Dynamics and Communication Structures of Nectar Foraging in Honey bees (*Apis mellifera*)

Dissertation zur Erlangung des

naturwissenschaftlichen Doktorgrades

der Bayerischen Julius-Maximilians-Universität Würzburg

vorgelegt von

Corinna Thom

Bonn

Würzburg, 2002

Eingereicht am:

Mitglieder der Promotionskommission:

Vorsitzender: Prof. Dr. R. Hedrich

Gutachter : Prof. Dr. J. Tautz

Gutachter: Prof. Dr. S. Fuchs

Tag des Promotionskolloquiums:

Doktorurkunde ausgehändigt am:

Table of Contents

General Introduction

The tremendous ecological success of social insects is based on the internal organization of their colonies. Social insects coordinate their action without central control. The mechanisms that organize their colonies are the division of labor, whereby members specialize in a subset of all tasks the colony has to perform, and the coordination and behavioral integration of individuals (Oster & Wilson 1978, Wilson 1987, Hölldobler & Wilson 1990, Bourke & Franks 1995). Division of labor allows individuals to concentrate their task performance, and thus to specialize to tasks. Integrating the specialized members into colony processes, and coordinating their combined action then allows the colony to efficiently master several tasks at the same time (Oster & Wilson 1978, Seeley 1995).

The most basic division of labor in social insect colonies exists between the reproductive and the non-reproductive individuals. A further division of labor exists between the non-reproductive individuals, the workers, of the colony. Two general patterns of division of labor among workers are recognized: 1. temporal polyethism, in which the worker's physiological state and its probability of task performance are correlated with age, and specializations are therefore temporary, and 2. physical polyethism, in which workers are morphologically adapted to certain tasks, and thus permanently specialized. While physical polyethism is observed only in relatively few species, temporal polyethism is widespread among social insects (Wilson 1971, Hölldobler & Wilson 1990, Oster & Wilson 1978). This is possibly because a system with flexible specializations can react more efficiently to environmental changes than a system with permanent specialization, and has thus an advantage in most environments (Seeley 1995).

The mechanisms that allocate a colony's labor among different tasks are various and operate on several levels. One principal factor that ensures an efficient allocation of labor is communication among colony members. Communication has been defined in several ways, but generally occurs when information given by one group member influences the behavior of another group member in a way that benefits the sender of the information (e.g. Wilson 1971, Bradbury and Vehrencamp 1998). A social insect usually benefits from releasing information to colony members, because the reproductive interests of the sender and receiver of information in a colony are closely related to each other and depend on the state of their common colony. Both sender and receiver of information can either be an individual or a group. Thus, information can

flow from individual to individual, from the individual to the group and from the group to the individual.

There are two general ways of how information can be transmitted: 1. via signals that are shaped by natural selection specifically to convey information (e.g. recruitment dances), and 2. via cues that convey information although they have not been shaped by natural selection to do so (e.g. degree of crowding at a food source) (Lloyd 1983, Seeley 1995). The process of group integration is largely a matter of information flow from the group to the individual, so that each individual can tune its activities in accordance with the activities of the other group members. When information flows from a group to an individual, cues are usually predominant over signals. A group will typically produce cues as a by-product of their combined actions that individuals can use to tune their behavior in on the group. On the other hand, specific signals that inform about the state of a group will only rarely evolve (Seeley 1995).

The modes of communication in social insects are extremely diverse and include chemical, visual, acoustical, and tactile signals (e.g. Hölldobler & Wilson 1990, Bradbury and Vehrencamp 1998). The best studied communication signal in social insects is probably the waggle dance of the honey bee (*Apis mellifera*). The waggle dance serves to recruit foragers to profitable food sources and is part of a complex feedback system. This system allows a honey bee colony to efficiently allocate workers among the two subtasks of foraging, nectar collection and nectar reception, and to concentrate the nectar collectors' effort on the most profitable food sites (e.g. v. Frisch 1967, Lindauer 1948, Seeley 1995). Thus, the foraging communication system allows honey bees to forage efficiently in an unpredictable environment. Although the foraging communication of honey bees has long fascinated researchers, we are only beginning to understand how the interactions of the system components organize the task of foraging (e.g. v. Frisch 1967, Lindauer 1948, Nieh 1993, Kirchner 1993, Kirchner & Lindauer 1994, Seeley 1995). In this thesis, I examine several aspects of honey bee foraging to achieve a better understanding of how communication serves to allocate labor and to integrate the behavior of colony members into the colony's collective foraging effort.

In the first of the thesis' four chapters, I address the basic question of how a honey bee colony organizes its nectar foraging effort from day to day. I do so by examining the dynamics of two factors that largely determine colony foraging effort, the number and activity of a colony's nectar foragers. The following three chapters are then devoted to the foraging communication system of the honey bees. In the second chapter, I investigate whether honey bees produce a

chemical signal to quickly activate nectar foragers. A fast volatile signal may complement as an activating signal the waggle dance, which activates foragers relatively slow and in a restricted area of the hive, but more specific as to quality and location of a food source. The third chapter introduces a signal that functions in the foraging communication system, the brief piping signal of nectar foragers (Nieh 1993, Kirchner 1993). I experimentally establish the context of the signal, examine its acoustic properties, and describe for the first time the unique behavior of the nectar foragers that produce brief piping signals. I then discuss cause and function of the signal in the foraging communication system.

Some of my observations on the behavior of piping nectar foragers have implications for another signal in the foraging communication system, the tremble dance. The tremble dance is regularly performed by nectar foragers to adjust and coordinate the colony's foraging effort (Lindauer 1948, Kirchner 1993, Seeley 1992, Seeley et al. 1996). The cause of the tremble dance was shown to be a long unloading delay for returning nectar foragers. Returning foragers unload their nectar not into cells, but to a receiver bee. A shortage of receiver bees will lead to long unloading delays, which stimulate nectar foragers to tremble dance and thus to recruit additional nectar receiver bees. In the fourth chapter of my thesis, I examine whether hive-external tremble dance factors can also directly stimulate tremble dancing, and discuss the implications this has for the function of the tremble dance in the foraging communication system of the honey bee.

Chapter I

Dynamics in the allocation of labor to nectar foraging in a honey bee colony

Abstract

In a honey bee colony, the adjustment of the labor devoted to nectar foraging is expected to be adaptive, because foraging conditions strongly fluctuate. The proportion of a colony's workers engaged in nectar foraging and the activity of each nectar forager are two parameters that might be involved in the adjustment. In this study I measured for the first time the proportion and activity of nectar foragers in a honey bee colony. Random samples of 50 bees were individually marked and observed during 6–13 day observation periods in late spring and early summer. Observations started at 0500 and lasted without interruption until 1900–2100 or until foraging activity stopped. The identity of each marked bee that left or entered the hive and both the departure and arrival times of the trips were recorded. A nectar forager was defined as a bee that, after a trip of 10 min or longer, unloaded at least once a day liquid to a receiver bee (Fig. 1). Between 0 and 67% of the workers engaged in nectar foraging on a given day, with a mean of 34 ± 18% per day. The percent nectar foragers in the colony changed significantly between days in 5 of 6 observation periods (Fig. 2). On average, 66% of the nectar foragers made 1–4 foraging trips per day, 34% made 5–10 trips, and no bee made more than 10 foraging trips per day (Fig. 3). The mean number of trips per nectar forager per day was 3.5 ± 1.3. The majority of the nectar foragers (over 70%) foraged for 4.5 h or less, even though there were approximately 15 h of daylight each day. The activity of the nectar foragers in the colony changed significantly between days in only 1 of 6 observation periods (Fig. 4). The results of this study suggest that a honey bee colony adjusts its daily foraging effort mainly by changing the number of nectar foragers rather than the activity of the nectar foragers. This might enable a colony to exploit nectar sources faster and more efficient. It is likely that the changes found in the allocation of labor to nectar foraging are due to changes in the nectar that is available to the nectar foragers.

Introduction

Division of labor is common to all insect societies and is regarded as one of the most important factors in their ecological success (Bourke & Franks 1995, Hölldobler & Wilson 1990, Wilson 1985, 1987). A key feature of the division of labor in insect colonies is its plasticity. Changing the allocation of workers among tasks enables a colony to respond adaptively to changes in external and internal conditions (Gordon 1989, Robinson 1992, Seeley 1995).

Flowers, the ephemeral food sources of honey bees, are subject to strong temporal and spatial fluctuations. Hence, adjustment of the foraging effort of a honey bee colony is likely to enhance it's ability to gather enough nectar for survival and reproduction. Although there are several ways by which a colony can adjust the allocation of its workers among different task (Robinson 1992), one of the most important is the behavioral flexibility of the individual workers in a colony. A colony's rate of nectar collection is a function of three variables: (1) number of active nectar foragers, (2) mean activity level of the nectar foragers, and (3) mean volume of the nectar loads (Seeley 1995). Each of these variables might be adjusted by the behavioral flexibility of the nectar foragers to cope with changing conditions. Some is known about the third variable: load volume increases the higher the quality of the nectar source, and it decreases the greater the distance of the source from the hive (Núnez & Giurfa 1996, Schmidt-Hempel et al. 1985). However, the mean volume of nectar loads probably varies only by a factor of two or three (Winston, 1987), while the number and the mean activity of nectar foragers may each vary by a factor of ten or more. Thus, changes in the mean volume of nectar loads probably contribute relatively little to the adjustment of a colony's nectar foraging effort. By counting and observing the nectar foragers in random samples of honey bee workers, I was able to monitor for the first time changes in the number and the activity of the nectar foragers in an undisturbed honey bee colony.

Material and Methods

Study site

The study was conducted in May, June and July, 1999, at the honey bee laboratory of the University of Würzburg, Germany. The laboratory is surrounded by fruit orchards and rape (*Brassica napus*) fields which provided nectar in May and June.

Bees and observation hive

Observations were made on one colony of the carniolan honey bee, *Apis mellifera carnica.* The colony (about 4000 bees) was housed indoors in a three-frame observation hive with internal dimensions of 65 cm * 45 cm * 5 cm, with an entrance tunnel leading outside. The colony inhabited the hive since May, 1998, hence was well established by the time I began the observations. No supplementary feeding was necessary. The queen and brood were restricted to

the lowest frame by a queen excluder, while the workers could move freely between the frames. The upper two frames of the hive always provided abundant storage room for the nectar that was gathered by the foragers.

Sampling and marking techniques

A random sample of 50 bees was marked at about 2300 the night before I began each 3-day period of observations. The random sample was obtained by placing paper grids, each with 150 rectangles, on both sides of the opened hive, and collecting from each rectangle the bee that was closest to the center. These 300 bees were put in two small cages; from each cage 25 bees were randomly selected, yielding a sub-sample of 50 bees. The other bees were then returned to the observation hive. The bees of the sub-sample were individually marked by gluing number tags (Opalithplättchen) to their thoraces. The marked bees were put back into their colony immediately after marking; no aggressive interactions with other workers that might influence the behavior of the marked bees were observed. The number of marked bees was recorded on each day of observation.

Behavioral observations

Observations were made for three consecutive days (one observation period) after the marking of the bees. Altogether, there were 6 observation periods. The data from 24 May 1999, the first day of the first period, were not included in the analysis, as the observation methods were still being refined. Observations started at 0500 and lasted without interruption until 1900-2100 or until foraging activity stopped. During observations, the entrance tunnel and the unloading area inside the hives' entrance were watched to determine the identity of each marked bee that left or entered the hive, and to record both departure and arrival times of these bees. The time records enabled me to determine the number of trips per worker and the length of each trip (time outside the hive). If only the departure or the arrival was observed, the number of trips per bee could still be calculated, while the time the bee spent on this trip could not. It is very unlikely that both the departure and the arrival of a marked bee were missed. Upon entry into the hive, each marked bee was observed until either she unloaded liquid, by allowing another worker to put its proboscis between her mandibles and suck up the regurgitated liquid, or she received food herself, without prior unloading.

Definition of a nectar forager

Nectar foragers had to be distinguished from other bees entering the hive, such as pollen foragers, water collectors, and bees returning from orientation flights. Only twice did I observe a pollen forager, easily recognized by the conspicuous pollen loads, in the sample of marked bees. Water collectors do not differ visibly from nectar foragers. However, water collecting trips typically require less than 5 min outside the hive when a water source is close to the hive (Park 1923, Robinson 1984, Kühnholz & Seeley 1997). The only water source in the vicinity of Würzburg's bee laboratory was about 15 m from the observation hive, hence water collectors could easily have completed trips to the source in 5 min or less. In any event, as long as water collectors did not take longer than 10 min for their flights, I avoided confusing water collectors with nectar foragers because I used a 10-min flight time as acceptance threshold for nectar foragers. I did so to distinguish nectar foragers from bees that made orientation flights. Our reasoning for using a 10-min acceptance threshold was as follows: when I compared the number of successful foraging trips (liquid was brought back) of a given duration to the number of unsuccessful foraging trips (no liquid was brought back) of the same duration, I found many more unsuccessful trips lasting 1-10 min than expected from the number of successful (hence confirmed) foraging trips (Fig. 1). This suggests that most of the orientation flights lasted 1-10 min. To be conservative, I considered only trips that lasted longer than 10 min as possible foraging trips. Definition A, which is more conservative than Definition B, defines as nectar foragers for a given day those bees that, at least once that day, unloaded liquid

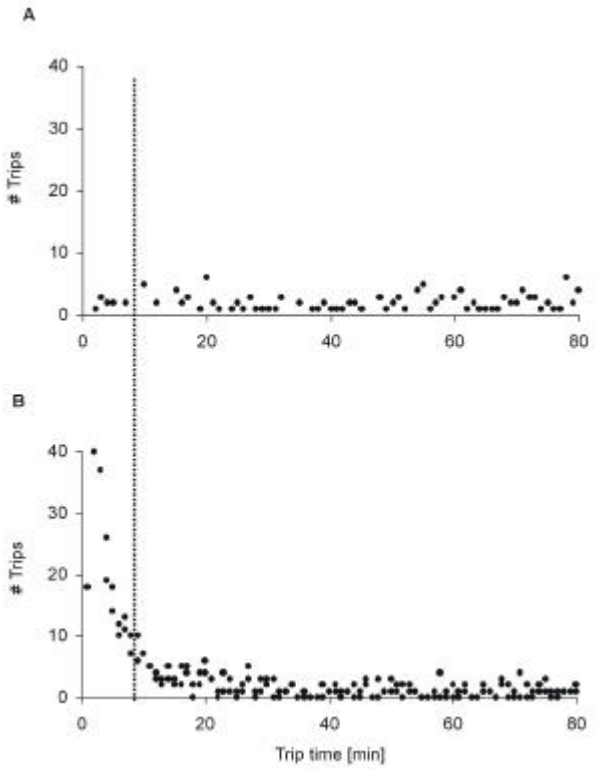

Fig. 1 The number of successful foraging trips of a certain duration (time outside the hive) (**A**), compared to the number of unsuccessful trips of the same duration (**B**).

after a trip longer than 10 min ("successful" nectar foragers). Thus, Def. A might exclude foragers that were not able to gather nectar although an attempt was made ("unsuccessful" nectar foragers). Definition B defines as nectar foragers for a given day those bees that, at least once that day, made a trip longer than 10 min. Thus, Def. B might include bees that, although out for longer than 10 min, did not attempt to forage for nectar. Since the more conservative Def. A includes only confirmed nectar foragers, it may reveal the dynamics of nectar foraging clearer than Def. B, although the estimates of the percent nectar foragers might be low. The values given in the Results are based on the data obtained with Def. A. When Def. B is used, this will be noted in the text. Def. B will be considered mainly in the Discussion.

Data analysis

The data obtained by observing the nectar foragers in the sample were used to estimate the percent nectar foragers in the colony and the activity of the nectar foragers, that is the number of foraging trips per nectar forager per day, as well as the time a nectar forager spent outside the hive per day.

Standard deviations for the percent nectar foragers on a day were estimated with the method given for binomial distributions by Sokal and Rohlf (1995). Confidence intervals (Sokal & Rohlf 1995) were used to detect significant changes in the percent nectar foragers between days within each 3-day observation period. Every foraging trip was categorized as either "successful" (when the bee unloaded liquid upon return), as "unsuccessful" (when the bee did not unload liquid before she received nectar herself), or as "unidentified" (when the bee could not be observed). The sum of all categories is the total number of foraging trips recorded. For each category, the average number of trips per nectar forager per day was calculated, as well as the average number of all trips per nectar forager per day. Means are given with one standard deviation. Kruskal-Wallis Tests (Heath 1995) were used to detect significant changes in nectar foraging activity between days within each 3-day observation period.

The mean daily temperature and the relative air humidity were recorded by the Deutscher Wetterdienst. The weather station and the apiary are about 15 km apart. Data about temperature and relative air humidity were used for Pearson Product Moment correlations with the percent nectar foragers. The data were analyzed using Microsoft Excel '98 and the '99 edition of Statistica.

Results

The percentage of the colony that foraged for nectar

The percent nectar foragers in the colony was estimated for each of the 17 days of observation (Fig. 2A).

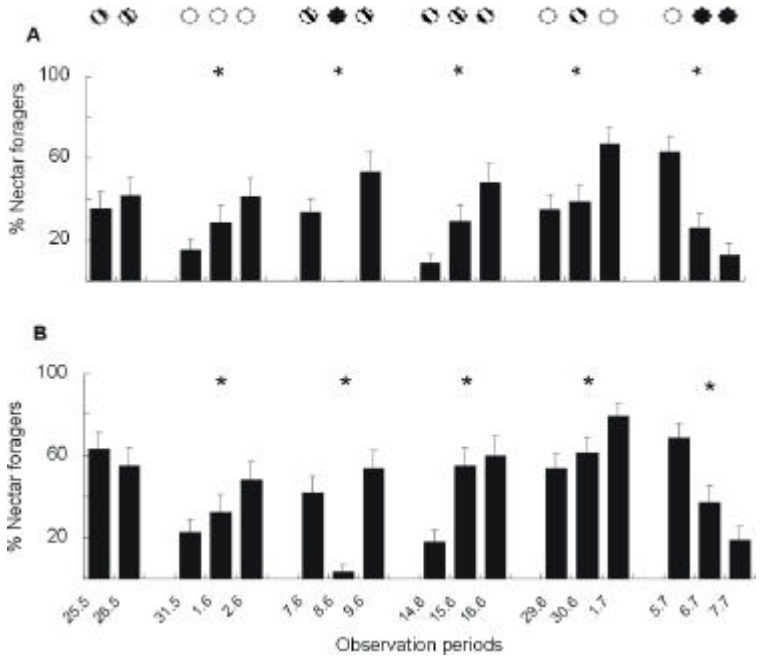

Fig. 2 **A** presents the percentage of the colony that foraged for nectar when Definition A is used. Good weather (>18°C, no rain), is indicated by a light sun, medium weather (15-18°C, light rain showers) by a half-filled sun, and bad weather (<15°C, rain) by a filled sun. **B** shows the percentage of the colony that foraged for nectar when Definition B is used. Stars indicate observation periods with significant changes ($p < 0.05$) of values between days. The measure of variability is the estimated standard deviation (Sokal&Rohlf).

Between 0 and 67% of the workers engaged in nectar foraging on a given day, with a mean of 34 ± 18% per day. The percent nectar foragers in the colony changed significantly between days in 5 of the 6 observation periods. No correlation was found between temperature and the percent nectar foragers ($p = 0.28$). The relative air humidity was negatively correlated with the percent nectar foragers ($r^2 = 0.34$, $p = 0.01$).

The activity of the nectar foragers

On average, 66% of the nectar foragers made 1-4 foraging trips per day, 34% made 5-10 trips, and no bee made more than 10 foraging trips per day (Fig. 3).

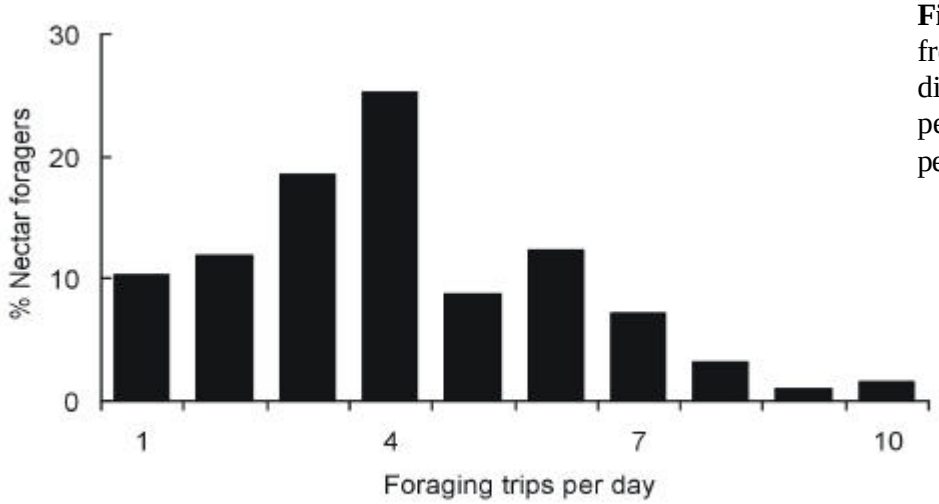

Fig. 3 The frequency distribution of trips per nectar forager per day.

The mean number of trips per nectar forager per day was 3.5 ± 1.3. On average, a forager made 1.9 ± 0.7 successful trips, 1.2 ± 0.8 unsuccessful trips and 0.5 ± 0.35 unidentified trips per day. It can be assumed that successful and unsuccessful trips were equally likely to be missed, therefore no systematic bias in the counts of successful or unsuccessful trips is expected. The majority of the nectar foragers (over 70%) foraged for 4.5 hrs or less even though there were approximately 15 hours of daylight each day.

Changes in the activity of the foragers, which might have influenced the colony's nectar foraging effort, occurred rarely: only in 1 (29.6.-1.7.) of the 6 observation periods did the mean number of trips per nectar forager vary significantly between days (Fig. 4).

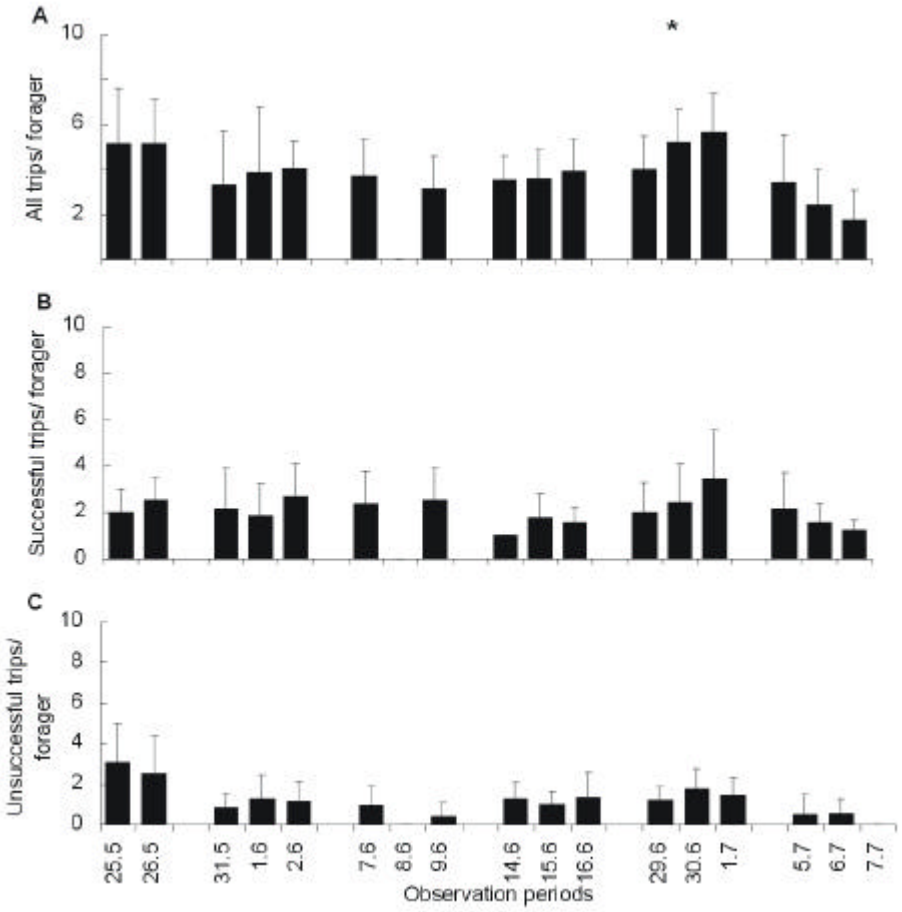

Fig. 4 The activity of the foragers (Def. A); means with one standard deviation. A number of all trips/nectar forager/day, B number of successful trips/nectar forager/day, C number of unsuccessful trips/nectar forager/day. Stars indicate a significant change in activity between days.

Neither the number of successful nor the number of unsuccessful trips per forager changed between days. The number of successful trips per forager was correlated with the percent nectar foragers of the colony ($r^2 = 0.63$, $p < 0.001$), while the number of unsuccessful trips per forager was not correlated with the percent nectar foragers ($p > 0.28$). No correlation was found between temperature and the activity of the nectar foragers ($p = 0.48$). The relative air humidity was negatively correlated with the activity of the nectar foragers ($r^2 = 0.38$, $p = 0.007$).

Discussion

Extensive work has been done on the foraging behavior of the honey bee (Seeley 1985, Winston 1987), but little is known about the daily adjustments of a colony to its internal needs and the external opportunities of foraging (Park 1929, Seeley 1995). By observing samples of workers of one colony over the course of late spring and early summer, I measured for the first time the percent nectar foragers in a colony. I also determined how the percent nectar foragers and the activity of the nectar foragers changed between days. The conclusions drawn from the results of this study are based on observations of one colony, and should thus be considered as preliminary conclusions. However, this study serves as a starting point to further investigations of the dynamics of nectar foraging in honey bees.

The percentage of the colony that foraged for nectar

The percentage of the colony that foraged for nectar was surprisingly high, up to 67% in one observation period (29.6.-1.7.). Hence, in that period, only 33% of the colony were hive bees, i.e. bees devoted exclusively to tasks within the hive. The high percent nectar foragers might have been due to the small size of the colony (approximately one–fifth the population of a full-sized colony (Winston 1987)). Small colonies of social insects have been found to put more energy into foraging than large colonies, to foster growth of the colony's population [Gordon 1991, Winston & Ferguson 1985]. However, the temporal pattern of foraging traffic over a day closely resembled the pattern found for full-sized colonies. Thus, it seems likely that the results are not simply due to small colony size, but rather that the percent nectar foragers in a colony is often higher than the 25% that were previously guessed (e.g. Seeley 1995).

To understand the dynamics in the allocation of labor to nectar foraging, not only the daily mean percentage of foragers, but also the variation between days, is of interest. The percent nectar foragers in the colony varied significantly between days (Fig. 2). It may be argued that the significant changes in the percent foragers were due to the fact that each sample of bees was no longer random on the second and third day of observation. Since a honey bee worker's age and task are strongly related to each other (Lindauer 1952, Oster & Wilson 1978, Rösch 1930, Seeley 1985), workers might have switched tasks as they were getting older in the three days of observation. Thus, on the second and third day of observation there may have been more foragers, typically the oldest workers, in the sample than was representative for the colony. However, the percent nectar foragers was not significantly higher on the second or third day ($p >$

0.1 for a comparison of the mean percent foragers on day 1 vs. day 2 or 3) than on the first day. Thus, it appears that the changes in percent foragers were not caused entirely by the aging of the workers during each 3-day observation period. Hence I believe that change in the percent nectar foragers was indeed involved in the adjustment of the colony's foraging effort.

The activity of the nectar foragers

The average number of trips per nectar forager per day, 3.5 ± 1.3, was low compared to prior studies which report means between 6-15 trips per day (Park 1929). In experiments involving artificial feeders that provide sugar water, nectar foragers may make 150 trips or more per day (Winston 1987). From this, it can be assumed that an average of 3.5 trips per forager per day did not exhaust the foragers' work capacity, especially as they spent on average only 4.5 hours outside the hive, not making use of the other approximately 10 h of daylight each day. The low number of hours per day that workers spent engaged in foraging raises the question of what nectar foragers do inside the hive. This question is especially interesting given the high percent nectar foragers in the colony. Great behavioral flexibility is generally assumed in honey bees, but the transition from in-hive tasks to foraging tasks is regarded as a permanent, one-way behavioral transition for a worker. In experiments, foragers could be induced to perform hive duties only after severe manipulations of the colony's age demography, that is, only after virtually all the younger hive bees were removed from the colony (Milojevic 1940, Robinson et al.1992, Rösch 1930, Winston & Fergusson 1985). More observations of individual nectar foragers in undisturbed colonies will be needed to show what nectar foragers do inside the hive, and hence to answer the question of whether the gap between hive bees and foragers is in fact as wide as assumed.

To learn about the dynamics of nectar foraging, not only the mean activity of the foragers has to be considered, but also whether the activity varied significantly between days. In fact, the results of this study show with just one exception, that there were no significant differences in mean foraging activity between the days of an observation period (Fig. 4). This suggests that a honey bee worker tends to make a binary decision to either forage or not, rather than a graded decision about the level of foraging activity.

Adaptive value of colony-level changes in the percent, not the activity, of the nectar foragers

The results reported here suggest that the percent nectar foragers of a colony is the most important variable in the daily adjustment of the nectar foraging effort of a honey bee colony. As this variable gets adjusted on the individual level by the behavioral flexibility of the workers, it adjusts the foraging effort on the colony level.

Why is the percent nectar foragers adjusted more than the activity of the nectar foragers? A colony should try to exploit the ephemeral nectar sources fast, to gain as much nectar as possible before the flow stops. Hence, it might be adaptive for a colony to allocate many foragers to a source, to exploit it quickly before unfavorable conditions stop the nectar flow, instead of allocating fewer foragers which would need longer to exploit the source fully even if they raised the level of their foraging activity.

It can also be hypothesized that coordinating the activity level of the nectar foragers would hinder, rather than foster, an efficient exploitation of the nectar sources. A colony-level adjustment requires coordinated actions at the individual level. For this, information has to be shared. Recruitment signals like the waggle dance broadcast information about the nectar availability and thus the need for more bees functioning as nectar foragers (v. Frisch 1967). Therefore, the nectar foragers can adjust their numbers to the present situation by responding to shared information. On the other hand, the activity of a forager should not depend on shared information, but on each bees' assessment of the quality of the particular source she is exploiting (Seeley et al. 1991). When the source quits producing nectar, a worker should stop spending energy on foraging trips, regardless of what the other nectar foragers are doing. Thus, in a situation where the nectar foragers of a colony exploit sources with different properties, the activity should differ between foragers. In a situation where most of the foragers exploit the same source, all foragers might have a similar activity. This later situation might have been the case on 1 July, the only day on which the activity of the foragers rose significantly (Fig. 4). This day also had the highest percent nectar foragers; it is probable that a nectar rewarding plant came into flower and was exploited by the majority of the foragers.

Comparison of the definitions of nectar foragers

For both definitions of a nectar forager it can be concluded that the percent nectar foragers in the colony is the main variable for the adjustment of the colony's foraging effort. When Def. B (a nectar forager made at least one trip longer than 10 min on a given day) was applied, the mean

percent nectar foragers in the colony was 45 ± 20% per day. The mean percentage changed significantly in 5 of 6 observation periods (Fig. 2B). The mean activity was 3.4 ± 1.4 trips per forager per day and significant changes in activity between days occurred in only 2 of 6 observation periods. Significantly more bees making fewer trips were defined as nectar foragers by Def. B as opposed to Def. A, possibly because unsuccessful foragers decided to stop foraging sooner than successful ones. Estimates of the percent nectar foragers and their activity that are based on the more conservative Def. A might be lower than they should be, but changes in the pattern of nectar foraging might be easier to detect, as only the behavior of confirmed nectar foragers was analyzed. The values found with the less conservative Def. B might be higher than they should be. To know whether bees that are classified as unsuccessful nectar foragers really are functioning as nectar foragers, it would be helpful to observe the bees for more than three days. With longer observation periods, unsuccessful foragers might have been clearly classified as nectar foragers on earlier days. Thus we might be able to understand better how unsuccessful foragers contribute to the daily dynamics of nectar foraging.

What causes the dynamics of nectar foraging?

Although the question of what caused workers to decide for or against nectar foraging on a given day cannot yet be fully answered, some clues are revealed by this study. Frequently, workers made a few short flights, often before they started foraging. These flights were not long enough to gather nectar, but it is possible that foragers checked former nectar sources and based their foraging decisions on the information about the nectar availability at these sources. Also, workers could have gathered information about the weather. Effects of the temperature on nectar foragers have often been reported (Bräuninger 1964, Schuà 1952), but sometimes, as in this study, no causal relationship is found. Both temperature and relative air humidity could have an indirect, rather than a direct effect on the foragers as they affect the state of flowers and hence the nectar availability (Núnez 1977, Winston 1987). Rain can wash the nectar out of flowers and it can take up to 24 h before enough nectar accumulates again to attract bees. I found a negative correlation between humidity and the percent nectar foragers, which might have been caused by the risk of rain for flying bees. In this study, direct and indirect effects cannot be separated from each other. Thus, no definite answer can be given as to the cause of the dynamics in the allocation of labor to nectar foraging, but fluctuations in the availability of nectar might have been the major reason.

Studies of the dynamics of nectar foraging under conditions of controlled nectar availability are needed, if possible on full-sized honey bee colonies, to reveal the link between the adjustment of a colony's foraging effort and the nectar availability. The number of recruitment dances over a day may be a good indicator of the availability of nectar that is relevant for the bees (not all available nectar is relevant for the bees, as not all nectar sources are discovered and because nectar foragers selectively exploit the best of the nectar sources that are discovered (Seeley 1986). If so, and if the number of dances on a given day can be related to the mean percent nectar foragers at the same day, then this would show that the percent nectar foragers in a colony gets adjusted in relation to the relevant nectar availability. Hence, the dance pattern of a day might provide an easy test of the ecological factor underlying the adjustment of the percent nectar foragers in a honey bee colony.

Chapter II

Do honey bees produce a volatile chemical to activate foragers?

Abstract

A honey bee colony frequently adjusts the number of its nectar foragers to changes in foraging conditions. It is possible that workers use a volatile substance, e.g. a pheromone, to quickly activate foragers in all regions of the hive. To test whether a foraging colony can activate foragers of a non- foraging colony via a volatile substance, I connected two colonies with a glass tube that allowed volatiles to drift between colonies. Each colony had access to a different green house. During the experiment, one colony was allowed to forage odor- less sugar water. I then recorded of the colony that did not have sugar water available the number of workers that each left the hive and arrived at an empty feeder station per time unit. In 50 % of all experiments did the foraging colony activate foragers of the non-foraging colony to visit an empty feeder station with a volatile substance. The results show that nectar foragers can be activated via a volatile substance. However, it remains to be investigated why foragers were not activated in all experiments. It is possible that negative results were due to the weak state of the non-foraging colony and cold weather at the time of experiment.

Introduction

Honey bee (*Apis mellifera*) colonies frequently adjust their foraging effort to changes in foraging conditions (Park 1929, Seeley 1995, Thom et al. 2000). It has long been known that waggle dancing honey bees can raise the number of foragers at nectar, pollen or water sources. Waggle dancers recruit both novice and experienced foragers by communicating information about the location and quality of a source (e.g. v. Frisch 1967). However, the recruitment rate of waggle dancers is relatively low. On average, a waggle dancer recruits 1 or 2 foragers per 15 min to a new source (v. Frisch 1967). Re-activation via the waggle dance of those nectar foragers that already know the advertised source may be more efficient. However, only those nectar foragers are likely to be reached by the signal that are on the dance floor, a small area close to the hive entrance where most waggle dances are performed (Tautz 1996).

While indispensable to a colony's foraging success, the waggle dance might serve to recruit foragers to a specific food source rather than to generally raise the foraging effort by activating foragers. Therefore, it is plausible to assume that honey bees employ other mechanisms than the waggle dance to quickly activate nectar foragers.

Honey bees, like all other social insects, strongly rely on pheromones and other chemical substances for communication (e.g. Winston 1987, Hölldobler and Wilson 1990). Several pheromones are used to mark food and water sources for higher attraction and better orientation, e.g. the Nasonov pheromone (v Frisch 1923, v. Frisch & Rösch 1926, Free & Racey 1966, Free & Williams 1970, 1972), foodprint pheromones (Ribbands 1955, Lecomte 1956, Butler et al. 1969, Ferguson & Free 1979, Williams et al. 1981), a sting- produced compound, (Z)-11-eicosen-1-ol (Pickett et al. 1982, Free et al. 1982), and food-marking pheromones (Ferguson & Free 1979). These substances are also used to mark flowers, although flowers often (not always) have an identifying odor themselves (v. Frisch 1967). The chemical marker may enhance flower odor or make it better perceptible.

It is possible that honey bees produce volatile substances inside the hive for a fast activation of the colony's forager force. This activation signal might be used whenever foragers inside the hive should be informed quickly about a favorable change of foraging conditions, but not about the location of a source. This is e.g. in the morning, after rain, or when an established but temporarily dried nectar source becomes suddenly available again.

In this study, I tested the hypothesis that volatile substances are produced in a foraging colony that raise the colonies nectar foraging effort. I did this by allowing volatile substances that were produced by a foraging colony to drift through a glass tube into another colony that did not have access to food. The colonies had each access to a different green house, to control foraging activity and to prevent e.g. flower odors from influencing the outcome of the experiment.

Material and Methods

Bees and observation hives

The study was conducted in August and September, 2001, at the honey bee laboratory of the University of Würzburg, Germany. Two colonies, Colony 1 and Colony 2, of the carniolan honey bee, *Apis mellifera carnica* were used for the experiments The colonies were housed outdoors in two-frame observation hives with internal dimensions of 45 * 45* 5 cm. A wedge in the entrance of the hive directed incoming bees to the front side of the comb. The hives were placed between two greenhouses. The front sides of the hives faced each other, so that the entrance area of the one hive was exactly opposite the entrance area of the other hive. The entrance tunnels of the hives led each into a different green house, and had each a length of

approximately 1 m. The colonies could at all times freely access their green house. The green houses provided a space of 4 x 4 x 7 m for Colony 1, and of 5 x 4 x 7 m for Colony 2. About 18 cm^2 of the glass near the junction of the hive and the entrance tunnel were removed and replaced with a cloth mesh. The cloth mesh allowed volatile substances to drift off the dance floor, but was too tight for bees to pass. A glass tube connected the meshed areas of the two hives. The glass tube was 20 cm long, had a diameter of 5 cm, and each end of the tube widened to tightly cover the meshed areas of one hive. A fan fitted in the middle of the glass tube controlled the direction of the air flow in the tube.

Training of bees

A week prior to the first experiment, nectar foragers of both colonies were each trained to a grooved-plate feeders located in the greenhouses (see v. Frisch, 1967 for description of training techniques). Colonies could collect at a random time of day an unscented 2 molar sugar water solution at the feeder. Random feeding times made food availability unpredictable for the colonies, and prevented the training of nectar foragers to a specific feeding time. To allow foragers to assess nectar availability at all times, the feeder was never removed from its location, but replaced with a clean empty feeder when necessary.

Experiments

Experiments were done at the 23.8., 27.8., 29.8., 31.8., 4.9., and 7.9.2001, and lasted from 0900 to 1100. Experiments were divided into a control phase that lasted from 0900-1000, and a manipulation phase that lasted from 1000-1100. During the entire experiment, the glass tube connected the insides of the two hives, and the air flow was directed from Colony 2 (C_2) to Colony 1 (C_1). Before and during the control of the experiment, no sugar water was available to either colony. During the manipulation, sugar water was available to C_2, but not to C_1. To confirm that C_2 was foraging during the manipulation, an assistant recorded the number of nectar foragers from C_2 that visited the feeder during the manipulation phase of all 6 experiments, and during the control phase of 3 experiments. During both the control and manipulation of the experiment, assistants counted for C_1 the number of bees exiting the entrance tunnel into the green house per 5 minutes (flight activity) as well as the number of bees landing on the empty feeder station per 5 minutes (nectar availability assessment rate or “foraging” activity).

To prevent that nectar foragers from C_1, acquired a negative association between the empty feeder and the volatile substances potentially coming from the foraging colony, I connected the colonies also during the daily feeding times.

Baseline counts

To correct the flight and foraging activity of C_1 for day-time fluctuations, I recorded the baseline activities of C_1. On the 22.8., 24.8, 28.8., 3.9., and 6.9.2001, I connected the two hives with the glass tube, but did not feed either colony during counts of the flight- and foraging activity of C_1. These counts of the colony's daily activity pattern (baselines) were done during the same time of the day as the control (0900-1000) and manipulation counts (1000-1100) of the experiments.

Statistical analysis

For comparison of the median numbers of bees per 5 min in control and manipulation, I subtracted the median of each time interval during the baseline count from the median of the same time interval during the experiment.

To compare the average medians of control and manipulation, I subtracted the median of the 0900-1000 baseline counts from the median of each experimental control count, and the median of the 1000-1100 baseline counts from the median of each manipulation count. I then averaged the corrected medians for each control and manipulation. Values are negative when the baseline count was higher than the experimental count.

For comparison of each flight and foraging activity between control and manipulation, I compared (a) the activity between *each time interval* of control and manipulation to account for delayed activation effects (therefore n = 12, because there were each 12 time intervals per control and manipulation), and (b) the *medians of all* controls and manipulations (therefore n = 6, because there were 6 experiments).

Measurements are given either as medians, or as average medians. Measurements of variability are given as one standard deviation unless otherwise noted. Statistical tests were performed according to Sokal and Rohlf (1995), and are given in the text. All data were analyzed using the 1998 edition of Microsoft Excel and the 2000 edition of Statistica.

Results

To confirm that C_2 was foraging during the manipulation when sugar water was available, we recorded the number of foragers/ 5 min from C_2 that visited the feeder during 3 control and 6 manipulation phases.

During the control of the experiment, 13.7 ± 11.6 foragers/ 5 min visited the empty feeder (n = 3). During the manipulation, 88.2 ± 23.2 foragers visited the filled feeder (n = 6). The number of foragers visiting the full feeder was significantly higher than the number of foragers visiting the empty feeder (Mann-Whitney- U Test, $p < 0.001$) (Fig. 1).

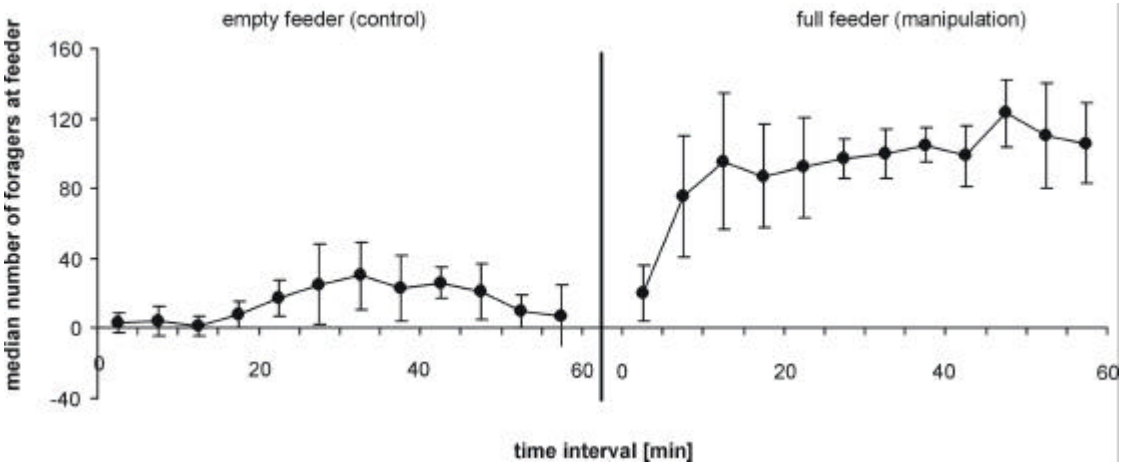

Fig. 1 The median number of bees from Colony 2 that landed on the feeder during each the control and the manipulation of the experiment.

To test whether volatile substances from C_2 raise the flight or "foraging" activity (nectar availability assessment rate) of C_1, we recorded the number of bees/ 5 min from C_1 that exited the hive or landed on the empty feeder. Fig. 2 shows the flight- and foraging activity per time interval for each the baseline and the experimental count. During the 0900-1000 baseline count, the average median flight activity of C_1 was 3.0 ± 1.4 bees/5 min, and the foraging activity was 3.5 ± 1.6 bees/5 min. For the 1000-1100 baseline count, the values were 5.8 ± 2.3, and 5.3 ± 1.8. During the control of the experiment, the flight activity of C_1 was 1.8 ± 0.8 bees/5 min, and the foraging activity 1.8 ± 1.1 bees/5 min. During the manipulation, these values were 5.4 ± 3.3 and 6.2 ± 3.5.

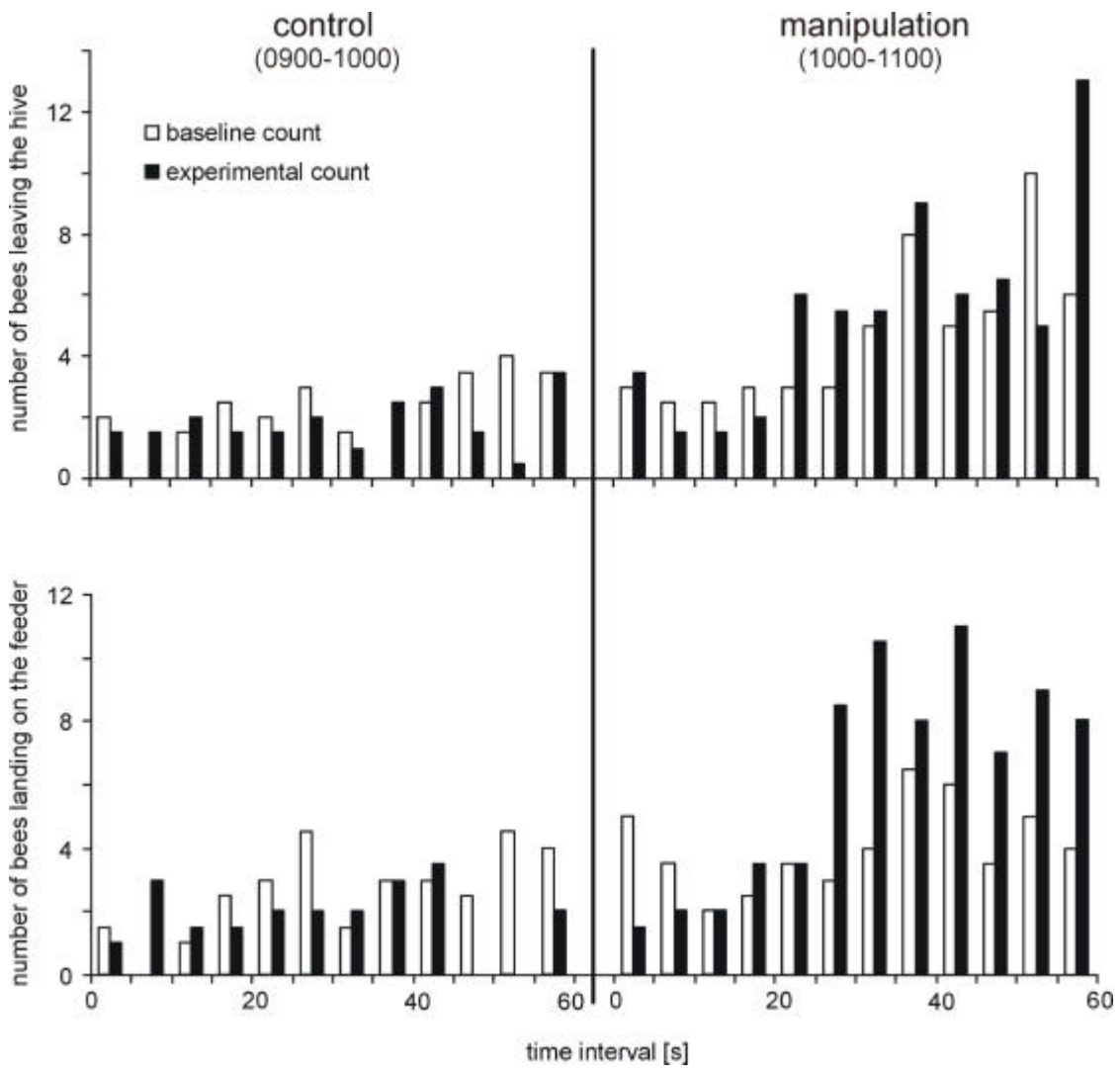

Fig. 2 The median number of bees from Colony 1 that exit the hive (upper panel) or land on the empty feeder (lower panel) for each baseline and experimental count. The black line indicates the end of the control and the according 0900 – 1000 baseline count, as well as the start of the manipulation and the according 1000 - 1100 baseline count.

The flight activity and foraging activity did not differ between the 0900-1000 baseline count and the control count of the experiment (G-test for goodness of fit, $p = 1.000$ and 0.115, respectively, $n = 12$). Flight activity did not differ between the 1000-1100 baseline count and the manipulation count of the experiment ($p = 0.133$, $n = 12$), but foraging activity was significantly higher during the manipulation count than was expected from the baseline count ($p = 0.004$, $n = 12$).

To correct for activity fluctuations that were due to the time of the day, I subtracted the baseline counts from the experimental counts. Fig. 3 shows the corrected values of the flight activity and the foraging activity of C_1 for each 5 in interval of the 6 experiments.

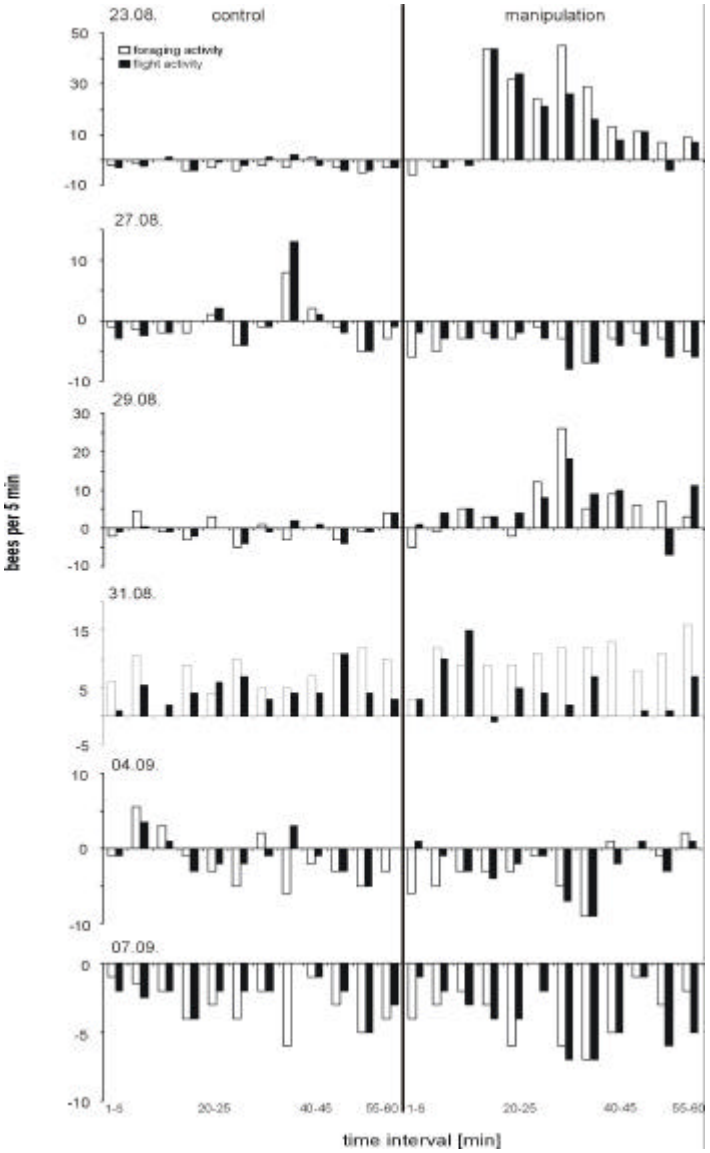

Fig. 3 The flight activity (black bars) and foraging activity (white bars) of Colony 1 for each of the six experiments.
To correct for fluctuations that were due to the time of day, the median baseline value of the interval was subtracted from the according value of the experimental count in each time interval.
The dates notify when each experiment was performed.

When the corrected flight and foraging activity was compared between control and manipulation of each experiment, the activity was significantly higher during the manipulation on August 23, 29 (Mann-Whitney- U Test, $p < 0.015$ for each comparison) and August 31 ($p = 0.0497$). On August 27, activity was higher during the control than during the manipulation ($p = 0.008$ for flight activity and 0.021 for foraging activity), and on September 4 and 7, activity did not change

between control and manipulation ($p > 0.080$ for each comparison). When the median flight and foraging activity were compared between control and manipulation, the flight activity did not differ between control and manipulation of the experiment (Mann-Whitney- U Test, $p = 0.328$, $n = 12$), but the foraging activity was significantly higher during the manipulation than during the control ($p= 0.032$).

During the control, the average median flight activity was -1.1 ± 3.8 bees/5 min, and the average median foraging activity was 0.7 ± 6.4 bees/5 min ($n = 6$). During the manipulation, the values were -0.9 ± 3.0 and 0.1 ± 5.3 bees/5 min, respectively. There was no difference in either flight activity or foraging activity when the medians of all controls were compared to the medians of all manipulations (Wilcoxon-test for paired samples, $p = 0.917$ for each comparison, $n = 6$).

Discussion

In this study, I investigated whether a foraging colony produced volatile substances that stimulated the flight or foraging activity of a non-foraging colony.

The results show that a foraging colony can produce volatile substances to activate nectar foragers. In 50 % of the experiments, the foraging colony stimulated the non-foraging colony to visit an empty feeder (Fig. 3). The unfavorable foraging conditions during experiments that were due to the late time of year, e.g. small colony size, low foraging motivation, low temperature (13.5 °C - 17.6 °C during the day), frequent rain and high humidity in the hive's entrance tunnels, might have lowered the motivation to produce, or to react to, an activating signal during some of the experiments, and possibly caused the differences in the degree of forager activation between experiments. The exact hive-external and internal conditions that support the production of, or reaction to, a foraging stimulating substance remain to be investigated and involve the questions about the chemical composition of the activating substance, its production and release.

In experiments where the non-foraging colony was stimulated to forage, the number of foragers from the non-foraging colony that landed on the empty feeder increased approximately 30 minutes after the foraging colony first had access to food (Fig 3). This delay in forager activation could reflect e.g. a feature of the production or release of the stimulating substance. It is also possible that the delay in the activation of honey bee foragers indicates that a certain

threshold concentration has to be reached before the information is reliable enough for foragers to decide to do energetically costly foraging trips. An example for this can be found in fire ants (*Soleonopsis invicta*), where a group of foragers has to release chemicals to activate another group of colony members to follow food trails (Wilson 1962, Hölldobler & Wilson 1990).

The volatile substance that activated the nectar foragers could be a signal or a cue. Signals evolve specifically to convey an information, while cues did not specifically evolve to transfer information, but nevertheless contain information. A forager activating substance would thus be a signal if it would have evolved specifically to convey the information that more foragers should be active. The substance would be a cue, if it would e.g. be used by foragers to mark the food source and remainders of the substance on returning foragers convey the information that there are food sources outside that are worthwhile marking for further exploitation.

To answer the question whether nectar foragers can be activated more reliably when foraging conditions are generally better than during the experiments of this study, the experiments should be repeated earlier in the honey bee season. A better understanding of the proximate mechanisms of the activation should be gained by closely monitoring the identity and behavior of the foragers in both the foraging and the non-foraging colony.

Chapter III

Worker piping in honey bees: the behavior of piping nectar foragers

Abstract

Honey bee nectar foragers often produce brief piping signals (“stop signals”) off the dance floor, suggesting that the current hypothesis for the function of these signals may be incomplete. The purpose of this study is to clarify the context and the acoustic properties of the signals, and to describe the behavior of the senders and receivers of the signal both on and off the dance floor. Piping was stimulated reliably by promoting a colony’s foraging activity, demonstrating a causal connection between worker piping and nectar foraging. Acoustic analysis revealed that piping signals are frequency-modulated. Observations of marked nectar foragers showed that the behavior of piping nectar foragers is unique. Piping tremble dancers spent more time in the hive, more time dancing, had longer unloading latencies, and spent less time on the dance floor. Piping nectar foragers sometimes unloaded their nectar directly into cells instead of searching for a nectar receiver, and often began tremble dancing immediately upon return into the hive. Most piping signals (approximately 99%) were produced by tremble dancers, yet not all (approximately 48%) tremble dancers piped, suggesting that piping and tremble dancing have related, but not identical functions in the foraging communication system. Many piping signals (approximately 43% of all pipes recorded) were produced off the dance floor, and thus were received by non-waggle dancing bees. We discuss the implications of these results for the current functional hypothesis and propose a new hypothesis for the function of piping by nectar foragers off the dance floor.

Introduction

The brief piping signals produced by honey bee nectar foragers have been referred to as begging signals (Esch 1964) and as stop signals (Nieh 1993, Kirchner 1993). Esch (1964) referred to the signals as begging signals because he observed that waggle dancers stopped dancing to provide food samples to piping waggle dance followers. In a more recent study, Nieh (1993) confirmed only that the piping signals stop waggle dances and therefore referred to the signals as stop signals. He suggested that the signal increases a colony’s foraging efficiency by retarding the recruitment of additional nectar foragers when the amount of nectar coming into the hive is already more than the colony can process.

This view of the piping signal produced by nectar foragers may be incomplete. If the piping signal produced by nectar foragers serves only to stop waggle dances, then nectar foragers should rarely pipe off the dance floor where there are no, or very few, waggle dancers (v. Frisch 1967, Seeley 1995). Also, receivers of this signal should with few exceptions be waggle-dancing bees. However, in pilot work we observed that piping signals are frequently produced off the dance floor where there are no waggle dancers, and are regularly targeted at non waggle-dancing bees. Thus, the piping signal produced by nectar foragers may have messages and meanings more complex than previously recognized. Therefore, we think that it is important to clarify the context and acoustic properties of these signals and to examine the behavior of the senders and receivers of the signal both on and off the dance floor.

In the first part of this study, we describe the acoustic properties of the piping signal. In the second part, we clarify the signal context by manipulating a colony's nectar supply and recording its piping activity. In the third part, we identify the signal's senders and receivers, and describe their behavior from the moment they enter to when they exit the hive.

Material and Methods

Study site and bee colonies

The study was conducted from May to August, 2000, at the honey bee laboratory of the University of Würzburg, Germany. The laboratory was surrounded by fruit orchards and rape (*Brassica napus*) fields which provided nectar in May.

Observations were made on two colonies (C_1 and C_2) of the carniolan honey bee, *Apis mellifera carnica.* Both colonies were installed in the laboratory in April, 2000, thus were well established by the time observations began. The colonies were housed indoors in three-frame observation hives with internal dimensions of 65 cm * 45 cm * 5 cm, with entrance tunnels leading outside. About 18 cm^2 of the glass near the junction of the hive and the entrance tunnel was removed and replaced with a cloth mesh. This enabled us to perceive piping sounds from the dance floor. The dance floor was the region of the hive, just inside the entrance, where all waggle dances were performed.

Training of bees

We trained bees to a grooved-plate feeder located 200 m from the observation hive (see v. Frisch, 1967 for description of training techniques). Each colony was trained to a different

feeder. During observational studies, only the colony under observation was trained to a feeder. The feeders provided a concentrated sugar solution ("Apiinvert®" [Südzucker], 2.4 mol/l, sugar composition 61 % glucose, 39 % fructose) and from June to August they supplied most of the food collected by the two study colonies, as natural nectar sources were scarce during this time. The feeder was tended by an assistant who kept it filled with sugar solution. As we did not restrict the number of recruits to the feeder, large numbers of foragers gathered often at the feeder, possibly from several colonies.

To recognize nectar foragers inside the observation hives, we marked bees that visited the feeder with a dot of paint on the thorax (for marking technique see v. Frisch, 1967, or Seeley, 1995). Nectar foragers were marked at least one day before observations began. We made sure that each bee was observed only once by marking with a second color each observed bee as she exited the hive.

Sound analysis

To record the piping signals of nectar foragers marked at the feeder, we held a custom-made microphone (5 mm diameter, flat frequency response from 20 to 6000 Hz) approximately 1 cm over the piping bee. The microphone's output was recorded on the audio track of a video camera (Sony 3CCd) that simultaneously filmed the nectar foragers. Recordings were analyzed with the *Avisoft SASlab* program [©R. Specht].

Context of worker piping

To determine whether worker piping occurs under natural conditions, we investigated the piping activity of a colony over the course of one day without providing the sugar-water feeder. We observed C_1 on 25 August 2000 from 0700 to 1900. We recorded every 30 min for 1 min the number of pipes heard from the dance floor of the observation hive and scanned the colony every 30 min for 1 min for waggle and tremble dances. We did not repeat this work-intensive count as one count sufficed to show that worker piping occurs frequently under natural conditions.

To determine if piping could be induced by stimulating foraging activity, we recorded in June and July, 2000 the number of audible pipes while providing a colony with a sugar-water feeder. Experiments were conducted once a day and started between 0830 and 1000. Data were recorded one time (13 June 2000) from one colony (C_1) and three times (29 June, 30 June, 2 July 2000) from a second colony (C_2) for a total of four trials. Both piping and foraging activity

measurements were taken for 50 min each before, during and after sugar water was provided at the feeder. Piping activity was measured as the number of pipes heard from the dance floor per 5 min. Dividing this number by 5 gave us an estimate of the number of pipes on the dance floor per minute. Foraging activity was measured by an assistant as the number of bees that left the hive every 5^{th} minute.

Behavior of piping and non-piping nectar foragers

To determine the identity of piping nectar foragers and investigate the behavior of nectar foragers, we observed nectar foragers that were marked at a sugar-water feeder. Observations started shortly after the feeder was filled with sugar solution, and lasted for 1-3 hours. The feeder station was set up as described above. We recorded the behavior of 80 foragers from C_1 and 63 foragers from C_2. We observed each nectar forager from the moment she entered the entrance tunnel until she exited the hive. We started observations the moment the bee entered the tunnel because foragers sometimes unloaded and danced there instead of on the dance floor. During observations, a custom-made electronic device was held on the cloth mesh or glass above the focal bee. This device detected sounds by turning signals of infrared light reflected from the studied object into an electric signal which was then amplified to allow reliable perception of the sound.

The identity of piping nectar foragers was determined by observing the behavior of nectar foragers throughout their stays in the hive. Nectar foragers performed waggle dances or tremble dances, or they did not dance. For each of these behavioral groups, we calculated the probability of a bee emitting a piping signal at least once during her time in the hive. Piping nectar foragers were identified as bees that piped at least once during a stay in the hive. To calculate the probability for a piping nectar forager to perform piping signals on sequential returns to the hive, we recorded the behavior of 8 piping nectar foragers from C_2 during 2-17 sequential stays in the hive.

The behavior of each nectar forager was examined by recording when she piped, whether or not she contacted a marked nectar forager or other bee during each pipe, her posture during each pipe, on which frame she emitted each pipe, and each time (if any) she received a pipe from another bee. We also recorded the duration of each forager's stay in the hive, how much time she spent on vs. off the dance floor, the time since entering the hive at which she started to dance, the type (waggle or tremble) and duration of her dance, the number of cells she inspected, and the

time and duration of each of her trophallactic contacts. Only contacts of 3 s or longer were considered to result in a transfer of nectar and thus to be unloading contacts.

To investigate the effect of the piping signal on nectar foragers, we compared the behavior of piping tremble dancers and waggle dancers 1 min before and 1 min after they received the signal. Specifically, we compared the durations of walking and dancing (waggle or tremble dancing), and the frequencies of piping, trophallactic contacts, and cell inspections 1 min before and 1 min after the bee received a signal. If a bee received several piping signals, the behavior of the individual was compared 1 min before and after each signal. The effect of only those signals were evaluated that were received at least 2 min apart from each other.

All behavioral data were recorded with the *Observer* program (Noldus Information Technology, version 3.0).

Statistical analysis

Measurements are given as means with one standard deviation, unless otherwise noted. Statistical tests are given in the text. All data were analyzed using the 1998 edition of Microsoft Excel and the 2000 edition of Statistica. Bonferroni corrections for multiple comparisons were performed according to Sokal and Rohlf (1995). The adjusted α–level for each statistical test is noted in the text.

Results

1. Piping signals are frequency modulated

The duration of 44 piping sounds was between 0.1 s and 0.7 s, with a mean of 0.23 $\pm$ 0.10 s (Fig. 1). The fundamental frequency of all piping signals we recorded was modulated.

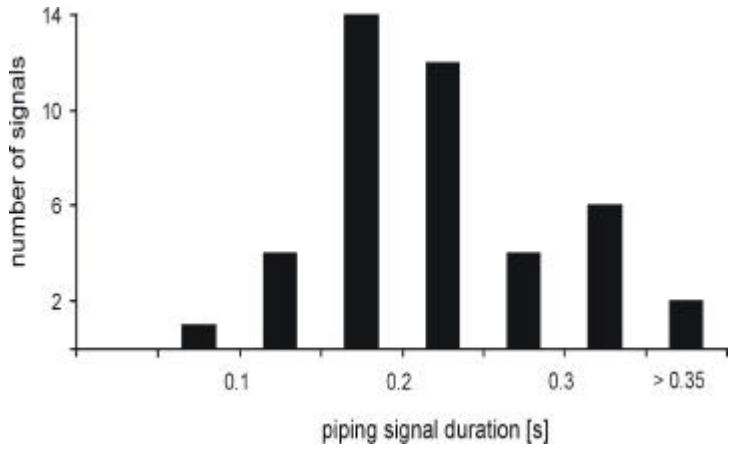

Fig. 1 Duration of piping sounds.

Typically, the frequency increased during the approximately 1st quarter of the signal, and then decreased steadily (for examples see Fig. 2). Because bees piped only inside busily foraging colonies, the level of background noise was very high, hence we could rarely distinguish the fundamental frequencies of a signal from the noise.

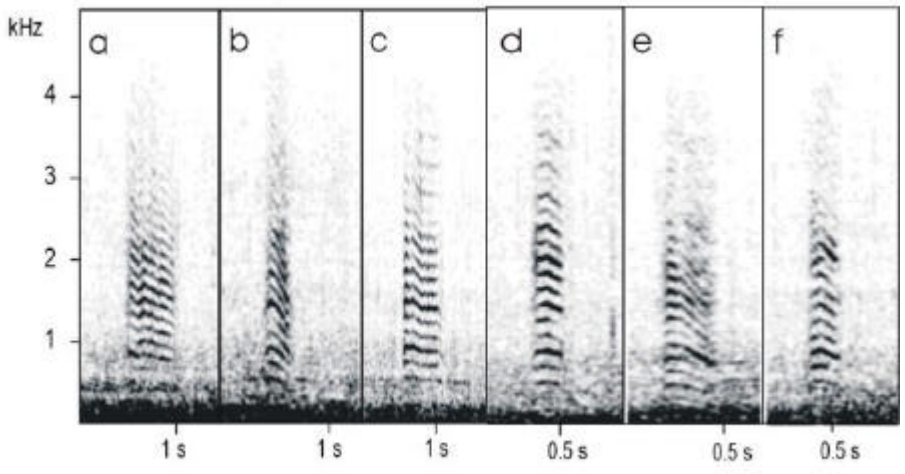

Fig. 2 Examples for the frequency modulation of the piping signals produced by nectar foragers. Time is at 0 s at the start of each box.

For one signal, we found at different times within the signal fundamental frequency peaks at approximately 270, 380 and 540 Hz. However, while the fundamental frequencies were rarely distinguishable from the colony's background noise, up to 11 harmonics were easy to perceive. Whether bees perceive the fundamental frequencies, or the harmonics, or both, has yet to be investigated.

2. Worker piping is associated with nectar foraging

The piping activity of a colony foraging from natural food sources is shown in Fig. 3. Piping activity ranged from 0 to 9.8 pipes/min, with an average of 4.0 ± 2.8 pipes/min. Piping activity and foraging activity, measured as the number of foraging dances, were positively correlated over time (Pearson-Product-Moment-Correlation, for waggle dances $r^2 = 0.48$, $p = 0.016$, for tremble dances $r^2 = 0.57$, $p = 0.003$).

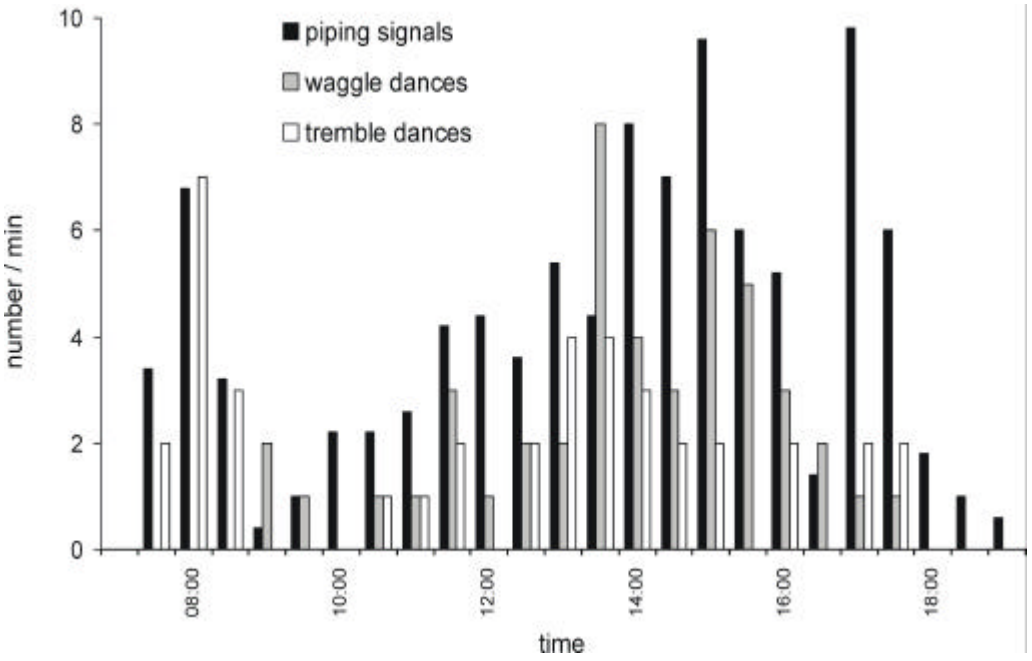

Fig. 3 The piping activity and nectar-foraging dances of a non-manipulated colony on a single day.

The foraging and piping activity of two colonies before, during, and after sugar water was made available is shown in Fig. 4. The number of bees leaving the hive increased immediately when sugar water was provided and decreased after the sugar water was removed. Piping activity increased approximately 10 min after sugar water was provided and decreased rapidly after the sugar water was removed from the feeder.

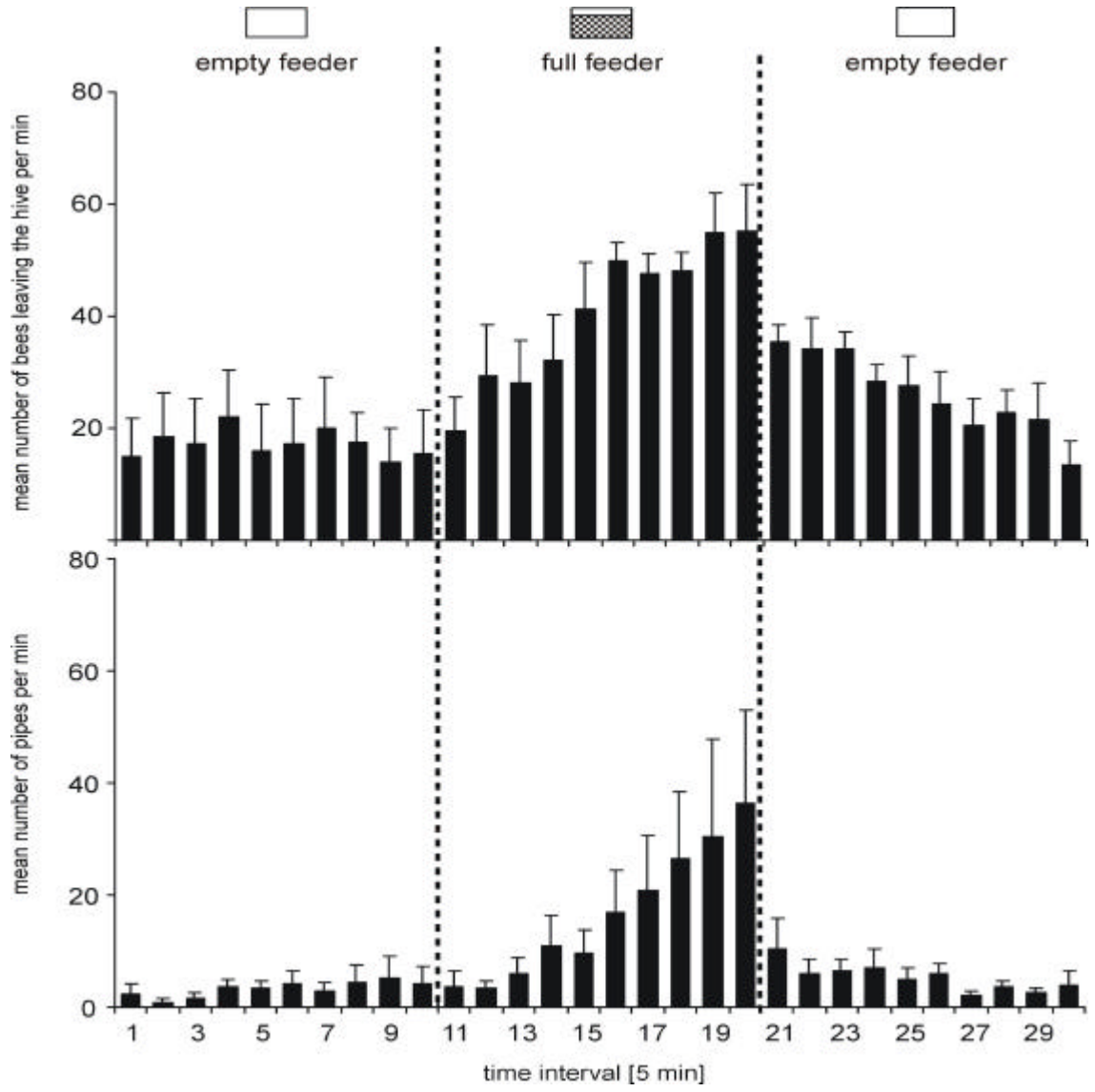

Fig. 4 The foraging and piping activity of two colonies before, during and after sugar water was available. Data were recorded for one min during each 5 min interval. For each interval, the upper panel shows the number of foragers leaving the hive/min, the lower panel the estimated number of pipes/min. Data were recorded once from one colony and three times from a second colony for a total of four trials. Data are pooled for both colonies. Error bars indicate the standard error of the mean.

3. Identity of piping nectar foragers

To determine the identity of piping nectar foragers, we observed 80 nectar foragers in C_1 and 63 nectar foragers in C_2. In total, these nectar foragers produced 204 piping signals (2.6 signals/bee/stay in the hive) in C_1 and 299 piping signals (4.8 signals/bee/stay in the hive) in C_2.

In C_1, 28 of the observed nectar foragers did not dance, 9 waggle danced and 43 tremble danced. In C_2, 23 nectar foragers did not dance, 7waggle danced and 33 tremble danced. The probability of piping at least once during a bee's stay in the hive was highest for tremble dancers (0.42 in C_1 and 0.55 in C_2), and lower for non-dancing nectar foragers (0.04 in C_1 and 0.09 in C_2) (Fig. 5). None of the 16 waggle dancers piped, but we occasionally observed non-focal waggle dancers piping on the comb in the turn of their waggle run. When 8 nectar foragers were observed for several sequential stays in the hive, we found that if a bee trembled and piped after one trip, then she repeated both piping and trembling after the next trip with a probability of 0.7 (n = 30).

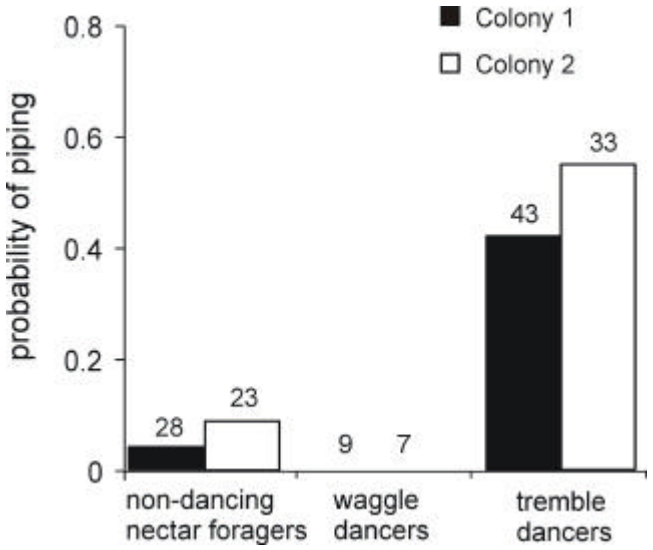

Fig. 5 The probability to pipe at least once during the stay in the hive for each behavioral group of nectar foragers. Numbers on top of the bars denote sample sizes.

Of 19 piping nectar foragers that were observed in C_1, 1 was a non-dancing nectar forager and 18 were tremble dancers. Of 20 piping nectar foragers observed in C_2, 2 were non-dancing nectar forager and 18 were tremble dancers. We recorded a total of 204 piping signals in C_1, 2 produced by the non-dancing nectar forager and 202 produced by tremble dancers. Of 299 signals recorded in C_2, non-dancing nectar foragers produced 2, and tremble dancers produced 297.

The higher probability of piping for tremble dancers relative to non-dancing nectar foragers and waggle dancers is not likely to be due to differences in lengths of stays in the hive. Most piping tremble dancers (81 %) emitted their first piping signal within 109 s of entering the hive. Since 88 % of the waggle dancers and 65 % of the non-dancing, non-piping nectar foragers stayed in the hive for at least 109 s, insufficient time in the hive is not likely to be the reason for their low probabilities of piping.

4. Behavior of piping nectar foragers

rate and pattern of signaling Piping nectar foragers produced in irregular intervals 1-47 piping signals/bee, with a mean of 10.7 ± 14.0, during a stay in the hive in C_1 (n = 19), and 1 - 91 signals/bee, with a mean of 15.0 ± 20.2, in C_2 (n = 20). The frequency of piping ranged between 0.3 – 3.0 pipes/bee/min, with a mean of 1.2 ± 0.9 in C_1 and 0.2 – 2.8 pipes/bee/min, with a mean of 1.1 ± 0.7 in C_2. These piping nectar foragers piped most during the first and second quarter of their stay in the hive. The number of pipes then decreased during the third quarter and further during the fourth quarter (χ^2 - test, $p < 0.001$ for both colonies). In C_1, 73, 77, 41, and 13 signals occurred in the 1st, 2nd, 3rd and 4th quarter, respectively. In C_2, the values were 97, 115, 57, and 30.

location of piping Piping nectar foragers (n = 19 in C_1 and 20 in C_2) produced piping signals on all three frames of the hive. The dance floor, where all recorded waggle dances were performed, was located on the lowest frame. The middle frame was mostly filled with brood and storage, while the top frame was mostly empty.

Many nectar foragers that piped more than once (n = 16 in C_1 and 18 in C_2) piped on more than one frame (25 % for C_1 and 67 % for C_2). Of 201 piping signals produced by nectar foragers that piped more than once in C_1, 71 % were produced on the bottom frame, 22 % on the middle frame and 7 % on the top frame. Of 297 piping signals in C_2, 47 % were produced on the bottom frame, 45 % on the middle frame and 8 % on the top frame. As piping nectar foragers produced most signals on the side of the frame that was closer to the hive entrance, almost all piping signals (100 % in C_1 and 93 % in C_2) on the bottom frame were produced on the dance floor that was located next to the entrance of the hive.

In both colonies, nectar foragers that produced more than one piping signal produced per unit time significantly fewer piping signals on the bottom frame and more signals on either the middle frame (C_1) or top frame (C_2) than expected after adjusting for time spent on each frame (χ^2 – test, in C_1 $p = 0.021$, number of pipes recorded 143, 44 and 14, and number of pipes expected 151, 31 and 19 on bottom, middle and top frame, respectively; for C_2 $p = 0.001$, number of pipes recorded 139, 133 and 25, and number of pipes expected 142, 143 and 12, on bottom, middle and top frame, respectively).

nectar foragers pipe in three ways Piping nectar foragers produced piping signals in three ways: 1. the piping bee pushed another bee with her head ("head butting") while pressing her thorax onto the comb, 2. the piping bee did not contact another bee while pressing her thorax onto the comb, or 3. the piping bee grasped another bee with the forelegs and pressed her thorax onto that bee. The grasping associated with the third way of piping resembles the grasping component of the shaking signal. All workers spread and lifted their wings to a varying degree during piping. Almost all foragers that piped more than once and for which we recorded the way of signaling (n = 11 in C_1 and 16 in C_2) piped in at least two ways (91 % in C_1, and 94 % in C_2), and often in all three ways. Fig. 6 shows the percentage of all piping signals performed in each way.

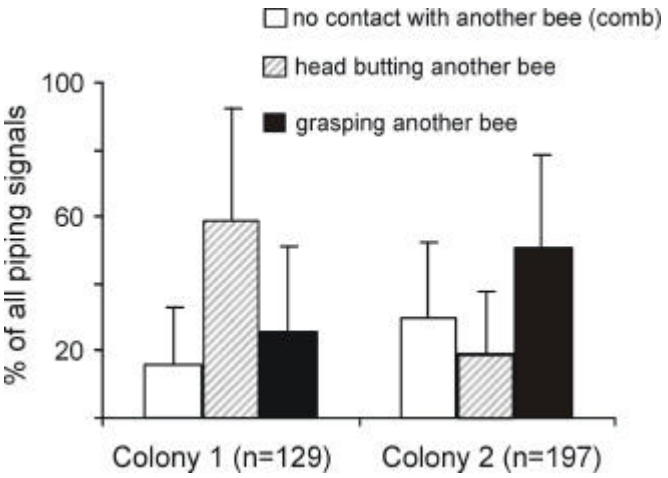

Fig. 6 The percentage of pipes performed in a given way. Sample sizes are given in brackets.

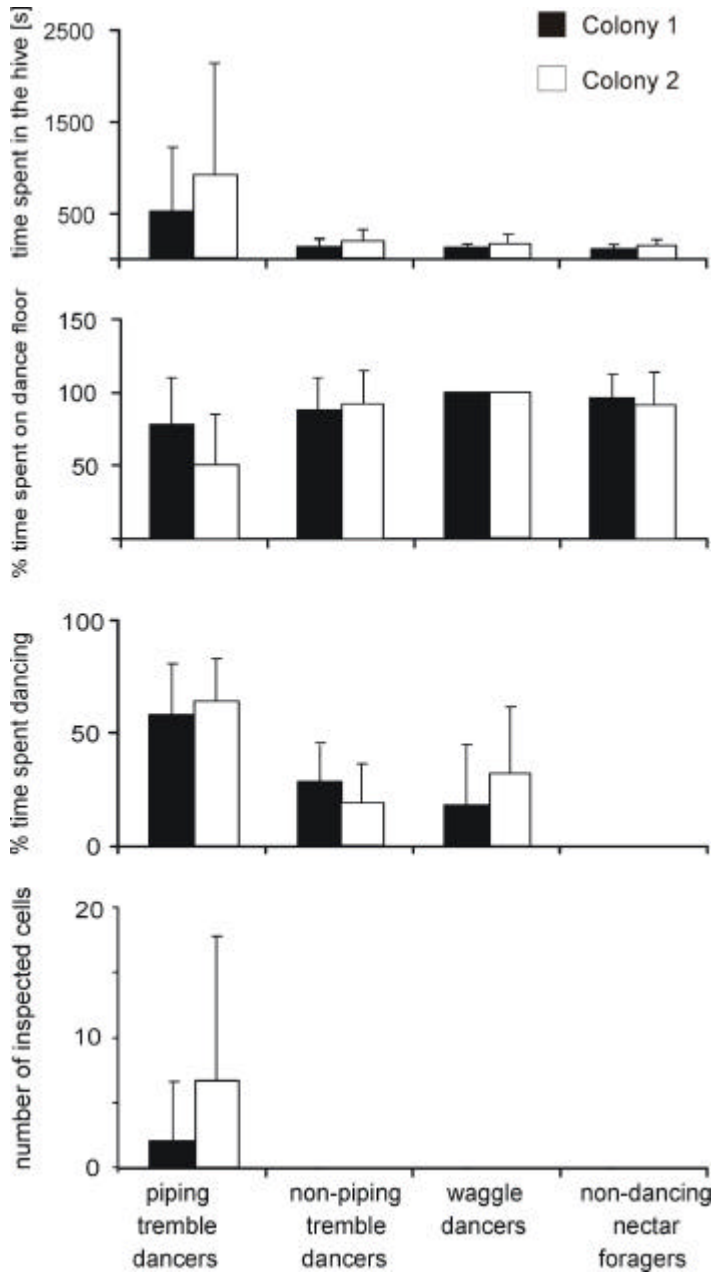

Fig. 7 The behavior of piping and non- piping nectar foragers. From top to bottom, the panels show the total time spent in the hive, the percentage of the total time spent on the dance floor, the percentage of the total time spent dancing, and the number of cells inspected by putting head and/or thorax into the cell. Sample sizes and statistics are given in the text.

5. Behavior of piping tremble dancers

The behavior of piping tremble dancers differed from the behavior of other nectar foragers. Fig. 7 compares piping tremble dancers (n = 18 in C_1 and 18 in C_2), non-piping tremble dancers (n = 25 and 15), waggle dancers (n = 9 and 7), and non-dancing, non-piping nectar foragers (n = 27 and 21) with respect to time spent in the hive, time spent on the dance floor, time spent dancing, and the number of cell inspections. Fig. 8 shows for each group the time until the first unloading

contact, the percentage of bees that danced before they had their first unloading contact, and the time until the first dance was started. To simplify the text, "non-dancing nectar foragers" is used as an abbreviation for "non-dancing, non-piping nectar foragers" .

time spent in the hive Piping tremble dancers remained for up to 1.5 h in the hive. On average, piping tremble dancers stayed in the hive for 8.9 ± 11.4 min in C_1 and 15.8 ± 20.3 min in C_2, non-piping tremble dancers stayed for 2.7 ± 0.9 and 3.4 ± 2.0 min, waggle dancers for 2.4 ± 0.5 and 2.9 ± 1.6 min, and non-dancing nectar foragers stayed for 2.1 ± 0.8 and 2.6 ± 1.0 min. Piping tremble dancers stayed significantly longer in the hive than non-piping tremble dancers, waggle dancers and non-dancing nectar foragers (Mann-Whitney-U Test, $\alpha = 0.017$, $p < 0.004$ for each comparison).

time spent on the dance floor The dance floor was located on the bottom frame near the entrance. Piping tremble dancers spent on average 77 ± 32 % of their time on the dance floor in C_1 and 51 ± 34 % in C_2, non-piping tremble dancers 88 ± 21 % and 91 ± 23 %, non-dancing nectar foragers 95 ± 17 % and 91 ± 22 %, and all waggle dancers spent 100 % of their time on the dance floor.

Piping nectar foragers spent a smaller proportion of their time on the dance floor than other nectar foragers, but this difference was significant only for C_2 (Mann-Whitney U-test, $\alpha = 0.017$, $p < 0.004$ for each comparisons in C_2 and $p > 0.065$ for each comparison in C_1). Piping tremble dancers in both colonies advanced deeper into the hive than other nectar foragers. 22 % of all piping tremble dancers in C_1 and 11 % in C_2 walked up to the top frame of the hive. No other nectar foragers reached the top frame.

time spent dancing Piping tremble dancers danced on average for 58 ± 23 % of their time in the hive in C_1 and for 64 ± 19 % in C_2, non-piping tremble dancers danced on average for 29 ± 17 % and 20 ± 17 %, and waggle dancers danced for 20 ± 14 % and 34 ± 30 %. Piping tremble dancers spent significantly more of their time dancing than non-piping tremble dancers and waggle dancers (Mann-Whitney- U Test, $\alpha = 0.025$, $p < 0.001$ for each comparison in C_1 and in C_2 for comparison with non-piping tremble dancers, and $p = 0.021$ in C_2 for comparison with waggle dancers). Non-piping tremble dancers and waggle dancers did not differ from each other in either colony ($p = 0.191$ in C_1 and 0.275 in C_2).

cell inspections Piping tremble dancers sometimes inspected cells by putting their head, or head and thorax, into a cell. 28 % of the piping tremble dancers in C_1 and 44 % of the piping tremble dancers in C_2 inspected cells. 63 % of the piping tremble dancers that were followed for 2 or more sequential stays in the hive (n = 8) inspected cells during at least one stay. No other nectar foragers inspected cells.

We counted up to 38 inspections per stay, with a mean of 6.4 ± 8.3 (C_1) and 9.4 ± 13.5 (C_2) inspections per cell-inspecting bee. In C_1, the duration of inspections ranged from 0.2 s – 23.9 s and inspecting bees spent 2.3 ± 2.7 % of their time in the hive with inspections (n = 5). In C_2, the duration of inspections ranged from 0.4 s – 50.5 s and inspecting bees spent 9.8 ± 12.0 % of their time in the hive with inspections (n = 8).

In C_1, all cell inspecting foragers had at least one unloading contact during their stay in the hive. In C_2, however, 38 % of the cell- inspecting foragers (equal to 17 % of all piping tremble dancers) did not have any trophallactic contacts during their stay in the hive, hence they did not unload to another bee. Coming from a feeder, it seems likely that these bees unloaded into cells. There was no obvious order in which cell-inspecting bees unloaded for the first time and inspected their first cell.

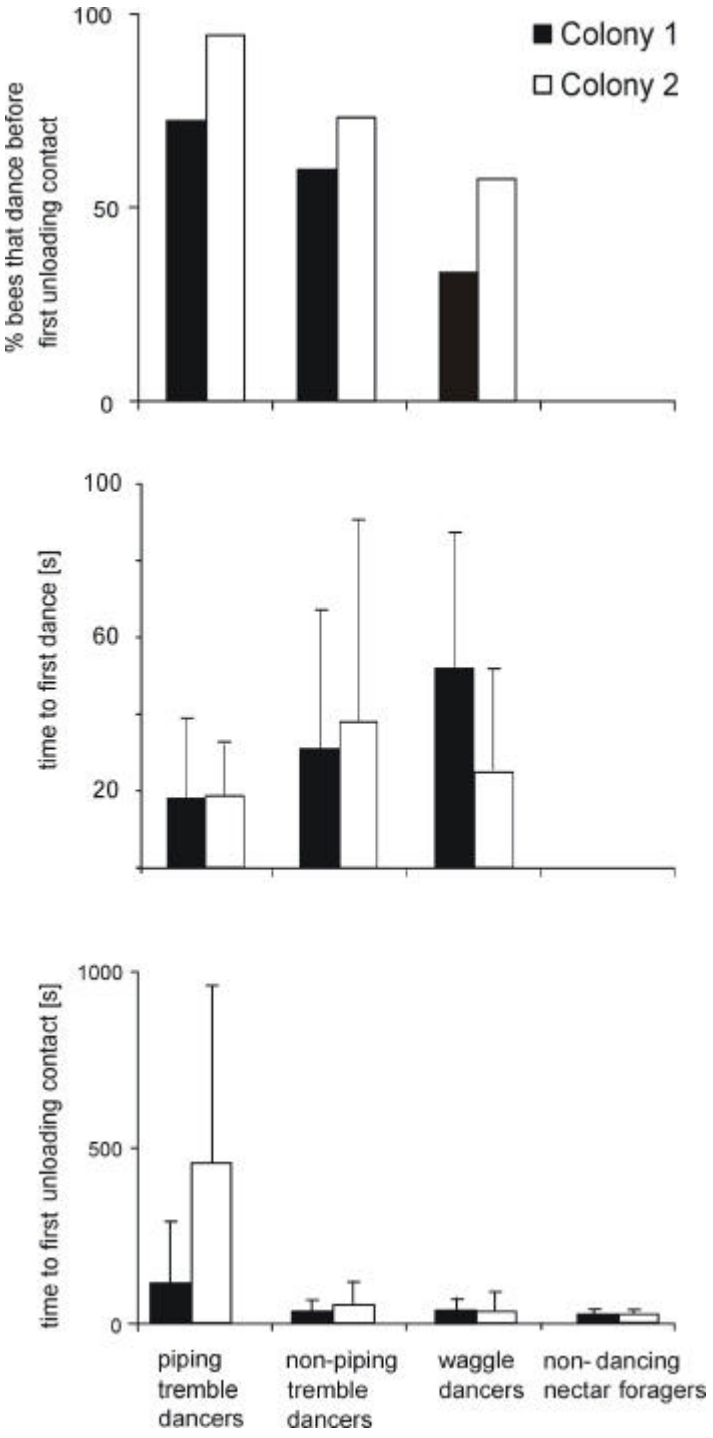

Fig. 8 The behavior of piping and non- piping nectar foragers. From top to bottom, the panels show the time until the start of the first trophallactic contact that lasted 3 s or longer, the percentage of bees that started a dance before they had a unloading contact, and the time until the start of a dance. Sample sizes and statistics are given in the text.

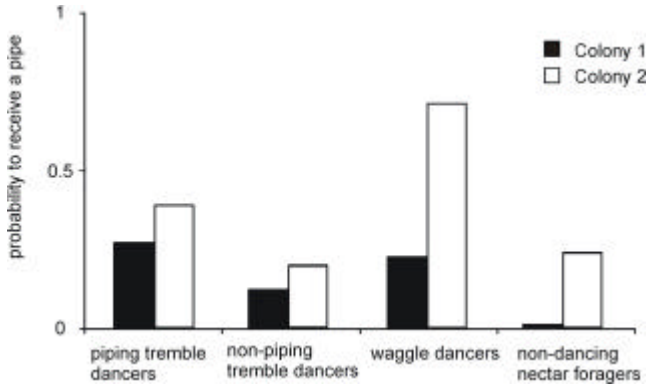

Fig. 9 The probability to receive at least one pipe during the stay in the hive for each behavioral group of nectar foragers.

time to start dancing The majority of piping tremble dancers started to dance before they had their first unloading contact. Piping tremble dancers danced first with a probability of 0.72 in C_1 and 0.94 in C_2. Non-piping tremble dancers danced first with a probability of 0.60 and 0.73, and waggle dancers danced first with a probability of 0.33 and 0.57.

Piping tremble dancers started to dance 18.3 ± 21.0 s after entering the hive in C_1 and after 18.7 ± 14.0 s in C_2. Non-piping tremble dancers started to dance after 31.3 ± 36.2 s and 38.3 ± 52.9 s, and waggle dancers started to dance after 54.5 ± 31.8 s and 25.3 ± 26.6 s. Piping tremble dancers, non-piping tremble dancers and waggle dancers in C_2 did not differ in the start time of their dance (Mann-Whitney- U Test, $\alpha = 0.017$, $p > 0.7$ for all comparisons). In C_1, and non-piping tremble dancers did not differ from piping tremble dancers ($p = 0.110$) or waggle dancers ($p = 0.044$), but piping tremble dancers started to dance significantly earlier than waggle dancers ($p = 0.006$).

time to first unloading contact The time to the first unloading contact (t_u) was measured as the time interval between when the forager entered the tunnel of the hive and her first trophallactic contact that lasted 3 s or longer.

Piping tremble dancers often started to dance soon after they entered the hive and rarely interrupted their dance for unloading contacts even when other bees tried to contact the tremble dancing bee by stretching the proboscis towards her. 17 of 18 piping tremble dancers in C_1 and 15 of 18 piping tremble dancers in C_2 unloaded to a bee. The mean t_u of piping tremble dancers was 122.2 ± 178.5 s in C_1 and 505.6 ± 512.8 s in C_2. The mean t_u of non-piping tremble dancers was 36.3 ± 30.8 s and 54.2 ± 56.5 s, and of waggle dancers 34.7 ± 32.0 s and 36.2 ± 55.2 s. Non-dancing nectar foragers had a mean t_u of 21.1 ± 18.7 s and 22.0 ± 16.7 s.

Piping tremble dancers had a longer t_u than other nectar foragers, but this difference was significant only in C_2 (Mann-Whitney- U Test, $\alpha = 0.013$, $p < 0.003$ for each comparison in C_2 and $p > 0.070$ for each comparison in C_1). Non-piping tremble dancers and waggle dancers did not differ from each other ($p = 0.654$ in C_1 and 0.245 in C_2).

6. Identity of bees that received piping signals

To determine the identity of the receivers of piping signals, we recorded the number of piping signals directed at non-marked bees, bees marked at the feeder (nectar foragers), or the comb. The majority of piping signals (74 % in C_1 and 62 % in C_2) for which we recorded the direction ($n = 129$ in C_1 and 197 in C_2), were directed at individual bees, not the comb. 33 % and 5 % of the piping signals that were directed at individual bees were directed at marked nectar foragers (for C_1 and C_2, respectively). The number of pipes directed at marked nectar foragers in C_2 is likely to be an underestimate resulting from crowding at the feeder that prevented marking of all nectar foragers. The feeder visited by C_1 was generally less crowded, and visiting nectar foragers could be marked more reliably.

To estimate for each group of nectar foragers the probability of receiving at least one pipe (Fig. 9), we recorded when a focal nectar forager (one of the 80 foragers in C_1, and 63 foragers in C_2 that were followed throughout a stay in the hive) received a pipe.

28 % of the piping tremble dancers in C_1 ($n = 18$) and 39 % of the piping tremble dancers in C_2 ($n = 18$) received a piping signal, 12 and 20 % of the non-piping tremble dancers ($n = 25$ and 15), 22 % and 71 % of the waggle dancers ($n = 9$ and 7) and 24 % of the non-dancing, non-piping nectar foragers ($n = 27$ and 21). One of the 3 non-dancing, piping nectar foragers received a piping signal. In both colonies, focal waggle dancers and piping tremble dancers received more pipes than expected, while non-piping tremble dancers and non-dancing, non-piping foragers received fewer pipes than expected (χ^2-test, $p < 0.004$ for each C_1 and C_2, expected values adjusted for time spent in the hive).

7. Behavior of bees after they received a pipe

To investigate the effect of the piping signal on the bees that received more signals than should be expected, we compared the behavior of piping tremble dancers and waggle dancers 1 min before and 1 min after they received the signal. We compared the behavior of only those focal bees that were observed for at least 1 min before they received the signal. We compared the

behavior before and after a total of 17 piping signals that were received by 8 piping tremble dancers, and of 4 piping signals that were received by 4 waggle dancers. The behavior of neither piping tremble dancers nor waggle dancers changed after they received a piping signal (Mann-Whitney U- test, $\alpha = 0.05$, $p > 0.121$ for all comparisons, data pooled for both colonies). However, the low sample sizes might have obscured any effects of the signal. We never observed the freeze response noted in previous studies (e.g. Nieh 1993), though bees were restricted in their movements as long as a piping bee grasped them with the forelegs or head-butted them. Such restriction of movement was never observed in bees that received the signal via the comb.

Discussion

The brief piping signals produced by honey bee workers in undisturbed, queenright colonies are associated with nectar foraging and were stimulated reliably by promoting a colony's foraging activity. This result demonstrates a causal connection between this form of worker piping and nectar foraging. The brief durations and frequencies of the piping signals elicited by stimulating a colony's foraging activity suggest that they are identical to the "stop signals" described by Nieh (1993). However, as we could analyze the characteristics of only a small sample of the piping signals produced during nectar foraging, we cannot be sure that all signals were stop signals. Piping signals with durations different from the stop signal were also suggested to be related to foraging (Pratt et al., 1996). To more precisely define the nectar foraging context of worker piping, the stimulus that causes a nectar forager to pipe needs to be determined.

The results of this study demonstrate that piping signals are an important part of the foraging communication system, as was suggested by Nieh (1993) and Kirchner (1993). That piping signals are part of the foraging communication system is demonstrated by the fact that almost all piping nectar foragers were tremble dancers and thus known to be participants in the foraging communication system (Seeley 1992, Seeley et al. 1996). It is not likely that tremble dancers interrupt their foraging tasks to perform non-foraging signals. The importance of piping signals in the foraging communication system is suggested by two results of this study. First, in a colony that was foraging on natural nectar sources, the rate of piping signals was 4 ± 3 pipes/min, a rate similar to the rate of foraging

dances (3 ± 5 dances/min, see Fig.3). Such frequent signals undoubtedly play an important role in foraging communication. Second, that although tremble dancers produced virtually all piping signals, piping signals are not automatically produced as part of the tremble dance. Only about half of all tremble dancers piped. Thus, the tremble dance and the piping signal appear to have related, but not identical, functions in the foraging communication system.

The results of this study have implications for the current hypothesis on the function of the brief piping signal of nectar foragers. Nieh (1993) and Kirchner (1993) have shown that this signal inhibits waggle dancing, thereby augmenting the function of the tremble dance, which recruits nectar receivers (Seeley, 1992, Seeley et al., 1996). According to Nieh and Kirchner, the signal retards the recruitment of additional nectar foragers when the amount of nectar coming into the hive is already more than the colony can process. Under this hypothesis, piping nectar foragers would need to assess the current state of the relative work capacities of foragers and receivers. Presumably, piping nectar foragers do this by using the same mechanism as tremble dancers, that is by measuring the time delay before unloading to a receiver bee (Seeley, 1992). In this study, the majority of piping tremble dancers started to dance immediately after they returned to the hive, often before even approaching a nectar receiver. Piping tremble dancers never offered droplets of nectar to attract nectar receivers, and sometimes never contacted nectar receivers at all, but instead unloaded their nectar directly into cells. This behavior of the piping tremble dancers suggests that they were not using a time delay cue after each return to the hive to assess the need to adjust the nectar-gathering effort. However, this does not disprove the hypothesis that piping nectar foragers assess and help adjust the relative work capacities of nectar foragers and nectar receivers. It is possible that piping tremble dancers based their decision to pipe on time delay cues from previous foraging trips. The bees we observed might have already experienced numerous foraging trips followed by long unloading delays before we recorded their behavior. Alternatively, piping tremble dancers might not use time delay cues to decide whether or not to pipe.

Instead of cues sensed inside the hive, nectar foragers might use cues sensed outside the hive to decide whether or not to pipe. Several authors have induced tremble dancing, and thus possibly piping, by disturbing nectar foragers at a food source (v. Frisch, 1967, Kirchner and Lindauer, 1994), which suggests that nectar foragers can use external cues to

decide whether or not to tremble dance, and possibly to pipe. In this study, foragers sometimes had to scramble for access to the crowded sugar-water feeder, because the number of recruits to the feeder was not restricted. Thus, it is possible that the piping nectar foragers were reacting to the external cue of crowding at the food source. Hence, the piping signal of nectar foragers might serve to stop recruitment to a low-quality food source and allowing reallocation of recruits to other, less exploited food sources rather than to retard the recruitment of additional nectar foragers. If this is the case, it may be expected that the signal is directed mostly at waggle dancers recruiting to the same low-quality food source that the piping nectar forager has visited. However, this hypothesis does not explain why piping tremble dancers signal at other location than the dance floor, and why many piping signals are directed at bees other than waggle dancers.

The piping signals produced by tremble dancers may serve other functions than the inhibition of waggle dances. If piping serves only to inhibit waggle dances, then piping bees should signal mostly on the dance floor, where the probability to target waggle dancers is highest, and rarely be at other locations in the hive. However, piping tremble dancers advanced deeper into the hive and spent less of their time in the hive on the dance floor than other nectar foragers, and produced many (29 – 56 %) of their piping signals off the dance floor. Waggle dancers, on the other hand, spent all their time on the dance floor and performed all dances there. One hypothesis for why tremble dancers pipe off the dance floor is that tremble dancers, who target potential nectar receivers that are mostly off the dance floor, use the piping signal as a modulatory signal. Modulatory signals alter the response threshold of the signal receivers towards other stimuli (Hölldobler and Wilson, 1990). The piping signal might lower a pipe receiver's response threshold to the tremble dance, and thus facilitate the recruitment of additional nectar receivers via the tremble dance. Piping tremble dancers seemed to be more motivated than non-piping tremble dancers, because they started to dance earlier after their return, danced longer and sometimes unloaded into cells. Highly motivated tremble dancers might employ the piping signal to enhance their recruitment success. Enhancing the recruitment success of the tremble dance might be beneficial when the need for additional nectar receivers is especially urgent, as was probably the case in this study. As tremble dancing without piping occurs frequently, but piping without tremble dancing only rarely, it is likely that the piping signal emphasizes the tremble dance rather than vice versa. This study suggests

that most of the piping signals that are directed at individual bees are directed at non-foraging bees (67 – 95 %), which are potential nectar receivers. If piping serves not only to inhibit the recruitment of additional nectar foragers, but also to modulate the recruitment of additional nectar receivers, it may be an example of a signal with one message and two meanings.

Chapter IV

Tremble dancing in honey bees can be stimulated directly by hive-external factors

Abstract

This study presents evidence that the tremble dance of honey bee nectar foragers can be stimulated directly by a high density of foragers at the food source. I increased the density of nectar foragers at an artificial food source by reducing the size of the feeder. When the size of the feeder was reduced, foragers had to crowd around the feeder and scramble for access to the food. Crowding at the feeder did not delay a foragers' first unloading contact with a nectar receiver bee, but increased the probability that returning foragers performed a tremble dance. A long unloading delay was until now the only factor known to elicit tremble dancing. Although the significance of crowding at the food source under natural conditions is not yet established, this study shows that nectar foragers returning from natural food sources often have short unloading delays, and thus suggests that hive-external factors directly stimulate tremble dancing under natural conditions. It is likely that tremble dancing can be elicited by several factors that each indicate a low foraging efficiency. Although tremble dancing was shown to recruit nectar receiver bees in earlier studies, no additional nectar receiver bees seemed to be recruited in this study. Possibly, not all situations that stimulate tremble dancing can be improved by recruiting nectar receivers, in which case the tremble dance may be supposed to have an additional function.

Introduction

The tremble dance of honey bee (*Apis mellifera*) nectar foragers is part of a feedback system that helps to regulate a colony's nectar foraging efficiency (Seeley 1992, Kirchner & Lindauer 1994, Seeley et al. 1996). Several studies have shown or suggested that hive-external factors, e.g. characteristics of the food source like degree of crowding, disturbances, or food quality, can stimulate foragers to tremble dance (v. Frisch 1967, Lindauer 1948, Schneider 1949, Schick 1953, Kirchner and Lindauer 1994, Thom et al. *submitted*). However, it has not been shown whether these factors stimulate tremble dancing directly, or indirectly by delaying in the hive the time when a returning forager can unload her nectar to a nectar receiver bee.

A long unloading delay, usually caused by a shortage of nectar receivers, is until now the only stimulus known to directly elicit tremble dancing in nectar foragers (Seeley 1992, Seeley et al. 1996). Because each returning forager unloads her nectar to a nectar receiver bee instead of into a cell before she leaves the hive on her next foraging trip, the duration of her unloading

delay provides her with information about how efficiently nectar foragers and nectar receivers work together. Queuing delays for either group lower the colony's foraging efficiency, and can be adjusted by recruiting workers to the group in shortage. If nectar foragers are in shortage, returning nectar foragers experience short unloading delays and are likely to perform waggle dances to recruit additional nectar foragers. If nectar receivers are in shortage, returning nectar foragers experience long unloading delays and are likely to perform tremble dances to recruit additional nectar receivers (Seeley 1992, Seeley et al. 1996).

As it is crucial for the understanding of a signal's function to recognize its causes, this study investigates whether a hive-external stimulus, the density of foragers at the food source, elicits tremble dancing directly, or indirectly by increasing unloading delay. For example, crowding at a food source could induce nectar foragers to release alarm pheromone that in the hive may repel nectar receivers and thus cause long unloading delays. To test whether crowding at the food source stimulates tremble dancing directly or indirectly, I manipulated the density of nectar foragers at the food source while controlling the duration of unloading delays inside the hive. To additionally examine whether in a non-manipulated colony tremble dancing is usually preceded by long unloading delays, I recorded the unloading delays of nectar foragers that returned from natural food sources.

Material and Methods

Study site and observation hives

The study was conducted from May to August, 2001, at the honey bee laboratory of the University of Würzburg, Germany. Observations were made on two colonies (C_1 and C_2) of the carniolan honey bee, *Apis mellifera carnica*. The colonies were housed indoors in two-frame observation hives, with internal dimensions of 45 x 45 x 5 cm and entrance tunnels leading outside. About 18 cm^2 of the glass covering the dance floors near the junction of the hive and the entrance tunnel were removed and replaced with a cloth mesh that allowed the marking of bees that exited the hive. To ensure that each bee was observed only once, each observed bee was marked wit a dot of paint on the thorax when she exited the hive. All observations and experiments were made with one colony at the time.

Measuring decision time and dances

The duration of a nectar forager's unloading delay can inform her about which workers (nectar foragers or nectar receivers) are in shortage and need to be recruited. If the delay to the first unloading contact exceeds a certain duration, it provides enough information for the nectar forager to make a recruitment decision without waiting any longer for the actual unloading contact. In this case, the bee starts to dance before she has had her first unloading contact. Thus, the time interval that contains the information about which workers need to be recruited, the nectar forager's "decision time" for a recruitment dance, is either the time interval between the entrance of a nectar forager into the tunnel of the hive and her first unloading contact (t_u), or, if the nectar forager starts to dance before she unloads, the time interval between the nectar forager's entrance into the hive and the start of her first dance (t_d). For nectar foragers that do not dance, decision time always equals t_u. The decision time of a dancing nectar forager can be either t_u or t_d. The median decision time of a group of nectar foragers represents the median of all t_u and t_d values of the nectar foragers in this group. Because t_u and t_d indicate different measures of decision time, I will present not only the median decision time of a group of foragers, but also the median t_d of those foragers in the group that danced first, and the median t_u of those foragers that unloaded first. The difference between the decision time and the "search time" sometimes used in earlier studies is that search time usually indicates the time until the first trophallactic contact with a nectar receiver bee, while decision time indicates the time until the first unloading contact *or* the time until the start of a dance.

To determine the decision times and dances of nectar foragers, I recorded when each observed bee entered the entrance tunnel of the hive, when she had her first unloading contact, when she started her first dance, and what type of dance she performed. Only trophallactic contacts that were equal or longer than 3 s were considered to result in food transfer and thus be unloading contacts. Nectar foragers that did not dance were observed during their entire stay in the hive.

Experiment

Nectar foragers returning from a crowded food source To determine whether crowding at the food source causes nectar foragers to tremble dance directly, or indirectly by increasing unloading delay, I manipulated the density of a fixed number of nectar foragers at a food source and recorded the unloading delays and dances in the hive during experiments in July and August,

2001. The food source was a grooved-plate feeder that was located 25 m from the hive. The feeder provided a concentrated sugar solution ("Apiinvert®" [Südzucker], 2.4 mol/l, sugar composition 61 % glucose, 39 % fructose) and supplied most, or all, of the food collected by the colonies, as natural nectar sources were scarce during this time.

I recorded the unloading delays and dances of foragers from C_1 and C_2 during 4 experiments with each colony for a total of 8 experiments. Experiments started between 0900 and 1300 and consisted of a control phase that was followed by a manipulation phase. Each phase lasted approximately 50 min. During the control, the feeder had a circumference of 79 cm (diameter 25 cm) and allowed all visiting nectar foragers to simultaneously access the food. During the manipulation, the feeder had a circumference of 16 cm (diameter 5 cm). This smaller feeder did not allow simultaneous access of all visiting nectar foragers to the food, so that nectar foragers had to scramble for access to the food.

To keep the demand for nectar receivers constant during the experiment, I allowed only a fixed number of foragers to visit the feeder. To do so, I trained 200 nectar foragers of the observation colony to the feeder one week before the experiments started. To recognize nectar foragers of the observation colony at the feeder, I first marked bees at the entrance of the hive with one color, and then added a second color at the feeder (for training and marking technique see v. Frisch 1967). During the time of training and experiments, an assistant captured with forceps all unmarked bees from the feeder. Captured bees were kept in a wood cage to prevent a build-up of alarm pheromone that captured bees might release. To compensate for loss of foragers between experiments, 10- 30 additional foragers from the observation colony were allowed to access the feeder after each experiment, and were then marked. Although the total number of marked foragers might have changed *between* experiments (i.e. decreased due to death or increased due to additional marking of foragers), it is unlikely that the number of foragers decreased significantly *during* an experiment and thus changed the number of foragers visiting the feeder between control and manipulation phase of the experiment. To check whether the approximately same number of foragers visited the feeder per unit time during each control and manipulation of the experiment, the feeder assistant recorded every 5th min the number of nectar foragers at the feeder during 3 control and 2 manipulation phases in experiments with C_1, and during 4 control and 3 manipulation phases in experiments with C_2. The assistant did not record the number of nectar foragers when the tending of the feeder needed undivided attention.

To check whether tremble dancing preceded a general decrease in the time to first unloading delay that was due to the recruitment of additional nectar receivers, I compared the time to the first unloading delay of nectar foragers before and after the 30^{th} min during the manipulation of the experiment.

Nectar foragers returning from natural food sources To determine the decision times and dances of non-manipulated nectar foragers, I observed nectar foragers from C_1 and C_2 that returned from natural food sources during May and June, 2001. No artificial food sources were set up during observations. Observations started at 0800 and lasted until 1500-1900. To reduce the probability of observing returning bees that were not nectar foragers, observations were interrupted during times of the conspicuous orientation flights of hive bees. Of the non-dancing bees, only those were considered to be nectar foragers that had at least one trophallactic contact before they exited the hive again. Although I could not distinguish between foragers for water and nectar, the probability for a forager to gather water is usually much smaller than the probability to gather nectar (Seeley 1986). Thus, it is not likely that decision times of water foragers introduced a large bias to the decision times of nectar foragers. Like nectar foragers, water foragers unload to hive bees and are thought to use unloading delay to assess the demand for water in the colony (Lindauer 1954, Kühnholz & Seeley 1997).

Statistical analysis

Measurements are given as medians and measures of variability are given as one standard deviation, unless otherwise noted. I chose the median over the mean because data were usually not normally distributed.

I calculated for each data set the probability for a returning nectar forager to waggle or tremble dance as a function of decision time. I calculated the probability to perform a dance for a given decision time interval only if the sample size for that decision time interval was equal or bigger than 5. Several time intervals may be pooled to reach a sufficient sample size to calculate dance probability.

Statistical tests are given in the text. All data were analyzed using the ME edition of Microsoft Excel and the 2002 edition of Statistica. Bonferroni corrections for multiple comparisons were performed according to Sokal and Rohlf (1995). The adjusted α–level is noted in the text.

Results

Dances and decision times of nectar foragers that visited a non-crowded versus a crowded feeder

To investigate whether nectar foragers tremble dance as a direct reaction to a high density of foragers at the food source, I recorded the decision times and dances of 210 nectar foragers during the controls, and of 230 nectar foragers during the manipulations of a total of 8 experiments. Table 1 gives an overview over the decision times of all nectar foragers observed in this study. To be able to estimate the number of nectar foragers returning to the hive, an assistant recorded every 5th min the number of nectar foragers at the feeder during 7 control and 5 manipulation phases of the experiment. The assistant did not record the number of nectar foragers when tending the feeder needed undivided attention.

Table 1

	Waggle dancers	Tremble dancers	Non–dancing nectar foragers
Natural food source			
Sample size n	41	25	31
t_d [s] (n for t_d)	9.3 ± 18.5 (27)	9.0 ± 15.9 (20)	
t_u [s] (n for t_u)	14.3 ± 11.5 (14)	15.1 ± 35.4 (5)	30.1 ± 26.4 (31)
decision time [s]	10.6 ± 16.4	10.3 ± 21.2	30.1 ± 26.4
Experiment control			
Sample size n	115	36	59
t_d [s] (n for t_d)	5.1 ± 6.8 (66)	4.7 ± 5.6 (33)	
t_u [s] (n for t_u)	4.2 ± 4.1 (49)	6.1 ± 4.4 (3)	5.1 ± 7.1 (59)
decision time [s]	4.7 ± 5.8	4.8 ± 5.5	5.1 ± 7.1
Experiment manipulation			
Sample size n	23	160	47
t_d [s] (n for t_d)	7.9 ± 10.3 (11)	2.7 ± 5.8 (144)	
t_u [s] (n for t_u)	3.8 ± 3.1 (12)	4.0 ± 9.3 (16)	4.8 ± 6.5 (47)

decision time [s]	6.7 ± 8.2	2.7 ± 6.3	4.7 ± 6.6
Comparison (p- value)			
control versus manipulation			
t_d [s]	0.013	< 0.001	
t_u [s]	0.899	0.491	0.463
decision time [s]	0.120	< 0.001	0.463

Number of nectar foragers at the feeder In C_1, the average number of nectar foragers at the feeder was 72.0 ± 9.5 foragers/min during the control (n = 3), and 76.3 ± 11.3 foragers/min during the manipulation (n = 2). In C_2, the average number of nectar foragers was 46.8 ± 11.2 foragers/min during the control (n = 4), and 56.6 ± 6.4 foragers/min during the manipulation (n = 3). The number of nectar foragers at the feeder was higher during the manipulation than during the control, but this difference was not significant for either colony (Mann-Whitney- U Test, p > 0.275 for each comparison).

Dances Fig. 1 shows for each colony the sequence of dances observed during the time course of the experiment.

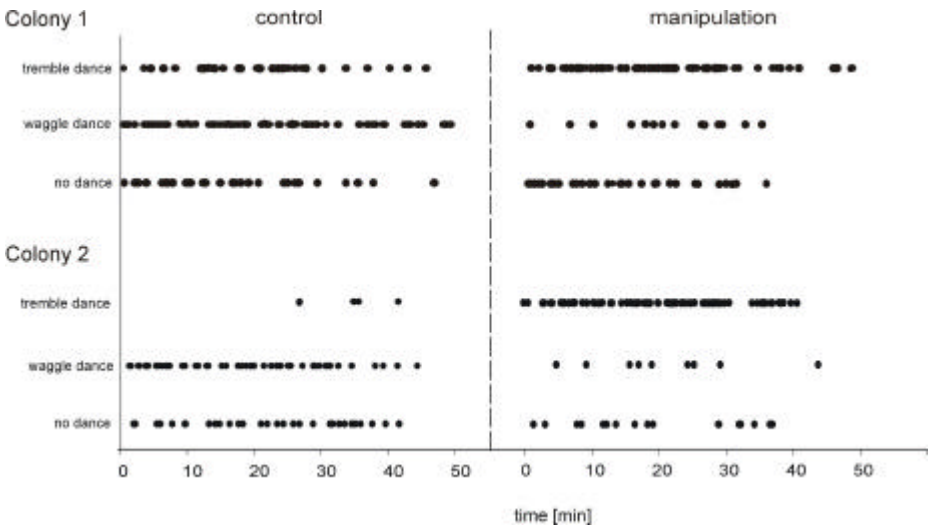

Fig. 1 The time sequence of dances (tremble dance, waggle dance or no dance) during control and manipulation of the experiment for C_1 and C_2. Dances are pooled for all 4 experiments of each colony. Each marker represents one dance.

For the following analysis, data from C_1 and C_2 were pooled for each control and manipulation of the experiment. Fig. 2 shows the probability for a nectar forager to waggle dance, tremble dance or not dance in both the control and the manipulation of the experiment. During the control phase, 115 foragers waggle danced, 36 tremble danced and 59 did not perform a dance. During the manipulation phase, 23 foragers waggle danced, 160 tremble danced and 47 did not perform a dance. The probability for a nectar forager to waggle dance was significantly higher during the control than during the manipulation of the experiment (Mann-Whitney- U Test, $p < 0.001$, $n = 8$). The probability to tremble dance was significantly lower during the control than during the manipulation ($p = 0.005$). The probability to not dance did not change between control and manipulation ($p = 0.431$).

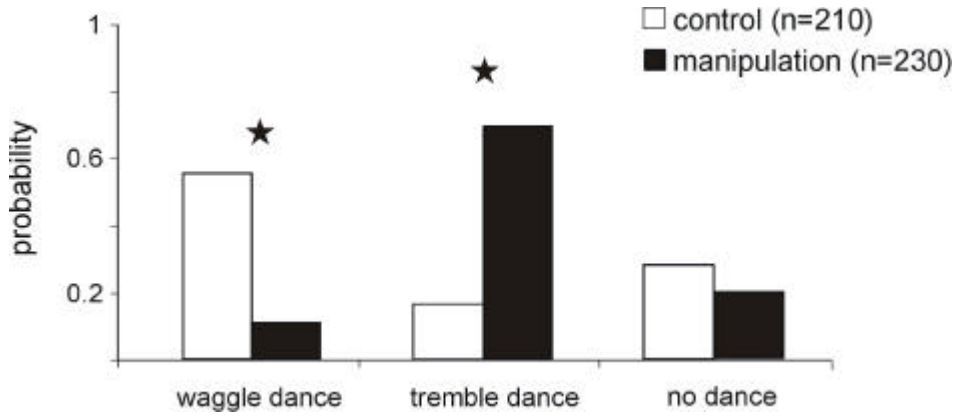

Fig. 2 The probability for a nectar forager to perform a waggle dance, a tremble dance or no dance during each control and manipulation of the experiment. Stars indicate significant differences between control and manipulation of the experiment. Statistics are given in the text.

Decision times Data of all waggle dancers, tremble dancers, and non-dancing nectar foragers were pooled for each control and manipulation from C_1 and C_2, because these groups did not differ in decision times between colonies (Mann-Whitney- U Test, $p \geq 0.165$ for each comparison).

During the control of the experiment, 57 % of the waggle dancers ($n = 115$) and 92 % of the tremble dancers ($n = 36$) started to dance before they had their first unloading contact. Waggle dancers that danced first had a t_d of 5.1 ± 6.8 s, and waggle dancers that unloaded first had a t_u of 4.2 ± 4.1 s. Tremble dancers that danced first had a t_d of 4.7 ± 5.6 s and tremble dancers that

unloaded first had a t_u of 6.1 ± 4.4 s. The t_d was not different from the t_u for each waggle dancers and tremble dancers (Mann-Whitney- U Test, $p > 0.102$ for each comparison).

During the manipulation of the experiment, 48 % of the waggle dancers (n = 23) and 90 % of the tremble dancers (n = 160) started to dance before they had their first unloading contact. Waggle dancers that danced first had a t_d of 7.9 ± 10.3 s, and waggle dancers that first unloaded had a t_u of 3.8 ± 3.1 s. Tremble dancers that danced first had a t_d of 2.7 ± 5.8 s and tremble dancers that unloaded first had a t_u of 4.0 ± 9.3 s. The t_d of waggle dancers that danced first was significantly longer than the t_u of waggle dancers that unloaded first (Mann-Whitney- U Test, $p = 0.025$). Tremble dancers did not differ in t_u and t_d ($p = 0.201$). The t_u of each waggle dancers and tremble dancers was shorter during the manipulation than during the control, but these differences were not significant (Mann-Whitney- U Test, $p > 0.490$ for each comparison). Waggle dancers had a significantly shorter t_d during the control than the manipulation ($p = 0.013$), and tremble dancers had a significantly longer t_d during the control than during the manipulation ($p < 0.001$).

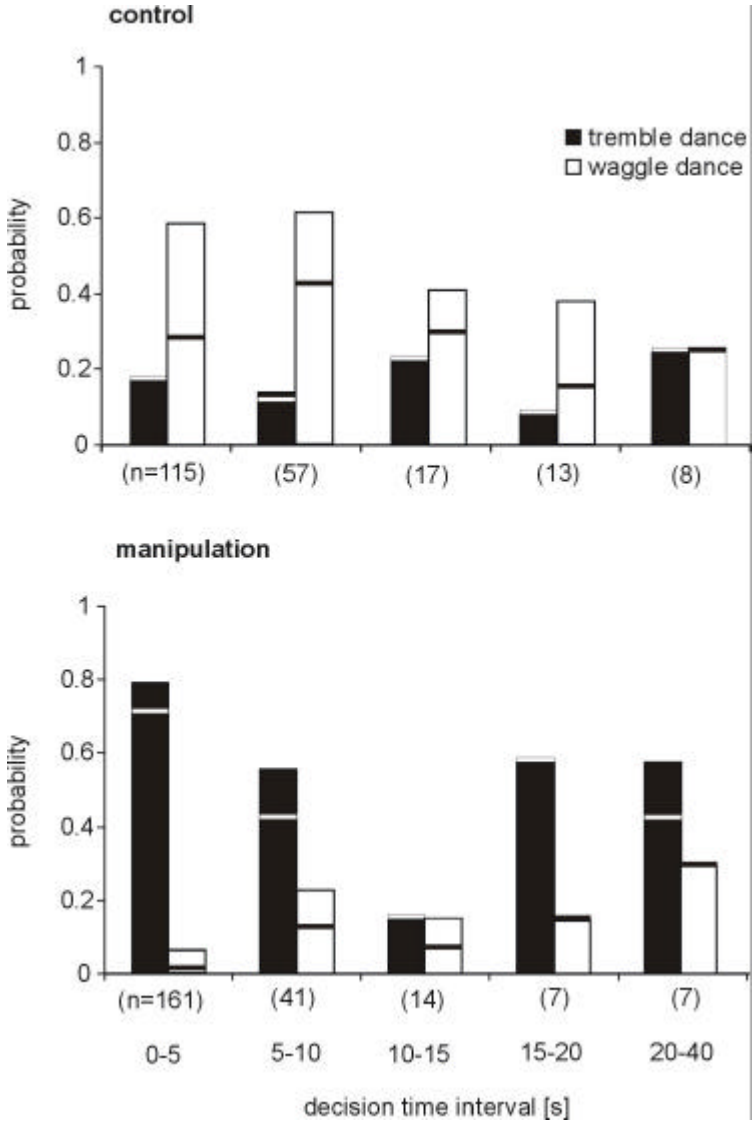

Fig. 3 The probability to perform a waggle- or a tremble dance for nectar foragers that returned from an artificial feeder as function of decision time. The section below the line within each bar shows the probability to dance before the first unloading contact, the section above the line the probability to unload before the first dance was started. A line on the top of a bar notifies a probability of 1 to dance first, and on the bottom a probability of 0. Data of C_1 and C_2 are pooled. Sample sizes are given in brackets.

Fig. 3 shows the probability for a nectar forager to perform a waggle dance or a tremble dance as a function of decision time during each control and manipulation of the experiment. Of all waggle dancers observed during the control of the experiment, 88 % had decision times between 0 - 10 s, 98 % between 0 - 20 s, and 99 % between 0 – 30 s. For tremble dancers, these values were 81, 94, and 100 %. For non-dancing nectar foragers, the values were 71, 93, and 100 %. Waggle dancers had a median decision time of 4.7 ± 5.8 s, tremble dancers of 4.8 ± 5.5 s, and non-dancing nectar foragers of 5.1 ± 7.1 s.

of all waggle dancers observed during the manipulation of the experiment, 78 % had decision time between 0 - 10 s, 91 % between 0 - 20 s, and 100 % between 0 – 30 s. For tremble dancers, these values were 94, 98, and 99 %. For non-dancing nectar foragers, the values were

72, 98, and 100 %. Waggle dancers had a median decision times of 6.7 ± 8.2 s, tremble dancers of 2.7 ± 6.3 s, and non-dancing nectar foragers of 4.7 ± 6.6 s. Tremble dancers had a significantly longer decision time during the control than during the manipulation (Mann-Whitney- U Test, $p < 0.001$), but the decision time of each waggle dancers and non-dancing nectar foragers did not differ between control and manipulation ($p = 0.120$ for each comparison). The decision times of tremble dancers, waggle dancers, and non-dancing nectar foragers did not differ from each other during the control (Mann-Whitney- U Test, α – level after Bonferroni correction for multiple comparisons 0.017, $p > 0.187$ for each comparison), but during the manipulation, tremble dancers had significantly shorter decision times than either waggle dancers or non-dancing nectar foragers (Mann-Whitney- U Test, $p < 0.001$ for comparison of tremble dancers with each waggle dancers and non-dancing nectar foragers, $p = 0.399$ for comparison of waggle dancers with non-dancing nectar foragers).

To check whether tremble dancing preceded a decrease in the time to first unloading delay, I compared the time to the first unloading delay of nectar foragers before and after the 30th min of the manipulation phase. Nectar foragers had a t_u of 7.1 ± 7.5 s ($n = 59$) before, and of 4.4 ± 2.7 s ($n = 16$) after the 30th min of the manipulation of the experiment. This difference was not significant (Mann-Whitney- U Test, $p = 0.335$).

Decision times of nectar foragers that visited natural nectar sources

I recorded the decision times of 41 waggle dancers, 25 tremble dancers and 31 non-dancing nectar foragers that had visited natural food sources. Data of C_1 and C_2 were pooled for each waggle dancers, tremble dancers and non-dancing nectar foragers, because these groups did not differ in decision time between colonies (Mann-Whitney- U Test, $p \geq 0.462$ for each comparison).

66 % of the waggle dancers and 80 % of the tremble dancers danced before they had their first unloading contact. Waggle dancers that first danced had a t_d of 9.3 ± 18.5 s ($n = 27$), and waggle dancers that first unloaded had a t_u of 14.3 ± 11.5 s ($n = 14$). Tremble dancers had a t_d of 9.0 ± 15.9 s ($n = 20$), and a t_u of 15.1 ± 35.4 s ($n = 5$). The t_d of nectar foragers that danced first was shorter than the t_u of nectar foragers that unloaded first, but this difference was significant only for waggle dancers (Mann-Whitney-U Test, $p = 0.038$ for waggle dancers and 0.154 for tremble dancers).

Fig. 4 shows the probability to perform a waggle dance or a tremble dance as function of decision time for nectar foragers that visited natural nectar sources.

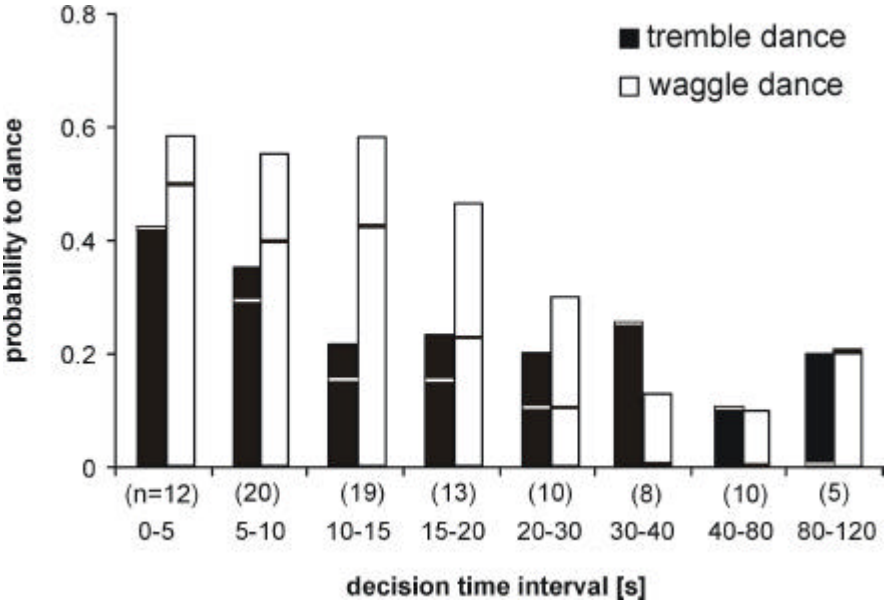

Fig. 4 The probability to perform a tremble dance or a waggle dance as function of the decision time for foragers returning from natural food sources. The section below the line within each bar shows the probability to dance before the first unloading contact, the section above the probability to unload first. Sample sizes are given in brackets. Data are pooled for both colonies.

Of all waggle dancers, 44 % had decision times between 0 - 10 s, 86 % between 0 - 20 s, and 93 % between 0 - 30 s. For tremble dancers, the values were 48, 76, and 84 %, and for non-dancing nectar foragers 7, 32, and 48 %. The median decision times were 10.6 ± 16.4 s for waggle dancers, 10.3 ± 21.2 s for tremble dancers, and 30.1 ± 26.4 s for non-dancing nectar foragers. Waggle dancers and tremble dancers did not differ significantly in decision times (Mann-Whitney- U Test, α- level after Bonferroni correction for multiple comparisons 0.017, $p = 0.947$), but non-dancing nectar foragers had significantly longer decision times than either waggle or tremble dancers ($p < 0.01$ for each comparison).

Discussion

This study shows that tremble dancing can be stimulated by a high density of foragers at the food source. A high density of foragers at the feeder was achieved by reducing feeder size, which forced nectar foragers to crowd at the feeder. Three non-exclusive hypotheses propose explanations for why more nectar foragers tremble danced when the feeder was crowded than when it was not crowded: 1. more nectar foragers returned per unit time to the hive and the

higher demand for nectar receivers caused longer unloading delays which stimulated tremble dancing, 2. nectar receivers were less motivated to unload foragers (e.g. because the foragers were sprayed with alarm pheromone) and thus caused longer unloading delays that stimulated tremble dancing, and 3. tremble dancing was a direct reaction to the quality decrease of the food source, and the unloading delays were not affected by the quality decrease of the food source.

Crowding at the food source did not cause longer median unloading delays for either tremble dancers, waggle dancers or non-dancing nectar foragers. Thus, hypotheses 1 and 2, which both propose longer unloading delays as the direct stimulus for tremble dancing, can be excluded for most nectar foragers. However, some tremble dancers did experience longer decision times than waggle dancers (Fig. 3 and 4), and thus could have decided to tremble dance because of long unloading delays. Principally, the results of this study support hypothesis 3 which proposes that tremble dancing is a direct reaction to stimuli at the food source. The short median decision time of tremble dancers and the early start of tremble dancing suggest that most tremble dancers did not assess unloading delay after their return to the hive, and thus did not tremble dance because their unloading delay informed them about a shortage of nectar receivers.

Although most tremble dancers experienced short decision times when they were observed and thus did not seem to react to a shortage of nectar receivers, it is possible that these tremble dancers (1) experienced many long unloading delays after earlier trips or (2) were informed about a shortage of receiver bees in the hive by the high density of foragers at the food source. However, the results of this study do not support either of the two hypotheses. It is very unlikely that many nectar foragers in this study experienced frequently long unloading delays before they were observed, because the demand for nectar receivers was low enough to not usually create long unloading delays for the foragers and did not change during the entire experiment. Also, nectar foragers switched from waggle dancing to tremble dancing immediately after the feeder size was reduced (Fig.1), which indicates that the switch was due to the manipulation of the feeder and not former experiences. Furthermore, Seeley (1992) reports that nectar foragers assess unloading delay after each return to the hive for at least 45-75 min after the start of foraging. The second hypothesis proposes that factors at the food source inform nectar foragers about a potential shortage of nectar receivers. For example, a high density of foragers at the food source might indicate that a high number of nectar foragers is going to return into the hive and to cause a shortage of nectar receivers. Thus, a high density of foragers at the food source might stimulate nectar foragers to recruit additional nectar receivers with the tremble dance. In this study,

however, the high density of nectar foragers at the feeder caused nectar foragers to stay longer at the feeder for a full load, and therefore lowered, or kept constant, the number of returning nectar foragers and thus the demand for nectar receivers. Nevertheless, it remains to be investigated in more detail whether hive-external stimuli can inform nectar foragers about the need for additional nectar receivers in the hive.

Although the significance of crowding at natural food sources has not been investigated, the short decision times of tremble dancers that visited a crowded feeder are not likely to be artifacts. Many tremble dancers that visited natural food sources had equal, or shorter, decision times than waggle dancers. This suggests that tremble dancing is an adaptive reaction to stimuli external to the hive. Little is known yet about naturally occurring tremble dance stimuli that are external to the hive. V. Frisch (1967) reported that tremble dancing can be elicited by sticky food sources like *Asclepias* flowers. Furthermore, several chemicals that, added to nectar, elicit tremble dancing (Lindauer 1948, Schneider 1949, Schick 1953) suggest that unwholesome or low-quality food stimulates tremble dancing. In the experiments of this study, nectar foragers often did not have immediate and uninterrupted access to the sugar water when the feeder was crowded, and thus loaded less efficiently than when the feeder was not crowded. Inside the hive, tremble dancing can be elicited by long unloading delays. All these factors have in common that they decrease foraging efficiency. Therefore, it is possible that tremble dancing is caused directly by factors that indicate a decrease in foraging efficiency either outside or inside the hive.

If tremble dancing can be stimulated by factors that decrease foraging efficiency, this should have implications on the function of the tremble dance. The recruitment of additional nectar receivers is evidently adaptive when the stimulus for the dance is a shortage of nectar receivers. When tremble dancing is caused e.g. by a stimulus at the food source, the recruitment of additional nectar receivers should be adaptive only if the stimulus indicates a shortage of nectar receivers in the hive. If, however, tremble dancing is caused by stimuli that do not provide information about a shortage of nectar receivers, the tremble dance may be expected to have an additional function to the recruitment of nectar receivers. Seeley (1992) observed that tremble dancing preceded 30-60 min a general decrease in unloading delay that was likely to be due to the recruitment of additional nectar receivers. In this study, the tremble dance did not seem to recruit additional nectar receivers, as tremble dancing did not precede 30 min a decrease in unloading delay. This finding is supported by Kirchner (1993), who found that an increased tremble dance activity did not decrease the time to the first trophallactic contact of a nectar

forager. One explanation for the different outcomes is that in this and the study by Kirchner (1993) the entire available workforce was already engaged in nectar receiving, so that no more nectar receivers could be recruited by the tremble dance. However, this is not likely at least for Kirchner's experiments, as he allowed only very few (10) bees to forage simultaneously. It is thus probable that in his experiment only very few nectar receivers were already engaged in unloading, and that many more bees in the hive could have been recruited as nectar receivers.

The results of this study suggest that if the hive-external factors that elicit tremble dancing do not indicate a shortage of nectar receiver bees in the hive, the function of the tremble dance may not be restricted to the recruitment of additional nectar receivers, but may be, like the brief piping signals often produced by tremble dancers (Nieh 1993, Kirchner 1993, Thom et al. *submitted*), involved in the inhibition or re-organization of nectar foraging in honey bees.

Summary

The ecological success of social insects is founded in the internal organization of their colonies, that is the division of labor and the coordination and behavioral integration of colony members. Division of labor and integration of colony members render a behavioral flexibility to social insect colonies that can cope with a wide range of internal and external demands. A high degree of flexibility is particularly rewarding when frequent changes in the environment challenge a colony's efficiency of task performance.

Honey bee colonies are regularly exposed to changes in their environment. To track these changes, honey bees constantly monitor their environment and communicate relevant information to other colony members. The colony members can then adjust in combined actions the colony's task efficiency to the new conditions. The efficiency of nectar foraging, a task that directly determines a colony's survival and reproductive success, highly depends on the exchange of information about environmental conditions and changes. According to the significance that efficient foraging has to a colony, honey bees evolved a complex feedback system that adjusts the colony's foraging efficiency by providing information about hive-internal and external foraging conditions.

In this thesis, I examined honey bee nectar foraging with emphasis on the communication system. In particular, I determined the daily dynamics of the number and activity of nectar foragers in an undisturbed honey bee colony, tested whether honey bees use a chemical signal to activate nectar foragers, and examined the cause and characteristics of the brief piping signal and the tremble dance of nectar foragers. To document how a honey bee colony adjusts its daily nectar foraging effort, I recorded on several days the number and activity (number of foraging trips per day per bee) of nectar foragers. To do so, I observed a random sample of individually marked workers during the entire day, and then estimated the number and activity of all nectar foragers in the colony. The total number of active nectar foragers in a colony changed frequently between days. This was likely due to changes in the availability of nectar. Foraging activity, however, did not usually change between days. Hence, the study suggests that a honey bee worker makes a binary decision to either forage or not, rather than a graded decision about the level of her foraging activity. Integrated in a colony response, this means that a honey bee colony adjusts its daily foraging effort by changing the number of its nectar foragers rather than their

activity. It might be adaptive for a colony to allocate many foragers to a source to exploit it quickly before unfavorable conditions stop the nectar flow, instead of allocating fewer foragers which might need longer to exploit the source fully even if they raised the level of their foraging activity.

If a honey bee colony relies to a large degree on the number of nectar foragers to adjust its daily foraging success, it seems plausible that honey bees possess mechanisms to quickly activate many nectar foragers. Although both novice and experienced foragers can be activated by waggle dances, waggle dances seem to activate only a few bees at a time and take mostly place in a restricted area of the hive where not all potential foragers may be reached (v. Frisch 1967). A volatile substance, however, could reach in a short time foragers in a large area of the hive. I tested whether volatiles produced by a foraging colony activated nectar foragers of a non-foraging colony by connecting with a glass tube two colonies. Each colony had access to a different green house. In 50% of all experiments, volatile substances from the foraging colony stimulated nectar foragers of the non-foraging colony to fly to an empty feeder. The results of this study show that honey bees can produce a chemical signal or cue that activates nectar foragers. However, more experiments are needed to establish the significance of the activating volatiles for the foraging communication system.

An antagonist to the forager activating signals in the honey bee communication system is the brief piping signal of nectar foragers. This signal inhibits forager recruitment by stopping waggle dances (Nieh 1993, Kirchner 1993). However, I observed that many piping signals (approximately 43%) were produced off the dance floor, a restricted area in the hive where most waggle dances are performed. If the inhibition of waggle dances would be the only function of the brief piping signal, tremble dancers should produce piping signals mainly on the dance floor, where the probability to encounter waggle dancers is highest. To therefore investigate the piping signal in more detail, I experimentally established the foraging context of the brief piping signal, characterized its acoustic properties, and documented for the first time the unique behavior of piping nectar foragers by observing foragers throughout their entire stay in the hive. Piping nectar foragers usually began to tremble dance immediately upon their return into the hive, spent more time in the hive, more time dancing, had longer unloading latencies, and were the only foragers that sometimes unloaded their nectar directly into cells instead of giving it to a nectar receiver bee. Most of the brief piping signals (approximately 99%) were produced by tremble dancers, yet not all tremble dancers (approximately 48%) piped. This suggests that piping and

tremble dancing have related, but not identical functions in the foraging system. Thus, the brief piping signals may not only inhibit forager recruitment, but have an additional function both on and off the dance floor. In particular, the piping signal might function 1. to stop the recruitment of additional nectar foragers, and 2. as a modulatory signal to alter the response threshold of signal receivers to the tremble dance.

The observation that piping tremble dancers often did not experience long unloading delays before they started to dance gave rise to a question. A nectar forager's decision to perform either a waggle- or tremble dance was shown to be determined by the delay the forager experiences before she can unload to a nectar receiver bee after returning to the hive (Seeley 1992). A forager's unloading delay provides reliable information about the relative work capacities of nectar foragers and nectar receivers, because each returning forager unloads her nectar to a nectar receiver before she takes off for the next foraging trip. Queuing delays for either foragers or receivers lower foraging efficiency and can be eliminated by recruiting workers to the group in shortage. Short unloading delays indicate to the nectar forager a shortage of foragers and stimulate waggle dancing which recruits nectar foragers. Long unloading delays indicate a shortage of nectar receivers and stimulate tremble dancing which recruits nectar receivers (Seeley 1992, Seeley et al. 1996). Because the short unloading delays of piping tremble dancers indicated that tremble dancing can be elicited by other factors than long unloading delays, I tested whether a hive-external stimulus, the density of foragers at the food source, stimulated tremble dancing directly. The experiments show that tremble dancing can be caused directly by a high density of foragers at the food source and suggest that tremble dancing can be elicited by a decrease of foraging efficiency either inside (e.g. shortage of receiver bees) or outside (e.g. difficulty of loading nectar) the hive. Tremble dancing as a reaction to hive-external stimuli seems to occur under natural conditions and can thus be expected to have some adaptive significance. The results imply that if the hive-external factors that elicit tremble dancing do not indicate a shortage of nectar receiver bees in the hive, the function of the tremble dance may not be restricted to the recruitment of additional nectar receivers, but might be, like the brief piping signals often produced by tremble dancers, involved in the inhibition or re-organization of nectar foraging.

References

Anderson C and F.L. Ratnieks, 1999. Task partitioning in foraging: general principles, efficiency and information reliability of queuing delays, in: *Information processing in social insects*. Claire Detrain et al. (eds) Birkhäuser Verlag Basel/Switzerland.

Bourke A.F.G. and N.R. Franks, 1995. *Social evolution in ants*. Princeton Univ. Press, Princeton, NJ.

Bradbury J.W. and S.L. Vehrencamp, 1998. *Principles of animal communication*. Sinauer Associates, Inc., Sunderland, MA.

Bräuninger H.D., 1964. Über den Einfluß meteorologischer Faktoren auf Entfernungsweisung im Tanz der Bienen. *Z. vergl. Physiol. 48*:1 - 130.

Butler C.G., D.J.C. Fletcher, and D. Walter, 1969. Nest-entrance marking with pheromones by the honey bee *Apis mellifera* L., and a wasp, *Vespula vulgaris* L. *Anim. Behav. 17*:142-147.

Dornhaus A., Brockmann A., and L. Chittka, 2002. Bumble bees alert to food with pheromone from a tergite gland. *Submitted to J. Comp. Physiol. A.*

Esch, H., 1964. Beiträge zum Problem der Entfernungsweisung in den Schwänzeltänzen der Honigbiene. *Z. vergl. Physiol. 48*: 534-546.

Ferguson A.W. and J.B. Free, 1979. Production of a forage-marking pheromone by the honeybee. *J. Apic. Res. 18*:128-135.

Free J. B. and P. A. Racey, 1966. The pollination of *Freesia refracta* in glasshouses. *J. Apic.res. 5*:177-182.

Free J.B. and I.H. Williams, 1970. Exposure of the Nasonov gland by honey bees (*Apis mellifera*) collecting water. *Behaviour 37*:286-290.

Free J.B. and I.H. Williams, 1972. The role of the Nasonov gland pheromone in crop communication by honey bees (*Apis mellifera* L.) *Behaviour 41*:314-318.

Frisch K. von, 1923. Über die Sprache der Bienen. *Zool. J. 40*:1-186.

Frisch K. von, 1967. *The Dance Language and Orientation of Bees.* Harvard University Press, Cambridge, MA.

Frisch K. von and G. A. Rösch, 1926. Neue Versuche über die Bedeutung von Duftorgan und Pollenduft über die Verständigung im Bienenvolk. *Z. vergl. Physiol. 4*:1-21.

Gordon D.M., 1989. Dynamics of task switching in harvester ants. *Anim. Behav. 38*:194 - 204.

Gordon D.M., 1991. Behavioral Flexibility and the foraging ecology of seed-eating ants. *Am. Nat.138 (2)*:379 - 411.

Heath D., 1995. *An introduction to experimental design and statistics for biology*. UCL Press, London.

Hölldobler B. and E .O. Wilson, 1990. *The Ants*. The Belknap Press of Harvard University, Cambridge, Mass.

Kirchner W.H., 1993. Vibrational signals in the tremble dance of the honey bee, Apis mellifera. *Behav. Ecol. Sociobiol. 33*:169-172.

Kirchner, W.H. and M. Lindauer, 1994. The causes of the tremble dance. *Behav. Ecol. Sociobiol. 35*:303-308.

Kühnholz S. and T.D. Seeley, 1997. The control of water collection in honey bee colonies. *Behav. Ecol. Sociobiol. 41*: 407 - 422.

Lindauer M., 1948. Über die Einwirkung von Duft- und Geschmackstoffen sowie anderer Faktoren auf die Tänze der Bienen, *Z. vergl. Physiol. 31*:348-412.

Lindauer M., 1952. Ein Beitrag zur Frage der Arbeitsteilung im Bienenstaat. *Z. vgl. Physiol. 34*:299-345.

Lindauer M., 1954. Temperaturregulierung und Wasserhaushalt im Bienenstaat. *Z. vergl. Phys*. *36*:391-432.

Lecomte J., 1957. Über die Bildung von "Strassen" durch Sammelbienen, deren Stock um 180° gedreht worden war. *Z. Bienenforsch. 3*:128-133.

Milojevic B.D., 1940. A new interpretation of the social life of the honey bee. *Bee World 21*:39-41.

Nieh J.C., 1993. The stop signal of honey bees: reconsidering its message. *Behav. Ecol. Sociobiol. 33*:51-56.

Núnez J.A.,1977. Nectar flow by melliferous flora and gathering flow by *Apis mellifera L.*. *J. Insect. Physiol. 23* :265-275.

Núnez J.A. and M. Giurfa, 1996. Motivation and regulation of honey bee foraging. *Bee world 77(4)*:182-196.

Oster G.F. and E.O. Wilson, 1978. *Caste and ecology in the social insect.* Princeton Univ. Press, Princeton, NJ.

Park O.W., 1923. Behavior of water carriers. *Am. Bee. J. 63*:553.

Park O.W., 1929. Time factors in the relation to the acquisition of food by the honey bee. *Res. Bull. Iowa Exp. Agric. Stat. 108*:184-224.

Pratt S.C., S. Kühnholz, T.D. Seeley, and A. Weidenmüller, 1996. Worker piping is associated with foraging in undisturbed, queenright colonies of honey bees. *Apidologie 27*:13-20.

Ribbands C.R., 1955. The scent perception of the honey bee. *Proc. Roy. Soc. London (B) 143*:367-379.

Robinson G.E., 1992. Regulation of division of labor in insect societies. *Annu. Rev. Entomol. 37*:637-65.

Robinson G.E., B.A. Underwood, and C.E. Henderson, 1984. A highly specialized water-collecting honey bee. Apidologie *15 (3)*:355-358.

Robinson G.E., Page R.E., Strambi C., and A. Strambi, 1992. Colony integration in honey bees: mechanisms of behavioral reversions. *Ethology 90* : 336-348.

Rösch G.A.,1930. Untersuchungen über die Arbeitsteilung im Bienenstaat 2. Teil: Die Tätigkeit der Arbeitsbienen unter experimentell veränderten Bedingunge. *Z. vgl. Physiol. 12*:1-71.

Schick, W., 1953. Über die Wirkung von Giftstoffen auf die Tänze der Bienen. *Z. vergl. Physiol. 35*:105-128.

Schmidt-Hempel P., A. Kacelnik, and A. I. Houston, 1985. Honeybees maximize efficiency by not filling their crop. *Behav. Ecol. Sociobiol. 17*:61-66.

Schneider F., 1949. Über die Vergiftung der Bienen mit Dinitrokresol. *Mitt. Schweiz. Entomol. Gesellsch. 33*:223-237.

Schuà L., 1952. Untersuchungen über den Einfluß meteorologischer Elemente auf das Verhalten der Honigbienen (*Apis mellifica*). *Z. vergl. Physiol. 34*:258-277.

Seeley T.D., 1985. *Honeybee ecology*. Princeton Univ. Press, Princeton, NJ.

Seeley T.D., 1986. Social foraging by honey bees: how colonies allocate foragers among patches of flowers. *Behav. Ecol. Sociobiol. 19*:343-354.

Seeley, T.D., 1992. The tremble dance of the honey bee: message and meanings. *Behav. Ecol. Sociobiol. 31*:375-383.

Seeley, T.D., 1995. *The wisdom of the hive: the social physiology of honey bee colonies.* Harvard University Press, Cambridge, MA.

Seeley T.D., S. Camazine, and J. Sneyd, 1991. Collective decision-making in honey bees: how colonies choose among nectar sources. *Behav. Ecol. Sociobiol. 28*:77-290.

Seeley, T.D., S. Kühnholz, and A. Weidenmüller, 1996. The honey bee's tremble dance stimulates additional bees to function as nectar receiver bees. *Behav. Ecol. Sociobiol. 39*: 419-427.

Sokal R. R. and F.J. Rohlf, 1995. *Biometry*. 3rd edition by W.H. Freeman and Company, NY.

Tautz J., 1996. Honeybee waggle dance: recruitment success depends on the dance floor. *J. Exper. Biol. 199*:1375-1381.

Thom C., Seeley T.D., and J. Tautz, 2000. A scientific note on the dynamics of labor devoted to nectar foraging in a honey bee colony: number of foragers versus individual foraging activity. *Apidologie 31*:737-738.

Thom C., Gilley D.C., Tautz J., 2001 *submitted.* Worker piping in honey bees: the behavior of piping nectar foragers. *Submitted to* Behav. Ecol. Sociobiol.

Williams I.H, J.A. Pickett, and A.P. Martin, 1981. The Nasonov pheromone of the honey bee, *Apis mellifera L.* (Hymenoptera: Apidae), II. Bioassay of the components using foragers. *J. Chem. Ecol. 7:225-237*.

Wilson E.O., 1962. Chemical communication among workers of the fire ant *Soleonopsis saevissima* (Fr. Smith), 1: the organization of mass-foraging, 2: an information analysis of the odour trail, 3: the experimental induction of social responses. *Animal Behaviour, 10 (1-2)*:134–147, 148-158, 159-164.

Wilson, E.O., 1971. *The insect societies*. Cambridge, MA: Harvard University Press.

Wilson E.O., 1985. The sociogenesis of social insect colonies. *Science 228*:1489-1496.

Wilson E.O., 1987. Causes of ecological success: the case of the ants. *J. Anim. Ecol. 56*:1-9.

Winston M.L., 1987. *The biology of the honey bee*. Harvard University Press, Cambridge, MA.

Winston M.L., and L.A. Fergusson, 1985.The effect of worker loss on temporal caste structure in colonies of the honeybee (Apis mellifera L.). *Can. J. Zool. 63* : 777-780.

APPENDIX

ZUSAMMENFASSUNG

Der überwältigende ökologische Erfolg sozialer Insekten basiert auf der Organisation ihrer Kolonien, d.h. auf Arbeitsteilung und der Integration individuellen Verhaltens zu sinnvollen Aktionen. Arbeitsteilung und Verhaltensintegration erlauben den Kolonien sozialer Insekten auf ein breites Spektrum von Bedingungen innerhalb und ausserhalb des Nestes zu reagieren. Dies ist besonders wichtig, wenn sich die Bedingungen, unter denen Kolonien überleben und sich fortpflanzen müssen, häufig ändern.

Die Kolonien der Honigbienen sind regelmässigen Änderungen ihrer Umwelt ausgesetzt. Um auf diese schnell und effizient reagieren zu können, tauschen die Mitglieder einer Kolonie dauernd entsprechende Informationen untereinander aus. Dadurch können sich die Individuen koordinieren und zusammen eine sinnvolle Koloniereaktion hervorrufen.

Das erfolgreiche Sammeln von Nektar, dem Hauptnahrungsmittel der Honigbienen, ist besonders abhänging von den jeweiligen Bedingungen, die Bienen in ihrer Umwelt vorfinden. Die Sammeleffizienz einer Kolonie beeinflusst direkt ihr Überleben und ihren Fortpflanzungserfolg, und hängt ab von der Gewinnung, Weiterleitung und Verarbeitung von Informationen über die Zustände inner-und ausserhalb des Nestes. Die grosse Bedeutung eines effizienten Nektareintrages spiegelt sich in einem hochentwickelten Kommunikationssystem wider, das die Sammeleffizienz der Kolonie optimiert.

In meiner Doktorarbeit habe ich die Charakteristika des Nektarsammelns bei Honigbienen mit spezieller Betonung des zugehörigen Kommunikationssytems untersucht. Im Einzelnen habe ich die täglichen Änderungen in der Aktivität und Anzahl der Nektarsammlerinnen einer nicht-manipulierten Kolonie verfolgt, habe getestet, ob Nektarsammlerinnen durch ein chemisches Signal aktiviert werden können, und habe die Auslöser und Charakteristika zweier Signale des Nektarsammelkommunikationssytems, dem kurzen Pipingsignal und dem Zittertanz der Nektarsammlerinnen untersucht.

Um die täglichen Änderungen des Sammelaufwandes einer Kolonie zu dokumentieren, habe ich an verschiedenen Tagen die Anzahl und Aktivität (Anzahl Fouragierflüge pro Tag und Biene) der Nektarsammlerinnen einer Kolonie gemessen. Dafür beobachtete ich jeweils den ganzen Tag eine zufällig ausgewählte Gruppe von individuell markierten Arbeiterinnen. Aufgrund der so gewonnen Daten konnte ich die Anzahl und Aktivität aller Nektarsammlerinnen in der Kolonie schätzen. Die Ergebnisse zeigen, dass sich die absolute Anzahl von

Nektarsammelerinnen regelmässig von Tag zu Tag änderte wahrscheinlich zurückzuführen auf die täglichen Änderungen im Nektarangebot, während sich die Aktivität der Sammlerinnen gewöhnlich nicht änderte. Die Ergebnisse zeigen, dass eine Arbeiterin eher die Entscheidung trifft zu sammeln oder nicht zu sammeln, statt eine abgestufte Entscheidung über die Anzahl ihrer Sammelflüge. Für eine Honigbienenkolonie bedeutet dies, das ihre Sammeleffizienz stärker durch die Anzahl der Sammlerinnen als durch deren Aktivität reguliert wird. Möglicherweise kann eine vergängliche Nektarquelle besser von vielen Sammlerinnen, die zeitgleich arbeiten, ausgebeutet werden als von weniger Sammlerinnen die zwar ihre Aktivität steigern, aber sequentielle Sammelflüge machen müssen und damit die Quelle vor ihrem Verschwinden nicht vollständig ausbeuten können.

Wenn eine Honigbienenkolonie ihren Sammelerfolg hauptsächlich durch die Anzahl der aktiven Sammlerinnen reguliert, könnte man erwarten, dass Honigbienen einen Mechanismus besitzen, um schnell Sammlerinnen zu aktivieren, wenn diese gebraucht werden. Es ist seit langem bekannt, das der Schwänzeltanz der Honigbienen Sammlerinnen aktivieren kann. Allerdings werden Schwänzeltänze meistens nur auf dem „Tanzboden“ des Stockes vollführt, einer relativ kleinen Fläche in der Eingangsregion des Stockes, wo kaum alle potentiellen Sammlerinnen erreicht werden könne n (v. Frisch 1967). Eine flüchtige Substanz dagegen könnte in relativ kurzer Zeit Sammlerinnen in grossen Teilen des Stockes erreichen. Ich habe daher untersucht, ob die flüchtigen Substanzen einer fouragierenden Kolonie die Sammlerinnen einer nicht-fouragierenden Kolonie aktivieren können. Um dies zu testen, verband ich die Eingangsbereiche zweier Kolonien mit einer Glasröhre, so das flüchtige Substanzen von einer zur anderen Kolonie geleitet werden konnten. Jede Kolonie hatte Zugang zu einem separaten Gewächshaus. Während eine der Kolonien gefüttert wurde, wurde die Aktivität der nicht-gefütterten Kolonie gemessen. In 50% der Experimente wurden die Sammlerinnen der Kolonie, die kein Futter zur Verfügung hatte, durch die flüchtige Substanzen aus der fouragierenden Kolonie zu dem Besuch Ihrer leeren Futterstation aktiviert. Die Ergebnisse zeigen damit, dass Honigbienen eine flüchtige Substanz produzieren können, die Sammlerinnen aktiviert. Die Fragen, ob es sich bei dieser Substanz um ein ‘signal’ (speziell für die Situation entwickelt) oder einen ‘cue’ (nicht speziell für die Situtation entwickelt, wirft aber brauchbare Information als Nebenprodukt ab) handelt, sowie die Bedeutung der Substanz für die Sammeleffizienz einer Honigbienekolonie, müssen jedoch noch etabliert werden.

Ein Antagonist zu den aktivierenden Signalen des Kommunikationssystems ist das kurze Pipingsignal der Nektarsammlerinnen. Dieses Signal hemmt die Rekrutierung von Sammlerinnen, indem es Schwänzeltänze stoppt (Nieh 1993, Kirchner 1993). Ich beobachtete, dass viele der kurzen Pipingsignale (ca. 43%) nicht auf dem Tanzboden produziert wurden. Wäre das Stoppen von Schwänzeltänzen die einzige Funktion des kurzen Pipingsignals, würde man erwarten, dass die meisten Signale auf dem Tanzboden produziert werden, wo die Wahrscheinlichkeit auf Schwänzeltänzerinnen zu treffen am höchsten ist. Um das kurze Pipingsignal genauer zu beschreiben, etablierte ich experimentiell seinen Fouragierkontext, characterisierte seine akustischen Eigenschaften und dokumentierte zum ersten Mal das Verhalten von pipenden Nektarsammlerinnen. Ich tat dies, indem ich Nektarsammlerinnen während ihres gesamten Aufenthaltes im Stock beobachtete.

Pipende Sammlerinnen führten gewöhnlich Zittertänze auf mit denen sie sofort nach ihrer Rückkehr in den Stock begannen. Pipende Zittertänzerinnen verbrachten mehr Zeit als nicht-pipende Sammlerinnen im Stock, tanzten länger, hatten längere Wartezeiten bis zu ihrem ersten Kontakt mit einer Bienen die ihnen den Nektar abnahm, und waren die einzigen Sammlerinnen, die ihren Nektar manchmal direkt in eine Zelle abluden statt an eine Nektarabnehmerin weiterzugeben. Obwohl die meisten kurzen Pipingsignale (ca. 99%) von Zittertänzerinnen kamen, produzierten nicht alle Zittertänzerinnen (ca. 48%) Pipingsignale. Dies lässt vermuten, dass die kurzen Pipingsignale und der Zittertanz ähnliche, aber nicht identische Funktionen im Nektarsammel-Kommunkationssystem haben. Es könnte sein, dass das kurze Pipingsignal nicht allein dazu dient Schwänzeltänze zu stoppen, sondern zusätzlich als modulierendes Signal die Reaktionsschwelle von Signalempfängern für den Zittertanz senkt.

Die Beobachtung das pipende Zittertänzerinnen häufig sehr kurze Wartezeiten bis zu ihrem ersten Nektarablade- Kontakt hatten, habe ich weiter verfolgt. Es ist bekannt, dass eine Sammlerin sich für einen Schwänzeltanz oder einen Zittertanz entscheidet je nachdem wieviel Zeit vergeht bevor sie ihre Nektarladung an eine Abnehmerbiene weitergeben kann (Seeley 1992). Diese Wartezeit auf eine Abnehmerbiene informiert eine Sammlerin zuverlässig über die relativen Arbeitskapazitäten von Nektarsammlerinnen und Nektarabnehmerinnen, da jede erfolgreiche Sammlerin ihren Nektar an eine Abnehmerin abgibt bevor sie ihren nächsten Sammelflug unternimmt. Lange Wartezeiten für eine der beiden Gruppen senken die Sammeleffizienz der Kolonie und können eliminiert werden, indem zusätzliche Arbeiterinnen in die schwächere Gruppe rekruitiert werden. Kurze Wartezeiten für Nektarsammlerinnen werden

durch zuwenige Sammlerinnen verursacht, und lösen Schwänzeltänze aus, die zusätzliche Nektarsammlerinnen rekruitieren. Lange Wartezeiten für Sammlerinnen werden verursacht, wenn es nicht genügend Nektarabnehmerinnen gibt, und lösen Zittertänze aus, mit denen zusätzliche Nektarabnehmerinnen rekrutiert werden (Seeley 1992, Seeley et al. 1996).

Die kurzen Wartezeiten der pipenden Zittertänzerinnen liessen vermuten, dass Zittertänze noch andere Auslöser haben als lange Wartezeiten. Da die beobachteten pipenden Zittertänzerinnen Zuckerwasser an einer häufig überfüllten Futterstation sammelten, untersuchte ich, ob die Dichte von Sammlerinnen an der Futterstelle Zittertänze direkt auslösen kann. Ich testete dies, indem ich eine konstante Anzahl Sammlerinnen an einer künstlichen Futterstation sammeln liess, und dann dort die Dichte der Sammlerinnen vergrösserte, indem ich den Umfang der Futterstelle verringerte. Im Stock nahm ich bei den unterschiedlichen Dichten die Wartezeiten und Tänze (Schwänzel – oder Zittertanz) der Sammlerinnen auf. Die Experimente zeigen, dass Zittertänze eine direkte Reaktion auf eine hohe Dichte von Sammlerinnen an der Futterstelle sein können. Dies lässt vermuten, dass Zittertänze eine generelle Reaktion sind auf Faktoren, die entweder innerhalb (z.B. durch lange Wartezeit) oder ausserhalb (z.B. durch Schwierigkeiten beim Trinken) des Stockes die Sammeleffizienz senken. Unter natürlichen Umständen scheinen Zittertänze regelmässig eine direkte Reaktion auf Stock-externe Faktoren zu sein, und werden daher einige Bedeutung im Sammelkommunikationssytem haben. Sofern die Stock-externen Faktoren nicht einen Mangel an Nektarabnehmerinnen im Stock anzeigen, könnte es sein, dass der Zittertanz nicht nur Nektarabnehmerinnen rekruitiert, sondern, ähnlich wie die kurzen Pipingsignale der Zittertänzerinnen, der Hemmung oder Re-organisation der Sammelaktivität einer Honigbienen Kolonie dient.

CURRICULUM VITAE

ADDRESS Zoologie II, Biozentrum,
Am Hubland, Universität Würzburg,
D-97074 Würzburg, Germany
Tel.: +49 0931 888 4328 Fax.: 4329
email: thom@biozentrum.uni-wuerzburg.de

AGE born 25th March 1974 in Bonn, Germany

RESEARCH AREA Sociobiology / Behavioral Ecology

EDUCATION

1999-2002	Ph.D. thesis, Würzburg University, Germany Advisors Prof. J. Tautz and Prof. B. Hölldobler
1998	Fellowship of the Graduierten- Kolleg "Grundlagen des Arthropodenverhaltens: Genetik, Neurobiologie, Verhaltensphysiologie", Würzburg University, Germany
1998	Diploma thesis, Cornell University, USA, and Würzburg University, Germany Advisors: Prof. T.D. Seeley and Prof. J. Tautz
1996-1998	Study of Biology, Würzburg University, Germany Major: Zoology Minors: Genetics, Biochemistry
1996-1998	Fellowship of the German National Merit Foundation (Studienstiftung des deutschen Volkes)

1993-1996 Study of Biology, Hamburg University, Germany

1993 Abitur, Friedrich- Ebert Gymnasium, Hamburg, Germany
Major: Biology, Philosophy

RESEARCH EXPERIENCE

05/99-09*/02 Ph.D. thesis: Dynamics and communication structures of nectar foraging in honey bees. Würzburg University, Germany (* estimated).

04/98-04/99 Diploma thesis: The allocation of labor to nectar foraging in honey bees (*Apis mellifera*). Cornell University, USA, and Würzburg University, Germany.

12/98 field assistant: body size evolution of marine iguanas in the Galapagos. University of Illinois, USA.

05/97-03/98 lab assistant: anatomy of mushroom bodies in ants. Würzburg University, Germany.

09/95-02/96 field assistant: mating strategies of marine iguanas in the Galapagos. Max-Planck Institute for Behavioral Physiology in Seewiesen, Germany.

10/93-09/96 lab assistant: fish ecology and marine fauna. Hamburg University, Germany.

04/93-10/93 lab & field assistant: fish ecology and marine fauna. Helgoland Institute for marine biology, Hamburg, Germany.

05/92-08/92 lab assistant: song learning in birds. Max-Planck Institute for Behavioral Physiology in Seewiesen, Germany.

07/91 lab assistant: evolution of plants. Institute for Botany, Hamburg University, Germany.

PUBLICATIONS

Full papers

Thom C., Seeley T.D., and J. Tautz, 2000. A scientific note on the dynamics of labor devoted to nectar foraging in a honey bee colony: number of foragers versus individual foraging activity. *Apidologie 31*:737-738.

Wikelski M. and C.Thom, 2000. Marine Iguanas shrink to survive El Niño. *Nature 403*: 37-38.

Submitted

Thom C., Gilley D.C., and J. Tautz, 2002. Worker piping in honey bees: the behavior of piping nectar foragers. *submitted to Behav. Ecol. Sociobiol.*

In prep.

Thom C., 2002. Tremble dancing in honey bees can be elicited by stimuli external to the hive. *In prep*.

Thom C., 2002. A new piping signal in honey bees? (*Short communication) In prep*.

Thom C. and A. Dornhaus, 2002. Do honey bees produce volatile chemicals to activate nectar foragers? *In prep.*

Esch H.E., Thom C., and J. Tautz, 2002. The movements of the tremble dance (*or similar title*) *In prep.*

Conference presentations

Thom C., Seeley T.D., and J. Tautz, 2000. Arbeitsteilung bei Honigbienen. AG Tagung der Bieneninstitute, Blaubeuren (*poster*).

Thom C., Seeley T.D., and J. Tautz, 2000. Dynamics of nectar foraging in a honey bee colony. TMR Florenz (*poster*).

Thom C., Seeley T.D., and J. Tautz, 2000. Dynamics of nectar foraging in a honey bee colony. ISBE Zürich (*poster*).

Wikelski, M. and C. Thom, 2000. Shrinking in marine Iguanas. International Congress of Osteology, Würzburg (*talk*).

Thom C., Gilley D.C., and J. Tautz, 2001. Das Piping-Signal der Honigbienen: Beobachtungen an Arbeiterinnen. AG Tagung der Bieneninstitute, Mayen (*poster*).

Thom C., Gilley D.C., and J. Tautz, 2001. Worker piping in honey bee colonies: observations on nectar foragers. TMR Granada (*poster*).

Thom C., Gilley D.C., and J. Tautz, 2001. Nectar foraging in honey bees: what makes bees tremble? IUSSI Berlin (*poster*).

ACKNOWLEDGEMENTS

I thank

My family for everything

Prof. Dr. Jürgen Tautz for his kind advise, support and encouragement,

Prof. Dr. Bert Hölldobler who enabled me to work in his department,

Prof. Dr. Stefan Fuchs for his friendly commitment in the revising of my work,

Prof. Dr. Thomas D. Seeley for spending much time considering my data,

David C. Gilley for constant help, encouragement and the work together,

Prof. Dr. Harald E. Esch for his advise, discussions, and the work together,

Dr. David J.T. Sumpter for helpful discussions and the work together,

Dr. Anna Dornhaus for discussions and the work together,

Dr. David R. Tarpy and Dr. Koos Bijsmejer for reading and discussing manuscripts,

Dr. Anna Dornhaus, Claudia Groh, Dr. Johannes Spaethe, and Dr. Anja Weidenmüller for being great colleagues and for willingly reading and discussing matters and manuscripts at all times,

Prof. Dr. Martin Wikelski for encouragement and the iguana part,

Elizabeth Tibbetts for helpful discussions,

Christian Stigloher for helping to start the project about forager activating signals,

Michael Eckart, Annett Endler, Stefanie Grüner, Susanne Reuther, Holger Steger and especially Kathrin Köppen-Schomerus for assistance in the field,

Heinrich Demmel for essential help with bees, hives and honey, and

everybody from Zoologie II and NB&B for discussions and the good times.

Erklärung

Hiermit erkläre ich, die vorliegende Arbeit in allen Teilen selbständig und nur mit den angegebenen Hilfsquellen angefertigt zu haben.
Diese Dissertation hat weder in gleicher noch ähnlicher Form in einem anderen Prüfungsverfahren vorgelegen.
Des weiteren erkläre ich, dass ich früher weder akademische Grade erworben habe, noch zu erwerben versucht habe.