From individual behavior to collective structure

Pollen collection and nest climate control in social bees

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Das Schönste, was wir erleben können, ist das Geheimnisvolle.

A. Einstein (1959)

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GENERAL INTRODUCTION

The social organization of insect colonies has fascinated naturalists for centuries. Insect colonies are groups of individuals that live together and reproduce as a unit. The colony presents a level of biological organization above the individual organism with its own characteristic morphology, internal organization and life history pattern (Wheeler 1928; Wilson 1971; Hölldobler & Wilson 1990; Seeley 1995).

The most thought provoking feature of insect colonies is their ability to achieve collective activity without central authority. For example, termites build complex nest structures thousands of times larger than the individuals who take part in construction (Lüscher 1961; Turner 2000). Honey bee colonies monitor an area of more than 100 km^2 around their hive and concentrate their foraging efforts on the most profitable food sources (Seeley 1995). Army ants collectively follow a precise compass bearing each day as they methodically sweep the forest floor for prey in enormous raids numbering up to 200 000 individuals (Hölldobler & Wilson 1990). Thus, colonies display a range of complex behaviors that far transcend those of the individual colony member and which can not be accounted for by any apparent central control or simple hierarchical structure.

This presents researchers with a challenging problem: what are the mechanisms that integrate the behavior of individuals into a functioning whole? Understanding how collective colony level patterns emerge from the behavior of the individual colony members is a major issue in the study of social insects.

One of the main features of colony organization is division of labor. Besides the primary division of labor between reproductive caste and worker caste, there exists a further division of labor among workers, whereby each member of the colony specializes in a subset of all tasks required for successful group functioning (Oster & Wilson 1978). Division of labor is fundamental to the efficient functioning of insects colonies and is believed to be a major determinant of their vast ecological success (Wilson 1987; Hölldobler & Wilson 1990). Two general patterns of division of labor are recognized. (1) Temporal polyethism, in which tasks are divided unequally between workers of different age and specializations are therefore temporary. (2) Physical polyethism, or polymorphism, in which tasks are divided unequally between workers of different size and/or shape. Here, specializations are permanent. Temporal polyethism is the most commonly observed pattern of division of labor in social insects while physical polyethism is found mainly in termites and about twenty percent of the ant genera (Wilson 1971; Oster & Wilson 1978; Hölldobler & Wilson 1990).

The most striking aspect of division of labor is its plasticity: the ratios of workers performing particular tasks can vary in response to internal perturbations and external challenges. Colony flexibility requires the behavioral flexibility of individual workers. Indeed, especially in colonies with temporal specialists, workers switch from one task to another as colony demands change (Robinson 1992; Gordon 1996). Workers of most social

Hymenoptera seem to be totipotent, that is they seem to be able to perform all tasks except reproduction (Wilson 1971).

How then does division of labor arise? How are certain individuals channeled into certain tasks, and why and when do they stop performing a task and switch to another one? Although there has been considerable work on division of labor and we know much about its evolutionary or functional advantages, the mechanisms by which different tasks are allocated among workers are poorly known (Gordon 1996).

Behavior is best understood by integrating analyses at multiple levels of biological organization (Robinson 1994). In order to unravel the coherent behavior of insect colonies, we need to look at both the colony level and the individual level. The first step is to characterize the group level pattern in detail. The next step is to identify the subunits involved in the collective pattern and to determine through empirical study their behavioral rules of thumb and the pathways of information flow among them (Seeley 1995). In other words, we need to know (i) how information about the colony's current needs is acquired by workers and (ii) how it is then acted upon. By dissecting and documenting the behavior of the colony and the colony's subunits in this way we can begin to understand the mechanisms that integrate dozens or thousands of insects into a higher-order entity. This is the approach I have taken in my thesis.

In the first chapter, I examine the control of pollen collection in honey bee colonies. Pollen foraging is an example of a collective behavior that is precisely organized and carefully regulated. While the ability of a honey bee colony to adjust its foraging effort around a homeostatic setpoint is well documented, the behavior of the individual pollen foragers that constitute this colony feature is not well understood. We know very little about the mechanisms that control pollen collection. It is still largely unclear how the foragers acquire information about how much pollen to collect and how they adjust their behavior accordingly. Working with marked bees in observation hives, I focus on the possible cues by which individual pollen foragers assess their colony's need for pollen. I carefully document changes in the in-hive experience of pollen foragers and compare the parameters constituting this experience before and after a change in the colony's pollen need. This approach is consistent with Lindauers' appeal for research in this area: 'The chief aim of future investigations must be to try to find out how the bee knows at this or that moment that a certain type of activity is needed' (Lindauer 1953, p 88).

I conclude this chapter with a discussion of the various information pathways that have been suggested in the literature to play an important role in informing pollen foragers about their colony's needs. Although we are still far away from a complete understanding of the process, some valuable progress has been made in unraveling the mysteries of pollen foraging.

I then turn to another homeostatic colony response in social bees that has so far not been well documented on either the individual or the colony level: the control of nest climate in bumble bee colonies.

In chapter two, I present a detailed analysis of the colony level pattern. I experimentally examine which parameters of nest climate a colony controls. Exposing bumble bee colonies to an increase in temperature, humidity and CO_2 under laboratory conditions I document the collective fanning response. Looking at the colony response across a wide range of colony sizes I examine the influence of group size on homeostasis. Based on data obtained from measurements in a field nest, I discuss the importance of the parameters under natural conditions.

In chapter three I then again turn to the subunits of the system, the individual workers and their behavior that constitutes the collective response. Compared to the control of pollen collection, information acquisition in control of nest climate is simple since all workers are directly exposed to parameters of nest climate. Therefore, in this chapter my focus on individual behavior moves away from the question of information acquisition. Rather, I concentrate on an idea that is currently hotly debated in search of the proximate mechanisms underlying division of labor, namely that interindividual differences in the responsiveness of a colony's members are responsible for the emergence of colony features such as division of labor. The concept of response threshold has been used to formulate behavioral rules that can account for specialization and flexibility in division of labor. Variation in response thresholds among workers represents a simple underlying mechanism that can begin to explain patterns of variation in task performance among workers. However, empirical studies evidencing differential response thresholds in workers of a colony are rare.

I present the control of nest climate in bumble bees as an ideal study system to investigate interindividual variability, since it is based on a simple, measurable stimulus-response pair: temperature intensity / CO_2 concentration and fanning response. (Honey bee pollen foragers presumably also exercise threshold-type decision rules for pollen foraging, however in this case interindividual variability is much harder to quantify since we can not measure the stimulus 'pollen need' as easily as temperature or CO_2). In light of my results, I discuss the role of behavioral thresholds in the emergence of polyethism and examine the link between individual and collective behavior.

I end chapter three with a discussion of self-organization concepts and of how natural selection may act upon such systems and present an outlook to future studies.

Classically, decision making is assumed to rely on the knowledge of a central unit or brain which collects all important information and then decides accordingly. However, the brain of an insect society *is* the insect society. The collective intelligence of insect colonies demonstrates that problems can be solved through decentralized information processing, based on the behavior of many independent individuals which interact with each other and with their environment. Insect colonies offer the possibility of studying such decentralized processes. By examining the range of complexity between the individual and collective levels we can begin to understand the emergence of efficient collective responses and hence ultimately to unravel the mechanisms that underlie the vast ecological success of social insects. This thesis hopes to contribute to this exciting quest.

CHAPTER I

Honey bee worker with pollen
Photo R. Sandemann

IN-HIVE BEHAVIOR OF POLLEN FORAGERS IN HONEY BEE COLONIES UNDER CONDITIONS OF HIGH AND LOW POLLEN NEED

ABSTRACT

Pollen collection in honey bees is regulated around a homeostatic setpoint. How the control of pollen collection is achieved is still unclear. Different feedback mechanisms have been proposed but little is known about the experience of pollen foragers in the hive. Here I present the first detailed documentation of the behavior of pollen foragers in the hive under different pollen need conditions. Taking a broad observational approach, I analyze the behavior of individual pollen foragers in the hive between collecting trips and quantify the different variables constituting the in-hive stay. Comparing data from two colonies and 143 individuals during experimentally induced times of low vs. times of high pollen need, I show that individual foragers modulate their in-hive working tempo according to the actual pollen need of the colony: Pollen foragers slowed down and stayed in the hive longer when pollen need was low and spent less time in the hive between foraging trips when pollen need of their colony was high. I discuss the possible information content of the different parameters constituting the in-hive stay by looking at those variables that change with pollen need. The number of cells inspected before unloading pollen load did not change and thus did not serve as cue to pollen need in our experiment. The trophallactic experience of pollen foragers changed with pollen need conditions: trophallactic contacts were shorter when pollen need was high and the number and probability of having short (<3s) trophallactic contacts increased when pollen need increased. Thus, the results of this study support the hypothesis that trophallactic experience is one of the various information pathways used by pollen foragers to assess their colony's pollen need.

INTRODUCTION

Honey bees collect pollen to satisfy the protein requirements of their colony. Pollen is converted into proteinaceous brood food by nurse bees (Crailsheim et al. 1992) and is crucial for the brood production of a colony (Morton 1950; Allen & Jeffree 1956; Kunert & Crailsheim 1988; Imdorf et al. 1998). It is not collected by the nurse bees, which ultimately make use of it in the hive, but by the forager bees. Thus there exists a division of labor between bees that work outside the hive collecting pollen and bees that work inside the hive consuming it (reviewed in Seeley 1995).

Pollen foraging in honey bees is an example of a collective behavior that is precisely organized and carefully regulated. The pollen need of a colony is closely correlated with the relative amounts of larval brood and stored pollen present in the hive (for review see Dreller & Tarpy 2000). If the number of cells containing larvae is high and the number containing pollen is low, then the colony's need for pollen is high.

Colonies adjust their pollen foraging effort in accordance with their colony's pollen need (Jeffree & Allen 1957; Free 1967; Fewell & Winston 1992) and try to maintain about 1 kg of stored pollen at any given time (Jeffree & Allen 1957). This regulation of pollen storage around a homeostatic set point provides the colony with a modest buffer against external fluctuations in pollen supply and ensures sufficient storage space for nectar to secure winter survival.

How do the foragers acquire information about how much pollen to collect? The mechanisms by which pollen foragers assess their colony's pollen need are not well understood. Two classes of models have been proposed.

According to the *direct assessment* models, each pollen forager makes decisions based on her own, direct assessment of colony need (Pankiw et al. 1998; Vaughan & Calderone 1998; Dreller et al. 1999; Dreller & Tarpy 2000). Unlike nectar foragers, which unload their nectar to receiver bees just inside the hive entrance (Seeley 1995) pollen foragers enter deep into the hive and unload their pollen directly into pollen storage cells (Winston 1987). As they do so, they probably come across pollen and brood areas, hence in principle they have the possibility of direct assessment of their colony's pollen needs.

The *indirect assessment* model proposes that foragers assess their colony's pollen need through cues transmitted by trophallactic interactions with hivemates. According to the model, nurse bees feed more of their proteinaceous brood food to adult bees when pollen stores are abundant and pollen foraging behavior is inhibited by consumption of proteinaceous food (Camazine 1993; Seeley 1995).

Surprisingly few details are known about the behavior of pollen foragers in the hive between foraging trips. For nectar foragers and for water foragers detailed descriptions of in-hive experiences and changes in variables constituting these experiences have provided insights into feedback cues influencing the behavior of the individual (Lindauer 1952; Seeley et al. 1996; Kühnholz & Seeley 1997). In contrast, we do not know which variables constitute the in-hive experience of returning pollen foragers and how these variables change following a change in the colony's pollen need.

In this chapter I provide the first complete observational data on the behavior and experience of individual pollen foragers in the hive between foraging trips. I quantify the parameters constituting an in-hive stay under different pollen need conditions and focus on those variables that change with pollen need and thus present potential indicators of this need. I discuss the results in light of the different feedback mechanisms proposed for the control of pollen collection.

METHODS

Honey bee colonies were exposed to different pollen need conditions by manipulating their pollen stores. The in-hive experience of returning pollen foragers was quantified under low and high pollen need conditions.

Study site and colonies

Experiments were conducted at the bee laboratory of the University of Würzburg in August 1998. Two colonies headed by naturally mated *Apis mellifera carnica* queens were established in three-frame observation hives and housed inside a building. Each colony

consisted of about 5000 bees. The bees could forage freely via a 30 cm Plexiglas tunnel connecting the lowest frame to the outside. The hive entrances faced the same direction and were positioned 3 m apart. The colonies were set up 4 weeks before the experiments started and were matched for amounts of brood, honey, and stored pollen. Each colony had 2 frames containing brood and honey, while the contents of the third, uppermost frame varied with each experiment, as described below.

Manipulation of pollen need

Before pollen stores were manipulated, the top frame in both colonies contained a mixture of brood and pollen. On the first day of the experiment, data on the behavior of pollen foragers in the unmanipulated colonies were collected. On subsequent days, the pollen need was manipulated twice in each colony and observations were repeated. Manipulations of pollen need were performed in the evenings. I experimentally induced the condition of high pollen need by replacing the top frame with one containing only uncapped brood (no pollen) and the condition of low pollen need by replacing it with one containing only pollen (no brood). Pollen and brood frames were derived from a colony not used in the experiment, they were matched for the size of the comb containing brood or pollen and for number of empty cells. To control for external effects (e.g. changes in pollen supply) on pollen forager behavior, manipulations were performed simultaneously and oppositely in the two observation colonies: while one colony's pollen need was increased, the other colony's pollen need was simultaneously decreased. The experiment lasted 7 days, data were collected every day except on day 4 because of poor weather conditions. Colony 1 received a comb of pollen on the evening of the first day and a comb of uncapped brood on the evening of the fifth day. Colony 2 received a comb of uncapped brood on the evening of the first day and a comb of pollen on the evening of the fifth day (see Table 1.1).

Day	1	2	3	4	5	6	7
Colony 1	no manip.	low	low	*no data*	low	high	high
Colony 2		high	high		high	low	low

Table 1.1 Pollen need conditions (low and high) experienced by the two observation colonies. Arrows denote manipulation of pollen need. During the first day of observation pollen need was not manipulated, the colonies then received opposed treatments twice. No data were taken on the fourth day.

Data collection

Returning pollen foragers were captured at the hive entrance and labeled with numbered plastic tags (Opalithplättchen) the day before observations started. 80 pollen foragers were individually marked in each colony. Observations started at 0800 and ended at 1300, when the number of returning pollen foragers fell below one per minute.

Pollen foragers in the two colonies were observed simultaneously by two observers that switched between the colonies every hour to minimize observational bias. A focal observation started when the forager entered the hive and ended either when the bee exited for her next collecting trip or when she stayed in the hive longer than 15 minutes. Observations were recorded using a laptop computer equipped with software for behavioral data capture and analysis (Observer, Noldus NL). From these records, I calculated the following variables:

- the latency for each behavior relative to when a bee entered the hive (time to inspecting the first cell, time to unloading pollen, time to first trophallactic contact, time to leaving the hive)
- the times of inspecting the first cell to unloading pollen and of unloading pollen to leaving the hive
- the number of cells inspected
- the number of waggle dance circuits performed
- the number of waggle dance circuits followed
- the number and durations of all trophallactic contacts. During a trophallactic contact the recipient bee puts her tongue between the mandibles of a donor bee and receives liquid food. Trophallactic contacts were classified as short when they lasted less than 3 s and as long when they lasted 3 s or more (see Crailsheim 1998; Kühnholz & Seeley 1997).

After the bee had departed, or 15 minutes had elapsed without departure, the observer closely watched the entrance tunnel for the next labeled pollen forager, which was then followed on her passage through the hive. During all observations, foraging activity was measured every hour by counting during 5 minutes the returning pollen and non-pollen foragers in the Plexiglas entrance tunnel while they were walking in.

Statistical analysis

Unless noted otherwise, values are given as mean ± one standard error (SE). If a forager was recorded more than once during one day, only the first set of observations on her was used for data analysis. Data of the days following each manipulation are pooled (days 2,3,5 and days 6,7). Data were analyzed using Student's *t*-test, Mann-Whitney U test and Chi-square tests. The chosen level of significance was 0.05.

RESULTS

Colony foraging activity

Both colonies showed a clear response to the experimentally induced state of high or low pollen need. The number of foragers that entered the hive with pollen increased significantly when a colony's pollen need was high and decreased significantly when it was low ($p<0.05$, see Table 2). There was, however, no significant effect of manipulation on the total number of foragers returning to the hive per 5 minutes (Table 1.2).

Individual behavioral response

I observed a total of 143 in-hive stays of returning pollen foragers. These bees showed a characteristic sequence of actions. In general, it consisted of self-grooming followed by inspecting cells and eventually unloading pollen into the last cell inspected. The sequence then continued with additional self-grooming, receiving food and finally departing for the next foraging trip. Rarely, the foragers groomed themselves extensively after unloading once and then unloaded into a second and sometimes even a third cell. Bees that showed this behavioral pattern always stayed in the hive longer than 15 min and presumably had stopped pollen foraging for the day. Most trophallactic contacts (receiving food) occurred after a pollen forager had unloaded her pollen.

Recruitment communication

Table 1.2 shows that only a small percentage of pollen foragers produces waggle dances. Of 143 pollen foragers, only 16 danced (11.2%). The probability of waggle dancing did not significantly differ between times of low and high pollen need. However, there seems to be a trend for a higher dance probability when pollen need is high.

Likewise, only 12 of the 143 pollen foragers followed waggle dances (8.9%). Never were these individuals themselves dancers. There was no significant change in the probability of dance following with different pollen need conditions.

The number of dance circuits followed or produced per dance varied between bees and showed no clear change with pollen need (Table 1.2).

Time budgets

Pollen foragers spent significantly less time in the hive between collecting trips when pollen need was high compared to when it was low (Fig. 1.1). In colony 1, pollen foragers spent 247 ± 22 s in the hive when pollen need was high compared to 331 ± 30 s when pollen need was low ($p<0.05$, *t*-test). In colony 2, the time spent in the hive decreased from 370 ± 43 s to 275 ± 15 s when pollen need was high ($p=0.01$, *t*-test).

When pollen need was low, a higher percentage of pollen foragers stayed in the hive longer than 15 min compared to when pollen need was high (Table 1.3, colony 1). These bees might have either abandoned pollen foraging, and thus would represent a subgroup within

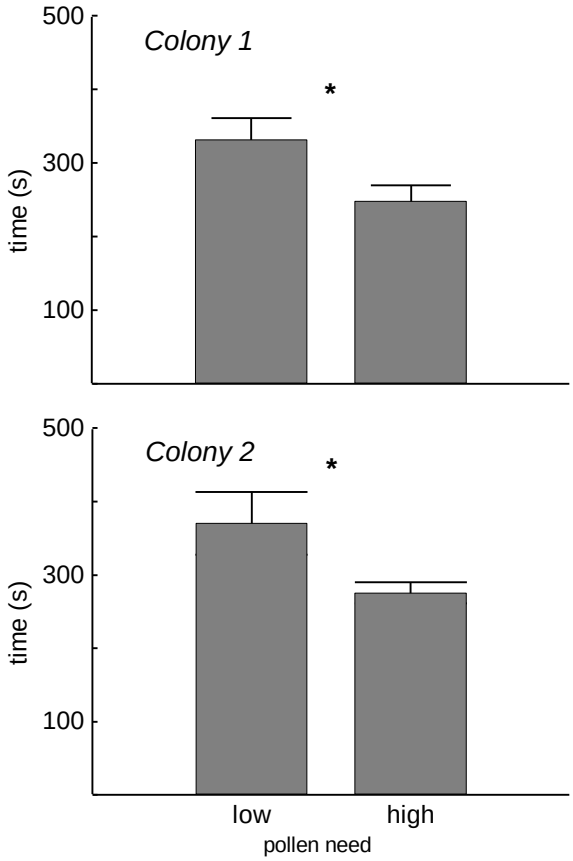

Fig. 1.1 Time (mean ± SE) pollen foragers spent in the hive between foraging trips when pollen need of their colony was low compared to when it was high (colony 1: $p<0.05$; colony 2: $p<.01$ *t*-test).

the observed foragers, or just slowed down considerably. Because the observational method did not allow to distinguish between these two cases, data from these bees were not pooled with data from bees that left the hive again within our observation period.

The in-hive stay of pollen foragers consists of several phases. Table 1.3 shows the data for each phase of the in-hive stay. The time from entering the hive to inspecting the first cell changed with pollen need: pollen foragers spent less time till inspecting the first cell when pollen need was high than when it was low. When pollen need was low the pollen foragers spent considerable time grooming and walking around slowly before inspecting the first cell.

Pollen foragers also showed a change in the last phase of their in-hive stay: they stayed in the hive longer after having unloaded pollen when pollen need was low than when it was high. This effect was significant in colony 2 and nearly so in colony 1.

The time spent for cell inspection (time from inspecting the first cell to unloading pollen into a cell) was not influenced by pollen need: in both colonies pollen foragers spent 30 to 40 s inspecting cells (Table 1.3). Time budgets for trophallactic behavior are presented below.

Thus I found a clear adjustment of the working tempo of pollen foragers depending on the pollen need of their colony. The longer in-hive stay of pollen foragers under low pollen need was caused by a delayed inspection of cells before unloading and by a prolonged stay in the hive after unloading.

Inspection of cells

The number of cells inspected before unloading pollen was not influenced by pollen need. When pollen need was low, pollen foragers inspected a mean of 5.7 ± 0.8 cells in colony 1 and a mean of 5.3 ± 1.2 cells in colony 2. When pollen need was high they inspected 5.1 ± 0.9 cells in colony one and 4.9 ± 0.6 cells in colony 2 (Table 1.3).

	Colony 1				*Colony 2*			
POLLEN NEED	(START)	LOW	HIGH	*p* (L vs H)	(START)	HIGH	LOW	*p* (L vs H)
Number of pollen foragers / 5 min	57.2 ± 59.2	22.9 ± 23.7	50.5 ± 40.9	**0.04**	46.2 ± 49.3	49.5 ± 45.8	15.3 ± 14.1	**0.04**
Total number of foragers / 5 min	152.6 ± 126.6	128.0 ± 59.4	166.1 ± 71.4	0.16	118.6 ± 73.4	177.3 ± 79.9	153.9 ± 37.8	0.41
Probability of dancing (%)	11.1	7.4	15.8	0.37	16.6	17.6	6.7	0.31
Number of dance circuits danced	(9, 9)	(22, 21)	(1, 9, 17)		(30)	(6, 4, 10, 2, 17, 11)	(1)	
Probability of dance following (%)	11.1	7.4	15.8	0.37	16.6	5.9	6.7	0.92
Number of dance circuits followed	(5 / 14)	(1 / 1)	(1 / 1 / 7)		(2)	(1 / 1)	(1)	
n	*18*	*27*	*19*		*6*	*34*	*15*	

Table 1.2 Foraging rates (mean ± SD) of colonies and probabilities of dancing and dance following of individual pollen foragers on one day before pollen need was experimentally manipulated (START) and at times of HIGH and LOW colony pollen need.

	Colony 1				Colony 2			
	(START)	LOW	HIGH	*p* (L vs H)	(START)	HIGH	LOW	*p* (L vs H)
in hive >15 min	3 of 21	7 of 34	0 von 19	**0.03**	1 of 7	6 of 40	7 of 22	0.12
time till first cell is inspected (s)	42.8 ± 4.1	78.8 ± 8.1	53.5 ± 7.2	0.05	90.8 ± 11.3	49.8 ± 5.6	66.3 ± 12.2	0.17
time till pollen is unloaded (s)	80.0 ± 10.4	106.7 ± 9.7	90.6 ± 9.6	0.26	139.6 ± 26.8	83.7 ± 7.6	101.5 ± 16.7	0.27
inspect first cell to unload pollen (s)	37.2 ± 9.1	30.9 ± 6.1	36.4 ± 6.8	0.56	48.8 ± 27.2	36.5 ± 6.0	34.3 ± 11.7	0.85
unload pollen to leave hive (s)	210.9 ± 29.8	206.5 ± 27.3	157.4 ± 16.0	0.08	235.9 ± 80.8	192.0 ± 14.0	262.0 ± 27.6	**0.02**
time till first troph. contacts (s)	98.0 ± 20.8	153.6 ± 16.3	100.9 ± 13.8	**0.02**	101.0 ± 33.1	109.6 ± 13.0	111.6 ± 26.8	0.94
time first to last troph. contact (s)	103.7 ± 24.4	35.9 ± 9.4	83.2 ± 17.0	**0.01**	94.7 ± 51.2	120.2 ± 15.0	162.9 ± 40.9	0.23
no. cells inspected	5.9 ± 0.9	5.7 ± 0.8	5.1 ± 0.9	0.68	4.5 ± 2.5	4.9 ± 0.6	5.3 ± 1.2	0.68
n	*18*	*27*	*19*		*6*	*34*	*15*	

Table 1.3 Parameters of the in-hive stay of pollen foragers on one day before pollen need was manipulated (START) and on days when their colony was experiencing LOW or HIGH pollen need. Values are given as means ± SE.

Trophallaxis

The time of entering the hive to first trophallactic contact changed significantly with pollen need in colony 1; under high pollen need conditions pollen foragers had their first contact earlier than when pollen need was low (Table 1.3). In colony 2 there was no change in time to first trophallactic contact.

The time between first and last trophallactic contact increased significantly in colony 1 (Table 1.2). This is the only time phase that increased when pollen need increased, even though pollen foragers spent significantly less time in the hive (Fig. 1.1). Time between first and last contacts did not change in colony 2.

By far, most of the trophallactic contacts observed were receiving contacts (625 of 633). Trophallactic contacts were usually terminated by the pollen forager who withdrew her tongue while the donor bee was standing still with spread mandibles.

A pollen forager made between 0 and 22 trophallactic contacts per in-hive stay. The number of contacts varied considerably between individuals. In colony 1 the mean number of trophallactic contacts a pollen forager made changed with colony pollen need: pollen foragers had more trophallactic contacts when pollen need was high than when it was low (mean low: 2.3 ± 0.4, high: 3.7 ± 0.5, $p<0.01$, Mann-Whitney U test). In colony 2 pollen foragers had more trophallactic contacts than in colony 1, the number of contacts did not change with pollen need (mean low: 7.4 ± 1.8, high: 7.4 ± 0.8, $p=0.71$, Mann-Whitney U test).

Trophallactic contacts varied considerably in duration, from 0.1 to 69 s. The median duration of the 625 receiving contacts was 2.8 s, the mean duration was 6.5 s. 317 receiving contacts lasted less than 3 s and were therefore classified as ‘short contacts’ (see methods section).

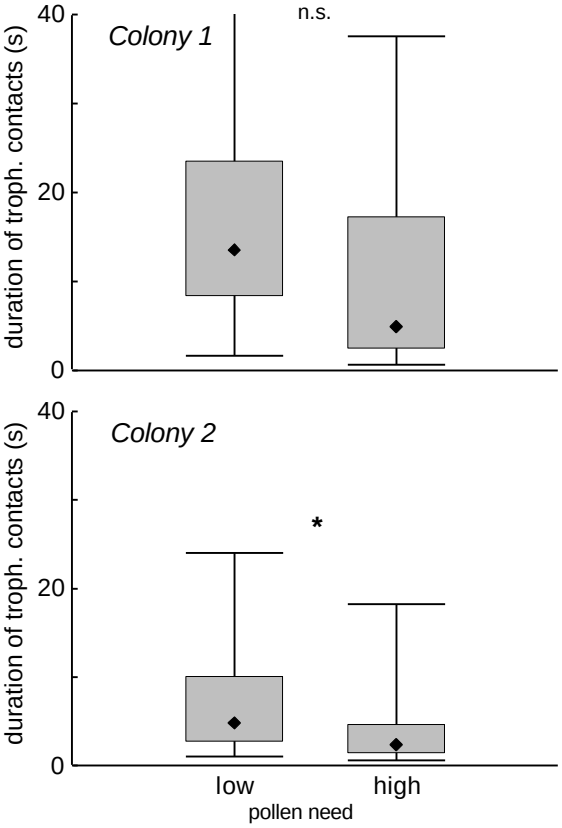

Figure 1.2 shows that the median duration of trophallactic contacts a pollen forager made changed with pollen need. When colony pollen need was high, trophallactic contacts were short compared to when pollen need was low. In colony 1 the median duration of trophallactic contacts was 4.9 s when pollen need was high and 13.5 s when pollen need was low ($p=0.05$, Mann-Whitney U test). In colony 2 trophallactic contacts had a median

Fig. 1.2 Duration of trophallactic receiving contacts during in-hive stay when pollen need of the colony was low compared to when it was high (col. 1: $p=0.05$; col. 2: $p=0.03$; Mann-Whitney U test). Diamonds: medians, boxes: quartile range, whiskers: range. In colony 1 (low pollen need) the maximum duration was 69 s.

duration of 2.4 s when pollen need was high and of 4.8 s when pollen need was low and ($p<0.05$, Mann-Whitney U test).

In colony 1, this change in duration of trophallactic contacts is reflected in the number of short (<3 s) contacts a pollen forager made (Fig. 1.3). While in both colonies the number of long trophallactic contacts did not change with pollen need (colony 1 low: 1.7 ± 0.2, high: 1.9 ± 0.2, $p=0.22$; colony 2 low: 3.2 ± 0.4, high: 3.1 ± 0.2, $p=0.74$, Mann-Whitney U test), the number of short contacts changed with pollen need in colony 1 (low: 0.7 ± 0.3, high: 1.7 ± 0.4; $p<0.05$). In colony 2 the number of short contacts did not change with pollen need (low: 4.1 ± 1.4, high: 4.2 ± 0.6, $p=0.46$, Mann-Whitney U test) (Fig. 1.3).
The probability of having a short trophallactic contact also changed with pollen need: When pollen need was low, only 30% of the pollen foragers in colony 1 experienced one or more short trophallactic contacts, compared to 69% when pollen need was high ($p<0.01$, Chi-square test). In colony 2 we found the same trend: when pollen need was low, 73% of the pollen foragers experienced short contacts compared to 84% when pollen need was high ($p=0.19$, Chi-square test).

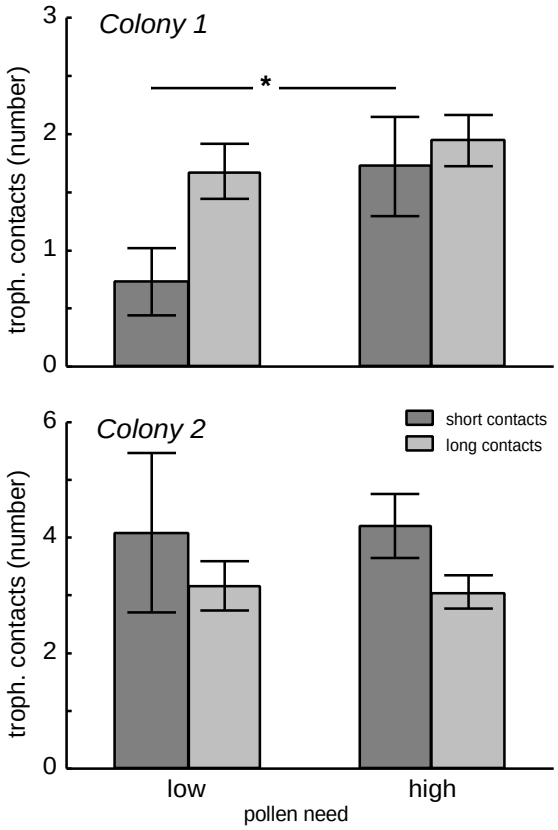

Fig. 1.3 Number of short (<3 s) and long (≥3 s) trophallactic receiving contacts a pollen foragers made during her stay in the hive (mean ± SE) when pollen need of the colony was low compared to when it was high. The number of short contacts changed significantly in colony 1 ($p=0.02$; Mann-Whitney U test).

Thus, I found a clear change in trophallactic experience of pollen foragers between times of low vs. times of high pollen need. In both colonies, the median duration of trophallactic contacts was significantly shorter when pollen need was high than when it was low. In colony 1, which went from low to high pollen need (see methods section), the number of trophallactic contacts increased significantly, caused by a significant increase in short contacts; pollen foragers had a significantly higher probability of experiencing short contacts when pollen need increased. In colony 2, which went from high to low pollen need, the number of trophallactic contacts did not change with pollen need.

DISCUSSION

The adaptive control of pollen foraging in a honey bee colony is one of many fascinating examples of functional flexibility in social insect societies. Understanding how this colony-level flexibility is implemented at the level of individual worker bees is crucial for a thorough understanding of how adaptive control is achieved.

How do honey bee workers obtain information about the pollen need of their colony and how do they then modulate their behavior accordingly? The present study adds to our understanding of this process by providing a detailed description of the in-hive behavior of pollen foragers between foraging trips. Pollen foragers presumably obtain information about the pollen need of their colony during their stay in the hive. Therefore, comparing the variables of behavior during an in-hive stay at times of low vs. high pollen need can provide insights into which variables provide this information.

Response to changes in pollen need

The ability of honey bee colonies to adjust their pollen influx to pollen need has been demonstrated numerous times (for review see Fewell & Winston 1992). One way of adjusting pollen influx is changing the number of pollen foragers. Two factors influencing this number are the rates of recruitment for and abandonment from the task of pollen foraging (Seeley 1995). My data on waggle dance behavior of the pollen foragers show that even when pollen need was extremely high only a relatively small proportion, less than one fifth of the pollen foragers, performed waggle dances. The probability of performing waggle dances did not show a significant change with pollen need, however, there was a trend for a higher likelihood of performing waggle dances when pollen need was high. This is in accordance with data by Camazine, who found an increase in dance probability when pollen need was high (reported in Seeley 1995).

This study provides no data on abandonment rates. However, in colony 1 the number of pollen foragers that stayed in the hive longer than 15 min and probably abandoned pollen foraging decreased significantly when pollen need was high.

Besides changing the number of pollen foragers, a colony can modulate its pollen collection by changing the per capita work effort of pollen foragers. Pollen foragers can adjust trip time and pollen load size to adaptively tune the pollen collection rate of their colony (Fewell & Winston 1992; Janmaat et al. 2000). My data show that pollen foragers also modify the time spent inside the hive. When colony pollen need was high, pollen foragers left the hive only a few minutes after having entered it. When pollen need was low the pollen foragers spent much more time in the hive between foraging trips. This change in mean time budgets has been reported before based on arrival and departure data of pollen foragers at the hive entrance (Fewell & Winston 1992). The data of this study however present a more detailed picture of what is actually happening in the hive. In particular, these data reveal when during their stay the pollen foragers slow down.

The in-hive stay of pollen foragers consists of several phases. A change in time budget occurred during the first phase of the in-hive stay, namely the time from entering the hive to inspecting the first cell. Pollen foragers spent more time in this phase when pollen need was low than when it was high. Note that the manipulations of pollen need were performed by replacing the top frame and that both the pollen and brood frames used for manipulation of pollen need contained only few empty cells. Therefore, the bees that I observed, which walked mainly on the lower two combs, encountered a comb area that had not changed in number of empty cells compared to when pollen need was high, and so were not experiencing greater difficulty in finding an appropriate location for inspecting cells. Rather, the reason for the increase in time was that they entered the hive walking rather than running and spent more time grooming and walking around slowly before inspecting a cell. Furthermore, the pollen foragers also slowed in the last phase of their in-hive stay, the time from unloading their pollen to leaving the hive for the next foraging trip. Thus, I am confident that the pollen foragers actually modified their working speed and were not in some way experiencing difficulties that restricted them.

Why don't pollen foragers always work at a high rate? 'Slow' individuals may serve their colony in two ways: First, they probably save energy. Increased work effort may increase the rate of physical deterioration (Neukirch 1982). Perhaps only when pollen is immediately and urgently needed by the colony does it pay for an individual to bear the higher costs of increased foraging effort (Eckert et al. 1994). Second, in staying in the hive longer, pollen foragers probably are more available for other more urgent tasks, thus providing a reserve of labor.

Perception of changes in pollen need

The adjustments of working speed at the individual level (Fig. 1.1) and the rate of returning pollen foragers at the colony level (Table 1.2) shows that the bees sensed the manipulation of pollen need. How did they obtain the information that pollen need had changed? Several cues have been suggested as potentially providing pollen foragers with information about pollen need. The time spent till finding an appropriate cell for unloading pollen has been suggested as one such possible cue (Dreller & Tarpy 2000). For nectar foragers it is well documented that the time spent searching for a receiver bee in the hive influences their foraging and recruiting decisions (Seeley 1989; Seeley & Tovey 1994). Thus, at first glance it may be tempting to think that pollen collection is controlled by a similar feedback mechanism. However, as discussed above, the data of this study suggest that at least under the experimental conditions examined the time spent till finding an appropriate cell cannot serve as a reliable cue for pollen foragers, since changes in times resulted from a self-controlled change in the bees' behavior rather than being imposed on the bees by changes in the hive environment.

The idea that under low pollen need the pollen foragers were not experiencing difficulties in finding cells for unloading their pollen is further supported by the fact that pollen foragers in my experiment sampled a constant number of cells during a constant

amount of time independent of pollen need. The number of cells a pollen forager must inspect before unloading her pollen is a second variable that has been suggested as potentially providing information about colony pollen need (Dreller & Tarpy 2000). The fact that pollen foragers in my experiment did not use this information pathway does not rule out the possibility that under different conditions a change in the ratio of empty cells to full cells (pollen or brood) can provide information about a change in pollen need. Camazine (reported in Seeley 1995) in manipulating the pollen need of a colony, changed the ratio of pollen cells to empty cells in the part of the hive encountered by the pollen foragers (providing either a comb of empty cells or a comb of pollen in the lowest position). He found an increase in the number of cells inspected when the empty frame was added.

How exactly bees could obtain information by checking cells is unclear. One possibility is that the bees look for an 'appropriate' (empty) cell for unloading pollen and count the number of 'inappropriate' (full pollen) cells while doing so. In this scenario pollen foragers would have to estimate the amounts of brood and pollen separately to obtain information about the colony's pollen need. Inspecting cells might, however, provide the bees with information in a different way. Sampling a certain number of cells might inform bees about the ratio of cells with and without pollen or provide information about the kind and quality of pollen stored in the colony. I observed that many of the cells inspected contained some pollen but were far from being full. Indeed, often a pollen forager under investigation would inspect a cell the previous pollen forager had just unloaded her pollen into and would continue inspecting other cells. To clarify whether pollen foragers are really simply looking for empty cells as has been suggested or whether they are collecting some other information while inspecting cells we need data on what kind of cells pollen foragers inspect and what makes a cell 'appropriate' for receiving pollen.

After unloading their pollen loads, the pollen foragers made numerous trophallactic contacts. The majority of these contacts were initiated and terminated by the forager bees. More than fifty percent of the trophallactic interactions lasted no longer than 3 seconds. During such short contacts no food transfer takes place (Korst & Velthuis 1982). However, they are probably sufficiently long for the pollen forager to find out whether the donor bee will provide food and if so what kind of food. The trophallactic experience of pollen foragers changed with their colony's pollen need. This is the strongest change in the parameters measured besides the changes in time budgets discussed above. Why does trophallactic experience change and how could information about pollen need be encoded in this experience?

Camazine demonstrated that trophallactic contacts between pollen foragers and bees on the brood nest are sufficient to induce a decrease in pollen foraging when pollen stores are experimentally increased (Camazine 1993). Obviously, pollen collection was inhibited through a cue received by the pollen foragers via trophallaxis. Camazine explained this phenomenon by hypothesizing a change in the trophallactic behavior of nurse bees: when a colony has ample pollen reserves nurse bees will feed more proteinaceous brood food to adult bees than when the colony is in need of pollen. Thus, under low pollen need

conditions protein is widely dispensed throughout the colony via trophallaxis and inhibits pollen foraging. Several studies provide support for this *indirect-inhibitor* hypothesis: (1) The transfer of protein from nurse bees to other hive mates including foragers has been indirectly demonstrated using radioactively labeled protein (Crailsheim 1991; Crailsheim et al. 1992). (2) Indeed, proteinaceous food transfer to foragers increases when pollen reserves are large (Camazine et al. 1998).

However, so far nothing was known about the actual trophallactic experience of pollen foragers in the hive and it remained unclear precisely which parameter of the trophallactic experience could encode information about pollen need. Various alternatives have been proposed: The quality or amount of protein received per in-hive stay and/or the ease of finding a protein dispensing trophallaxis partner could change with pollen need (Camazine et al. 1998).

My study demonstrates that the trophallactic contacts a pollen forager made were consistently shorter when pollen need was high compared to when it was low. This supports the idea that the amount of protein received by a pollen forager per in-hive stay changes with the pollen need of the colony: As the colony need for pollen increases, nurse bees have less excess proteinaceous food, pollen foragers therefore receive less protein per trophallactic contact and thus experience protein hunger.

In colony 1, which experienced a change from low to high pollen need, pollen foragers had a higher probability of experiencing short trophallactic contacts and experienced more short contacts per in-hive stay when their colony was in need for pollen. This supports the idea that compared to conditions of low pollen need, when it is easy to locate hive bees that are willing and able to transfer large amounts of proteinaceous food, pollen foragers have to sample more hive bees in order to find a protein dispensing bee when the pollen need of their colony increases. This would imply an active search for protein on the part of protein-hungry pollen foragers, not merely the perception of protein as a byproduct of trophallaxis as originally proposed by Camazine (Camazine 1993). Foraging aged bees have a considerable protein turnover rate (Crailsheim 1986) but their capability to digest pollen is limited (Moritz & Crailsheim 1987; Szolderits & Crailsheim 1993); they therefore rely on liquid proteinaceous food received from hive mates to fulfill their protein requirements (for review see Crailsheim 1998). The idea that pollen foragers experience protein hunger and actively search for protein dispensing trophallactic partners when the pollen need of their colony increases is further supported by the fact that a greater proportion of pollen foragers are found in the brood nest where they are more likely to encounter nurse bees when pollen need is high compared to when it is low (Camazine et al. 1998).

Thus, perception of pollen hunger might be responsible for switching on pollen collection. A comparable indirect feedback mechanism is described for the control of water collection, where foragers start collecting water when they are thirsty (Lindauer 1954; Kiechle 1961).

There is increasing evidence that the behavior of pollen foragers is influenced not only by indirect cues (Camazine 1993) but also by direct cues (Pankiw et al. 1998; Vaughan & Calderone 1998; Dreller et al. 1999; Dreller & Tarpy 2000; Pankiw & Page 2001).

Workers in insect societies often utilize more than one information pathway to increase the reliability of information (Franks 1999). Several cues, direct and indirect, may be used by pollen foragers to adjust their foraging behavior according to their colony's need. The information sources used may differ depending on whether a colony needs to decrease or increase its pollen collection and whether this change has to be small or large. While protein hunger is probably important in starting pollen collection, direct cues may play an important role in shutting down pollen collection. This may explain the difference found between the two colonies tested: the duration of trophallactic contacts changed with pollen need in both colonies, but only in colony 1 which experienced an increase in pollen need did the number and probability of short contacts increase.

Conclusions

In summary, this study shows that pollen foragers adjust their working speed in the hive to the pollen need of their colony: when pollen need is high they spend less time in the hive. The duration of trophallactic experience changes with pollen in both colonies. When pollen need increases, the pollen foragers experience many short contacts. These findings support the hypothesis that protein hunger is one possible information pathway informing pollen foragers about the pollen need of their colony. In order to reveal the relative importance of the different trophallactic parameters, experiments aimed at directly testing the influence of trophallactic interaction rates and protein transfer on pollen foraging behavior are needed.

CHAPTER II

Bumble bee colony with marked workers

THE CONTROL OF NEST CLIMATE IN BUMBLE BEES: PARAMETERS INDUCING VENTILATION RESPONSE AND THE INFLUENCE OF COLONY SIZE

ABSTRACT

Two main questions concerning the social control of nest climate in bumble bees colonies were addressed. First, I examined which parameters of nest climate bumble bees actively down-regulate by fanning. Second, I analyzed the dynamic of the colony response as colony size increased. Colonies of *Bombus terrestris* were exposed to an increase in carbon dioxide, temperature or relative humidity. Data of 112 trials with four colonies showed that an increase in CO_2 concentration and temperature level elicited a fanning response whereas an increase in relative humidity did not. This is the first report of fanning in bumble bee colonies to control respiratory gases. The fanning response was graded; the number of fanning bees increased with stimulus intensity. The colony response to a CO_2 concentration of 3.2 % was comparable to the colony response to a temperature of 30°C. A marked fanning response already occurred at 1.6% CO_2, a concentration never exceeded in a large field nest during a pilot measurement of 10 days. I investigated the colony response over a wide range of colony sizes (between 10 and 119 workers). The number of fanning workers increased as colony size increased, but the proportion of total work force invested by the colony into nest ventilation did not change. The dynamics of the colony response changed with colony size; larger colonies showed a faster response to perturbations of their colony environment than smaller colonies.

INTRODUCTION

Most social insects posses elaborate regulatory capabilities that enable them to control climatic conditions within their nests (Seeley & Heinrich 1981). These capabilities provide a certain degree of independence from the environment and thus promote growth and survival of the colony. Control over nest microclimate is achieved through a combination of nest design and worker activities. While nest site choice and nest architecture present long term adjustments, short term control of nest climate involves behavioral and physiological responses of individual colony members resulting in co-operative activities. These activities aim at returning conditions inside the nest to the state prior to perturbation, a phenomenon known as social homeostasis (Emerson 1956).

Bumble bees live in colonies that are founded by a single queen and grow to a worker population of several hundred within one summer. Due to their enormous incubating capacities colonies are able to maintain high nest temperatures even under extremely cool ambient conditions (Heinrich 1979). As colony size increases and during times of high ambient temperatures, colonies may sometimes face the problem of their brood nest overheating. Like other winged hymenopterans, bumble bees have the ability of fostering nest ventilation and thus heat loss by fanning out their nests with their wings (Vogt 1986a). Using the thermoregulatory measures of incubating and wing fanning, bumble bee colonies are able to maintain exceedingly stable temperature levels inside their nests.

The ability to maintain stable temperature conditions changes with colony size; small colonies undergo larger fluctuations in brood temperature than larger colonies (Seeley &

Heinrich 1981). An increase in nest climate homeostasis with increasing colony size has also been reported for honey bees (Seeley 1974) and hornets (Gibo et al. 1974).

Besides temperature, respiratory gases presumably are important parameters of nest climate. The majority of bumble bee species nests in underground cavities originally excavated by small mammals. The gas exchange properties of such cavities may not always fit the colony needs. Many tasks e.g. incubating require high metabolic activity (Heinrich 1979) and insufficient gas exchange is likely to result in a decrease in O_2 levels and an increase in CO_2 levels. Thus, especially when population size is large, bumble bee colonies presumably face the problem of insufficient exchange of respiratory gases. However, the requirements of bumble bee colonies concerning the concentration of respiratory gases are unclear. Nothing is known about the concentration of respiratory gases in bumble bee nests and whether they are actively controlled by colony members. Similarly, it is unknown whether bumble bees actively control a third parameter of nest climate, relative humidity.

In this chapter I address two main questions concerning the social control of nest climate in bumble bees: First, I experimentally test which parameters of their nest climate bumble bee colonies actively control. I expose colonies to increasing levels of temperature, CO_2 and relative humidity and carefully document the collective response. Second, I address the question why under natural conditions large colonies undergo smaller fluctuations in nest temperature than small colonies. To this aim, I analyze the colony response over a wide range of colony sizes.

METHODS

Lab colonies

I measured the response of bumble bee colonies to increasing levels of temperature, relative humidity and CO_2 under controlled laboratory conditions.

Colonies

Queen bumble bees (*Bombus terrestris*) obtained from a commercial breeder were allowed to establish colonies in Plexiglas-covered nest boxes (14 x 14 x 10 cm) in the laboratory. The nest boxes were divided into an upper compartment (8 cm high) containing the nest and a lower compartment (2 cm high) which could be opened and closed from one side for manipulations. The two compartments were separated by a wire mesh. The nest boxes had three screened ventilation holes (∅ 1.5 cm) and connected via a 60 cm Plexiglas tunnel (∅ 2 cm) to a foraging chamber (30 x 40 x 30 cm) where sugar solution was provided ad libidum. Pollen was fed directly into the nest. Colonies were kept at room temperature of 22°C. All newly emerged workers were marked with numbered plastic tags (Opalitplättchen), thus all workers of a colony were individually age marked.

Manipulation and measurement of nest climate

Temperature was increased by regulating a commercially available IR lamp (150 W) positioned 70 cm above the nest. Radiation was slowly increased during the experiment following a fixed temperature regime. Since colonies repeatedly experienced manipulations of their nest climate they were not exposed to temperatures above 30°C in order to avoid damage to the brood. Relative humidity was manipulated by placing a dish of dried silica gel in the lower compartment of the nest box 3 hours before the experiment started. This caused relative humidity to drop to levels of 40-50%. At start of manipulation the silica gel was exchanged for a dish of water. This induced a steady increase in relative humidity up to 90%. Carbon dioxide was increased by successive closing of the three ventilation holes. The first ventilation hole was closed at the start of the manipulation, the second and third were closed 10 and 20 minutes later, respectively, causing a gradual increase in CO_2 concentration. The fanning response of the colony was shown to be caused exclusively by the self-induced increase in CO_2 concentration by comparing it to the response to an artificial (injected) increase of CO_2.

Temperature and humidity were measured using a Vaisalla temperature and humidity probe (Vaisalla HMP 36B) inserted into the upper compartment of the nest box, 4 cm above the wire mesh. The CO_2 concentration was measured by IR-absorption with a gas sensor type GS 20 ED/ CO_2 (Sensor Devices, Germany). Air from the nest box was drawn into the gas sensor by an open loop circulation, driven by a membrane pump (12 V Wisa, Germany) at a flow rate of 1.5 l/min. Exchange of air in the circulating air current was achieved by two plastic tube openings covered by a fine wire mesh which was inserted into the nest box.

Data collection

Before an experiment started the entrance to the foraging chamber was closed with a wire mesh, thus confining all bumble bees during data collection to the nest box and entrance tunnel. Manipulations were performed daily between 1200 hours and 1500 hours. Either temperature, relative humidity or CO_2 concentration was experimentally increased while the other parameters remained constant. An experiment lasted 75 minutes, divided into 15 observation periods of 5 minutes each. Manipulations started after 15 minutes (3 observation periods) and lasted 45 minutes (9 observation periods). Thus, manipulations ended 60 minutes after the start of the experiment. Climatic conditions then gradually returned to starting conditions. Temperature, humidity and CO_2 values were noted at the beginning of each observation period. The number of individuals showing fanning behavior during an observation period was recorded. Fanning behavior was defined as steady fanning with extended wings while standing still for at least 10 seconds.

Four colonies were tested, each over a period of several weeks. Colony response to an increase in temperature / CO_2 was measured 9-16 times per colony; colony response to an increase in relative humidity was measured only 2 times in two colonies, resulting in a total of 112 trials (50 temperature, 58 CO_2, 4 relative humidity). Worker populations of the tested colonies ranged from 10 to 119 workers.

The controlled increase in nest temperature allowed me to analyze the dynamics of the colony response over the whole range of colony sizes tested. The time to maximum increase in number of fanning bees in each trial (for temperature only) was determined using a sigmoidal fit (model: y=a+b/(1+exp(c-x)) between time (x) (corresponding to temperature) and proportion of bees showing fanning behavior (y). The turning point c of the sigmoidal fit was used as a measure for the maximum increase in the number of fanning bees only if the model described more than 75% of the data. A correlation between colony size and turning points was used to investigate the delay of colony response.

Field colony

A field nest of *Bombus terrestris* was opened 30th June 1999. The colony inhabited an abandoned mouse nest, 20 cm beneath the surface with a 40 cm tunnel leading to the entrance hole (∅ 3 cm). I carefully opened the nest cavity from one side, leaving the tunnel undamaged. The colony consisted of more than 100 individuals, and already contained some drones. The nest canopy had several holes. Into one of these I inserted a temperature probe (Testo 175). One end of a probing tube (∅ 0.4 cm) was covered by fine wire mesh and also inserted into the nest for taking air samples. The other end of the probing tube was closely sealed. A second temperature probe was installed in the soil next to the nest cavity. I placed a Plexiglas sheet vertically in front of the nest and refilled the hole with soil. A third temperature sensor was placed 20 cm above ground-level in the shade. Starting on 1st of July, CO_2 data were taken for 10 days, temperature data were taken for 5 weeks. Temperature data of all three sensors were logged every 30 minutes. In order to reduce artificial ventilation of the nest, CO_2 concentration was measured no more than every three hours (3-5 times per day) using the pump and sensor described above. Air was drawn from the nest for 3 minutes at a flow rate of 1.5 l/min. The maximum CO_2 concentration measured was recorded and the tube was then immediately closed again. Observational data such as flight traffic, presence of drones and young queens, weather conditions etc. were recorded daily. CO_2 was again measured in September after the nest was abandoned.

Statistical analysis

Except for the examples shown in Figure 2.1, data are reported normalized for colony size (percentage occurrence: number of fanning workers / total worker population). Result are given as mean ± standard deviation. Statistical tests of the difference between two means were performed using Student's *t*-test. Pearson's correlation was used to analyze the dynamic of the colony response. The chosen level of significance was 0.05.

RESULTS

Lab colonies

Figure 2.1 shows examples of the fanning response of a colony exposed to an increase in CO_2 concentration, temperature and relative humidity. As CO_2 concentration rises (Fig. 2.1A) there is a gradual increase in the number of fanning bees. Fanning decreases as soon as stimulus intensity declines. Colonies showed the same graded fanning response when exposed to an increase in nest temperature (Fig. 2.1B) but did not show a clear fanning response to an increase in relative humidity (Fig. 2.1C). I performed three more trials with increasing relative humidity but never observed a fanning response. Therefore, only the colony response to an increase in CO_2 and temperature was studied further.

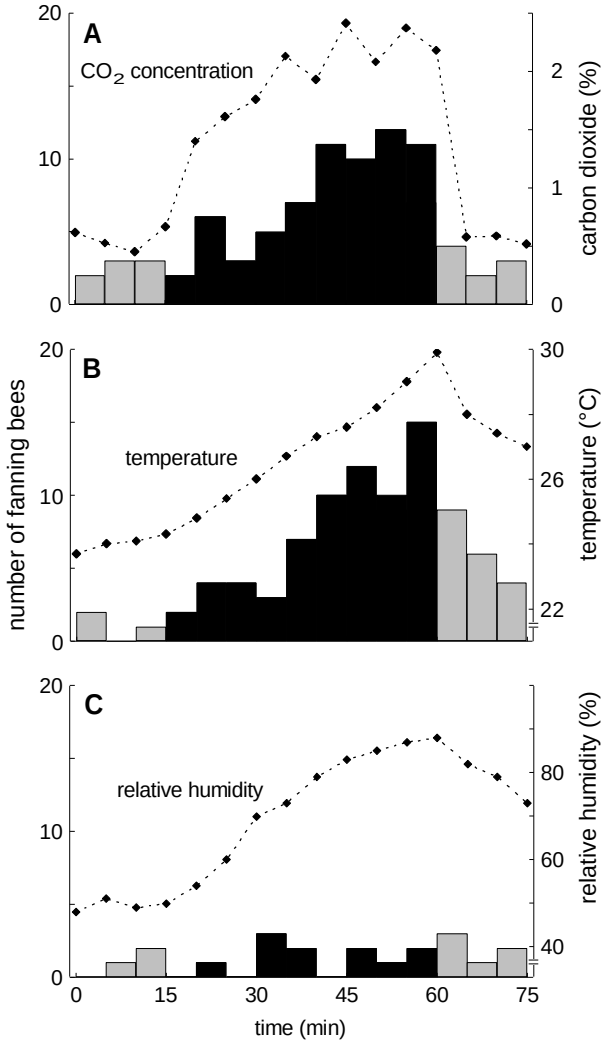

Fig. 2.1 Example of the fanning response of a *Bombus terrestris* colony exposed to an increase in A) CO_2, B) temperature and C) relative humidity. Grey bars denote number of fanners before and after manipulation, black bars denote number of fanners during manipulation of nest climate. The colony consisted of 78 worker bees.

Colony size increased over the course of the experiments. When first tested, colonies had between 10 and 33 individuals. During the last experiments (approximately 4 weeks later) the colony size ranged between 91 and 119 workers. Starting conditions (conditions before manipulation, during the first three observation periods) remained constant within each

colony throughout all experiments and with colony size. Temperatures at beginning of the experiments ranged between 22°C and 24°C. Since the experimentally induced temperature increase was controlled it was steady across all experiments and between colonies, independent of colony size. The maximum temperature was reached 45 minutes after the heating lamps were switched on. CO_2 concentrations at beginning of the experiments ranged between 0.3 - 0.8%. Maximum concentrations were reached 30 - 45 minutes after closing the first ventilation hole. Fanning as a response to high temperature and CO_2 levels was performed exclusively by worker bees. Only in one colony did the queen sometimes fan. Drones were never observed fanning.

The graded colony response to increasing CO_2 concentrations or temperature levels shown in Figure 2.1 was found in all colonies tested throughout all trials (Fig. 2.2). A marked increase in the number of fanning workers was usually observed at CO_2 concentrations of more than 1.6% or temperatures above 26°C. The four colonies differed in the intensity of their response. Differences between colonies were consistent over the whole range of intensities tested and for both temperature and CO_2 manipulations (except for colony X at a CO_2 concentration of 0.8 - 1.6 % CO_2). Colonies responded to temperatures of around 30 °C with the same intensity as to CO_2 levels of about 3%.

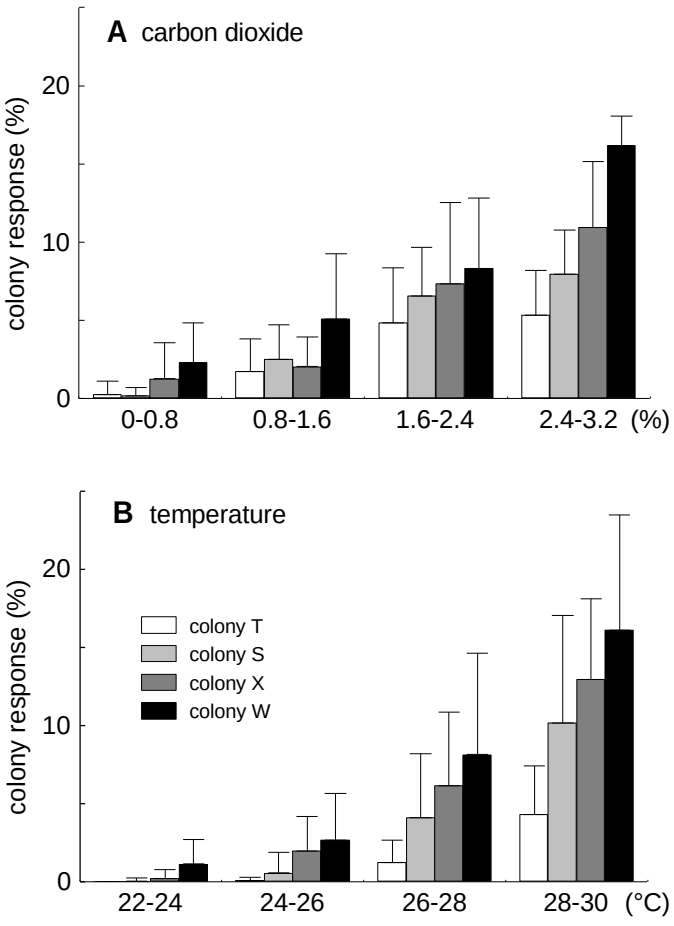

Fig. 2.2 Colony response (in % of workers fanning) of four colonies to different A) CO_2 concentrations and B) temperature levels. Columns indicate means, error bars are standard deviations. Pooled data from 58 CO_2 and 50 temperature trials are shown, presented in bins of 0.8% CO_2 / 2°C.

I analyzed two parameters of the colony response as function of colony size: The maximum response to a manipulation and the dynamic of the response to temperature stress.

The maximum response (maximum percentage of workers fanning per observation period) to CO_2 or temperature stress (2.5 – 3.2 % CO_2 or 28-30°C) was highly variable, with between 3 and 32% of worker population fanning (Fig. 2.3). The proportion of available work force maximally invested into nest ventilation did not show a clear change with colony size. In most cases there was no correlation between maximum response and colony size. Only in colony S did the maximum percentage of workers fanning under CO_2 stress slightly increase with colony size (slope: 0.08, r_s=0.55, $p<0.05$) and decrease with colony size under temperature stress (slope: -2.05, r_s=0.64, $p<0.01$). In colony X the maximum percentage of workers fanning under temperature stress increased with colony size (slope: 3.33, r_s=0.63, $p<0.05$).

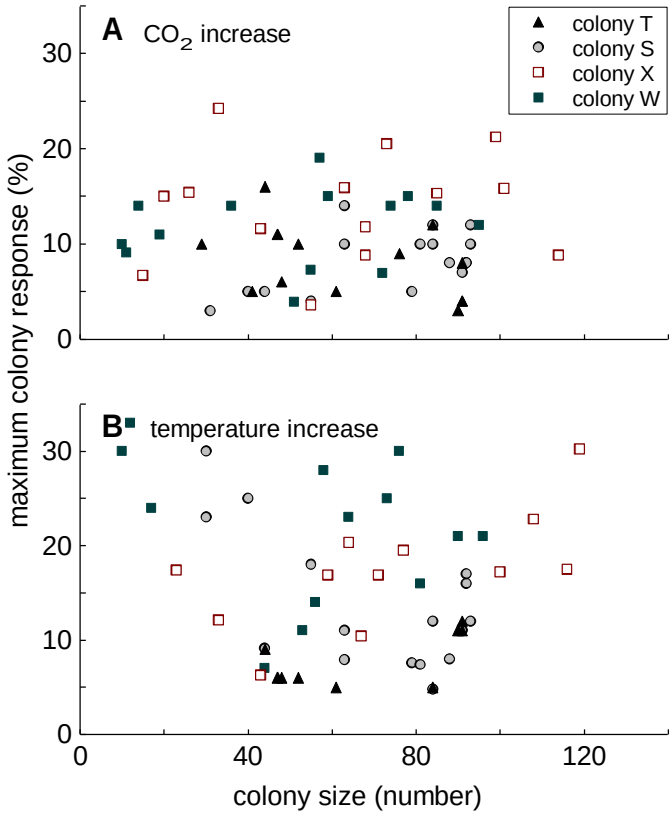

Fig. 2.3 Response of four colonies (in maximum percent of workers observed fanning during one trial) to a) CO_2 and b) temperature stress versus colony size.

The second parameter of colony response analyzed as function of colony size was the delay in the colony's response to an increase in temperature. Only temperature trials were analyzed because the course of the temperature increase followed a strict temperature regime, whereas CO_2 concentrations could sometimes drop during measurements as a result of massive fanning.

Using the 75% criterion (see methods), the turning points in the sigmoidal fit (Fig. 2.4A) of 11 and 12 trials, respectively for three colonies were pooled and used for further analysis (Fig. 2.4B). In one colony (colony T), the 75% criterion was met by only 5 out of

14 trials and the colony was therefore not included into the analysis. Figure 2.4B shows that as colony size increased the response delay (time to maximum increase in number of fanning bees, see methods and Fig. 2.4A) decreased (slope: -0.20, R=0.58, p<0.001). Larger colonies increased their fanning population earlier than smaller colonies. Colonies with less than 30 individuals had a maximum increase in response on average 38 minutes after manipulations started whereas colonies containing more than 90 individuals had a maximum increase already after 22 minutes.

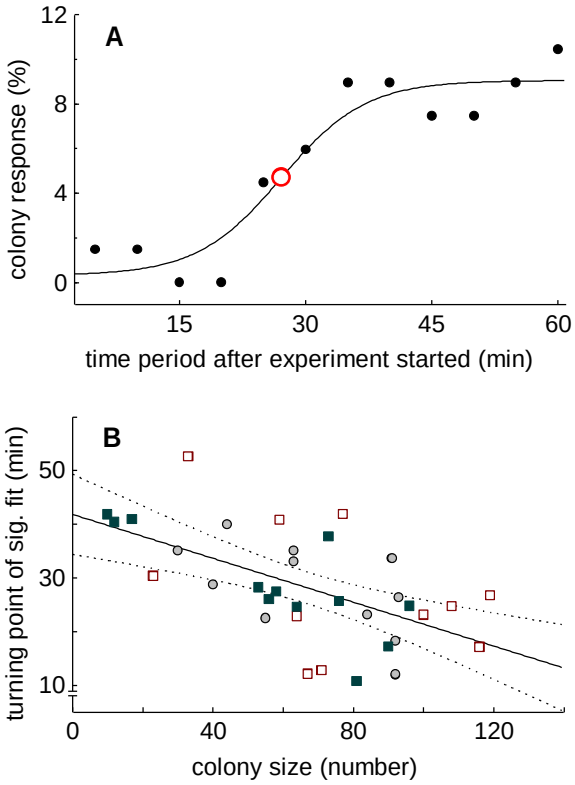

Fig. 2.4 Decrease in response delay with increasing colony size. The turning point of a sigmoidal fit (○ in A) was determined for each temperature trial. Turning points of 34 trials from three colonies were plotted against colony size (B). Turning points are plotted as minutes after start of manipulation. Response delay correlated with colony size. Linear regression with 95% confidence interval (dashed line). Symbols in lower graph denote different colonies (for legend see Fig. 2.3).

Field colony

In order to evaluate whether the range of stimulus intensities used in the laboratory experiments is comparable to the range experienced by a bumble bee colony under natural conditions, and since no data on the concentration of respiratory gases in bumble bee nests are available in the literature, I measured nest climate in a large subterranean field nest of *Bombus terrestris*.

Figure 2.5 shows data recorded during a period of 10 days. The nest had a mean CO_2 concentration of 1.27 ± 0.1% (range 0.91-1.51%). CO_2 concentrations showed slight daily fluctuations with higher concentrations during the night (1.33 ± 0.1%) than during the day (1.19 ± 0.1%) ($p<0.001$; *t*-test). The abandoned nest cavity measured 0.28% CO_2 (data not shown in Fig. 2.5).

Ambient air temperature showed daily fluctuations ranging between 13.2°C and 34.4°C (20.3 ± 4.0°C), whereas nest temperature underwent almost no fluctuations, ranging between 31.3 and 33.4°C (32.3 ± 0.4°C). The soil surrounding the nest was cooler and averaged 20.1 ± 0.8°C, fluctuating between 18.9 and 22.2°C. Figure 2.6 shows that temperature regulation broke down end of July, shortly after the first queens had emerged.

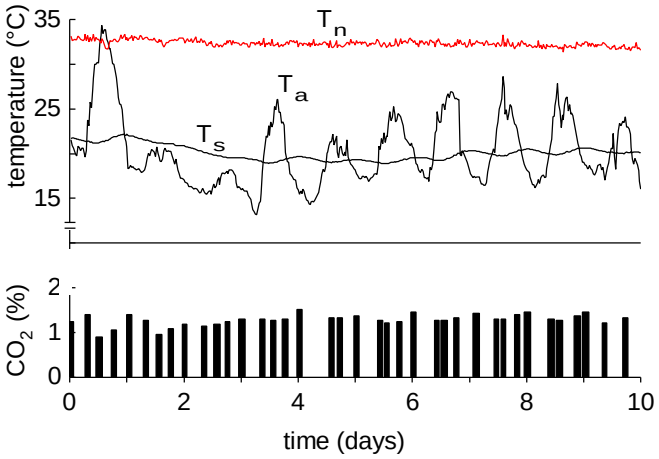

Fig. 2.5 Temperature and CO_2 data from a field nest of *Bombus terrestris* measured during 10 days in early July. CO_2 concentration in the nest, nest temperature (T_n), ambient temperature (T_a) and the temperature of the soil surrounding the nest (T_s) are shown.

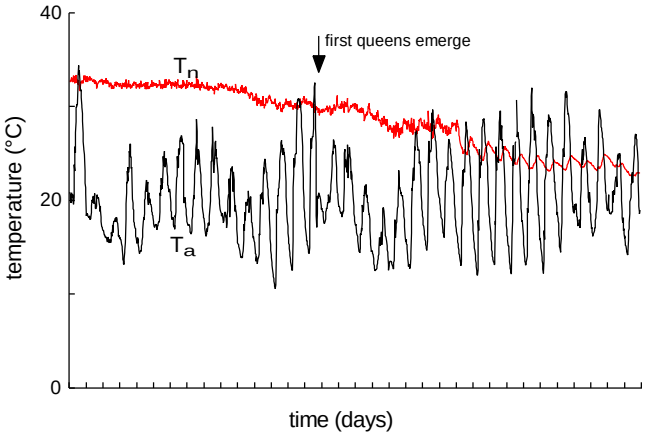

Fig. 2.6 Temperature data from a field nest of *Bombus terrestris* measured during 34 days in July/August. Nest temperature (T_n) and ambient temperature (T_a) are shown.

DISCUSSION

In this study I examined two aspects of the social control of nest climate in bumble bee colonies. First, I addressed the question which parameters of their nest climate bumble bee colonies actively control by fanning. Second, I analyzed the dynamic of the colony response to increasing temperatures in dependence of colony size.

While the thermoregulatory abilities of bumble bee colonies are well documented, it is now clear that colonies control a further climatic parameter: the concentration of carbon dioxide. I have shown that the colony response to increasing CO_2 concentrations is fast, graded and fine tuned. I have further demonstrated differences in colony response between large and small colonies. The number of fanning workers increased as colony size increased, but the proportion of the total work force invested into nest ventilation by the colony did not change. The dynamics of the colony response changed with colony size, larger colonies showed a faster response to perturbations of their colony environment than smaller colonies.

Responses to manipulation of nest climate

Wing fanning is a behavioral mechanism that promotes evaporation and convection. Thus it influences all three parameters of nest climate tested: temperature, CO_2 concentration and relative humidity. However, it is utilized by bumble bees only to lower nest temperature and to meet the colony's needs for gas exchange; high relative humidity levels do not elicit fanning behavior.

Colonies responded to high CO_2 levels and high temperatures in a similar way: as soon as stimulus intensity increased, individual bees started fanning their wings. The number of fanning bees increased with stimulus intensity. This graded response ensures an adequate answer to environmental perturbations. The finding of an active down-regulation of high CO_2 concentrations in bumble bee colonies is in close agreement with findings on honey bees (Seeley 1974).

Why do colonies try to avoid high CO_2 concentrations? Like super-optimal temperatures which e.g. disrupt metamorphosis and lower eclosion rate (Himmer 1927; Heinrich 1979; Vogt 1986b), high levels of CO_2 may be detrimental to the colony. Various physiological effects of high CO_2 levels have been reported, e.g. changes in acidity of the haemolymph and in hormone titers (Röseler & Röseler 1984; Nicolas & Sillans 1989). Furthermore, and maybe more importantly, since CO_2 production and oxygen depletion are directly linked, the CO_2 concentration in subterranean dwellings is a reliable indicator of oxygen availability. Insects are unable to measure oxygen concentrations; in honey bees oxygen depletion alone evokes no fanning response (Seeley 1974). However, as has been shown for ants (Kleineidam & Tautz 1996; Kleineidam et al. 2000), social insects are able to measure absolute CO_2 concentrations with specialized antennal sensilla (Lacher 1964; Dumpert 1972; Ågren & Hallberg 1996). A rapid fanning response to increasing CO_2 concentrations thus ensures sufficient oxygen supplies for the colony. When exposed to

high CO_2 concentrations, bumble bee workers have been reported to eject larvae from their colony. Obviously, when poor gas exchange properties cannot be compensated for by nest ventilation, colonies reduce the metabolic mass in their nest cavity in order to decrease CO_2 levels and increase oxygen availability (Kukuk et al. 1997).

Exactly which CO_2 concentrations or accompanying levels of anoxia are harmful to a bumble bee colony is unknown. In contrast, the influence of super-optimal temperatures has been studied in great detail. A comparison between the colony response to temperature stress and CO_2 stress therefore allows a speculative evaluation of the possible impact of the CO_2 concentrations measured. In my experiments, the intensity of the fanning response to CO_2 concentrations of about 3 % corresponded to the intensity of the colony response to a temperature stress of about 30°C (Fig. 2.2). Bumble bee colonies are known to respond much stronger when temperatures increase further: Vogt reports up to 60 % of a colonies' workers fanning when ambient temperatures rise above 35°C (Vogt 1986a); a temperature condition that is known to have lethal effects on the brood (Himmer 1927). Thus, the colony response to CO_2 concentrations of about 3% suggests that concentrations of this magnitude present a moderate stress level to the colony.

To date, no information on CO_2 concentrations in natural nests of bumble bees exists in the literature. My fist pilot measurement of CO_2 concentrations in a field nest suggests that under natural conditions down-regulation of CO_2 through fanning is quite effective. CO_2 concentrations measured in the subterranean field nest did not exceed 1.5% even though the colony was large and the cavity had a long, narrow entrance tunnel. Presumably, even few fanning individuals can create a massive ventilation effect.

An increase in relative humidity did not elicit a fanning response. Obviously, high humidity levels do not comprise colony development and colonies therefore do not invest valuable work force into down-regulating them. Whether relative humidity is up-regulated by colonies living in an arid habitat in order to avoid desiccation of the brood remains to be examined. Honey bees have been reported to down-regulate high humidity conditions (Winston 1987). In contrast to bumble bees, honey bee colonies are perennial. In order to secure winter survival honey bees need to collect enormous quantities of nectar and transfer it into storable honey by evaporating water. This concentration process is promoted by low relative humidity in the nest and strong nest ventilation by fanning (Reinhardt 1939). Fanning as a measure to concentrate nectar also keeps CO_2 concentrations below 1% during summer (Simpson 1961; Seeley 1974). During winter, in swarms, and in small colonies CO_2 concentrations of 2-6% have been measured (Simpson 1961; Nagy & Stallone 1976) and fanning in winter seems to occur in order to control respiratory gases (Simpson 1961).

Social insects that lack wings and are therefore unable to actively ventilate their nests, e.g. ants and termites, have developed elaborate nest designs which facilitate air circulation (Lüscher 1961). CO_2 concentrations found in such nests are usually below 3% (Lüscher 1961; Kleineidam & Roces 2000; Korb & Linsenmaier 2000). This again supports the idea that CO_2 concentrations of more than 3% or the oxygen conditions accompanying such concentrations are harmful and successfully avoided across species by means of active and / or passive ventilation mechanisms.

Influence of colony size

Under natural conditions the requirements of a bumble bee colony in controlling the climatic conditions inside the nest will presumably change with colony size. While small colonies face the main challenge of heating their brood nest, large colonies are more likely to experience both overheating and insufficient nest ventilation caused by crowding. However, depending on the location of their nests, super-optimal temperature conditions caused by external heat may be experienced both by small and large colonies. Thus, the condition of a temperature increase up to 30°C experienced by the lab colonies may well represent a natural stress situation.

Social homeostasis, the control of the physical environment of a colony through co-operative activities, is a phenomenon found in most species of social insects. In many cases, the degree of stability achieved increases with colony size. Honey bee colonies containing 35000 bees undergo smaller CO_2 fluctuations than colonies containing 10000 bees (Seeley 1974). In species with annual colonies highest precision in regulating brood temperature is usually reached in the middle of the colony cycle when colonies have large contingents of workers (Seeley & Heinrich 1981). The mechanisms underlying this increase in stability are not well understood.

I looked at the question why small colonies undergo stronger fluctuations, focusing on the underlying regulatory process, that is the number of bumble bees showing fanning behavior as response to changes in stimulus intensity and on the dynamics of this response over a wide range of colony sizes.

In theory, colonies can increase the stability of a parameter regulated by adjusting the intensity of the response and / or decreasing the delay in their response. I found a change in both parameters as colony size increased. Larger colonies responded to one stimulus intensity with more fanners than smaller colonies. Additionally, colony size influenced the delay of the response, larger colonies responded to increasing temperatures faster than small colonies.

Previous studies have explained strong fluctuations of brood temperature in small colonies by the fact that small colonies have less provisions and thus ‘run low on fuel’ more often than large colonies, or that small colonies need to invest more heavily into foraging or brood care than large colonies and can thus simply spare fewer individuals (Seeley & Heinrich 1981). These parameters may often influence the control of nest climate under natural conditions. In my experiments, however, colonies had sufficient nectar supplies and all workers were confined to the nest. Thus, the small colonies could have easily allocated more than 30% of their work force to fanning. Apparently only a certain percentage of the colony work force is susceptible to the task-related stimulus at one time.

Conclusions

I have shown that bumble bee colonies are well adapted to fluctuating environmental conditions: by increasing nest ventilation through wing fanning they are able to respond quickly to increases in temperature and carbon dioxide levels and thereby avoid unfavorable climatic conditions, thus promoting growth and survival of the colony. The colony response to perturbations is faster in larger colonies, leading to an increase in degree of homeostasis as colonies grow.

The results of this study raise questions about the rules governing the behavior of the individual and the degree of flexibility in individual behavior. Although nest environment is controlled by a colony-level response, it is the sum of individual worker behavior which actually regulates it. Understanding the functioning of the whole requires an understanding of how, why, when, and to what the individuals respond. In this context, questions concerning individual response thresholds (Detrain et al. 1999), feedback loops and amplifying phenomena (Turner 2000), and the role of learning in task efficiency remain to be investigated. In my experiments, larger colonies were also more experienced colonies. Certain fanning positions in the nest may be more effective than others (Southwick & Moritz 1987) and learning of effective positions by single individuals may massively influence the overall efficiency of the colony response. Thus, the next step in understanding the collective response to nest climate perturbations will involve an investigation of the rules governing individual behavior and the mechanisms that integrate individual behaviors into a collective response. These questions will be addressed in chapter three.

CHAPTER III

Bumble bee worker fanning at the nest entrance

INTERINDIVIDUAL VARIABILITY IN FANNING RESPONSE AND THE CONTROL OF NEST CLIMATE IN BUMBLE BEES

ABSTRACT

Interindividual variability is believed to have a major impact on collective behaviors in social insects. Here I present a detailed investigation of interindividual differences in fanning behavior underlying the collective control of nest climate in bumble bee colonies. Four colonies were repeatedly exposed to increasing temperature and CO_2 levels. The response threshold of each worker involved in the collective fanning response was determined. Temperature response thresholds of 118 workers and CO_2 response thresholds of 88 workers were analyzed. I show that workers differed in their response thresholds. Some consistently responded to low stimulus intensities, others consistently responded to high stimulus intensities. Further, I demonstrate that workers of a colony differed in two other parameters of responsiveness: response probability and fanning activity. My data suggest that response threshold, response probability and fanning activity are independent parameters of individual behavior. Workers were stimulus specialists rather than task specialists; no correlation between temperature and CO_2 thresholds was found within individuals. Additionally, my data evidence specialization through reinforcement. Response thresholds of fanning bees decreased over successive trials. I discuss the importance of interindividual variability for specialization and the collective control of nest climate and present a general discussion of self-organization and selection in social insects.

INTRODUCTION

An insect colony faces the same challenges to survival that confront a single organism – foraging, defense and protection against environmental extremes. The colony's collective solutions to these challenges have prompted a view of the society as a functional unit, capable of adaptive decision making and coordinated behavior. In contrast to a multicellular organism, the colony lacks mechanisms such as a nervous system that physically integrate its subunits. Thus, the question arises which analogous mechanisms coordinate the activities of the colony's members. In order to understand the complex collective behaviors of insect colonies we need to understand how they are generated by the actions and interactions of the individual colony members. As Wilson declared, 'the reconstruction of mass behavior from a knowledge of the behavior of single colony members is the central problem of insect sociobiology'(Wilson 1971, p.227).

Division of labor and self-organization

Division of labor, one of the most prominent organizational principles of insects colonies, has been studied in great detail for nearly a century, the main focus being on its evolutionary or functional significance (Oster & Wilson 1978; Seeley 1982). Within the last two decades the focus has shifted from the ultimate causes to the proximate mechanisms that underlie colony organization and generate division of labor (Robinson 1992). What are the mechanisms that control the adaptive allocation of individuals to the various tasks required for successful group functioning? The challenge here is to

understand the rules that govern individual behavior and the mechanisms that integrate the behavior of individuals into a functioning whole.

In search of the proximate mechanisms underlying colony organization, a number of models have emerged. They have been used to formulate behavioral rules that can account for specialization and flexibility in division of labor in the attempt to link patterns of task performance at the individual and the colony level. A common approach has been to view social insect societies as self-organized, decentralized systems in which behavior emerges from independent actions and decisions of workers (Bonabeau et al. 1997). The theory of self-organization, originally developed in the context of physics and chemistry, is now widely applied in studies of proximate mechanisms of social life in animals and social insects in particular (Bonabeau et al. 1997). The basic idea is that structures (e.g. division of labor) appear at the global level of a system (the colony) from actions and interactions of its lower level components (the workers of a colony). This is in contrast to concepts proposing the guidance of well-informed leaders or sets of predetermined templates that specify the structure. Instead, structure is an emergent property of the dynamic interactions in the system and the 'collective intelligence' of a colony requires only limited and local knowledge by its members.

Response threshold models

Which are the rules governing individual behavior? One simple rule could be: 'perform a task when the task related stimulus exceeds your internal threshold for that stimulus'. The idea that simple stimulus-response relationships underlie animal behavior is an old concept in ethology. For example, it played an important role in early research on instinctive behavior (Tinbergen 1952; Beshers et al. 1999). However, only recently have sociobiologists begun to view features of insect colonies such as flexible division of labor as emergent properties resulting from interindividual variation (e.g. in response thresholds) in the members of a colony.

Response threshold models make two basic assumptions: First, each task that needs to be performed is associated with a stimulus or set of stimuli (signals and cues correlated with specific labor requirements). Second, workers perform tasks in response to specific task-related stimuli. Each worker of a colony is characterized by a set of response thresholds to various stimuli and performs a task when the corresponding stimulus exceeds her internal threshold. The 'default state' of a worker is to not perform the task.

Thus, behavior expressed by an individual is a function of (at least) two variables: the exogenous labor needs of the society, coded in stimulus intensities, and the endogenous individual response thresholds, influenced by e.g. genetic, physiological, and learned components. Additionally, all threshold models incorporate a negative feedback loop: performing a task decreases stimulus intensity for that task. Workers with low response thresholds not only respond to lower stimulus intensities but by decreasing stimulus intensity through their response further remove those workers with higher response thresholds from the task. Hence, relatively small inter-individual variance in response

thresholds may cause large inter-individual differences in task performance and division of labor results as emergent property of the system.

The idea that division of labor is based on 'caste specific differences in sensitivity to task-associated stimuli' first appeared in the 1970's in Wilson's work on ants (Wilson 1976), see also (Wilson 1984) and (Wilson 1985). Soon, others followed and proposed to consider division of labor from the viewpoint of hormonally (Robinson 1987c; Robinson 1987b; Robinson et al. 1989) or genetically influenced response thresholds in honey bees (Calderone & Page 1988; Frumhoff & Baker 1988; Robinson & Page 1988; Calderone & Page 1991), wasps (Jeanne et al. 1988) and ants (Calabi & Rodengaus 1988; Detrain & Pasteels 1991; Detrain & Pasteels 1992). The concept of variation in thresholds as proximate mechanism and primary driving force in colony organization has since then received strong interest, shown by the numerous review papers on this subject published within the past ten years (Page & Mitchell 1991; Robinson 1992; Tofts & Franks 1992; Bonabeau et al. 1997; Page 1997; Calderone 1998; Page & Mitchell 1998; Detrain et al. 1999; Sendova-Franks & Franks 1999; Bonabeau et al. 2000; Beshers & Fewell 2001).

The published threshold models differ mainly in their assumptions concerning the nature of the thresholds. The first threshold models assumed response thresholds to be fixed within an individual (Calabi & Rodengaus 1988; Page & Mitchell 1991; Bonabeau et al. 1996; Page & Mitchell 1998). A more recent model suggests that response thresholds are dynamic rather than static: performing a task induces a decrease in the corresponding response threshold, while not performing a task induces 'forgetting' and an increase in response threshold – this combined reinforcement increases the variance in thresholds and functionally walls off those individuals with low thresholds from the other members of the colonies (Theraulaz et al. 1998).

Even though the theoretical framework concerned with the possible role of variability in response thresholds has received a lot of interest, few studies have aimed at showing the existence and distribution of response thresholds in social insects. Some authors have reported differences in the responsiveness of groups of workers from different patrilines or worker castes (Wilson 1984; Wilson 1985; Detrain & Pasteels 1991; Detrain & Pasteels 1992) or of several selected individuals (Seeley 1994). However, studies that quantify stimulus intensities and the corresponding individual responses of all colony members involved in a collective response are lacking. Such experiments require controlling or at least being able to measure the intensity of the stimulus workers are responsive to. This has turned out to be extremely difficult for most tasks. In order to test the models and assess the importance of interindividual variance for colony level behaviors, we need to know how workers vary in their responsiveness, how variation in responsiveness contributes to colony efficiency, and the mechanisms by which colonies can regulate their behavior by regulating worker response thresholds.

Control of nest climate in bumble bee colonies as model system

In this chapter I fill this gap by analyzing the response of individual bumble bee workers in the context of nest climate control. This study system allowed me to (1) control and measure the intensity of a task-associated stimulus and to (2) simultaneously measure the individual task-related responses of all colony members involved.

Bumble bee workers are able to manipulate nest climate by actively increasing air circulation through the nest and in this way control temperature and CO_2 levels in their colony (see chapter two). As discussed earlier, control of nest climate is an example of a colony level response that is fast, flexible and highly adaptive. Figure 3.1A shows the colony fanning response to an overheating of the nest. Figure 3.1B shows the same response but focuses on the individual fanning responses that give rise to the collective pattern.

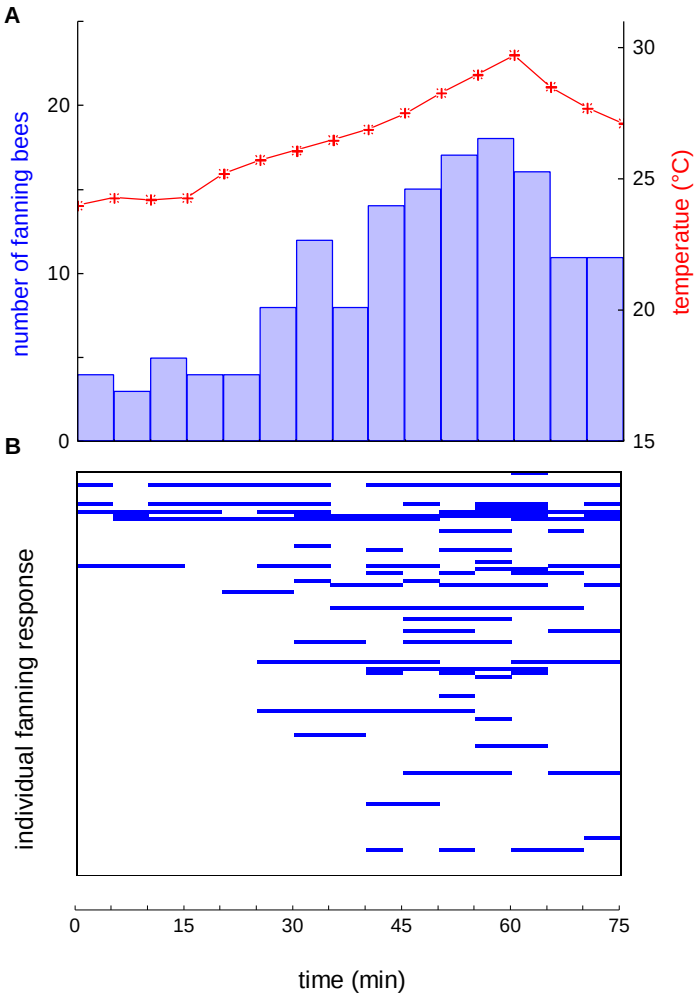

Fig. 3.1 Fanning response to an increase in temperature. A) Colony level response: number of fanning workers per five minute observation period. B) Individual fanning responses: blue lines denote fanning activity of individual workers.

By studying the rules governing the fanning behavior of individual workers important questions concerning individual response thresholds and interindividual variance can now be addressed:

- Do workers differ in their response – do individual response threshold exists - or do workers show a stochastic response distribution?
- How are response thresholds distributed among workers of one colony?
- Are response thresholds fixed or reinforced?
- How are response thresholds to different stimuli arranged within an individual? Are they somehow linked or clustered, or is each threshold independent of the others?
- Are there other factors of interindividual variability, besides response thresholds, that are important in constituting the colony response?

METHODS

The response of bumble bees colonies to an increase in temperature and CO_2 levels was studied. In contrast to chapter two, the focus in this chapter is not on the colony response but on the individual behaviors constituting the colony response. To this aim, individual fanning behavior of all workers of four colonies that were repeatedly exposed to super-optimal temperature and CO_2 levels was analyzed.

Data collection

Bumble bee colonies (*Bombus terrestris*) were housed and nest climate was manipulated as described in chapter two. All workers were age marked with number tags (Opalithplättchen) so that they could be recognized and analyzed individually. During an experiment (for details of manipulation procedure see methods chapter two) the colony was closely scanned. Data were recorded in five minute observation periods as described in chapter two. Whenever a worker started fanning, defined as steady fanning with spread wings while standing still for at least 10 s, the momentary stimulus intensity (°C or % CO_2 at onset of fanning) and the position of the fanning worker in the nest were recorded. Fanning positions were categorized as (i) on nest (ii) in nest entrance and (iii) in entrance tunnel outside nest (see Fig 3.7). For every observation period I recorded whether a worker was still fanning (and if so where), had stopped, or had re-started fanning (and if so where). Stimulus intensity was recorded only at first onset of fanning for every individual.

Experiments were performed repeatedly and over several weeks in four colonies as described in chapter two. After experiments ended all colonies were killed by deep-freezing and worker size was measured. Size measurements were carried out using a Wild^{TM} M3Z (Heerbrugg, Switzerland) stereomicroscope at 50x magnification. Bees were mounted on a

table that could be moved with a micrometer screw for measurement. Maximal head width and length of the left wing were determined to the nearest micrometer.

Data analysis

Fanning parameters

Only workers that experienced at least five trials per manipulation (temperature or CO_2 increase) were used for analysis. I analyzed the following parameters of individual fanning behavior:

- response threshold: Based on all trials a worker fanned in, her response threshold was calculated as the mean stimulus intensity at onset of fanning. Only workers that responded in at least three trials were assigned response thresholds.
- activity: Based on all trials a worker fanned in, her fanning activity was calculated in two ways: First, for every worker the mean number of observation periods (5 minute blocks, see above) she fanned in per trial was calculated. Second, activity data were normalized for remaining time (time after a worker had started fanning till stimulus intensity decreased again): the percentage of remaining time fanned in was calculated (normalized value). Only workers that responded in at least three trials were assigned activity values.
- response probability: Based on all trials a worker experienced, her response probability was calculated as the proportion of trials she fanned in.

Workers that showed fanning behavior before stimulus intensity increased (during the first 15 minutes, see methods chapter two) were not included into the analysis.

Reinforcement

In order to test whether individual response thresholds were fixed or reinforced, I analyzed the change in response threshold over time. Only thresholds of workers that responded in at least six trials were analyzed. For every trial I calculated the deviation as difference between stimulus intensity responded at and mean individual response threshold.

Thus a positive value was obtained when a worker responded at a stimulus intensity above her mean response threshold and a negative value when she responded below her mean response threshold. The individual response values were ordered successively (skipping non-responded trials), data of workers from a colony were pooled and the mean deviation in first, second etc. responded trial was calculated.

Individual activity was analyzed in the same way, again calculating for every worker the deviation from mean activity for every responded trial. This was done for activity data and for normalized activity data.

Only temperature trials were analyzed because the course of the temperature increase followed a strict temperature regime, whereas CO_2 concentrations could sometimes drop during measurements as a result of massive fanning (see chapter two). Also, sample sizes from CO_2 trials were small since only few workers fanned six or eight times (see Table 3.1). Data from colonies X and W were analyzed, since individual responses were documented without interruption only in these two colonies.

Fanning positions

For every trial I analyzed the number of fanning events (fanning position during one five minute observation period) occurring at the different locations. I compared fanning positions in CO_2 trials with fanning positions in temperature trials.

Size

In order to test whether size influenced any of the analyzed parameters, each measure of individual fanning performance (response threshold, response probability and response activity) was plotted against size.

Statistical analysis

Differences in response thresholds between workers of a colony were tested with a one-way Anova for a subset of workers (for details see Appendix). If the Anova revealed significant differences between workers these were analyzed by multiple comparison using the Fishers-LSD test (least significant difference test). Differences in activity (normalized data) between workers of a colony were tested with a nonparametric analysis of variance. The Kruskal-Wallis test was used. If the Kruskal-Wallis test revealed significant differences between workers, these were analyzed by nonparametric multiple comparison using the Dunn test.

Differences between colonies were tested with a one-way Anova where data were shown not to differ from normality (Shapiro-Wilks W test), otherwise the Kruskal-Wallis test was used.

Correlations between two parameters were tested using a nonparametric test for association (Spearmann's rank correlation coefficient). Correlations were performed separately for each colony. The chosen level of significance was 0.05.

RESULTS

Differences in fanning behavior among the workers of a colony

A total of 303 workers from four colonies experienced an increase in temperature at least five times, 326 workers experienced an increase in CO_2 at least five times. Only these workers were considered for further analysis.

Workers differed in their responsiveness. Around 40% of the tested workers never showed fanning behavior. About 45% fanned at least three times when temperature or CO_2 was increased and could thus be assigned a mean threshold value for at least one parameter. Roughly 20% repeatedly responded to both temperature and CO_2 manipulations and could therefore be assigned mean threshold values for both parameters (classified as general fanners in Table 3.1). 12% of the workers responded regularly to temperature stress but only once or twice to CO_2 stress (temperature fanners). Another 6% of all workers showed fanning behavior exclusively under temperature stress (exclusive temperature fanners). 6% of all workers were CO_2 fanners, 2% were exclusive CO_2 fanners (Table 3.1).

Response thresholds

Temperature response thresholds of 118 workers and CO_2 response thresholds of 88 workers were analyzed. Figure 3.2 shows the response thresholds to temperature and CO_2 of workers from the four tested colonies. Workers differed in their response thresholds. Some workers started fanning when stimulus intensity was still comparatively low, others consistently started fanning under high stimulus intensities (for statistics of differences see Appendix and Table 3.2). Interindividual differences were apparent for both temperature and CO_2 thresholds.

	Colony S		Colony T		colony X		colony W	
	Temp	CO_2	temp	CO_2	temp	CO_2	temp	CO_2
No. workers exp. ≥5 trials	84	84	62	85	85	85	72	72
Max no. trials exp.	16	11	9	11	12	13	13	14
No. of trials fanned in (median)	6.0	5.0	4.0	4.0	5.0	4.0	4.5	4.0
Workers fanning in ≥3 trials (%)	39.3	20.2	17.7	18.8	41.2	32.9	54.2	36.1
General fanners (%)	14.5	14.3	9.7	7.1	20.5	23.5	33.3	33.3
Temp. fanners (%)	15.7		6.4		10.5		13.9	
Exclusive fanners (%)	9.6		1.6		7.1		6.9	
CO_2 fanners (%)		4.7		8.2		8.2		1.4
Exclusive CO_2 fanners		1.2		3.5		1.2		1.4

Table 3.1 Sample sizes, experienced and responded trials and categorized fanners in the four tested colonies. General fanners: workers that responded at least three times in both temperature and CO_2 trials. Temp. / CO_2 fanners: workers that responded in at least three trials of one parameter and in less than three trials of the other. Exclusive fanners: workers that responded in at least three trials of one parameter and in no trial of the other parameter.

	S	T	X	W
CO_2	n.s.	-	9 of 9	9 of 9
Temperature	5 of 9	-	5 of 9	9 of 9

Table 3.2 Results of the comparison of thresholds of three low threshold bees against three high threshold bees (LSD test, for details see Appendix). Numbers denote pairs that differed significantly.

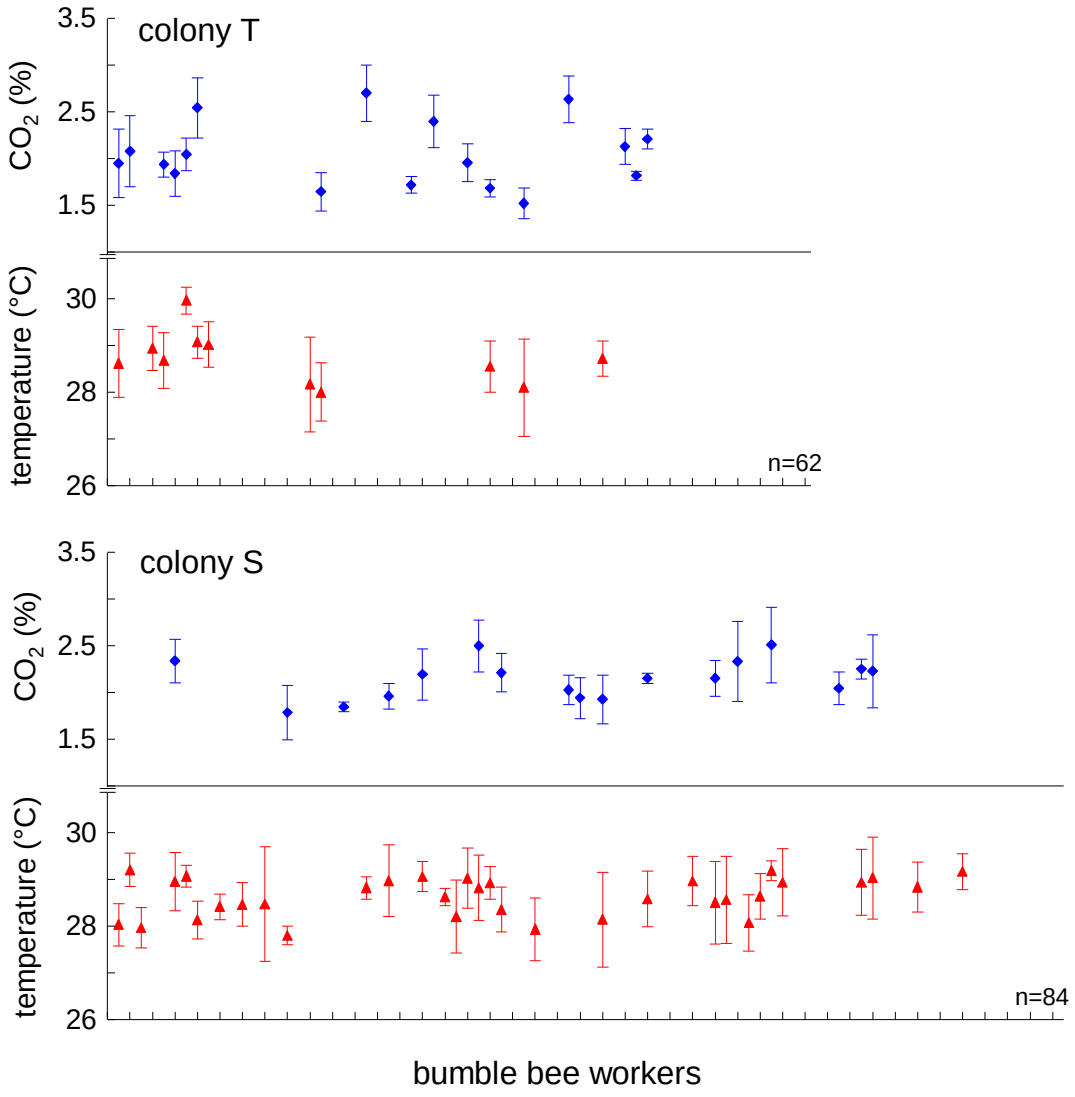

Fig. 3.2A Individual response thresholds (mean ± SE) for CO_2 (blue) and temperature (red) of workers form colony T and colony TS. On the x-axis, workers are plotted in order of their hatching; n denotes number of workers that experienced at least five trials.

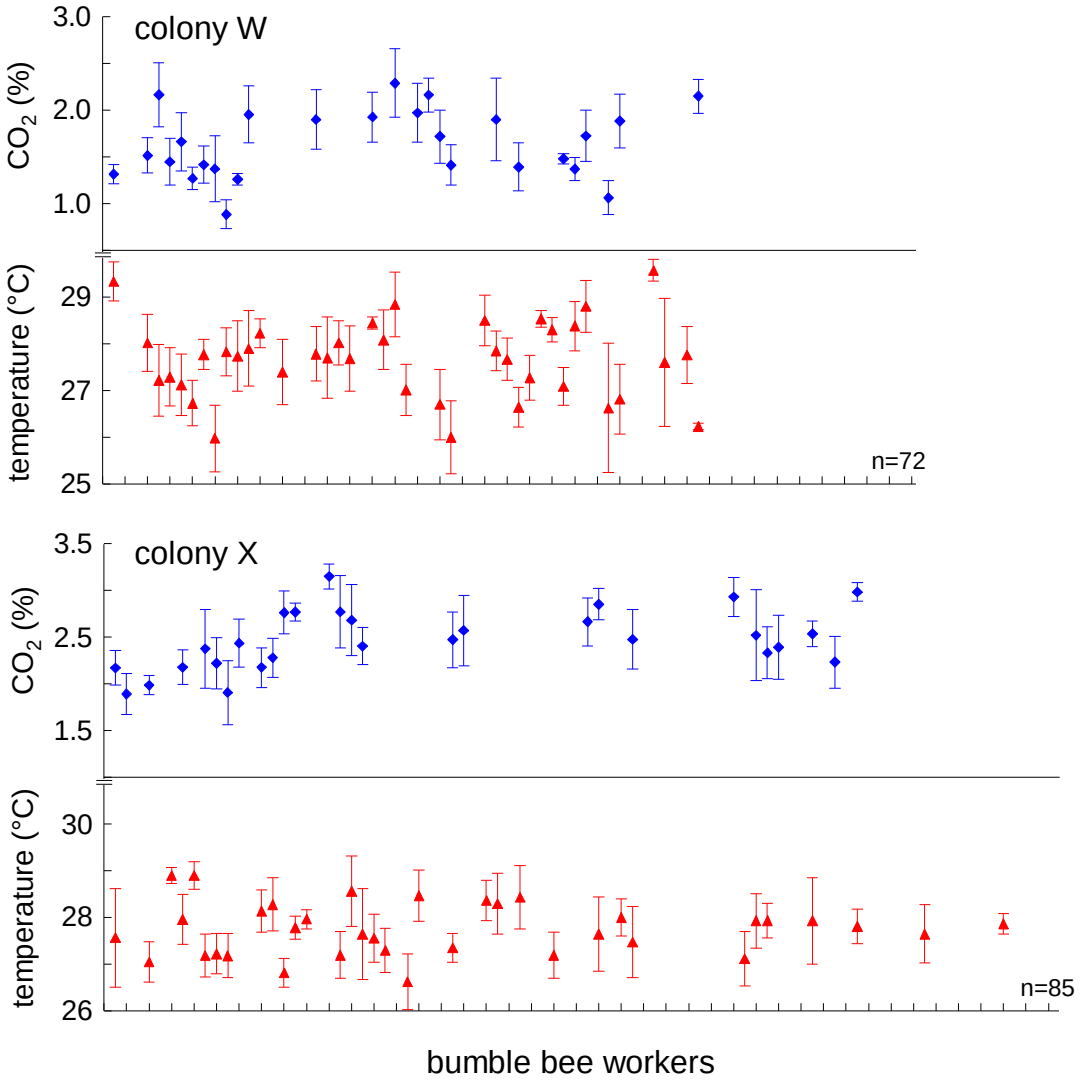

Fig. 3.2B Individual response thresholds (mean ± SE) for CO_2 (blue) and temperature (red) of workers from colony W and colony X. On the x-axis, workers are plotted in order of their hatching; n denotes number of workers that experienced at least five trials.

Within the general fanners, that is those workers that repeatedly responded to both parameters, no correlation between temperature threshold and CO_2 threshold was found in two out of the four colonies (Fig. 3.3 and Table 3.3). These were the colonies with the largest sample sizes (colony X and W). A low temperature threshold did not necessarily imply a low CO_2 threshold or vice versa.

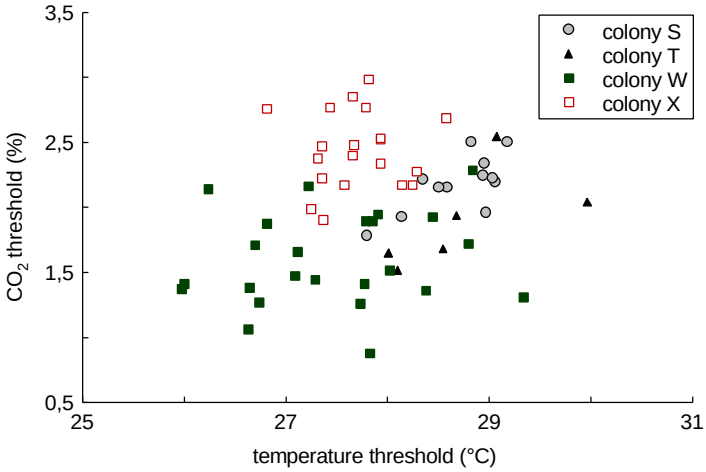

Fig. 3.3 Temperature and CO_2 thresholds of workers that showed fanning as response to both parameters. A correlation between the two thresholds was found in colonies S and T (see Table 3.3).

Colony	n	r_s	p
S	12	0.59	**0.04**
T	6	0.88	**0.02**
W	24	0.22	0.30
X	20	0.09	0.69

Table 3.3 Spearmann's rank correlation coefficient r_s of individual temperature thresholds against individual CO_2 thresholds. Note the larger sample sizes in colonies W and X.

The distribution of response thresholds within colonies did not differ from normality ($p>0.2$, Shapiro-Wilks'W test). The mean response threshold for temperature within colonies ranged between 27.7 and 28.7°C. The mean response threshold for CO_2 within colonies ranged between 1.6 and 2.5% (Fig. 3.4).

Colonies differed in their response thresholds (Anova: $F_{temp}=20.3$, df=3, $p<0.001$; $F_{CO2}=29.4$, df=3, $p<0.001$). The mean response threshold for temperature was significantly higher in colony S and T than in colony X and W respectively ($p<0.001$ for all pairs, LSD test). There was no difference between colonies S and T and between colonies X and W ($p>0.3$ for both pairs, LSD test). The mean response threshold for CO_2 differed significantly between all colonies ($p<0.01$ for all pairs, LSD test) except between colony S and T ($p=0.33$, LSD test). Thus, besides intracolonial variance in response thresholds my data evidence intercolonial variance. Because of the differences in thresholds, data were not pooled and correlations (see below) were performed separately for each colony.

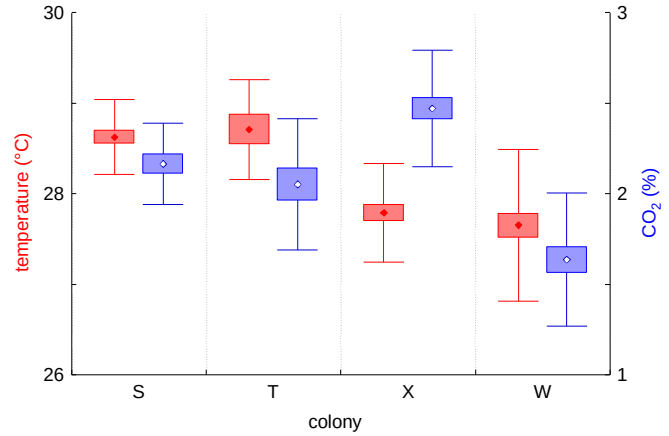

Fig. 3.4 Response thresholds for temperature (red, filled diamonds) and CO_2 (blue, open diamonds) in four colonies. Diamonds denote means, boxes denote standard error, whiskers denote standard deviation. For sample sizes see Table 3.1.

Response probability

Differences in individual responsiveness were not sufficiently described by response thresholds. I therefore analyzed a second parameter describing individual responsiveness: response probability. Response probability of all workers varied between 0% and 100%.

Within colonies, the distribution of response probabilities was skewed towards lower probabilities in five out of eight cases (Shapiro-Wilks'W test). The median ranged between 0 and 33% for temperature and between 0 and 19% for CO_2. Response probability of fanners, that is those workers that fanned in at least three trials and thus could be assigned response thresholds, varied between 19% and 100%. The median response probability of these fanners ranged between 45% and 50% for temperature and was usually lower for CO_2, ranging between 34% and 50% (Table 3.4).

Colonies did not differ significantly in response probability of their fanners during temperature trials (H_{temp}1.16, df=3, p=0.76; H_{CO2}=6.51, df=3; p=0.09; Kruskal-Wallis test).

Response activity

Workers of a colony differed not only in *when* they responded to an increase in temperature or CO_2, they also differed in *how* they responded. Since observations were plotted on a five minute grid (see methods) my data give only a coarse representation of individual fanning activity. However, inter-individual differences are evident. Some workers fanned on average in 5 observation blocks (equaling 25 minutes) while others fanned in only one or two observation blocks (5-10 minutes).

Workers with low thresholds started fanning earlier and thus had more time left till stimulus intensity decreased again compared to workers with high thresholds. In order to exclude the influence of variable response thresholds on activity data, normalized activity data were analyzed. Normalized activity data, that is the proportion of remaining manipulation time a worker fanned once she had started, revealed interindividual differences (for statistics of differences see Appendix). Some workers stopped soon after they had started fanning or fanned intermittently while others continuously fanned until stimulus intensity decreased. The median normalized activity of fanners ranged between 50 and 73 % (Table 3.4).

The distribution of activity within colonies was not significantly different from normality (Shapiro-Wilks' W test) except in colony T for CO_2. Colonies did not differ in the fanning activity (normalized) of their workers under temperature stress (H_{temp}=6.20, df=3, p=0.10; Kruskal-Wallis test). Colonies differed in fanning activity of their workers under CO_2 stress (H_{CO2}=18.42, df=3, p<0.001; Kruskal-Walis test). In general, individual fanning activity during CO_2 trials tended to be higher than during temperature trials (Table 3.4).

Colony		S	T	X	W
Response probability					
Median (%)	temp	45.5	44.4	50.0	50.0
25-75% quartile		33.3 - 62.5	33.3 - 55.5	36.4 - 62.5	36.4 - 62.5
	CO_2	50	36.4	41.0	34.5
		36.4 - 63.6	27.3 - 50.0	30.8 - 57.8	30.0 - 42.9
Fanning activity					
Mean ± SD (no. of 5 min blocks fanned in per trial)	temp	2.0 ± 0.5	2.0 ± 0.2	2.6 ± 0.2	2.3 ± 0.1
	CO_2	3.5 ± 0.2	2.2 ± 0.2	3.2 ± 0.2	2.6 ± 0.1
Normal. fanning activity					
Median	temp	61.7	73.2	67.0	61.5
(% remaining time fanned in)		52.1 – 67.5	64.0 – 80.1	56.8 – 71.6	54.5 – 74.7
25-75% quartile	CO_2	70.1	50	60.3	51.9
		66.1 – 77.2	43.8 – 67.8	51.4-70.8	44.8 – 59.7

Table 3.4 Response probability and fanning activity of workers in the four tested colonies.

Independent parameters?

Clearly, the pattern shown in Figure 3.1B is not merely the result of a stochastic variation in response but is based on interindividual differences in at least three parameters of fanning behavior: response threshold, response probability and fanning activity. I further analyzed these parameters in order to find out whether they are independent or linked.

Threshold - Probability

Figure 3.5 shows that no correlation between individual response threshold and response probability was found. This was true for temperature and for CO_2. Only in colony X did workers with low temperature thresholds have a higher probability of responding than workers with high thresholds (r_s=-0.43, $p<0.01$; Spearmann's rank correlation).

Threshold - Activity

There was a positive correlation between the response threshold of a worker and the duration of her fanning activity when exposed to an increase in temperature, with the exception of colony S (col. T: r_s=-0.82; col. X: r_s=-0.77; col. W: r_s=-0.56, <0.001 for all colonies). For CO_2 stress a correlation between response threshold and individual activity was found in colonies S and X (col. S: r_s=-0.40; col. X: r_s=-0.39; $p<0.05$ in both colonies). Workers with low thresholds tended to spend more time fanning than workers with high thresholds. As mentioned above, this is not surprising since those workers that had lower thresholds started fanning earlier and had more time left till stimulus intensity decreased again. When thresholds were plotted against normalized activity a correlation was found only in colony W (temp: r_s=0.45; CO_2: r_s=0.45; $p<0.05$), in all other cases no correlation was found.

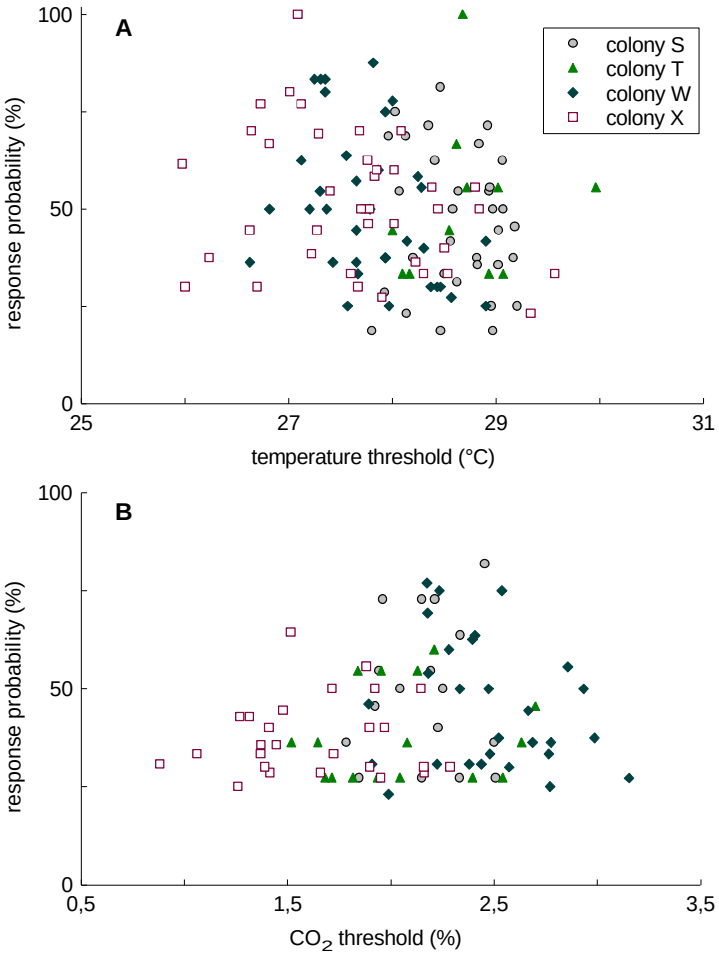

Fig. 3.5 Individual response thresholds versus response probabilities for a) temperature and b) CO_2.

Probability - Activity

A within-colony correlation between response probability and individual fanning activity when temperature increased was found in two of four colonies (col. X: r_s=0.33, $p<0.05$; col. W: r_s=0.44, $p<0.01$). Workers with higher response probability tended to spend more time fanning under temperature stress than workers with low probability of response. For CO_2 a correlation between response probability and fanning activity was found in two of four colonies (col. S: r_s=0.52, $p<0.05$; col. X: r_s=0.46, $p<0.01$).

When probability was plotted against normalized activity data, no correlation between the two parameters was found.

Reinforcement?

An important question concerning response thresholds is whether they are fixed or change with experience. Figure 3.6 shows the deviation from mean temperature response threshold over the first six to eight trials each worker fanned in. Temperature thresholds decreased from trial to trial in both colonies analyzed ($p<0.001$; colony W: r_s=-0.58; colony X: r_s=-0.44).

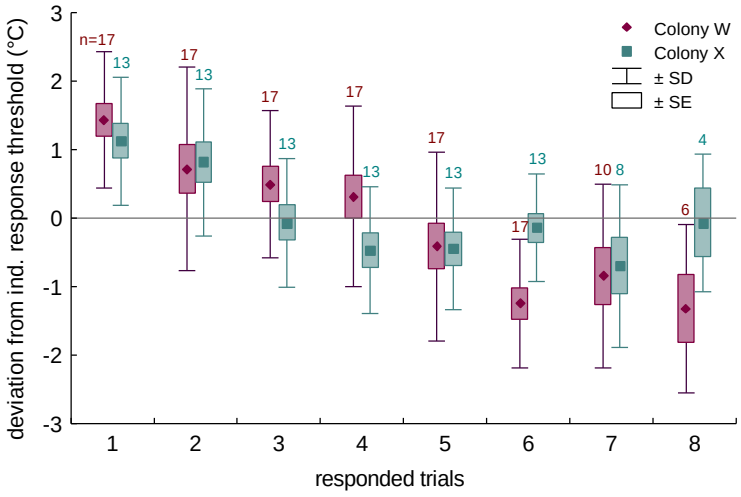

Fig. 3.6 Reinforcement of individual response thresholds during the first 6-8 eight temperature trials a worker fanned in (colony W: r_s=-0.58, colony X: r_s=-0.44; p<0.001). Diamonds/squares denote means, boxes denote standard error, whiskers denote standard deviation.

The duration of fanning increased from trial to trial (p<0.001, colony W: r_s=0.46; colony X: r_s=0.30). This increase in fanning duration seems to be caused by the decrease in temperature thresholds. Normalized fanning activity in temperature trials did not change; workers spent a constant proportion of their time fanning.

Fanning positions

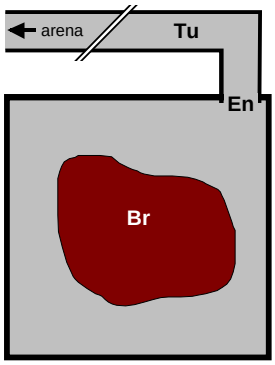

Fig. 3.7 Fanning positions: on the brood (Br), in the nest entrance (En) and in the entrance tunnel (Tu).

Fanning can be performed at various places, in the nest and in front of the nest (Fig. 3.7). Fanning positions differed between temperature and CO_2 manipulations (Fig. 3.8). When colonies experienced an increase in temperature the majority of fanning was performed in the nest on the brood (median of 75% of fanning events per trial in colony X, 55% in colony W) and less than 10% took place in the entrance tunnel outside the nest. In contrast, during CO_2 manipulations a significantly larger percentage of fanning was performed in the entrance tunnel outside the nest (col. X: 19%, col. W: 31%; p<0.05) and significantly less fanning occurred on the brood (col. X: 52%, col. W: 25%; p<0.01, Mann-Whitney U test). Around 30% of fanning per trial was performed in the nest entrance under both temperature and CO_2 manipulations.

The probability of an individual worker to fan at the 'correct' position seemed to increase with experience (Fig 3.9).

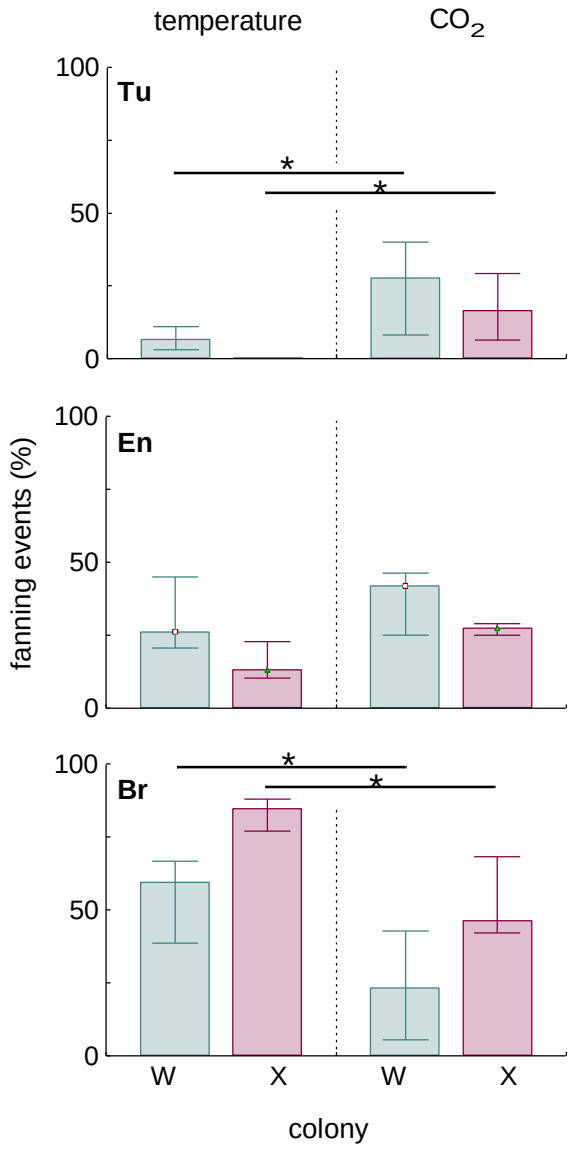

Fig. 3.8 Fanning positions during temperature and CO_2 increase. Fanning occurred in the entrance tunnel (Tu), in the nest entrance (En), and on the brood (Br). Bars denote median, whiskers denote quartile range. Stars denote significant differences in fanning positions between the two treatments ($p<0.05$, n=12-14 trials, Mann-Whitney U test).

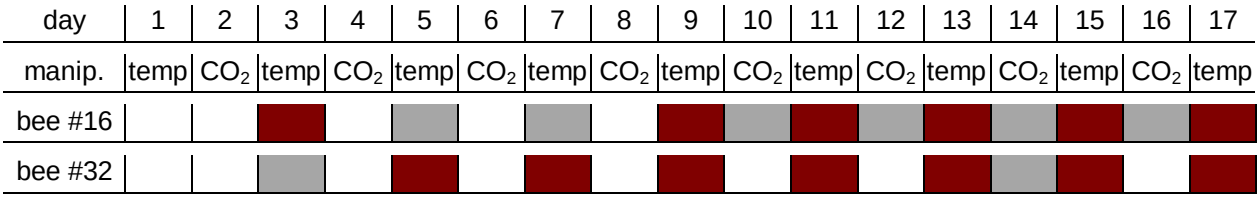

Fig. 3.9 Fanning positions of two workers during 17 successive trials. Brown bars: fanning on the brood (Br), grey bars: in the entrance (En) or entrance tunnel (Tu), white bars: no fanning activity.

Size

Workers varied in size from 2.9 mm to 4.8 mm head width (3.7 ± 0.3) and 6.7 mm to 13.3 mm wing length (10.1 ± 1.0). Workers that fanned three or more times did not differ in size from those that never fanned or fanned less than three times ($p>0.05$ for all colonies, *t*-test). Within the fanners, body size influenced none of the parameters of individual fanning behavior (response threshold, response probability, activity and normalized activity); ($p>0.05$ for all colonies).

DISCUSSION

In this chapter I have analyzed a collective behavior, the control of nest climate in bumble bee colonies, at the level of the individual colony members. I have focused on the question whether workers of a colony show consistent and different responses to parameters of nest climate. The results of my study demonstrate that bumble bee workers differ in at least three parameters of their fanning response: response threshold, response probability, and fanning activity. In the following, I will discuss the importance of interindividual variability for the collective response; I will analyze my results concerning mechanisms of specialization; and will end the chapter with a general discussion of self-organization and selection.

Interindividual variability in fanning response

My study documents large variability in worker responsiveness. Workers that repeatedly responded to a manipulation of nest climate differed in their response thresholds, that is the mean stimulus intensity that elicited fanning behavior. Some consistently responded at low stimulus intensities, others consistently started fanning when stimulus intensities were already quite high. Some fanned only when temperature increased, others fanned only during CO_2 manipulations. Although differential response thresholds among the members of a colony have been frequently assumed and discussed, to my knowledge this is the first study that documents response thresholds of a large number workers, namely of all workers involved in a collective behavior. We can now examine the nature of these response thresholds and address the question how the distribution of thresholds within a colony gives rise to the collective response described in chapter two.

A small proportion of a colony's workers had relatively low thresholds. At low stimulus intensities, only these low-threshold bees will respond. Their fanning will often suffice to down-regulate stimulus intensities, thus leaving the majority of the work force to other important tasks. If however stimulus intensity increases further and presents a real danger to the colony, the number of workers who's response threshold is exceeded increases dramatically and the colony responds with massive fanning. In other words, the

graded colony response to increasing stimulus intensities shown in chapter two is based on the distribution of individual response thresholds. This threshold distribution allows the close matching of the number of workers to current task needs.

Theoretically, a graded colony response could also be achieved by workers with a stochastic distribution of response. The advantages of individual thresholds will become apparent below.

The mean of the threshold distribution ranged between 27.7-28.7°C air temperature and 1.6-2.5% CO_2 concentration. As discussed in chapter two, only little is known about the effects of excessive CO_2 levels or the accompanying suboptimal O_2 levels. For temperature, the found mean of response thresholds seems to be an adaptive value: brood temperatures usually range 1-2°C above air temperature (Vogt 1986b and pers. obs.) and temperatures exceeding 32°C are known to impair brood development (see chapter two). Hence, in order to avoid such temperature levels and protect the brood, the colony needs to respond massively at temperatures below 30°C. This is achieved by a large number of workers with response thresholds around 28°C.

The distribution of thresholds for all tasks that need to be performed in a colony (colony threshold distribution *sensu* Beshers et al. 1999) should affect patterns of behavioral specialization, i.e. which tasks are likely to be found in a workers repertoire. My experiments allowed the measurement of two thresholds within an individual: temperature and CO_2 response threshold. Linked thresholds for temperature and CO_2 should result in general fanning specialists, that is, a certain subset of workers should respond with fanning to low stimulus intensities irrespective of whether the colony was experiencing heat stress or insufficient oxygen supply. However, I found no general correlation between the two thresholds. Clearly, workers are 'stimulus specialists' rather than 'task specialists'. This independence of thresholds in the context of microclimatic control and the occurrence of 'stimulus specialists' may be of biological significance when the colony faces trade-offs in regulating the two parameters. Furthermore, stimulus specialists may be of advantage when different nest climate parameters pose different requirements to fanning and when the efficiency in task performance increase with experience (see discussion of specialization and reinforcement below).

Other studies documenting individual, task-related response thresholds are rare. Some authors have demonstrated differences in the response of groups of workers from different patrilines or worker castes (Wilson 1984; Wilson 1985; Detrain & Pasteels 1991; Detrain & Pasteels 1992). In honey bees, dance thresholds of several selected workers have been shown to differ (Seeley 1994). A correlation has been found between the threshold concentration of sucrose solution for extending the proboscis in honey bees and forager preferences for water, nectar, or pollen (Page et al. 1998; Pankiw & Page 1999; Pankiw & Page 2000). However, it is not yet clear how the sucrose stimulus is related to the task of foraging for water or for pollen. In contrast, temperature/CO_2 intensity and fanning response examined in this study present simple stimulus-response pairs.

Response thresholds alone are not sufficient to accurately describe the variability in worker responsiveness. Workers also differed in their response probability. Nearly half of the workers never responded to a manipulation of nest climate. Within the fanners, some workers fanned nearly every time they were exposed to a stimulus intensity exceeding their individual response threshold, others responded only very rarely. This was the case even though all workers were confined to the nest during manipulations and were therefore inevitably exposed to the increase in stimulus intensity. Response threshold and response probability were two independent parameters of individual responsiveness.

What is the effect of interindividual differences in response probability? A response probability below 100% distributes the task of nest climate control more broadly among the workers of a colony: The group of fanners will be composed of different individuals every time the colony experiences climatic stress conditions. This increases the pool of workers to draw from when the need arises. Thus even when workers are outside the nest or occupied with some other urgent task within the colony there will always be a reliable work force present. (Especially if the response probability of a worker is influenced by other individuals already performing the task, see below). In other words, decreasing the probability of response decreases the importance of a single individual for the fulfillment of a certain task. Furthermore, and maybe more importantly, if workers learn certain tasks and increase their efficiency by doing so, as is commonly assumed (Oster & Wilson 1978) and discussed below, intermediate response probabilities ‘train’ more workers, this way again increasing the overall efficiency and reliability of the colony response. Thus, variable response probabilities enhance flexibility in that a reserve of workers becomes trained to efficiently perform multiple tasks.

Workers differed in a third parameter, namely in how actively they responded to a given stimulus intensity. While some workers fanned until stimulus intensity decreased, others showed only very short or intermittent fanning behavior. Workers of all colonies on average fanned during 60% of the time they were exposed to a stimulus exceeding their threshold. Interrupted fanning activity may serve the flexibility of the colony; workers that frequently resample the stimulus they are responding to (see sampling rate below) or other task-related stimuli remain responsive to changes and available for other urgent tasks.

Specialization and reinforcement

Different terms have been used in the literature to describe interindividual variance in task performance. The term ‘specialist’ usually describes workers that perform a subset of tasks more frequently than their nestmates (Oster & Wilson 1978). ‘Elitism’ describes the existence of individuals who are exceptionally active or entrepreneurial within age-size cohorts and 'do almost all the work’ (Plowright & Plowright 1988, p.420) or show an unusually high frequency of task performance, either as a specialist or a generalist (Oster & Wilson 1978; Jeanne 1999). Both terms have been used as descriptors, without reference to any underlying mechanism or social process (Robson & Traniello 1999). Considering the parameters introduced in this chapter, under natural conditions, that is conditions where

fanning will have the effect of decreasing stimulus intensity (note that this effect was counterbalanced in my experiments!), the observed fanning specialists of a colony will be those workers with low response thresholds and high response probabilities, while elite workers will be workers that additionally have exceptionally high activity rates.

Specialization is believed to be a key element of colony organization that increases the overall colony efficiency and thus ultimately the ecological success of social insects (Oster & Wilson 1978). Reinforcement has been discussed as a mechanism that sharpens the differentiation between specialists and the remaining work force. The concept of reinforcement proposes that the impact of a single worker on stimulus intensity increases with experience. This can be achieved in one of two way: First, response threshold for task associated stimuli may decrease with experience in performing the task (Theraulaz et al. 1998). Second, the efficiency of a worker may increase with experience e.g. because individuals learn to perform a task. Learning and increase in task efficiency have often been considered as the main reason for the efficiency of division of labor (Oster & Wilson 1978; Seeley 1982; Jeanne 1986). However, studies documenting any form of reinforcement are extremely rare (O'Donnel & Jeanne 1992).

The results of this study show that reinforcement may play an important role in specialization. Response thresholds of those workers that repeatedly fanned decreased over time. Under natural conditions, low-threshold bees will fan more often than high threshold bees, since they will sometimes down-regulate stimulus intensities and thus remove higher threshold from the task of fanning. This will lead to an increase in variance of the colony level distribution through the decrease in threshold of the low thresh bees.

My data also suggest that control of nest climate will prove a good system to study the second form of reinforcement: increase in task efficiency with experience. Workers choose different fanning positions in dependence of the stimulus. When temperature was high the majority of fanning took place directly on the nest, hardly any fanners were seen in the entrance tunnel. In contrast, when CO_2 was high, the percentage of fanning on the brood decreased drastically and more than 20% of all fanning events occurred in the entrance tunnel. This finding suggests that the two parameters require different fanning responses: fanning on the brood may locally increase evaporative cooling while fanning in the entrance tunnel may increase general air exchange (Southwick & Moritz 1987). The efficiency of an individual worker may increase with experience because her probability of adopting ‘correct’ positions increases. However, this remains to be investigated in more detail.

The combined effect of decreasing thresholds and increasing efficiency of a small subgroup of workers will result in strong specialization. Examples of ‘task fixation’ or ‘habituation’ described in ants and bees may be based on similar mechanisms of reinforcement. Ants become increasingly entrained on certain tasks they practice, and perform such tasks in preference of others (Sendowa-Franks & Franks 1993). In bumble bees and honey bees, the probability of reversion form e.g. foraging to nursing is a decreasing function of time spent performing a task (Free 1955; Seeley 1982).

In conclusion, my data present first evidence for positive reinforcement through decreasing response thresholds. Furthermore, they suggest that reinforcement through increasing efficiency may occur. Whether those individuals that perform a task only rarely experience negative reinforcement, e.g. increase in their response threshold as proposed in the reinforcement model (Theraulaz et al. 1998), remains to be tested.

Summary: Rules of behavior underlying self-organized control of nest climate

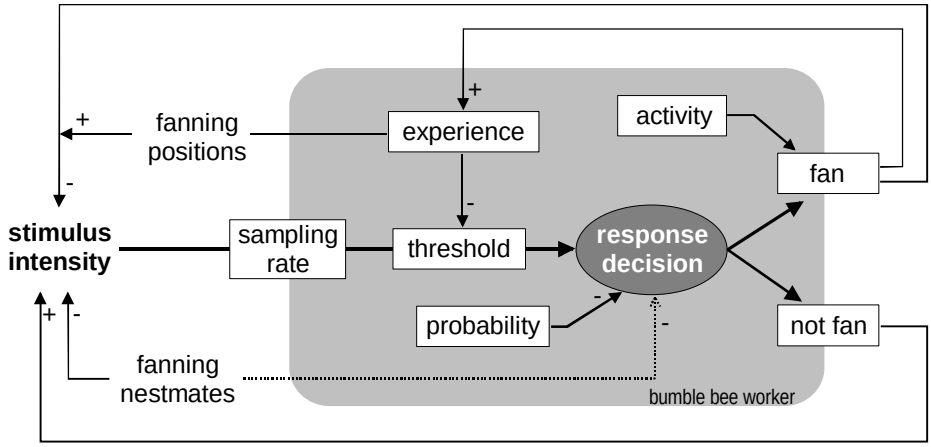

Fig. 3.10 Schematic diagram of the factors influencing individual fanning behavior.

In summary, the question whether workers show consistent responses to stimulus levels and differ in their response can now be clearly answered. My study has evidenced individual response thresholds and has shown interindividual variability in two other parameters of fanning behavior: response probability and fanning activity. Figure 3.10 gives a schematic summary of the factors influencing individual fanning behavior.

A worker responds to a stimulus (temperature or CO_2) with a certain probability and activity when her threshold is exceeded. By responding, she decreases stimulus intensity. Her impact on stimulus intensity increases with experience, since her response threshold decreases. Additionally, her efficiency may increase with experience by her adopting highly efficient fanning positions. She will thus respond earlier and her impact on stimulus intensity will increase.

Figure 3.10 also outlines the open questions that remain concerning the rules of behavior. Organization of insect colonies generally includes multiple pathways of negative feedback for the control of key variables of a colony's physiology (Seeley 1995). Such feedback loops also have to exist in the collective control of nest climate. Here I can only offer tentative speculations.

One important factor in control of nest climate are individual information sampling rates. How and how often does a fanning worker measure the stimulus she is responding to? For example, in temperature control we need to know whether workers measure air temperature, brood temperature or even receive some kind of stimulus directly form the brood. Furthermore, we need to know whether fanning behavior is interrupted in order to resample stimulus intensity. Workers were often observed fanning outside their nest at the end of the entrance tunnel where stimulus intensity was low. These workers would rush back to the hive every few minutes and then resume their fanning position in the tunnel after a few seconds. One worker was observed fanning in front of the field nest described in chapter 2 on July 4th, 1999. Her behavior was timed during the next three hours. She stayed outside fanning for 2.5 ± 0.2 minutes and then rushed back into the nest, reappearing after 0.5 ± 0.06 minutes (Fig. 3.11). Observations like these may provide insights into sampling rates in the future.

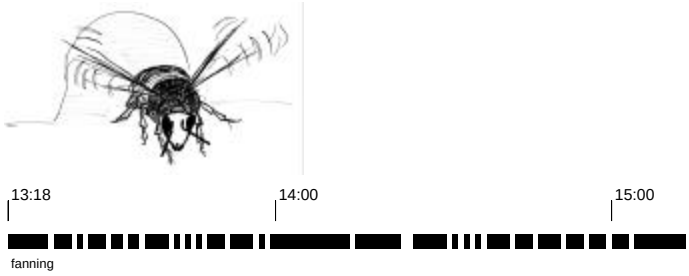

Fig. 3.11 Activity of a worker fanning in front of a field nest during a hot summer day. Black bars denote fanning in front of the nest entrance, white bars denote times when the worker disappeared into the subterranean nest.

Further, it remains to be investigated if and how workers receive feedback on the efficiency of their nestmates. A pilot experiment suggests that the decision to fan is influenced by the behavioral outputs of other workers. When those workers that fanned repeatedly (the fanning specialists) were removed from the colony shortly before the experimental increase of CO_2 during three consecutive days, the response probability of the remaining workers increased (data not shown). One possible information pathway for such feedback loops could be air currents.

Observations like the ones outlined here may contribute to a full understanding of feedback loops involved in the control of nest climate.

Self-organization and selection in insect societies

Why do individuals differ? From a proximate point of view, various factors are known or hypothesized to be responsible for interindividual variance. They can be roughly categorized as exogenous and intrinsic factors (reviewed in Beshers et al. 1999). Exogenous factors include nest environment, social environment (e.g. interactions with other workers), colony size and colony state. Intrinsic factors are genetics, ontogeny, experience, morphology, state (hunger, parasites) etc. Exogenous and intrinsic factors are often interrelated and their influence remains to a large extent unexplored. In my experiments, I

reduced the influence of exogenous factors as far as possible. Queens were singly mated, all workers had the same climatic experience during their ontogeny and the colony was supplied with enough food. In short, the proximate reasons for interindividual differences in responsiveness to nest climate parameters remain unknown.

What are the ultimate reasons for interindividual variance? Do the workers of a colony differ to a certain degree because natural selection favored variance over uniformity, or is interindividual variability simply inevitable noise in any system comprised of different subunits? Postulating response thresholds as an explanation for division of labor has important consequences for our understanding of the behavioral programs of workers, and the behavioral organization and adaptive design of colonies. In this section I offer a speculative discussion of some of these consequences, to illustrate the potential of the threshold concept for illuminating our understanding of insect societies and to suggest some possible directions for further research.

Many colony features have been studied with the assumption that they are colony-level functional adaptations, evolved by means of natural selection on available variants. Recently however, some authors have suggested that if all groups of organisms demonstrate colony level phenomena like e.g. division of labor as a consequence of group living, then these characteristics are not themselves adaptations. In other words, division of labor may be an inescapable property of groups of individuals (Page & Mitchell 1998). This view is supported by empirical evidence. For example, when pairs of young queens of the ant *Pogonomyrmex barbatus* are forced to cofound a nest there is an almost spontaneous division of labor (Page 1997). Thus, eusocial life may originally have arisen directly within solitary species without any intervening species (Michener 1985).

However, the specific features of colony level phenomena are adaptations to specific environments. In contrast to abiotic self-organized structures like e.g. ripples in the sand, biological structures have natural selection as their driving force in building increasingly complex subunits. Insect colonies are the product of group-level adaptations, and their subunits and the rules governing the behavior of the subunits are tuned by natural selection (Moritz & Southwick 1992; Seeley 1997; Page & Mitchell 1998; Ronacher & Wehner 1999). Natural selection must act on colony-level components that through self-organization processes result in specific behavioral patterns (Page & Mitchell 1998). To name only a few of these colony-level components, natural selection may act on the mean colony response threshold, the variance of thresholds within a colony, the degree of fixation within an individual, the degree of clustering of various thresholds within an individual, information sampling rates, response probability and response activity (Beshers et al. 1999).

How can we study the evolution of self-organized systems? One promising approach are comparative studies. For example, it would be interesting to know whether solitary insects vary similarly in respect to response thresholds and whether they show reinforcement of thresholds. Furthermore, for social insects, if there is an optimal mix of tasks performed in a colony of social insect, we would expect natural selection to prefer

certain threshold distributions for specific task needs or within specific environments (Moritz & Page 1999). The colony threshold distribution should affect patterns of specialization, the proportion of active to inactive workers, the latency of the collective response and the flexibility or resiliency of the colony response (Beshers et al. 1999). Flexibility in honey bee colonies has been shown to be high for some tasks (Calderone & Page 1992; Fewell & Page 1993) and low for others (Robinson 1987a). Colony flexibility should be high if many workers have low thresholds for most tasks.

A narrow distribution of thresholds will result in a rapid colony response, whereas a wide distribution will result in a slow and graded colony response. Thus, for tasks that require massive and fast colony response, e.g. colony defense, we would expect to find comparatively narrow distributions of thresholds, whereas for tasks that undergo only small and gradual changes in their immediate importance to the colony the corresponding thresholds should be distributed over a broad range of stimulus intensities.

Similarly, the degree of fixation within an individual may depend on the kind of task. Fixed thresholds are believed to occur in tasks that are important in response to short term changes in the colony environment (Beshers et al. 1999). In many cases however, adaptive tuning of thresholds in response to changing colony need may be essential to colony fitness. Modulation of thresholds should occur when the importance of the corresponding task gradually changes with e.g. season, colony size or colony state. For example, dance thresholds of honey bee workers are modulated by the amount of nectar brought into the hive (Seeley 1994). Sucrose response thresholds in honey bees are modulated by hunger (Page et al. 1998) and brood pheromone (Pankiw & Page 2001).

Often, thresholds are modulated through modulatory communication (Hölldobler 1995; Hölldobler 1998). Signals may act as additional stimuli, to exceed the thresholds of workers that would not otherwise perform these tasks, or they may serve to lower response thresholds to task related stimuli. Thus, modulatory signals may cause a short-term adjustment of colony labor allocation by 'pulling' additional workers to perform specific tasks (Seeley et al. 1996). Examples of modulatory signals are the shaking signal and the piping signal in honey bees (Pratt et al. 1996; Nieh 1998; Seeley et al. 1998) and alarm drumming in carpenter bees (Raub 1998). Future studies will presumably reveal a high degree of flexibility and modulation of thresholds for most tasks.

In species with pronounced age-polyethism, shifts in responsiveness to task-associated stimuli with age are found (Robinson 1987b). These can be due for example to age dependent differences in hormonal titer (Robinson 1987c).

Besides comparative studies of response thresholds for different types of tasks or in different environments, a further important step in our understanding of self-organization and selection will be to experimentally show that there is a heritable component to variability, e.g. strong environment-gene interaction and / or large plasticity in genotype for behavioral thresholds (Moritz & Page 1999).

Conclusions

The goal in developing hypothesis for understanding division of labor should be to find unifying explanations for phenomena at each of the two levels: individual organism and colony. Models of self organization meet this requirement. Especially in tasks where stimulus intensity is directly assessed by individual workers as is the case in control of nest climate, the concept of response thresholds has proven valuable in understanding the dynamics of a complex system such as an insect colony. However, one has to be aware that biological systems are bound to be much more complex and varied than the model. Therefore, in order to truly understand a behavioral system as complex as division of labor, one needs to know how the 'real' subunits behave and one needs to discover the many pathways of feedback and information flow between them.

This chapter has contributed to this quest by demonstrating that individual bumble bee workers differ in their response thresholds and that besides response thresholds, response probabilities and activity are important parameters to consider. Further, in this chapter I have presented first evidence for a mechanism of specialization: reinforcement. Future experiments should aim at unraveling the feedback loops in the control of nest climate. The next steps will also involve formulating new models, based on the empirical findings, and using the models to test whether the sets of behavioral rules and processes identified through empirical analyses do indeed produce the actual performance of an intact group.

Differences in response thresholds for task related stimuli are not the only possible mechanism underlying division of labor. Other self-organization models propose worker-worker interactions (Huang & Robinson 1999) or differences in local environment experienced by workers as driving mechanisms (Tofts & Franks 1992). None of these models are mutually exclusive and all may eventually contribute to our understanding of division of labor. The advantages of self-organized systems are that they are decentralized, robust to perturbations, largely independent of colony size and age structure and based on relatively few and simple rules (Bourke & Franks 1995). Self-organization models have been used to describe numerous aspects of social insect behavior, from development of foraging systems and mass action responses to nest building and food distribution (Deneubourg & Goss 1989; Deneubourg et al. 1989; Seeley 1989; Camazine 1991; Camazine & Sneyd 1991; Seeley et al. 1991; Deneubourg & Franks 1995; Watmough & Camazine 1995; Cassill et al. 1998; Pratt 1998; Bonabeau et al. 1999; Camazine et al. 1999; Millor et al. 1999; Sumpter & Broomhead 2000). Self-organization with its ability to generate complex patterns and behaviors from simple processes may underlie not only division of labor in social insects, but also be the origin of complexity in many other systems.

Understanding the nature of individual variability is critical to understanding the integration and organization of insect colonies. Careful observations of both colony and individual behavior are a promising approach to unravel the mechanisms behind various colony features. Additionally, self-organization models will prove useful tools in our understanding of complex systems. Studies of division of labor usually address the question

why has a certain structure been favored by natural selection. Studies of self-organization are more often concerned with the question how is a certain pattern or structure created (Sendova-Franks & Franks 1999). Linking these why and how question of ultimate and proximate causation shows great promises for a deeper understanding of the evolution of biological forms from cells to animal societies.

APPENDIX

Statistical analysis of interindividual variability

Interindividual differences in thresholds

In order to test for differences among the workers of a colony the following procedure was chosen: Fanners in each colony were sorted according to their mean response threshold values (Fig. A1 and Fig. A2). A subset of 6 individuals was chosen for further analysis. Three workers were selected from the low side of the distribution (‘low-threshold’ bees) and three workers were selected from the high side of the distribution (‘high-threshold’ bees). The criterion for selection was that the assigned threshold resulted from at least 5 repeated measurements for each individual. The selected bees are marked in Fig. A.1 and Fig. A.2 with red diamonds.

A multisample analysis (one-way Anova) was used in order to test for differences between the 6 selected individuals. Using a subset of individuals takes into account (at least partially) that the data were obtained from multiple groups (k: individuals). It has the advantage that the power of the Anova is the same for all colonies and manipulations analyzed. In addition, with increasing number of groups the power of the Anova decreases drastically.

An alternative statistical analysis for analyzing interindividual differences would be a comparison of a control mean (control individual) to each other group (individual) mean according to the Dunnett test. However, this test could not be applied, since there was no control group and to assign one individual as control with respect to the others presupposes that this individual has about twice the measurements as the others. There were individuals who’s threshold value was based on a larger number of measurements, but their threshold was, of course, not necessarily at the lower or the upper end of the distribution. Thus, these individuals would represent an inhomogeneous control group.

Following an successful Anova (differences between individuals were detected), a multiple comparison between each pair was performed. The results of the Fisher's-LSD test for each of the 'low-threshold' bees with each of the 'high-threshold' bees are shown in the tables below Fig. A.1 and Fig. A.2 for the two manipulations respectively. Significant differences ($p<0.05$) between individuals are marked in red.

Interindividual differences in activity

For the analysis of differences in activity between individuals, in general, the same procedure as described above was used. Since the data differed from a normal distribution, nonparametric statistics were used. The Kruskal-Wallis test was used instead of the Anova; for the multiple comparisons the Dunn test was used instead of the Fisher’s LSD test (Zar 1984). The Q-values which show significant differences between individuals are marked in red. Note that because of the coarse grid in which observation were recorded (5 minutes blocks) the normalized activity data of those bees that started fanning late in the trial (high-threshold bees) were skewed towards higher values; an effect which could not be accounted for in the analysis.

Zar, J. H. 1984. *Biostatistical Analysis.* Englewood Cliffs: Prentice-Hall.

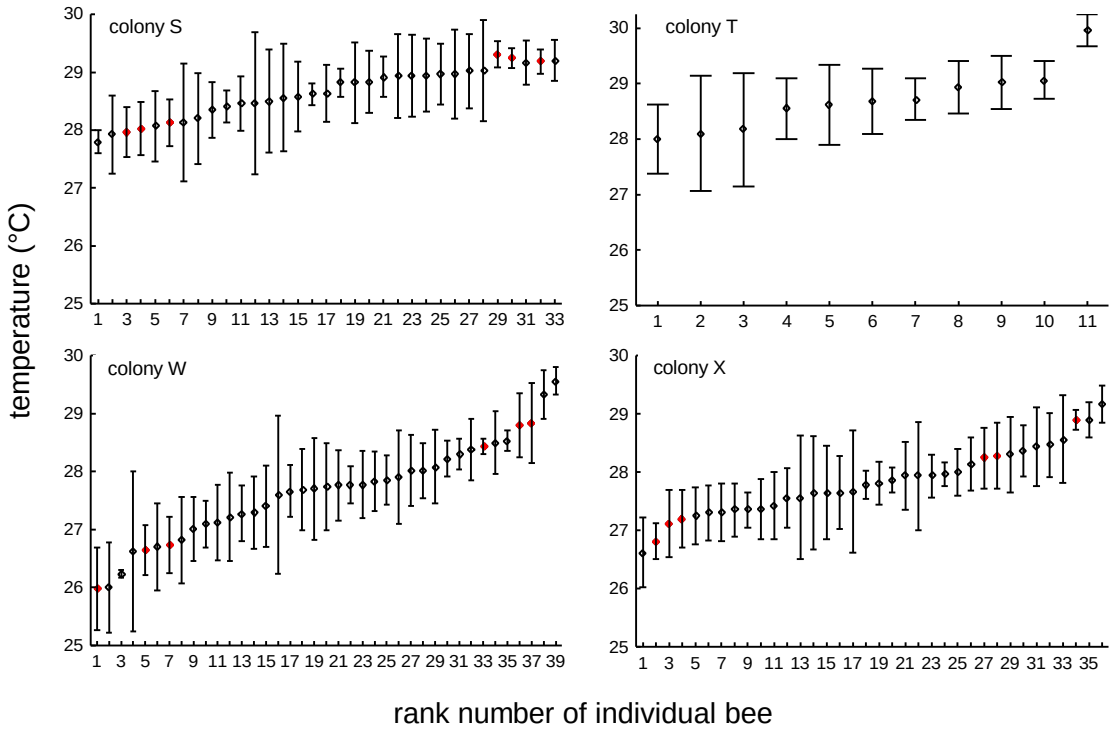

Fig. A.1 Individual response thresholds (mean ± SE) for temperature, sorted. Red diamonds denote those workes that were included into the analysis.

Colony S

One-Way-Anova	Fisher-LSD-Test		high threshold bees		
		mean (°C)	29.31	29.24	29.18
F=2.6832	low	27.96	.0163	.0329	.0673
p=0.0320	threshold	28.03	.0193	.0387	.0780
	bees	28.13	.0337	.0615	.1118

Colony T

the criterion of 5 repeated measurements was met in only 5 individuals. No Anova was performed for that reason.

Colony W

One-Way-Anova	Fisher-LSD-Test		high threshold bees		
		mean (°C)	28.44	28.80	28.84
F=4.6505	low	25.98	.0057	.0018	.0016
p=0.0024	threshold	26.64	.0437	.0168	.0151
	bees	26.73	.0404	.0144	.0128

Colony X

One-Way-Anova	Fisher-LSD-Test		high threshold bees		
		mean (°C)	28.24	28.28	28.90
F=3.0187	low	26.82	.0282	.0376	.0043
p=0.0272	threshold	27.12	.0941	.1086	.0169
	bees	27.20	.1187	.1340	.0219

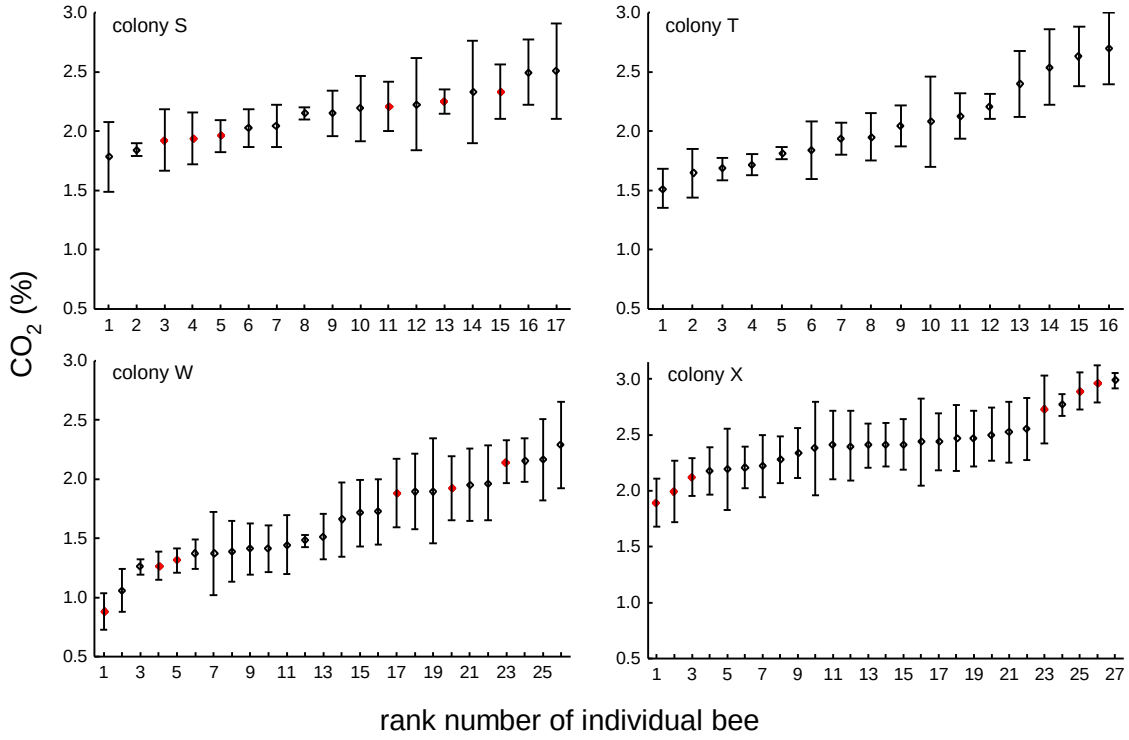

Fig. A.2 Individual response thresholds (mean ± SE) for CO_2, sorted. Red diamonds denote those workes that were included into the analysis.

Colony S

One-Way-Anova	Fisher-LSD-Test		high threshold bees		
		mean (%)	2.21	2.25	2.34
F=0.8088	low	1.92			
p=0.5518	threshol d bees	1.94	not tested, since the Anova revealed no significant differences		
		1.96			

Colony T

the criterion of 5 repeated measurements was met in only 5 individuals. No Anova was performed for that reason.

Colony W

One-Way-Anova	Fisher-LSD-Test		high threshold bees		
		mean (%)	1.88	1.92	2.15
F=5.5879	low	0.88	.0022	.0015	.0004
p=0.0015	threshol	1.27	.0285	.0200	.0045
	d bees	1.31	.0411	.0291	.0066

Colony X

One-Way-Anova	Fisher-LSD-Test		high threshold bees		
		mean (%)	2.73	2.89	2.96
F=4.6282	low	1.89	.0135	.0037	.0022
p=0.0032	threshol	2.00	.0354	.0112	.0070
	d bees	2.12	.0471	.0131	.0077

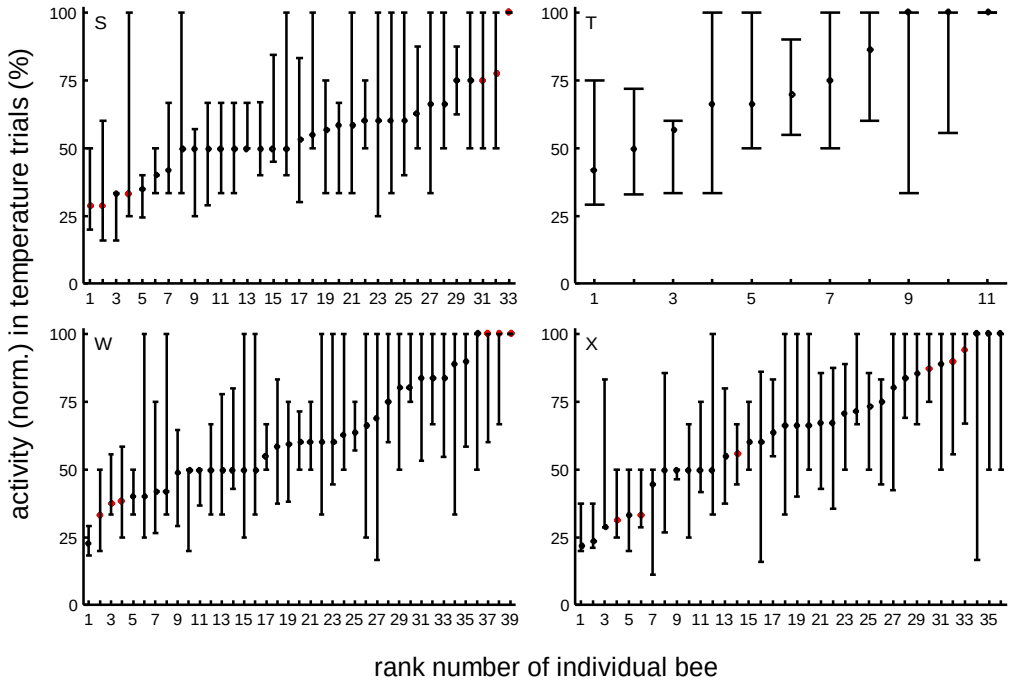

Fig. A.3 Individual response activity (normalized) for temperature, sorted. Median and quartile are shown. Red diamonds denote those workes that were included into the analysis.

critical values for k=6: p<0.05: Q=2.936; p<0.01: Q=3.403 R_i:mean ranks

Colony S

Kruskal-Wallis-test	Dunn-Test		high threshold bees		
N=34		mean R_i	21.30	21.58	27.08
H=14.2662	low	7.90	2.3644	2.5217	3.5353
p=0.0140	threshold	9.08	2.2514	2.4161	3.4792
	bees	16.10	0.9175	1.0105	2.0241

Colony T

the criterion of 5 repeated measurements was met in only 5 individuals. No Kruskal-Wallis-test was performed for that reason.

Colony W

Kruskal-Wallis-test	Dunn-Test		high threshold bees		
N=44		mean R_i	26.50	26.72	28.29
H=12.2591	low	9.20	2.7568	2.9312	3.0414
p=0.0314	threshold	9.93	2.8928	3.1094	3.2045
	bees	16.10	1.6573	1.7769	1.9418

Colony X

Kruskal-Wallis-test	Dunn-Test		high threshold bees		
N=44		mean R_i	29.667	29.792	30.167
H=20.8064	low	9.0833	2.9386	3.4138	3.01
p=0.0009	threshold	11.071	2.755	3.2444	2.829
	bees	20.214	1.4004	1.6599	1.4745

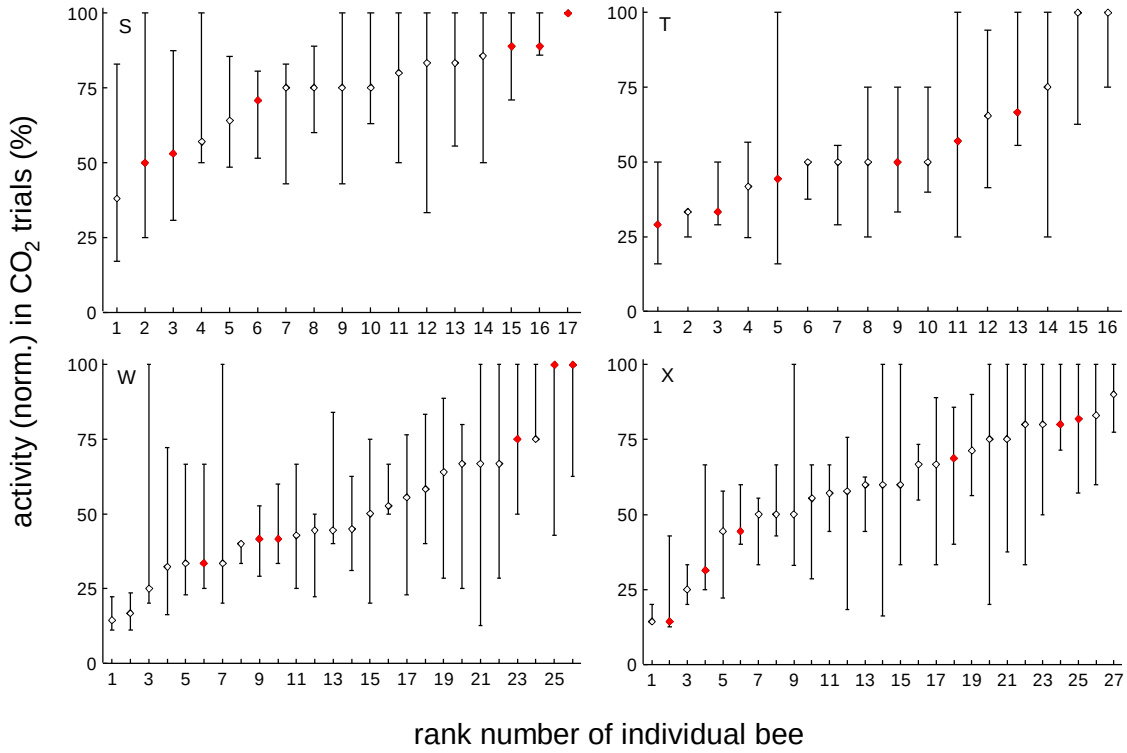

Fig. A.4 Individual respones activity (normalized) for CO_2, sorted. Median and quartile are shown. Red diamonds denote those workes that were included into the analysis.

critical values for k=6: p<0.05: Q=2.936; p<0.01: Q=3.403 R_i:mean ranks

Colony S

Kruskal-Wallis-test
N=38
H=4.6553
p=**0.4594**

Colony T

Kruskal-Wallis-test
N=26
H=5.8653
p=**0.3196**

Colony W

Kruskal-Wallis-test
N=30
H=6.1369
p=**0.2932**

Colony X

Kruskal-Wallis-test
N=39
H=14.1140
p=0.0149

Dunn-Test		high threshold bees		
	mean R_i	19.83	25.83	27.41
low	8.00	1.729	2.6057	3.1838
threshold	13.83	0.9195	1.8389	2.3666
bees	16.30	0.5163	1.3929	1.8223

SUMMARY

The social organization of insect colonies has long fascinated naturalists. One of the main features of colony organization is division of labor, whereby each member of the colony specializes in a subset of all tasks required for successful group functioning. The most striking aspect of division of labor is its plasticity: workers switch between tasks in response to external challenges and internal perturbations. The mechanisms underlying flexible division of labor are far from being understood. In order to comprehend how the behavior of individuals gives rise to flexible collective behavior, several questions need to be addressed: We need to know how individuals acquire information about their colony's current demand situation; how they then adjust their behavior according; and which mechanisms integrate dozens or thousands of insect into a higher-order unit.

With these questions in mind I have examined two examples of collective and flexible behavior in social bees. First, I addressed the question how a honey bee colony controls its pollen collection. Pollen foraging in honey bees is precisely organized and carefully regulated according to the colony's needs. How this is achieved is unclear. I investigated how foragers acquire information about their colony's pollen need and how they then adjust their behavior. A detailed documentation of pollen foragers in the hive under different pollen need conditions revealed that individual foragers modulate their in-hive working tempo according to the actual pollen need of the colony: Pollen foragers slowed down and stayed in the hive longer when pollen need was low and spent less time in the hive between foraging trips when pollen need of their colony was high. The number of cells inspected before foragers unloaded their pollen load did not change and thus presumably did not serve as cue to pollen need. In contrast, the trophallactic experience of pollen foragers changed with pollen need conditions: trophallactic contacts were shorter when pollen need was high and the number and probability of having short trophallactic contacts increased when pollen need increased. Thus, my results have provided support for the hypothesis that trophallactic experience is one of the various information pathways used by pollen foragers to assess their colony's pollen need.

The second example of collective behavior I have examined in this thesis is the control of nest climate in bumble bee colonies, a system differing from pollen collection in honey bees in that information about task need (nest climate parameters) is directly available to all workers. I have shown that an increase in CO_2 concentration and temperature level elicits a fanning response whereas an increase in relative humidity does not. The fanning response to temperature and CO_2 was graded; the number of fanning bees increased with stimulus intensity. Thus, my study has evidenced flexible colony level control of temperature and CO_2. Further, I have shown that the proportion of total work force a colony invests into nest ventilation does not change with colony size. However, the dynamic of the colony response changes: larger colonies show a faster response to perturbations of their colony environment than smaller colonies. Thus, my study has revealed a size-dependent change in the flexible colony behavior underlying homeostasis.

I have shown that the colony response to perturbations in nest climate is constituted by workers who differ in responsiveness. Following a brief review of current ideas and models of self-organization and response thresholds in insect colonies, I have presented the first detailed investigation of interindividual variability in the responsiveness of all workers involved in a collective behavior. My study has revealed that bumble bee workers evidence consistent responses to certain stimulus levels and differ in their response thresholds. Some consistently respond to low stimulus intensities, others consistently respond to high stimulus intensities. Workers are stimulus specialists rather than task specialists. Further, I have demonstrated that workers of a colony differ in two other parameters of responsiveness: response probability and fanning activity. Response threshold, response probability and fanning activity are independent parameters of individual behavior. Besides demonstrating and quantifying interindividual variability, my study has provided empirical support for the idea of specialization through reinforcement. Response thresholds of fanning bees decreased over successive trials. I have discussed the importance of interindividual variability for specialization and the collective control of nest climate and present a general discussion of self-organization and selection.

This study contributes to our understanding of individual behavior and collective structure in social insects. A fascinating picture of social organization is beginning to emerge. In place of centralized systems of communication and information transmission, insect societies frequently employ mechanisms based upon self-organization. Self-organization promises to be an important and unifying principle in physical, chemical and biological systems.

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ANHANG

ZUSAMMENFASSUNG

Ein besonderes Merkmal sozialer Insekten ist die Arbeitsteilung. Die Mitglieder einer Kolonie führen jeweils unterschiedliche Arbeiten aus und wechseln, je nach Bedarfslage der Kolonie, flexibel zwischen den verschiedenen Tätigkeiten. Die Mechanismen dieser flexiblen Arbeitsteilung sind bislang weitgehend unverstanden. Wie erfahren einzelne Arbeiterinnen welche Tätigkeiten gerade notwendig sind? Nach welchen Regeln ändern sie ihr Verhalten, wenn sich die Anforderungen an die Kolonie ändern? Wie wird das Verhalten vieler Einzelindividuen so koordiniert, daß die Kolonie als Ganzes sinnvoll auf eine sich verändernde Umwelt reagieren kann? In der vorliegenden Arbeit bin ich diesen Fragen an zwei unterschiedlichen Systemen nachgegangen.

Im ersten Kapitel dieser Arbeit untersuchte ich die Regulation des Pollensammelns bei Honigbienen. Pollen ist für Honigbienen eine wichtige Proteinquelle zur Aufzucht der Brut. Sowohl die Menge an Brut als auch die bereits im Stock vorhanden Menge an Pollen beeinflußt die Sammelaktivität. Bislang ist unklar, wie die Sammelbienen Information über den Pollenbedarf ihrer Kolonie erhalten und wie sie ihr Verhalten dementsprechend ändern. Meine Versuche zeigten, daß Pollensammlerinnen ihr Arbeitstempo der aktuellen Bedarfslage anpassen: Ist der Pollenbedarf der Kolonie hoch, verbringen sie wenig Zeit im Stock, ist ausreichend Pollen vorhanden, gehen sie ihrer Sammeltätigkeit langsamer nach.

Während ihres Aufenthalts im Stock haben die Sammlerinnen eine Vielzahl trophallaktischer Kontakte mit anderen Bienen. Die Anzahl solcher Kontakte änderte sich mit dem Pollenbedarf der Kolonie: Bei hohem Pollenbedarf sind die trophallaktischen Kontakte kürzer und die Anzahl sehr kurzer Kontakte hoch. Diese Ergebnisse unterstützen die Hypothese, daß Änderungen in der trophallaktischen Erfahrung eine wichtige Informationsquelle über den aktuellen Pollenbedarf einer Kolonie darstellen.

Das zweite Beispiel flexibler Arbeitsteilung, welches ich in dieser Arbeit untersucht habe, ist die Regulation des Nestklimas in Hummelkolonien. Dieses System unterscheidet sich von dem oben dargestellten grundlegend, da Information über Änderungen im Bedarf an Arbeitskraft jedem Koloniemitglied zugänglich ist. Jedes Koloniemitglied im Nest kann direkt erfahren wie sich das Nestklima ändert. Ich konnte zeigen, daß Hummelkolonien auf einen Temperaturanstieg und eine Zunahme der Kohlendioxidkonzentration im Nest mit Ventilationsverhalten reagieren. Einzelne Hummeln fächeln dabei mit ihren Flügeln und sorgen so für Evaporationskühlung bzw. eine verstärkte Belüftung des Nestes. Erhöhte Luftfeuchtigkeit löste diese Reaktion nicht aus. Die Anzahl fächelnder Hummeln war abhängig von den Temperatur/CO_2 Werten, die Kolonie reagierte fein abgestimmt auf die aktuellen Bedingungen. Unabhängig von ihrer Größe investierten die untersuchten Kolonien einen bestimmten Anteil ihrer Arbeiterinnen in die Ventilation des Nestes. Große Kolonien unterschieden sich jedoch von kleinen Kolonien in ihrer Antwortgeschwindigkeit: Große Kolonien antworten schneller auf einen Temperatur / CO_2 Anstieg als kleine.

Die flexible und fein abgestimmte Kolonieantwort auf Veränderungen im Nestklima basiert auf dem Verhalten vieler Einzelindividuen. Im dritten Kapitel dieser Arbeit stellte

ich aktuelle Ideen und Hypothesen zu Selbstorganisation und dem Einfluß interindividueller Variabilität auf Kolonieverhalten dar. Regulation des Nestklimas in Hummelkolonien ist ein ideales System um interindividuelle Variabilität und ihre Auswirkungen zu untersuchen. Ich konnte zum ersten Mal Unterschiede im Antwortverhalten aller an einem kollektiven Verhalten beteiligten Koloniemitglieder quantifizieren. Neben Unterschieden in Antwortschwellen, die in der Literatur zwar viel diskutiert, aber noch nie schlüssig nachgewiesen wurden, konnte ich zeigen, daß sich Arbeiterinnen einer Kolonie in zwei weiteren Parametern unterscheiden: Die Wahrscheinlichkeit auf einen Stimulus zu reagieren und die Dauer, mit der die Arbeiterinnen das Verhalten ausführen (Aktivität) ist zwischen Individuen unterschiedlich. Diese drei Parameter (Reaktionsschwelle, Antwortwahrscheinlichkeit und Aktivität) sind vermutlich unabhängige Parameter individuellen Verhaltens. Neben diesen interindividuellen Unterschieden konnte ich nachweisen, daß sich die Antwortschwellen verändern, je häufiger eine Hummel fächelt: Arbeiterinnen reagieren von Mal zu Mal auf niedrigere Stimulusintensitäten. Diese Ergebnisse sind für unser Verständnis von Arbeitsteilung und Spezialisierung bei sozialen Insekten von besonderer Bedeutung.

In dieser Arbeit habe ich sowohl das Verhalten individueller Arbeiterinnen als auch die daraus resultierende kollektive Antwort der Kolonie untersucht. Es wird zunehmend deutlicher, daß dem faszinierenden Verhalten sozialer Insekten häufig nicht zentrale Informationsverarbeitung sondern Selbstorganisation zugrunde liegt.

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PUBLICATIONS

full papers

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conference presentations

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Weidenmüller A, Kleineidam C, Tautz J (2000) The response threshold concept and nest climate control in bumble bees, TMR Social Evolution Conference, Firenze *(poster)*

Weidenmüller A, Kleineidam C, Tautz J (2000) Control of nest climate in bumble bee colonies, AG Tagung, Blaubeueren *(poster)*

Weidenmüller A, Kleineidam C, Tautz J (1999) Individual fanning response and the control of nest climate in bumble bee colonies. EU TMR network workshop 'Social insects as model systems', Sheffield *(talk)*

Weidenmüller A, Kleineidam C, Tautz J (1999) Fächelnde Hummeln: Individuelle Reaktionsschwellen und deren Bedeutung für die Nestklimatisierung. IUSSI, Hohenheim *(talk)*

Weidenmüller A, Tautz J (1999) Pollen, immer nur Pollen? Wie spezialisiert sind sammelende Honigbienen? AG Tagung, Marburg *(poster)*

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Diese Dissertation hat weder in gleicher noch in ähnlicher Form in einem anderen Prüfungsverfahren vorgelegen.
Des weiteren erkläre ich, dass ich früher weder akademische Grade erworben habe, noch zu erwerben versucht habe.

Würzburg, den 20.03.01