nectar synchronously at different places. Furthermore, this strategy might be advantageous under certain conditions even if the food quality of the revisited food patch is inferior to the one advertised by dances. If the chance of finding previously visited food patches is considerably higher than the chance of finding the advertised flower patch, then it could still be worthwhile to fly to a potentially inferior but known food patch. Both the average numbers of waggle runs followed by foragers (Table 8.1) and reactivation success of dances did not differ for dancers that collected familiar scents but indicated unknown food locations (O-dances: 87.2% reactivation success) and dancers providing attuned information (OV-dances: 87.9% reactivation success). Thus, we cannot confirm von Frisch's statement that dances providing "wrong" vector information are of little interest to experienced foragers (von Frisch 1967) and have a much lower reactivation success (37.5% compared to 92% in his experiment) (von Frisch 1946). In von Frisch's experiments foragers either collected unscented food or he used two forager groups, which performed either round or waggle dances. Foragers might perceive round dances and waggle dances as intrinsically different. Our waggle data is to some degree ambiguous, because dances indicating the known feeder location (OV-dance vs. NN-dance and V-dance vs. O-dance) were followed less long than dances indicating the unknown feeder location. It is doubtful that this result is ecologically relevant since foragers flew back to the previously visited feeder in most cases in all situations.

Reactivated bees usually started drinking immediately at the feeder even when they encountered a new food scent at the known feeding site. This shows that an odour mismatch does not prevent a bee from landing on a feeding location that has the expected visual display (Galizia et al. 2005; Vladusich et al. 2006).

Dances are the most important type of interaction for foraging reactivation (von Frisch 1923; Gil & Farina 2002). However, inactive experienced foragers also engage in trophallaxes or simple contacts with antennation (Gil & Farina 2002). The occurrence of trophallactic contacts was affected by olfactory field experiences. This could be a consequence of bees preferring to receive food containing a known food scent (Goyret & Farina 2005). Since dancers frequently distribute food samples to their followers (von Frisch 1967), it could also be a by-product of the preference to follow dancers that collected food with a known scent.

The "dance language", i.e. the transfer of vector information from dancers to followers, has clearly been demonstrated (von Frisch 1967; Riley et al. 2005). It is normally assumed that foragers use the "dance language" when finding a food source after following a dance (e.g. Seeley 1983; Seeley & Visscher 1988). Biesmeijer and Seeley (2005) assumed that the location information provided by dancers is used whenever foragers follow at least 5-10 waggle runs. However, in our experiment a substantial number of bees fell into this range, and most bees relied on self-acquired navigational information. So when do foragers actually use the "dance language"? Can bees flexibly choose between private and social information after dance following, depending on ecological conditions and their own experience or are they constrained to rely on private information if olfactory cues carried by the dancer are associated with navigational memories in followers? The three cases of bees that apparently used the vector information strongly suggest that bees can switch strategy. We would expect that foragers switch their strategy if either the quality of the private information or the quality of the visited food patch is below a certain threshold. Information could be of low quality if it is outdated or not reliable (Leadbeater & Chittka 2007); a food patch is of low quality if it is of low relative profitability (Seeley 1995). Accordingly, van Bergen et al. (2004) showed that the quality of private information influences the use of social information in a situation of conflicting information in nine-spined sticklebacks. The three bees that used the social vector information were all from H3 and were trained to collect anise solution. Observers at the feeder noted that our anise solution was somewhat less preferred compared to other odours and so might have been of inferior quality for bees. These bees followed on average 17 waggle runs, which is similar to values reported by Michelsen (2003).

Experiments investigating the waggle dance are often performed at the end of the flowering season (e.g. Riley et al. 2005; this study) or at places, where there are few alternative food sources, because it is otherwise difficult to train bees to artificial feeders (Seeley 1995). In such environments, bees might use the vector information of the waggle dance more often than during times of nectar abundance in spring and summer, because private information is likely to be outdated and natural food patches are of lower quality. In other words, bees might rely more on private information in times of nectar abundance, which would help to explain why colonies with misdirected dances often perform equally well in temperate habitats during times of nectar abundance (Sherman & Visscher 2002; Dornhaus & Chittka 2004).

However, even if follower bees often ignore the vector information of the waggle dance, the long-term consequences of the vector information are not well understood. If bees are recruited to a food patch and subsequently forage for several days (up to 21 days at the same patch (Ribbands 1949)), then even these rare events might be of considerable ecological importance. The question of when honeybees use either private or social information under natural conditions needs further examination. This will most certainly reveal that the waggle dance modulates collective foraging in more complex ways than is currently assumed.

Acknowledgments

We thank Gonzalo Corti Bielsa, Gabriela Ramírez, Severine Loosli, Dolores Schütz and Francisca Segers for help with data collection, Daniel Rankin and Francisca Segers for comments on the manuscript, Michael Taborsky for logistic support, Hector Verna and Peter Stettler for technical help. CG was financed by the Janggen-Pöhn Stiftung and the Berner Hochschulstiftung. This study was supported by funds from ANPCYT, CONICET and University of Buenos

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9

Social experiences affect interactions between foragers and hive-mates in the honeybee (*Apis mellifera*)

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9.1 Abstract

Social insect colonies face the challenge of adjusting the behaviour of individuals performing various tasks to the given environmental situation. In order to achieve this, individuals often respond to local information, which is provided during interactions between individuals. Characteristics of interaction patterns provide information as a by-product and adjust individual behaviour in adaptive ways. Factors affecting interaction patterns are the number of individuals in a group, activity levels or spacing behaviour. A well-studied example is the modulation of recruitment dancing in honeybees (*Apis mellifera*) in response

to the time the foragers have to wait until unloading starts and the number of unloading bees. Here we tested if social experiences of hive bees established during interactions in the past affect interaction patterns perceived by honeybee foragers during hive stays. Honeybee foragers returning with a scent, which was familiar to the hive bees from previous interactions had more food receivers during unloading compared to forgers returning with unscented food or food containing a novel scent (+ 37.5% compared to the latter cases). These foragers were also followed by more bees if they were performing recruitment dances. We confirm that the number of receivers during unloading is positively related to the motivation of foragers to dance immediately after unloading. Our results demonstrate that social experiences in the past affect the ways in which individuals interact and, therefore, also the behavioural adjustments that are caused by these interactions.

9.2 Introduction

Social insect colonies need to adjust the numbers and activity levels of individuals performing various tasks, such as foraging or brood care in response to changing environmental conditions. These adjustments happen without central control or planning (Gordon 1996,1999). It has been shown for several social insect species that colony level responses to environmental changes often are the result of individuals responding to simple local information (Seeley 1995; Gordon 1999; O'Donnell & Bulova, 2007). Nest-mates are a rich source of such information. Individuals can respond either to signals, e.g. the vibration signal (Schneider & Lewis 2004; Cao et al. 2007) or to inadvertent social information cues (ISI) (Danchin et al. 2004; Dall et al. 2005). Interaction patterns provide the latter kind of information and help individuals to adjust individual behaviour in ways that allow the colony to function efficiently in a variable environment.

For example, during nest construction in *Polybia occidentalis* wasps, the right proportions of individuals performing the different tasks are maintained as individuals respond to the experienced time delays between interactions with each other (Jeanne 1986,1999). In the red harvester ant (*Pogonomyrmex barbatus*), the interaction rate between foragers and patrollers determines the forager's decision to leave the nest for foraging. If patrollers do not return safely, foragers will not leave the nest (Gordon 2002; Greene & Gordon 2003,2007). In the ant *Temnothorax albipennis*, workers assess the suitability of a new nest site by assessing the rate of encounters with nestmates at potential new nest sites

(Pratt 2005).

One of the best-studied examples of how the perceived interaction pattern affects behaviours is nectar unloading and subsequent dancing in honeybees (*Apis mellifera*). When a honeybee forager returns from a profitable food source, she transfers her food to one or several food processor bees (von Frisch 1923,1967; Park 1925) and, if the food source was very profitable, she performs a recruitment dance, which provides information about food source characteristics such as the location to dance followers (von Frisch 1967). Lindauer (1948,1954) observed that the nectar abundance outside the nest correlates negatively with the time a returning forager has to wait until food processor bees start unloading nectar or water. If foraging conditions are good, more foragers collect food and as a consequence most of the food processor bees are busy receiving and handling food. This increases the time-delay experienced by foragers. In times of poor foraging conditions only a few foragers collect food. It is therefore easy for them to find a food processor.

In this way, waiting time and the number of receiving food processors during unloading reflects the balance between the nectar collection rate and its processing capacity (Seeley et al. 1991; Seeley & Tovey 1994). The waiting time (Lindauer 1948,1954; Seeley 1989; Seeley et al. 1991; Seeley & Tovey 1994) and the number of receiver bees (Farina 2000; De Marco 2006) affects the motivation to dance after unloading: The longer a forager has to wait and the fewer receiver bees unload food, the less motivated she is to dance and as a consequence the less foragers she will recruit to her own food patch. This social feedback mechanism helps colonies to keep an optimal balance between collection and processing (Seeley 1995).

In these examples, individual decision rules are based on interaction patterns. The interaction patterns, on the other hand, depend on the number of individuals in groups (Gordon 1996,1999), their general activity level (Cao et al. 2007) and their spacing behaviour (Gordon et al. 1993).

Prior social experiences might be a factor affecting interaction patterns irrespective of the number of individuals in an area and the general activity level of individuals. If certain individuals have a socially acquired preference to interact with a particular class of individuals, for example because the latter carry a familiar type of material such as food, then interaction patterns and social feedbacks could be affected by social experience. In honeybees, for example, food-receiving bees socially learn the food scent during food transfer (Gil & De Marco 2005,2006; Farina et al. 2005,2007; Grüter et al. 2006). Bees learn to associate a scent with the sweet food. As a consequence of extensive food sharing (Nixon & Ribbands 1952; Grüter & Farina 2007), large proportions of bees of all castes learn the food scent (Grüter et al. 2006). Food processor bees prefer to unload foragers if the offered food has a scent, which is known to the food processor from previous interactions (Goyret & Farina 2005). Therefore, the type of collected food could affect the reception of foragers by food processors, i.e. the interaction patterns experienced by foragers. Here we tested if foragers returning with food containing a scent that is familiar to hive-bees from previous interactions (1) find food processor bees faster, (2) are unloaded by more food processors and (3) are followed by more potential recruits during dancing.

9.3 Methods

9.3.1 Study site and animals

The experiment was conducted at the ethological field station near Bern, Switzerland. We used two two-frame observation hives containing a colony of about 2'500 Buckfast honeybees (a cross between *Apis mellifera ligustica* and *A. m. mellifera*) each. Colonies had a queen, brood and honey reserves.

9.3.2 Experimental procedure

A group of bees (group 1) was trained to collect unscented sucrose solution of 15% w/w sucrose concentration at an ad libitum feeder (F1) located 5 m from the hive. Bees were marked individually and about 10 to 15 foragers were trained during 2 days of training. Simultaneously, a second group (group 2) of foragers was trained to collect 56% w/w sucrose concentration at an ad libitum feeder (F2) located 100 m from the hive. Group 2-foragers (between 30-100 different foragers) were allowed to freely collect a total amount of 200 ml of solution scented with the treatment scent (known scent; KS) during the two days of training. On day 3, individual foragers of group 1 were videotaped while they collected food at F1. One forager at a time was allowed to collect 56% w/w sucrose solution. The foragers performed three foraging cycles with unscented solution (US), three cycles with solution containing the known scent (KS) and three cycles with solution containing a novel scent (NS) (a total of nine foraging cycles). Novel scent means that this odour has never been used as treatment odour before for the tested colony. The sequence always started with unscented solution; afterwards we first offered the KS in half of the cases and the NS in the other half of the cases.

Because we wanted focal foragers to be equally motivated to collect both the KS and the NS, we fed these foragers at the end of training day 2 with small amounts of both scents at the feeding place (a few drops of both scents per forager). Without this treatment, foragers would have encountered and learned only the KS in the hive during mouth-to-mouth contacts (Grüter et al. 2006), which could cause differences in the motivation to collect solution containing the KS and the NS. So while the majority of all hive bees experienced only the KS, the focal foragers had experience with both the KS and the NS.

We filmed about 3-5 bees on a test day with a particular combination of treatment odour and novel odour. We used orange, peppermint, anis, jasmine, lavender, eucalyptus (essential oils) and the pure odours linalool and phenylacetaldehyde (Sigma-Aldrich, Steinheim, Germany).

9.3.3 Behavioural observations

We recorded the following forager behaviours and in-hive interactions with a digital camera: total time dancing, time dancing before and after unloading, unloading delay (time between entering the hive and first food offering of > 5 sec), number of receivers during unloading, number of dance followers (number of followers 10 sec after the beginning of dancing; see Božič & Valentinčič 1991 for definition of "following"). Furthermore, foragers sometimes interrupted drinking solution or left the feeder after a first approach. We recorded whether this happened and for how long foragers left the feeding place. The videotapes were analysed with the JWatcher 1.0 software (Blumstein et al. 2006).

9.3.4 Statistical analysis

For data analysis we used generalized linear mixed-effects models (GLMM) in R 2.6.0 (R Development Core Team 2006). Since we had more than one observation per bee, both bee and hive were used as random effects. Dependent variables had either a Poisson or a binomial distribution.

Treatment was used as a fixed effect. We tested the significance of the fixed effect by using a parametric bootstrap method (Faraway 2006). This method estimates the probability of observing the value of the likelihood-ratio test (LRT) of the actual model or a higher value. Therefore, the LRT of the model is compared to a randomly generated LRT distribution obtained by 10000 permutations of the model. The estimated probability corresponds to the p-value (Faraway 2006; pp. 158-161). When the fixed effect had more than two levels, pair wise comparisons between levels were performed if a significant overall effect was found. We corrected for multiple testing of a data set and adjusted the significance level by using the sequential Bonferroni method (Sokal & Rohlf 1995). Values of $p < 0.05$ after correction are indicated with one asterisk, results no longer significant are indicated with two asterisks. The N given in the text refers to the number of bees for which data was available. Descriptive statistics are given as mean ± SE (standard error).

9.4 Results

Since we fed focal foragers with both the KS and the NS the day before testing, we expected them to be equally motivated to collect the two scents. However, this was not the case. When approaching the feeder offering the known scent for the first time, foragers often were reluctant to land on the feeder or they interrupted drinking for longer periods. If foragers left the feeder after an initial approach for at least 30 seconds or interrupted drinking for 30 seconds we considered them to be hesitating to accept the food. This was the case in 38% of the cases when foragers visited the KS-feeder for the first time, in 14% of the first visits of the NS-feeder and never with the US-feeder. This led to a significant difference between the three situations (Permutation test: $p < 0.001$). Comparisons between the treatments showed that foragers hesitated most when landing on the KS-feeder (KS vs. US: N = 39/39, $p < 0.001$; KS vs. NS: N = 39/39, $p < 0.001$). There was no significant difference between US and NS (N = 39/39, p = 0.039**).

9.4.1 Unloading delay and dancing

In 30.1% (83 of 276) of all recorded hive stays, bees danced already before unloading. Since we were interested in the relationship between unloading delay and the dance duration afterwards, we exclude these cases this analysis. There was a negative relationship between unloading delay and the dance duration after unloading (Permutation test: N = 38, p = 0.009).

9.4.2 Receiver number and dancing

We tested whether the number of receivers during unloading was related to the amount of dancing after unloading. Cases, in which bees danced before unloading were again excluded, because dancing attracts receivers (Farina, 2000) and, therefore, including these cases would confound the outcome. Foragers had between 1-7 receivers (2.67 ± 0.11). We found a positive relationship between the total number of receivers and the probability to dance (N = 31, p = 0.007; effect on dance duration: p = 0.18).

9.4.3 Unloading delays in the different treatments

Our first hypothesis was, that foragers returning with a familiar scent start unloading sooner because receiver bees in the hive are more interested in this food. Therefore, we tested if bees that did not dance before unloading started unloading sooner when collecting the familiar scent, but we found no significant effect of treatment (Permutation test: $p = 0.77$; Fig. 9.1) on the unloading delay.

9.4.4 Number of receivers in the different treatments

We hypothesised that more receivers unload foragers returning with a familiar scent. There was a significant treatment effect on the number of receivers during unloading (overall treatment effect: $p = 0.001$; Fig. 9.2a). When foragers collected the known scent they had in total more receivers during unloading than when foragers collected unscented solution ($p = 0.001$*) or the novel scent ($p = 0.024$*). Foragers also tended to have more receivers when they collected a novel scent compared to the unscented situation ($p = 0.071$).

9.4.5 Number of followers in the different treatments

Our third hypothesis stated, that foragers returning with familiar scents have more followers compared to instances when foragers collected unscented solution or solution with a novel scent. Treatment had a significant effect on the number of followers ($p < 0.001$; Fig. 9.2b). As hypothesised foragers collecting food containing a known scent were followed by more bees during dancing than when they collected unscented solution ($p < 0.001$) or solution containing a novel scent ($p = 0.001$). There was no difference between the unscented situation and the novel scent situation ($p = 0.16$).

9.4.6 Dance probability and duration in the different treatments

We compared the dance probability and duration in the different treatments. There was no treatment effect on dance probability before unloading ($p = 0.071$; Fig. 9.3a), after unloading (no dancing before, $p = 0.151$; Fig. 9.3b) and overall ($p = 0.524$). However, we found that the dance duration differed significantly between treatments (Permutation-test: $p = 0.007$) when bees were dancing. Pair-wise comparisons showed that bees danced less when collecting the known scent compared to instances when collecting an unscented solution ($N = 28/30$, $p = 0.012$*). The other two comparisons were not significant (US vs. NS: $N = 28/31$, $p = 0.11$; KS vs. NS: $N = 30/31$, $p = 0.23$). We suspected that this

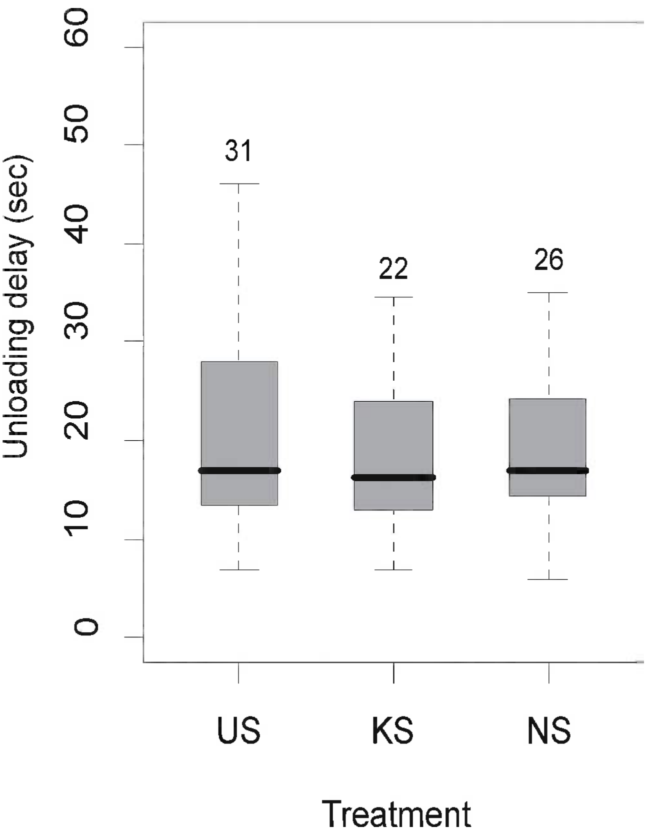

Figure 9.1: Unloading delays in different treatments. US = no scent in solution, KS = known scent in solution, NS = novel scent in solution. The three trips per treatment were averaged for this figure. The boxplots show medians, quartiles, 5th and 95th percentiles. Numbers above bars represent the number of bees, for which data was available.

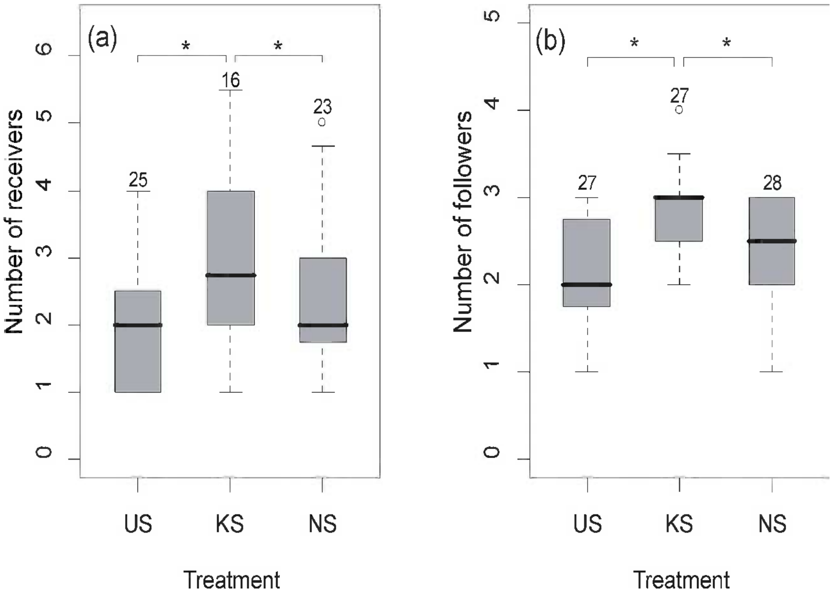

Figure 9.2: Number of receivers and dance followers in the different treatments. (a) Number of receivers during unloading, (b) number of followers in case bees were dancing. Maximum values outside of the percentiles are shown as empty dots. Significant differences are indicated with asterisks. Boxplots as in Fig. 9.1.

negative effect of the KS on the dancing motivation of foragers is less pronounced after unloading, because foragers had the opportunity to experience the social feedback during unloading. Therefore, we plotted the effect of the treatment on the dance duration in case foragers started to dance before unloading (without the experience of the social feedback; Fig. 9.3c) and the same effect in case foragers danced only after unloading (with the experience of the social feedback; Fig. 9.3d). When bees started to dance before unloading, there was again a significant treatment effect ($p = 0.003$, Fig. 9.3c). Bees danced more when collecting unscented solution, than when collecting a solution containing a scent (US vs. KS, $p = 0.019^*$; US vs. NS: $p = 0.01^*$). There was no difference between the two odour situations ($p = 0.23$). When we considered only bees that danced after unloading, we no longer found an effect of treatment on dancing duration ($p = 0.228$; Fig. 9.3d).

9.5 Discussion

We found that foragers collecting a scent, which has previously been fed to the colony, did not initiate unloading faster than when collecting an unknown scent. However, they had more receivers during unloading and were followed by more bees when they performed recruitment dancers. This shows, that social experiences affect characteristics of the interaction pattern perceived by foragers during hive stays. It has been shown that there is a positive relationship between the number of receivers during unloading and the motivation to dance and recruit other bees to the food source immediately after unloading (Farina 2000; De Marco 2006; this study).

Why was there no treatment effect on the unloading delay, i.e. the time between entering the hive and starting with the unloading contact? The unloading delay not only depends on the behaviour of food processor bees in the hive, but is also affected by the motivation of the forager. This is obvious when a forager starts dancing before unloading. This was the case in 30.1% of all hive stays (see also Thom 2003). In other cases foragers started a trophallactic contact, but broke off food transfer after a few seconds (< 5sec). Since our treatment had an effect on the motivation of collecting foragers, unloading delay might be a confounded measure of social feedbacks.

The fact that foragers were less motivated to collect the solution containing the KS than collecting the NS is surprising and puzzling. It is likely that focal foragers encountered the KS in the hive during mouth-to-mouth contacts during the two days of training (Grüter et al. 2006). This should cause a preference

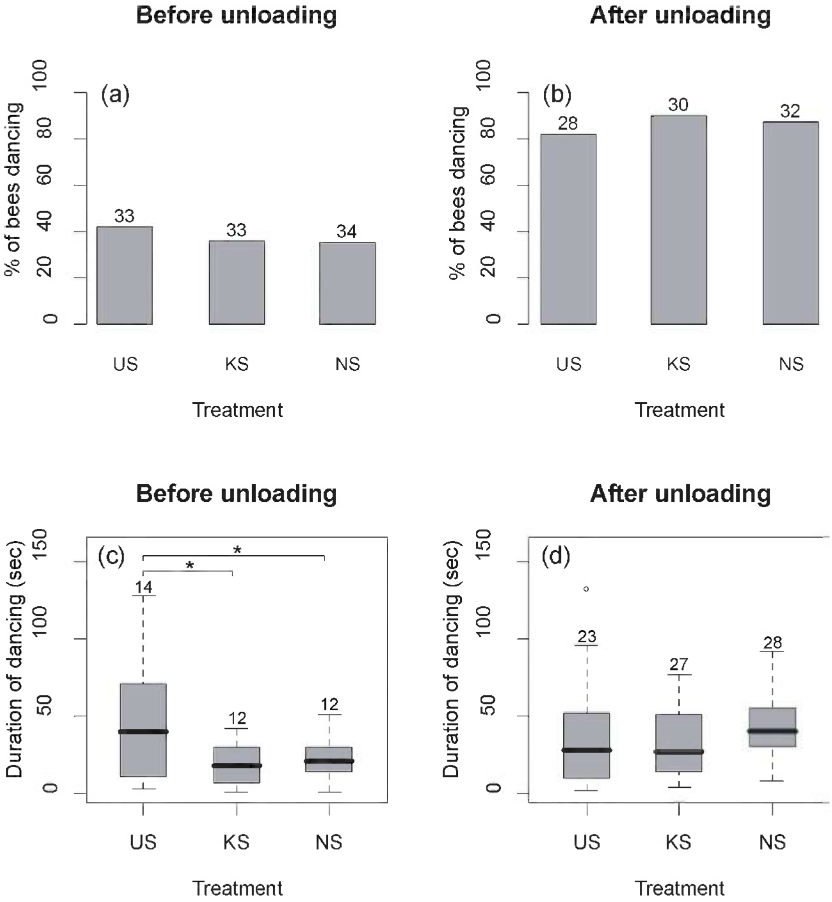

Figure 9.3: Dancing probability and duration before and after unloading in different treatments. (a) Percentage of bees dancing before unloading, (b) percentage of bees dancing after unloading, (c) duration (in sec) of dancing before unloading and (d) duration of dancing after unloading. Boxplots as in Fig. 9.1.

for this scent, rather than an aversion (Arenas et al. 2007). On the other hand, focal foragers were collecting unscented solution during the two training days and they might have encountered the KS mostly passively on the bodies of other hive bees or in the hive atmosphere and only rarely associated with food. The permanent passive exposure to the odour can make the learning of the association between odour and food more difficult (Sandoz et al. 2000), a phenomenon called latent inhibition (LI) (Chandra et al. 2000). However, it is not known whether LI could have inhibiting effects on landing and dancing behaviour.

The interaction pattern informs foragers about the balance between food collection and processing capacities (Seeley 1995). Social learning in hive bees alters this pattern, which means that the information about this balance is likely to become less accurate. Can this be an adaptive behaviour? Three reasons might explain, why food processor bees show a preference to interact with certain foragers based on olfactory cues. First, honeybee colonies are able to direct their foraging activities to highly profitable food sources. They achieve this by selectively recruiting foragers to the best food patches (Seeley 1995). If many bees of a colony know a particular floral odour, then this is a consequence of the high profitability of this plant species. It might be beneficial to enhance the dance motivation of foragers collecting at this plant species, compared to foragers returning from a plant species largely unknown to the colony. This on the other hand could mean that there is relatively less recruitment to newly emerging superior food sources, because hive bees initially do not know the odour of such plant species and returning foragers are welcomed with less interest. It has been suggested earlier that social learning can cause sub-optimal or even maladaptive patterns of behaviour because it can lead to the preservation of inferior alternatives (Laland et al. 1996).

Second, this socially acquired preference to interact with a particular group of foragers can also be seen as a form of task specialization. Specialization to perform a task on the other hand is likely to affect working efficiency (Ratnieks & Anderson 1999). The association between particular groups of food processors and groups of foragers, based on experience, could increase the efficiency of nectar collection compared to completely random interactions between foragers in food processors.

Third, it might simply be an inevitable side effect of associative learning. Once bees have learned the association between food and odour, the presence of the odour causes a conditioned response like the extension of the proboscis and the attempt to reach the food (Bitterman et al. 1983).

Foragers collecting the known scent had also more followers when they were dancing. These additional foragers could be foragers that were attracted to these dances because they were already collecting this scent during the training phase at a different feeding location. At least some of these additional followers should become recruited to the advertised food source location under natural conditions, which means that there is another factor positively affecting the exploitation of plant species already familiar to the colony.

Given the positive effect of the number of receivers during unloading on dance motivation and the positive effect of our treatment on receiver number, bees collecting the KS should also have danced more. This, however, was not the case. In our experiment it was not possible to prevent foragers from being affected directly by our treatments (see above). Additionally, the presence of a scent in a sugar solution has complex concentration and time dependent effects on the dance motivation of foragers collecting the solution (Lindauer 1948; Kaschef 1957). However, when separating the treatment effect on dancing before unloading and after unloading, evidence for a positive effect of our treatment can be found: While the KS had an inhibitory effect on dance duration before unloading, there was no longer a difference in dance duration after unloading, i.e. after the bees had the opportunity to acquire information about the interaction pattern.

Our results demonstrate that social experiences in the past affect the ways in which individuals interact. Further research using different species is needed to investigate the effect of experience and learning on interaction patterns and on the perception of these patterns by individuals.

Acknowledgments

We thank Peter Stettler for technical help and Michael Taborsky for logistic support. CG was financed by the Janggen-Pöhn Stiftung and the Berner Hochschulstiftung. This study was supported by funds from ANPCYT, CONICET and University of Buenos Aires. The present study complies with the current laws of the state country in which experiments were performed.

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10

Does pollen function as a reward for honeybees in associative learning?

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10.1 Abstract

The ability of foraging bees to learn an association between floral characteristics such as its odour, colour and shape and a reward such as nectar is key to honeybee foraging success. Here, we tested if also pollen could function as a reward for associative learning in honeybees. We found that large proportions of foragers and bees without any field experience showed an unconditioned response, the extension of the proboscis, after touching the bees' antennae with bee-collected pollen. Furthermore, bees readily learn to associate an odour with pollen in a classical conditioning assay. We suggest that pollen might play an important role as a reward for free flying bees.

10.2 Introduction

Associative learning plays an important role in foraging for honeybees (*Apis mellifera*). When a forager discovers a source of nectar, it learns to associate surrounding visual and olfactory cues with the reward (unconditioned stimulus; US) (von Frisch 1967). This helps bees to return to previously visited food sources or to discover new ones displaying similar characteristics. Bees do not only learn food source characteristics during foraging, but also during social interactions inside the hive (Farina et al. 2005).

However, often bees collect pollen exclusively even if plant species offer both nectar and pollen (Scheiner et al. 2004). Some plant species that are visited by bees, like *Papaver*, *Rosa* and *Solanum*, do not offer nectar at all (Vogel 1983). Moreover, bees that are recruited by dancing pollen foragers successfully learn the pollen odour during these interactions (von Frisch 1967). This raises the question of how bees learn relevant colours, shapes and odours in the field or food odours in the hive if there is no nectar that could function as a reward? Here we test if pollen itself could function as an unconditioned stimulus for associative learning. We test if bees show the proboscis extension response (PER), after touching their antennae with pollen. In honeybees, the PER is an unconditioned response, which is elicited after contact with a US such as a sucrose solution. Bees were either captured at the entrance of the hive (with or without pollen loads) or when they were reared under controlled conditions (caged bees) without ever experiencing pollen. Furthermore, we use a classical olfactory conditioning procedure with pollen as reward to test if foragers learn to respond to a previously neutral odour after pairing the odour with the putative US.

10.3 Methods

10.3.1 Caged bees

Combs with pre-emerging brood were maintained in an incubator (temperature: 36° C, relative humidity 55%). On the day of emergence, bees were introduced into wooden boxes (10cm x 10cm x 10cm). They were fed exclusively 1.8 M unscented sucrose solution ad libitum. The boxes were kept in an incubator (25°C, 55% relative humidity, darkness) for 17 days. Around that age, bees usually become foragers (Seeley 1982).

10.3.2 Foragers

Bees were captured with plastic tubes when they tried to enter the colony. We captured similar numbers of bees returning with pollen packages and bees returning without pollen packages, which are likely to be nectar foragers.

10.3.3 PER testing

One hour before testing, bees were harnessed in plastic tubes so that they could move freely their mouthparts and antennae (Cook et al. 2005). First, the antennae of bees were touched with water and bees extending the proboscis were fed until satiation. Both antennae were touched with pollen and a 1.8 M sucrose solution. One half of the bees was tested first with pollen, the other half with sucrose solution. We checked if bees showed the PER towards the pollen scent immediately before physical contact with the pollen preparation, but observed no such case. Bees were tested with a commercial bee-collected pollen mix (Apícola Calandri). We added 30-50% w/w water to the dried pollen packages.

10.3.4 Olfactory conditioning

A device that delivered a continuous airflow was used for odorant application (see Cook et al. 2005). Foragers that showed the reflexive extension of the proboscis after applying the pollen mix and that did neither respond to the mechanical airflow stimulus nor to the first presentation of the odour were used for the olfactory conditioning. For conditioning we used either a 1.8 M sucrose solution or the pollen mix as a reward and Linalool (LIO) as conditioned odour. The inter-trial interval was about 15 min. Test trials lasted for 46 s and consisted of 20 s of airflow, 6 s of odour (CS) and 20 s of airflow. Only during the first three trials (conditioning trials; C1-C3), the reward was delivered upon the last 3 s of the CS to both antennae and proboscis. Otherwise; only the CS was presented (test trials; T1-T5).

10.4 Results

Figure 10.1a shows that high proportions of all bees extended their proboscis after contacting their antennae with both pollen and a 50% sucrose solution (Pollen vs. 50% sucrose: caged bees, McNemar-Test, $N = 32$, $p = 0.13$; pollen foragers, $N = 86$, $p = 0.63$, no-pollen foragers, $N = 112$, $p < 0.001$).

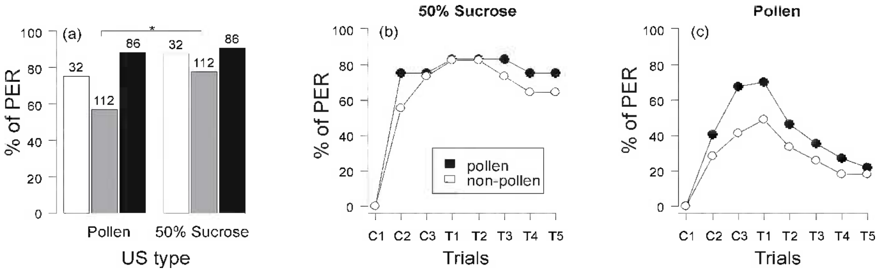

Figure 10.1: Percentage of bees showing the PER. a) Percentage of PER in caged bees (white bars), no-pollen foragers (gray bars) and pollen foragers (black bars) showing the PER after touching the antennae with pollen or a 50% sucrose solution. b) Percentage of PER in no-pollen foragers (white circles) and pollen foragers (black circles) in a classical conditioning procedure with 3 conditioning trials (C1-C3) and 5 test trials (T1-T5) using a 50% sucrose solution as a reward. c) as in b) but with pollen as reward.

Pollen foragers were more sensitive to both pollen (G-test, $\chi^2 = 24.44$, N = 86/112, $p < 0.001$, significant after Dunn-Sidak correction for multiple comparison) and sucrose ($\chi^2 = 6.16$, N = 86/112, p = 0.013, significant after correction) than no-pollen foragers.

Figure 10.1b is an example of a curve for pollen foragers and no-pollen foragers using a 50% sucrose solution as US.

Figure 10.1c shows that 70.3% of all pollen foragers and 48.7% of all no-pollen foragers learn to associate LIO with Pollen as a reward after three conditioning trials (T1, $\chi^2 = 3.62$, N = 37/39, p = 0.057). At T1, more bees respond to LIO if a 50% sucrose solution was used as US (sucrose vs. pollen, $\chi^2 = 4.48$, N = 23/76, p = 0.034, not significant after correction).

10.5 Discussion

A high proportion of bees with and without foraging experience show the reflexive extension of the proboscis after touching the antennae with pollen (Fig. 10.1a). We used bee-collected pollen in our experiment, but Scheiner et al. (2004) showed that foragers also extend the proboscis when using hand-collected pollen. We also found, that bees learn to respond to an odour using pollen as the US in a classical conditioning assay. This strongly suggest, that pollen can play a potentially important role as a reward for associative learning in free-flying bees and for recruits inside the hive. The odour-pollen combination can be quickly perceived by foragers when they work pollen with forelegs and mouthparts during collection or when they antennate and lick pollen from the corbiculae of a returning forager (von Frisch 1967).

The main constituents of pollens are proteins, carbohydrates and water. The relative amounts vary greatly between species and bee-collected pollens usually contain more sugars, which are added by the foragers (Solberg & Remedios 1980). It is likely that it is mainly the sugars the bees respond to, but for example also amino acids, which are common in pollen, can positively affect learning in honeybees (Kim & Smith 2000). More studies with different types of pollen are needed to determine the importance of the different pollen constituents for learning. Furthermore, the role of pollen as a reward for the learning of ecologically relevant cues such as shapes and colours should be investigated under more natural conditions.

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11

Trophallaxis – a mechanism of information transfer

Farina, W.M. and Grüter, C. In: *Food Exploitation by Social Insects: An Ecological, Behavioral, and Theoretical Approach* Eds: Jarau, S. and Hrncir, M. (*in preparation*)

11.1 Introduction

Trophallaxis is the exchange of liquid material between individuals, mostly members of the same colony. Wheeler (1918) was the first to propose the term trophallaxis for describing these interactions between nest-mates in ant colonies. He interpreted them as being "clearly cooperative and mutualistic relationships" and thereby separated his term from the term trophobiosis, which had been suggested earlier by Roubaud (1916) to indicate oral food transfers based on a "trophic exploitation" in social wasps (for review and historical background see Sleigh 2003).

There are two main kinds of intra-specific liquid food transfer in social insect nests; in one, adults exchange liquids with larvae (they imbibe brood larval saliva and transfer glandular secretions, honey and pollen), and in the other, the liquid is transferred between two adults. The stomodeal (or oral) trophallaxes are the most common ones. Donors regurgitate a drop of food from their crops while one or more receivers drink the liquid (Wilson 1971; Michener 1974). During these mouth-to-mouth contacts, intensive antennation (Montagner & Galliot 1982; Hölldobler 1985; Goyret & Farina 2003; Mc Cabe et al. 2006), occasional foreleg movements of both partners (Hölldobler 1974; Mc Cabe et al. 2006) or, as in stingless bees, the transmission of pulsed vibrations (Hrncir et al. 2006) accompany the oral contacts. Abdominal or anal trophallaxes are also used to distribute material such as intestinal symbionts for wood digestion in termite colonies (Grass & Noiret 1945).

The evolutionary origin of trophallaxis might have been related to aggression regulation in group-living insects (Roubaud 1916). Aggressive behaviours often stimulate trophallaxis, which ceases aggression after a food offering (Wilson 1971; Hölldobler 1977; Wcislo & González 2006). However, adult-adult food-sharing may also enhance survival prospects where bad weather prevents foraging for extended periods (Wcislo & González 2006).

The occurrence and high frequency of trophallaxis among adult individuals is a common characteristic of highly social insect species (Michener 1969; Wilson 1971). While eusocial insects such as honeybees and many other bee, ant, termite and wasp species engage in frequent oral interactions inside their nests; oral contacts in communal insects are rare (e.g. halictine bees: Kukuk & Crozier 1990).

During the course of evolution, trophallaxis probably became more important in species for which it considerably improved the efficiency of the performance of vital tasks, e.g. food collection or nest construction. The partitioning of tasks is assumed to increase overall colony task performance (Ratnieks & Anderson 1999). Once material transfer became an important aspect of work organization, it offered an opportunity for both food donors and receivers to acquire information about internal and external environmental parameters via incidental cues, such as searching delays (Seeley 1995), numbers of receivers (Farina 2000), chemosensory cues present in the solution (Gil & De Marco 2005; Farina et al. 2007) and gustatory cues (Martínez & Farina 2007).

The frequency of trophallaxis is highly variable between social insect species and is particularly high in honeybees (Michener 1974; Hölldobler 1977), where trophallaxes occur between bees of all castes and ages (Free 1957; Moritz & Hallmen 1986; Crailsheim 1998; Grüter & Farina 2007). Its observation in ex-

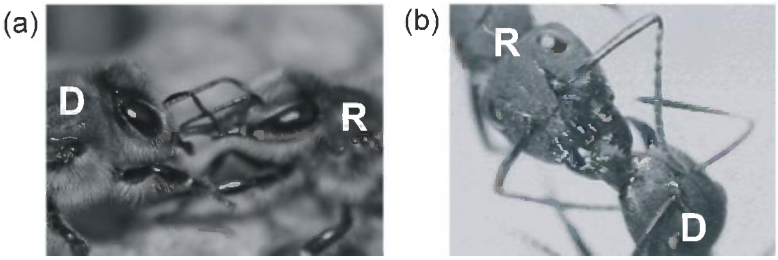

Figure 11.1: Trophallaxis in (a) the honeybee *Apis mellifera* and (b) the carpenter ant *Camponotus mus*. In honeybees, the role of the donor (D) and the receiver (R) of food are easily distinguishable. The receiver bee protrudes her proboscis and contacts the donor's prementum, which causes different head position of the trophallactic partners. In the carpenter ant *C. mus*, the position of the heads and differences in the antennation intensity give information about the trophallactic roles (photos by C. Grüter in (a) S. Mc Cabe in (b)).

perimental hives allows for an easy identification of the bees performing trophalaxes: the food donor opens her mandibles and regurgitates the food; the recipients protrude their proboscis to contact the donor's prementum. Other eusocial insects such as ants and wasps do not have similar feeding-channel structures and the distinction between donor and receiver has to be made either by the head position or by differences in the antennation between partners (Fig.2.1).

As central place foragers, honeybees perform successive feeding trips to a profitable food source, interrupted by hive-stays. This allows the researcher to analyse their trophallactic behaviour in observation hives in the context of foraging (von Frisch 1967; Núñez 1970; Seeley 1986,1989; Farina 1996; De Marco & Farina 2001; Farina & Wainselboim 2001a). In this review we will discuss the role of trophallaxis for the transfer of information in the context of nectar foraging in honeybees. We will denote all mouth-to-mouth contacts as trophalaxes, because even short interactions with a low probability of an effective food transfer potentially provide important chemosensory information.

11.2 Returning to the nest after foraging

Division of labor, i.e. the formation of groups specialized in different tasks, is supposed to improve the efficiency of collective activities in a constantly changing environment (Wilson 1971; Michener 1974). In honeybees, young and middle-aged workers perform in-hive duties (e.g. cleaning cells, caring for brood, grooming, receiving and processing of nectar), while older workers forage outside (Rösch 1925; Lindauer 1952; Seeley 1982). Nectar foraging in honeybees is a partitioned task: foragers collect nectar and transfer the food to bees of middle age (often called food processors, receivers or food storers) in the delivery area close to the hive entrance (Park 1925; Lindauer 1954; von Frisch 1967; Seeley 1995). These bees of middle age are then mainly responsible for processing nectar into honey and storing it in cells (Park 1925).

Foragers returning from a profitable food source sometimes display dance maneuvers (von Frisch 1967; Seeley 1995), which encode information about the location of a food source (von Frisch 1967). The dance also communicates the presence of an attractive food source by increasing the attention and the activity of bees in the vicinity and subsequently the propensity to leave the hive and search for the food source (von Frisch 1923,1967; Božič & Valentinčič 1991; Thom et al. 2007). However, the dance display not only attracts potential foragers but also food processor bees, which unload the forager (Farina 2000). Foragers do not only inform other individuals, they also receive information during these interactions. They seem to use both the time to find a bee for unloading and the number of unloading bees as cue to adjust their dance behaviour after unloading (Lindauer 1954; Seeley 1992; Seeley & Tovey 1994; Farina 2000). Thus, social interactions experienced by the active forager on the delivery area provide foragers with information that helps them to adjust the dance levels according to the general "interest" of hive bees in a particular food source and the availability of food processor bees (Seeley 1995).

Waggle dances are often performed immediately after arrival and before food unloading (Thom 2003). This shows that unloading delays not only depend on the availability of food processor bees but also on the motivation of foragers themselves, which in turn depends for example on the profitability recently experienced at the food source (von Frisch 1967, Seeley 1986). Laboratory studies similarly suggested an important role of the donor: the delays to initiate trophallaxis between worker pairs in experimental arenas are longer for reduced crop loads in donors (Farina & Núñez 1993) and for decreasing sugar concentrations (Tezze & Farina 1999). Thus, the interplay between hive-internal and hive-external information available to the bee determines the amount of dancing (see also Seeley 1995).

Previous experiences of donors and receivers might be another factor affecting queuing delays and the social feedback inside the hive. Experiments with arenas showed that if foragers had prior odour information, new scents present in the crop of the donors negatively affected the occurrence of trophallaxis (Gil & Farina 2003). Similarly, food processor bees show a preference to unload foragers that collect sugar solution with a previously experienced food scent (Goyret & Farina 2005a).

11.3 The offering behaviour of active foragers

Foragers often perform several offering-trophallactic contacts with a wide range of durations after returning from a nectar source (from milliseconds up to more than 60-s in some cases, De Marco & Farina 2001; Farina & Wainselboim 2001a,2005) (Fig. 11.2a). In general, nectar foragers perform one or two trophallaxes longer than 2-3 seconds per hive stay and a much more variable number of shorter offering contacts (De Marco & Farina 2001). During the interactions of at least 2 sec duration, effective food transfers take place between foragers and receivers (Farina & Wainselboim 2001a). Under constant reward conditions the frequency of offering-trophallactic contacts between different foraging trips (short and long trophallaxes) is fairly constant (De Marco & Farina 2001) and is similar for different reward rates offered at the feeder (Fernández et al. 2003). Under such conditions, most of the short-offering contacts occur at the beginning of a hive stay (De Marco & Farina 2001). However, when reward conditions fluctuate, an adjustment of the number of short trophallactic contacts occurs (Farina 199; De Marco & Farina 2001). An increase in profitability causes a rapid increase in the number of the short interactions (Fig. 11.2b). There is therefore a clear and positive relation between the profitability of a food source (i.e. sugar concentration) and the number of short contacts as well as the dance duration in a changing environment (De Marco & Farina 2001). A similar correlation between offering contacts and spinning behaviours was observed in the stingless bee Melipona beeichei (Hart & Ratnieks 2002). In honeybees, short contacts can take place before, during and after dancing (Park 1925; von Frisch 1967; De Marco & Farina 2001; Farina & Wainselboim 2005; Díaz et al. 2007), which leads to a rather equal distributing of contacts during hive stays, at least after an increase in food profitability (De Marco & Farina 2001). This shows that short offering contacts do not simply represent failures to unload all the food at once due to a high motivation for dancing. But why do foragers perform several short offering contacts if the chance to transfer food is very low (Farina & Wainselboim 2001a)? The context in which they occur suggests that they might play a role in providing information about fluctuating resources. Short contacts provide chemosensory information, such as the taste and the scent of the exploited food source (see section 11.7 of this chapter for more details).

While the number of short-offering contacts is highly variable, the number of long trophallaxes (longer than 2-3 sec, Fig. 11.2b) is quite constant and does not seem to depend on the amount of food collected by the forager (Fernández et al. 2003), but rather on the colony nectar influx (Gregson et al. 2003; Huang & Seeley 2003,). Additionally, a mismatch between the crop loads of foragers and the crop capacities of hive bees receiving the nectar seems to explain why foragers perform more than one long unloading trophallaxis (Gregson et al. 2003; Huang & Seeley 2003).

11.4 Dynamics of food transfer

As bees modulate their crop-loading behaviour at the feeding place according to the food source profitability (Núñez 1966,1970,1982), they also adjust their crop-unloading behaviour during the long trophallaxes according to food source profitability (Farina & Núñez 1991). In observation hives, the estimated transfer rate increases with higher crop loads, which in turn depends on the reward rates of the food source (Farina 1996; Farina & Wainselboim 2001a). In an experiment using small interaction arenas, the transfer rate was additionally affected by the sugar concentration of the transferred food (Farina & Núñez 1991; Tezze & Farina 1999) and the reward rate experienced at a food source by a food donor (Wainselboim & Farina 2000a,b). Disturbance of foragers during food collection also affects the transfer rate. In summary, bees seem to evaluate the profitability of the source by integrating an overall flow rate throughout the entire visit, instead of measuring only the current flow rate delivered at the feeder (Wainselboim et al. 2003; Wainselboim & Farina 2003). Furthermore, foragers also seem to be able to detect sudden changes in the delivered flow of solution within a single foraging bout and subsequently adjust the transfer rate in relation to these changes (Wainselboim et al. 2002; Wainselboim & Farina 2003).

But does the modulation of trophallactic behaviour by donors actually modify the behaviour of food-receiver bees? Infrared thermal analysis of foraging bees showed that if a feeder offered food with a higher reward rate, foragers initiate unloading inside the hive at higher thoracic temperatures compared to low reward rates. During the food transfer, receivers actively heat up their thoraxes. The heating rate positively correlates with forager temperatures and the reward rate exploited by the donor (Farina & Wainselboim 2001a; Fig. 11.3a).

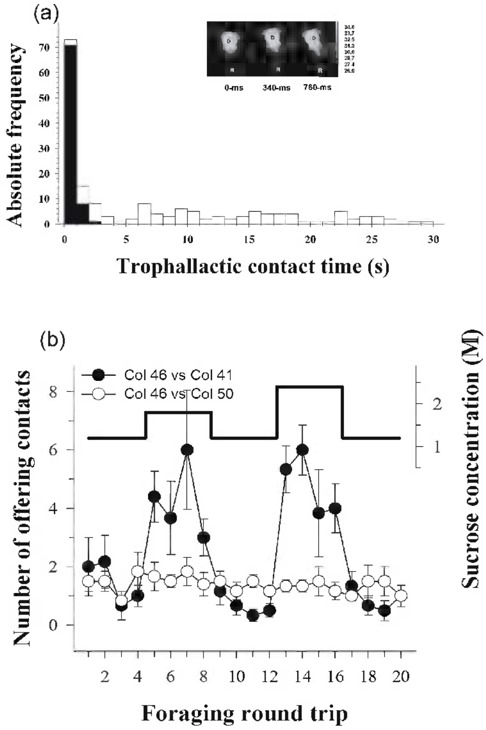

Figure 11.2: Trophallactic-offering behaviour by active foragers. a) Frequency distribution of the offering contacts performed by a donor forager in relation to the contact duration. Bees collected 50% w/w sucrose solution from a rate-feeder which provided a constant flow rate of 8.2 μl/min. Black bars: contacts without increase in the receiver's proboscis temperature; white bars: contacts with an increase in proboscis temperature. A temperature increase represents an effective transfer of the liquid food amongst partners. Insert figure: Thermograms showing the surface temperatures of a donor forager (D) and a receiver (R) at three different times during one trophallaxis. The proboscis temperature of the receiver rapidly changes while ingesting the "hot" liquid from the donor. Donor head and thorax temperatures remained at 39.4°C during the sequence. The grey scale at the right indicates the temperature ranges measured (After Farina and Wainselboim 2001b). b) The number of offering contacts of the incoming foragers performed during their hive stays were categorized according to their length: shorter (filled circles) or longer than 2-s (empty circles). A variable reward program with constant flow rate (5 μl of sugar solution per minute) during 20 consecutive round trips was used. The sucrose concentration changed after four foraging bouts (modified from De Marco and Farina 2001)

These heating rates also depend on the orientation of the receiver towards the donor. Receivers positioned frontally to the donor forager warm up faster and attain higher proboscis temperatures than those positioned laterally (Farina & Wainselboim 2001a; Fig. 11.4a). This would not be expected if receivers were evaluating forager temperature or if they were receiving equal proportions of the unloaded solution. Receiving bees (mostly food processors) also adjust their nectar processing behaviour in accordance to the profitability of the nectar source. After receiving nectar, food processors perform offering contacts or cell inspections and often both behaviours before returning to the delivery area (Pírez & Farina 2004). When they performed a single task, either the occurrence of cell inspections increased or contact offerings decreased for the highest reward rate offered to the donor forager in that study. These results strongly suggest that first-order receivers acquire quantitative information about the nectar source exploited by foragers.

Another factor, which correlates with aspects of trophallaxis, is the intensive antennal contacts performed by trophallactic partners during food transfer (Montagner & Galliot 1982). Antennal movements of donor and receiver during food transfer are rapid in honeybees (mean frequency of 13 Hz) and they seem to vary according to the reward rate experienced by the food donor, showing a positive correlation in both trophallactic partners (Goyret & Farina 2003; Fig. 11.3b). Information related to the food supply of the colony might also be encoded in the tactile stimulation during trophallaxis, as has been suggested for the carpenter ant *Camponotus mus* (Mc Cabe et al. 2006).

11.5 Changing the trophallactic role

After crop unloading, foragers walk across the delivery area to the hive entrance and often protrude their proboscis touching the mouthparts of their nestmates. It has been suggested that these begging contacts are refueling events for the forthcoming foraging trip (Beutler 1950; von Frisch 1967). After leaving the hive, foragers carry more food if they do not know the feeding site well (Brandstetter et al. 1988) or if they collect far from the nest (Istomina-Tsvetkova 1960). This could be explained either by refueling inside the hive or by unloading only a part of the collected crop. However, begging behaviour can also be found in foragers, which have a lot of foraging experience at a feeder with a constant (and short) distance from the hive (Núñez 1970; Farina 1996; De Marco & Farina 2001). So, why do these bees beg for food? It would seem much more efficient, if experienced bees would simply retain the amount, which

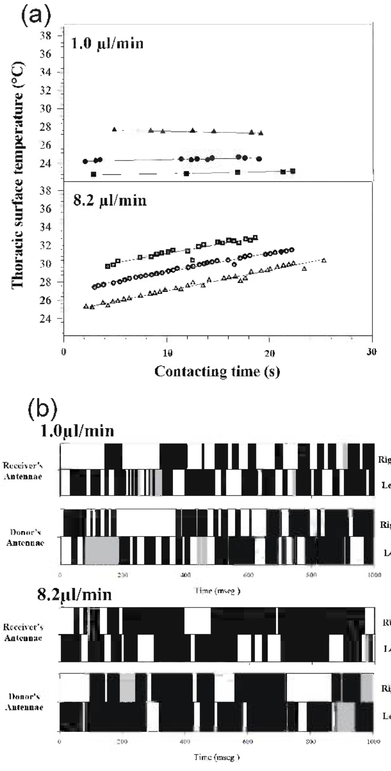

Figure 11.3: Thermal behaviour and antennation during trophallaxis in honeybees. a) Thermal behaviour of food receivers during a trophallaxis with a forager trained to collect either 1 or 8.2 μl of a 50% sucrose solution per minute at a feeding station (After Farina and Wainselboim 2001a). b) Antennal contacts (strokes, black bars) of a donor and a single receiver during a long trophallaxis inside the nest. The donor forager returned from a rate-feeder offering one of the two reward rates mentioned in a). Temporal resolution: 5-ms. Grey bars represent moments where the position for the antenna could not be precisely determined (After Goyret & Farina 2003). Under these experimental conditions, the mean values of the thoracic temperature of the donor foragers at the beginning of the trophallaxis were 31.8°C for a reward rate of 1 μl/min and 37.5°C for 8.2 μl/min respectively; while the estimated transfer rates of the donors were 1.1 and 2.1 μl/s for both reward rates respectively.

is necessary for the flight, during unloading.

Food source profitability affects the forager's begging behaviour as it affects short offering contacts, but in the opposite direction. Nectar foragers increase their frequency of begging-contacts after food unloading when they return from a low profitable source (Farina 1996; De Marco & Farina 2001) or if the diversity of odour cues and food qualities encountered in the exploited food patch is enhanced (De Marco & Farina 2003). These contacts often last less than 1 second (De Marco & Farina 2003), which means that there is a very low probability of actual food transfer takes place (Farina & Wainselboim 2001b). Hence, it is very unlikely that foragers are refueled during these begging interactions.

An alternative hypothesis proposed that begging might be a means of information acquisition (Núñez 1970; Farina 1996; De Marco & Farina 2001,2003). Incoming foragers may direct their begging behaviour to other employed nectar foragers in order to obtain resource related chemosensory information, which facilitates the reevaluation of their food source. Employed foragers could then decide either to continue exploiting their food source, to switch to a previously exploited one, which reappeared (indicated by the presence of its scent in the hive), or to abandon it and stay in the hive. However, until now there is no evidence for these hypotheses and begging contacts remain a puzzling phenomenon.

Experimenting with low reward-rate conditions can help us to understand communication strategies in a more natural context. Natural flowers normally offer minute amounts of nectar with variable flow rate (Núñez 1977; Vogel 1983) and bees often visit several hundred flowers per foraging trip (Ribbands 1949). Many of the modulatory effects described above become apparent only when bees collect food at a low reward rate.

11.6 The distribution of the nectar inside the hive

After receiving food from foragers, a majority of processor bees offers food to other bees on their way to the honey cells, sometimes large parts of their load (von Frisch 1923; Park 1925; Seeley 1989; Pírez & Farina 2004; Grüter & Farina 2007). Compared to about 0.25-0.75 trophallactic contacts per 10 minutes of average workers that are not involved in food processing (Istomina-Tsvetkova 1953a,b cited in Free 1959; Grüter & Farina 2007), food processor bees have been shown to perform between 4.3-10.5 contacts per 10 minutes (Seeley 1989), which highlights their role in the rapid distribution of collected food amongst hive bees.

The bees receiving food from processors (second-order receivers) can be other foragers, food processors but mainly nurse bees (Grüter & Farina 2007). These second-order receivers perform about 4 contacts per 10 minutes; most of them are offering contacts. As a consequence the incoming nectar is rapidly distributed among bees of all ages. Nixon and Ribbands (1952) measured food distribution using radioactive tracer in the sugar solution (^{32}P). They fed between 5 and 9 foragers belonging to colonies of different population sizes with 10-20 ml of radioactive solution and found that four hours later a majority of hive bees received food samples within that time. Similarly, DeGrandi-Hoffman and Hagler (2000) found a rapid distribution of food amongst young hive bees by using a protein marker.

Characteristics of food processing such as rate of trophallactic events or the food storing behaviour of the hive bees depend on many factors such as the nutritional state of the colony, the amount of brood, genotype, the amount of incoming nectar or the season (Free 1959; Kloft et al. 1976; Hillesheim 1986; Seeley 1989; Crailsheim 1998). While experiments with caged bees also showed an effect of age on the trophallactic activity (Moritz & Hallmen 1986), in beehives the trophallactic activity of workers seems to depend more on the performed task than on the age (Free 1957).

Another interesting aspect of trophallaxis in honeybees is that the transfer rates of subsequent trophallaxes positively correlate (Goyret & Farina 2005b). In other words, trophallactic experiences of bees affect their trophallactic behaviour in the immediate future in similar ways as nectar flow rates affect the unloading rate of foragers. So, receiving bees that are not directly unloading foragers might still be able to acquire information about the colony's foraging situation.

Given the extensive sharing of food amongst bees of all ages, cues conveying information about food source characteristics present in the collected food can reach most hive individuals in a relatively short time. Information available to most or all members of a colony, or "global" information (Mitchell 2006), potentially affects the behaviour of most nestmates, thereby causing a "global" response (Moritz & Southwick 1992; Pankiw et al. 2004). For example, the sugar concentration of incoming nectar affects the sugar response thresholds (SRT) of nectar receivers (Martínez & Farina 2007) and even of young hive bees, that are not involved in foraging (Pankiw et al. 2004) and have little direct contact with engaged foragers (Seeley 1995). This result not only indicates a fine-tuning of sensory thresholds in hive bees, but also highlights the role of trophallaxis as a mechanism to transfer gustatory information in honeybees (Martínez & Farina

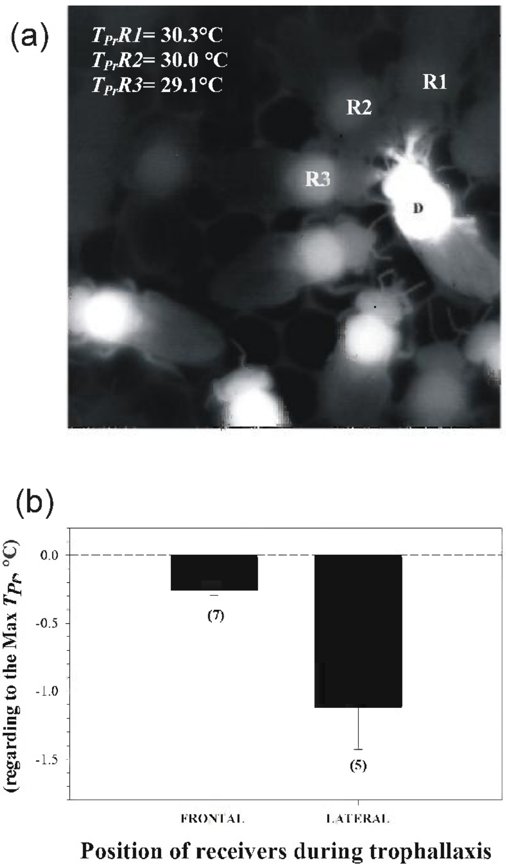

Figure 11.4: A trophallaxis with multiple food receivers. a) A thermogram showing the food donor (D), which was an active forager trained to collect at a rate feeder that offered 8.2 μl of a 50% sucrose solution per minute. The frontal receivers (R1 and R2) attained higher proboscis temperatures than the other receiver R3 did (see temperature in the insert). The heating of frontal receivers was independent of the number of simultaneous receivers. b) The mean differences between one receiver's proboscis temperature and the maximal proboscis temperature found in the simultaneous receivers according to their position during trophallaxis with an incoming forager. The receiver placed in front of the donor forager had lower temperature differences compared to the maximal proboscis temperature of lateral receivers (Farina, unpublished data).

2007).

11.7 Odour learning through trophallaxis

Bees are excellent learners and readily establish associations between odours (or other cues) and a reward such as a sugar solution (e.g. von Frisch 1967; Koltermann 1969; Menzel 1999). During olfactory conditioning the sugar solution functions as an unconditioned stimulus (US), while the odour becomes the conditioned stimulus (CS).

Olfactory learning has a strong effect on foraging decisions (von Frisch 1967). In a series of simple and elegant experiments, von Frisch (1923) showed that bees recruited by a forager later showed a strong preference for the food odour brought back by the recruiting bee. In the meantime, this has been shown also in other social insects such as bumblebees (Dornhaus & Chittka 1999), stingless bees (Lindauer & Kerr 1960), wasps (Maschwitz et al. 1974) and ants (Roces 1990). It has been suggested that recruits learn food odours while receiving food samples from foragers, i.e. during trophallaxis (von Frisch 1967). The transferred food samples could function as a reward for learning in recruits. Dirschedl (1960) found, that more than 95% of the recruits arriving at a feeder that offered stained food received small food samples inside the hive before leaving the colony.

The proboscis extension response (PER) test as been used with big success to study associative learning and to analyse the physiology and the memory process rules underlying learning in honeybees under controlled laboratory conditions (Kuwabara 1957; Bitterman et al. 1983; reviewed in Menzel 1999; Menzel et al. 1993). Bees extend their proboscis when antennal, tarsal or proboscis chemoreceptors are stimulated with a sucrose solution (US). If an odour (CS) is presented simultaneously, the odour itself becomes capable of eliciting the proboscis extension as a conditioned response (CR), often after only one learning trial.

More recently, the PER method was also used to test learning during trophallaxis. Honeybees associatively learn food odours while receiving food from other bees under different behavioural contexts (e.g. within an experimental arena: Gil & De Marco 2005; within an observational hive: Farina et al. 2007). These experiments showed, that increasing the concentration of either the CS or the US results in better learning during trophallaxis (Gil & De Marco 2005). Interestingly, successful learning does not seem to depend much on the duration of the oral contact (trophallaxes as short as 1.2 sec led to learning; Gil & De

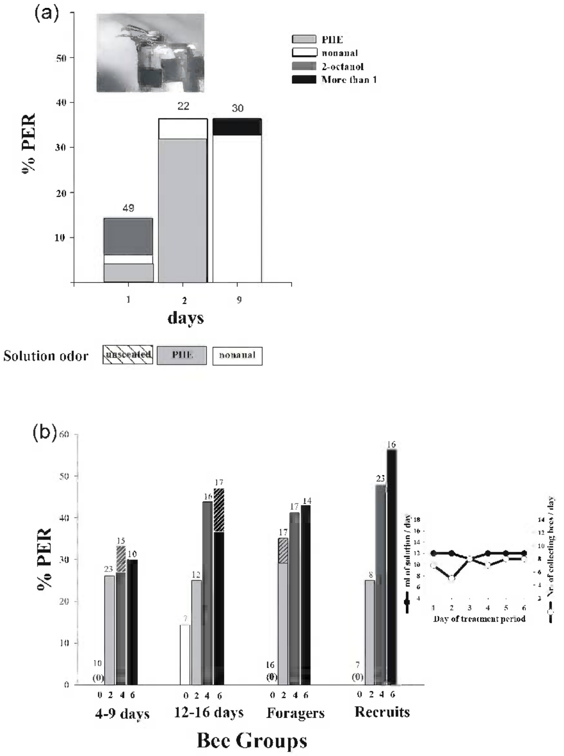

Figure 11.5: Olfactory memories formed within the hive during the sharing of scented food. a) Proboscis extension response (PER) percentages for food-receiver bees that extended the proboscis on the first presentation of an odour. A group of foragers collected a 2.0-M sucrose solution which was either unscented (day 1), scented with phenylacetaldehyde (PHE) (day 2) or scented with nonanal (day 9). Responses for PHE (grey), nonanal (white), 2-octanol (dark grey), and for more than one test odour (black). Number of tested bees above bars (After Farina et al. 2007). b) The percentage of bees showing the PER on the first presentation of a treatment odour and a novel odour for 4-9 days old bees, 12-16 days old bees, foragers, and recruits. Solution odour (filled bars), novel odour (crossed bars), or both odours (hatched bars). PER frequencies were measured on days 0, 2, 4, and 6 after starting to feed a colony with a sucrose solution scented with Linalool (LIO). The insert figure shows the daily quantity of the collected sucrose solution in ml (emptied circles) and the number of trained foragers (filled circles) (After Grüter et al. 2006)

Marco 2005). For this kind of learning it should be of little importance if the odour is perceived in the solution or on the bees bodies, most important is the contiguity between CS and US (Rescorla 1988; Menzel et al. 1993).

Furthermore, the PER test showed that bees that are recruited to a food source associatively learn food odours inside the colony (Farina et al. 2005; Grüter et al. 2006; Fig. 11.5). Similarly, food processor bees that unloaded foragers also show an elevated probability to show the PER for a particular scent after unloading food containing this scent (Farina et al. 2007; Fig. 11.5a).

Grüter et al. (2006) fed scented sucrose solution to marked foragers during about a week and measured the PER of bees of 4-9 days, of 12-16 days and of a sample of foragers during this period (Fig. 11.5b). During the feeding period, the proportion of bees extending their proboscis upon presentation of the solution scent increased for all age groups, which shows that olfactory information about the flower species exploited by foragers propagates within the entire colony as a consequence of food sharing. This potentially has long-term consequences, since olfactory memory acquired inside the hive can be stored in long-term memory (LTM) (Farina et al. 2005; Gil & De Marco 2006; Arenas et al. 2007), a form of memory, which affects the behaviour of bees for several days (e.g. Menzel 1999).

In the described experiments, the PER frequencies were somewhat lower than expected, if comparing them to learning performances under controlled lab conditions, where one learning trial often leads to about 50% of bees responding to the odour (Menzel et al. 1993). However, there are several problems with such a comparison. First, in the previously mentioned experiments, bees were tested in a context, which was different from the context the bee experienced when learning the odour. This can cause a considerable reduction in performance (Bouton & Moody 2004) and lead to an underestimation of information acquisition. Second, bees in a hive do probably not always perceive odours associated with a reward, but potentially often without it, e.g. when contacting a dancing bee without receiving a food sample. These CS-only experiences (retrieval trials after acquisition) often result in a reduction of the conditioned response (CR) (Stollhoff et al. 2005).

For olfactory information propagation to work, the scent must be present in the nectar. But are nectars scented at all? Unfortunately, very little is known about the presence of scents in nectars of most plant species visited by bees. Apparently, many types of nectar contain scents but systematic studies on the distribution of scented floral nectar are needed (reviewed in Raguso 2004).

The propagation of olfactory information among hive bees has important ecological and behavioural implications. Novice foragers without foraging experience leave the hive with socially acquired olfactory information. Olfactory information acquired this way can help the bees of a colony to discover new plants of the same species. This is the basic principle of attempts to guide bees to certain plant species by feeding colonies with scented sugar solution in order to increase visitation rates and seed production of these plant species (von Frisch 1943,1967).

Olfactory memory also affects unloading decisions of food processor bees, as discussed above (Goyret & Farina 2005a). As a consequence, the flower constancy observed in honeybee foragers (von Frisch 1967) can already be observed in nectar processors during unloading, but in a lesser degree. As a consequence, a forager returning with a new odour might be received with less interest by nectar processors, which in turn could affect the foragers' motivation to perform dances (see above). However, studies are needed to investigate the consequences of olfactory experiences for in-hive interactions. Additionally, research should be extended to other social insects species to evaluate the general importance of trophallaxis for learning. In the context of olfactory learning, the bee dance might have an amplifying effect. As we mentioned, dancers attract both foragers and food processors and thereby increase the number of trophallaxes (Farina 2000). In this way the dance creates an environment for the acquisition for olfactory information (von Frisch 1923,1967; Díaz et al. 2007).

In a system without central control, understanding how a colony coordinates individuals in order to efficiently obtain and process food represents a fascinating challenge. Trophallaxis seems to be one of the means by which individuals belonging to different worker castes are rapidly informed about characteristics of the collected resources. Hence, trophallaxis is an important mechanism not only to transfer food, but also to inform individuals about fluctuating foraging opportunities, adjust foraging-related in-hive tasks and create informational networks that connect different groups of workers.

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12

General Summary

This project investigated how honeybees acquire and use socially transmitted food odour information in the context of foraging and food processing.

12.1 The acquisition of olfactory information

Food odours are important information cues, which affect foraging decisions of honeybees. We show that honeybees can associatively learn food odours that were brought back by foragers, during mouth-to-mouth interactions inside the hive. The liquid food functions as an unconditioned stimulus, the food odour as a conditioned stimulus in this associative learning (Chapters 3-5). The information can be stored in long-term memory and potentially affects the behaviour of bees for several days (Chapter 3).

We found that not only bees that directly interact with returning foragers learn the food odour, but also bees belonging other working groups and corresponding age-classes, like nurse bees and food processors (Chapter 5). This seems to be a consequence of extensive sharing of food that has been collected by foragers (Chapter 2). This shows that a honeybee learns food odours during her entire lifetime and not only at foraging age. There are many factors that can affect the speed and extent of this information propagation, like the season, the amount of stored honey or the strength and the identity of the odour. One major factor affecting associative learning and therefore the dynamics of information sharing in a honeybee colony is the strength of the unconditioned stimulus or in other words, the sugar concentrations. Correspondingly, we found an effect of the food sugar concentration on the extent of information propagation (Chapter 6). In other words, more bees learn food odours of plant species offering highly concentrated nectar, which in turn is a simple way how colonies can preferentilly forage on high quality food sources.

12.2 The use of olfactory information

While the use of social information by foragers in the field has been extensively studied, little is known about how olfactory memory affects in-hive behaviours. We found that the presence of food odours per se affects the way bees are distributed around foragers (Chapter 7). Dance followers contact dancers where olfactory cues are most intense. Hence, bees following dancers do not only try to acquire information regarding the location of the food source but also to optimise the acquisition of olfactory information.

In chapter 9, we show that memories established during social interactions affect the propensity of food processor bees to unload liquid food offered by returning foragers. Bees prefer to receive food containing an odour, which has been experienced during previous interactions. As a consequence, foragers returning with a scent, which is known to many hive bees find more unloading partners and their dances are followed by more bees compared to foragers returning with a novel scent (Chapter 9). This is has consequences for the recruitment to these food sources because foragers use the number of receiver bees to adjust their amount of dancing.

If an animal uses different sources of information, informational conflicts can occur. For example, private and social information might often be available simultaneously for honeybee foragers. The waggle dance can recruit new foragers to an advertised food source and at the same time reactivate experienced foragers to visit known food sources. This double function sets the stage for a conflict between social and private information. We found that in such a situations foragers used private information in 93% of all cases, i.e. they were not recruited to the indicated food site, but flew to previously visited food locations instead (Chapter 8). This shows, that dance followers are often not interested in the location information encoded in the waggle dance even if they follow dancers sufficiently long to decode the offered information. However, there might be other ecological circumstances under which foragers pursue a different strategy.

In chapter 10, we tested if pollen could function as an unconditioned stimulus in associative learning and found that honeybee foragers readily learn to associate pollen with a floral odour. If a plant species offers only pollen, this reward-function of pollen could help foragers to learn plant characteristics like the plant odour, colour or shape.

Acknowledgements

Many people helped me during the last 3 1/2 years and made the project a very exciting adventure. First, I would like to thank Walter Farina for giving me the opportunity to work in his lab, for his encouragement, his interest in my ideas and his enthusiasm. He always had time to discuss problems and to help me adjusting to the life in Buenos Aires.

I thank the crew-members in Buenos Aires for being both very motivating colleagues and friends and for making the time in the lab fun. I'm especially grateful to Andres Arenas for all he taught me about bees and for helping in moments of crisis. Especially the experiment presented in chapter 6 was close to failure more than once, e.g. after swarmings, but his expertise saved the experiment.

Paula Díaz, Sol Balbuena and Luis Acosta put a lot of effort into the experiments and their work was fundamental for the success of many of the experiments. Discussing experiments or statistical problems with Vanesa Fernández and Sofi Mc Cabe was always helpful and instructive. Gabi Ramírez and Gonzalo Corti Bielsa helped with data collection, Hector Verna provided technical help, Roxi Josens and Agustina Falibene provided many useful suggestions during the entire period.

I'm deeply indebted to Michael Taborsky for allowing me to work at Hasli and providing me with the necessary infrastructure to perform experiments in Hinterkappelen.

I would not have been able to perform the experiments at Hasli without the help and bee-keeping experience of Peter Stettler. He did all the bee-keeping work, helped moving colonies and bred the queens for my observation hives. He always had time to chat about the practical aspects of my work.

I'm very grateful to Roger Schürch for all the discussions about my experiments, science and life in the last 3 1/2 years. I enjoyed every meal he cooked and his help with statistics and software saved a lot of time.

Many people helped me with data collection in Switzerland. Without any prior experience, they showed no fear to closely observe and capture bees. Spe-

cial thanks go to Séverine Loosli who helped me during two experimental seasons, but also Sabina Joos, Rebecca Schreier, Dolores Schütz, Francisca Segers, Andrea Woodtli, Adrian Britschgi, Jürg Logue and Tomi Reichlin contributed to the presented work.

Many thanks go to Francisca Segers who commented on ideas, collected data, critically read parts of the thesis and helped me staying calm in moments of stress towards the end of my thesis.

The comments and suggestions of Dik Heg, Ian Hamilton and Daniel Rankin helped to improve some of the manuscripts considerably. I also would like to thank the rest of the "haslians" for many discussions during the last years, Marlis Gerteis for helping me with all the administrative aspects of my PhD and Marcel Moser for IT support. Thanks also to Prof. Lars Chittka who kindly agreed to act as my external referee and to Prof. Heinz Richner who offered to chair the defence of this PhD thesis.

The following organisations provided money for this project:

- Janggen-Pöhn Stiftung
- Dr. Joachim De Giacomi Stiftung
- Basler Stiftung für biologische Forschung
- Verein deutschsprachiger und rätoromanischer Bienenfreunde (VDRB)
- Swiss Academy of Sciences (SAS)
- Berner Hochschulstiftung
- Association for the Study of Animal Behaviour (ASAB)

Finally, I would like to thank my parents for always supporting me patiently in all possible ways on my way into science.

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Education

1998 – 2001	1^{st} and 2^{nd} Vordiplom (Bachelor) in Biological Sciences at the University of Bern, Switzerland, with main concern on Zoology.
2001 – 2002	Studies at the Universitá RomaTre in Rome, Italy, with main concern on Zoology and Molecular Biology.
2002 – 2003	Master in Behavioural Ecology at the ethological field station Hasli, University of Bern, Switzerland, about biparental care and monogamy in the cichlid fish, *Eretmodus cyanostictus*.
2004 –	PhD-Thesis in Behavioural Ecology, University of Bern, Switzerland and the Social Insect Group, Universidad de Buenos Aires, Argentina. Supervisor: M. Taborsky, W.M. Farina, Titel: Social learning of food odours in Honeybees (*Apis mellifera*).

Practical training

1999	Volunteer in a project about dispersal and behaviour of several shark species in Florida, USA.
2000	Working as a volunteer in a monitoring project about capercaillie abundance in Germany.
2001	Marine-biological field course on the island of Elba, Italy at the Hydra Institute for Marine Sciences.
2002 – 2003	Laboratory assistant at the University of Bern, for the Division of Behavioural Ecology in a project on life history decisions in a cichlid fish species.
2003	Volunteer in a project about the physiology of hibernating Marmots in the Swiss Nationalpark.
2004	Assistant at the Universidad de Buenos Aires, Argentina in a project about trophallaxis in the honeybee, *Apis mellifera.*
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Teaching	
2001	Mathematics teacher at the Kantonsschule Sursee LU, Switzerland.
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Publications

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Grüter, C., Acosta, L.E. & Farina, W.M. 2005 Propagation of olfactory information within honeybee hives. 3^{rd} European conference on social learning in animals, St. Andrews, Scottland.

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