

**The influence of developmental temperatures
on division of labour in honeybee colonies**

D i s s e r t a t i o n

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D i s s e r t a t i o n

zur Erlangung des akademischen Grades
Doctor rerum naturalium (Dr. rer. nat.)

vorgelegt der
Naturwissenschaftlichen Fakultät I
Biowissenschaften
der Martin-Luther-Universität Halle-Wittenberg

von
Diplom-Biologe Matthias Adolf Becher
geb. am 26.07.1974 in Heidelberg

Gutachter

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Halle (Saale)

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CHAPTER 1

Introduction

1.1. Division of labour

One of the primary characteristics of eusociality in insects is the division of labour among members of the same colony, in which sets of workers specialize in different sets of tasks (Michener 1969, Wilson 1975, Beshers et al. 2001). While reproductive individuals specialise in the production of offspring, a functionally sterile worker caste becomes responsible for brood rearing and foraging, and maintains the nest homeostasis (Schmickl and Crailsheim 2004). However, division of labour in social insects also takes place within the worker caste (Robinson 1992, Page and Erber 2002). Whereas several ant and termite species show an impressive worker polymorphism like the leaf-cutter ant *Atta sexdens* (Wheeler 1986, Winston 1980a,b), in the majority of social insects including the honeybees the worker caste is monomorphic and task allocation strongly depends on the age of the workers (Seeley 1995, Beshers and Fewell 2001). A general pattern of this temporal polyethism is that younger workers perform in-hive tasks like nest building and brood care, whereas older ones take the risk of foraging outside the colony (Rösch 1925, Beshers et al. 2001). However, this mechanism of task allocation does not follow a rigid pattern, and allows for flexible responses to environmental and intracolony requirements, which may even reverse the behavioural development from outdoor to indoor worker (Rösch 1930, Robinson et al. 1989, Robinson 1992, Page et al. 1992, Robinson 2002). A loss of foragers in a colony leads to an earlier onset of foraging in workers, whereas the presence of brood and the lack of young nurse bees delays the behavioural development of older workers (Huang and Robinson 1996, LeConte et al. 2001, Leoncini et al. 2004). Plasticity in age polyethism is also achieved by genetic components. Due to the multiple mating of honeybee queens the colony is structured in several subfamilies (Taber 1958, Laidlaw and Page 1984) of which some generate a higher proportion of precocious foragers than other subfamilies if older bees are lacking (Calderone and Page 1988, Giray and Robinson 1994, Robinson and Huang 1998). The shift in the behavioural patterns of honey bees goes along with physiological changes as it can be observed in glandular development. Wax glands for example are most active in bees with an age of three to 21 days (Rösch 1927, Lindauer 1952, Muller and Hepburn 1992) and the hypopharyngeal gland produces brood food in workers aged between three to 16 days (Lindauer 1952, Winston 1987). Physiological and behavioural developments are coordinated by hormones and in honeybees the juvenile hormone (JH) plays a central role for division of labour. It has

been shown that the interplay of vitellogenin and JH is important for the transition from the in-hive worker to the forager, where JH acts as „behavioral pacemaker“ reducing the age of first foraging (Jaycox et al. 1974, Robinson 1987, Huang et al. 1997, Robinson and Vargo 1997, Sullivan et al. 2000).

1.2. Thermoregulation

Although insects in general are ectotherms and their temperature depends on the environment many of them are able to regulate their body temperature to a certain degree. A favorable body temperature can be achieved passively by moving to a location with an adequate microclimate (Steiner 1929, Cloudsley-Thompson 1962), but it can also be achieved by actively generating metabolic heat (May 1979, Casey 1981, Tschinkel 1985, Jones and Oldroyd 2007). In social insects, the cooperation of many individuals allows temperature regulation not only on the individual level but as a feature of the whole colony, where especially the brood nest is under particular control (Jones and Oldroyd 2007). Endowed with strong flight muscles, social wasps and bees have the ability to directly incubate their brood (Himmer 1927, Jones and Oldroyd 2007). A highly precise regulation of the nest temperature is found in honeybees. The western honeybee (*Apis mellifera*) shows a mean brood temperature of around 35°C within a range of 32-36°C (Hess 1926, Himmer 1927, Dunham 1931, Wohlgemuth 1957, Koeniger 1978, Kronenberg and Heller 1982, Ritter 1982, Heinrich 1993). Honeybees sense temperature with thermo-receptors in their antennae (Heran 1952, Lacher 1964, Yokohari 1983) and it is assumed that a worker engages in nest thermoregulation, if the temperature it is exposed to exceeds an individual threshold (Jones et al. 2004, Weidenmüller 2004, Graham et al. 2006, Fehler et al. 2007, Jones and Oldroyd 2007). Honeybees increase their body temperature by activation of the flight muscles without moving the wings (“shivering“) which can result in thorax temperatures exceeding 40°C (Esch 1960, Esch and Bastian 1968, Esch et al. 1991, Kleinhenz et al. 2003, Stabentheiner et al. 2003). Most workers and even the drones contribute to the colonial thermogenesis (Harrison 1987, Kovac et al. 2009). Older workers with strong flight muscles have a higher heating capacity than younger workers (Himmer 1925, Allen 1959, Stabentheiner and Schmaranzer 1987, Vollmann et al. 2004), but younger workers are usually located close to the brood nest (Seeley 1982) and hence are directly stimulated for brood heating. Heating workers press their warm thoraces firmly on capped brood cells to incubate the brood (Bujok et al. 2002). Sometimes empty cells, scattered in the sealed brood area, are entered by a heating workers, which is assumed to efficiently warm the pupae in the neighbouring cells

(Kleinhenz et al. 2003, Fehler et al. 2007). Basile et al. (2008) showed that heating workers are supplied with energy via trophallaxis by their nestmates.

High environmental temperatures often require cooling of the brood. For cooling bees ventilate by wing fanning, evaporate water by tongue lashing or spreading droplets of water on the brood nest (Lindauer 1954, Kiechle 1961, Lensky 1964, Southwick and Moritz 1987). Moreover Starks and Gilley (1999) describe a behaviour termed „heat shielding“, where workers position themselves on hot interior regions especially on the broodnest to prevent overheating.

Additionally to the thermoregulation during the brood rearing period, honey bees maintain temperatures between 18 and 32°C also in the winter cluster with ambient temperatures far below 0°C (Hess 1926, Southwick and Mugaas 1971, Southwick 1987, Southwick and Heldmaier 1987). The individuals of the colony form a cluster with a warm core temperature and lower temperatures in the insulating mantle while the stored honey is consumed to metabolically produce heat (Owens 1971, Southwick 1985, Fahrenholz et al. 1989, Sasaki et al. 1990, Stabentheiner et al. 2003). Size, shape and tightness of a honey bee cluster depends on the ambient temperatures (Simpson 1961, Heinrich 1981, Myerscough 1993, Watmough and Camazine 1995, Sumpter and Broomhead 2000). Although overwintering as a colony is costly, it allows to start brood rearing already in early spring and is hence one reason for the ecological success of the honey bees (Seeley 1985, Winston 1987).

1.3. Impact of developmental temperatures

Brood nest temperatures in general are well regulated to about 35°C, but temporary deviations from the optimal temperature inevitably occur, especially at the edge of the brood nest (Rosenkranz and Engels 1994). Stronger deviations result in the death of the brood or in malformations of wings, stinger, proboscis or legs of the adult bees (Himmer 1927) or preclude ovary development in workers (Lin and Winston 1998). Smaller variations between 32 and 36°C, as it occurs in the brood nest, do not cause visible defects (Himmer 1927, Groh et al. 2004, Jones et al. 2005) but can affect wing morphology (Ken et al. 2005) or pigmentation of thorax and abdomen in *A. cerana* (Tsuruta et al. 1989). Additionally, developmental temperature influences the behavioural traits of adult workers. High temperatures of 36°C during pupal development have been shown to improve the olfactory learning ability of adult workers in proboscis extension reflex (PER) tests. Pupal developmental temperatures of 32°C however reduced the probability to dance as well as the

number of performed waggle dance circuits (Tautz et al. 2003). Jones et al. (2005) confirmed these results and found improved short-term learning and memory abilities of adult workers experienced higher temperatures during their pupal development.

Groh et al. (2004) detected significant changes in the mushroom bodies of the honeybee's brain in response to the developmental temperature. As mushroom bodies are involved in learning and memory and influence division of labour in honeybees (Withers et al. 1993, Heisenberg 1998), developmental temperatures may have far reaching consequences for the social organization of the colony.

1.4. Study question

As developmental temperatures affect behavioural traits - particularly such involved in the outdoor activities of honey bees - they may well be an instrument for fine-tuning division of labour in the colony. Changes in environmental temperatures as well as a fluctuating colony size can influence brood temperatures and hence foraging thresholds of individual workers. Increasing proportions of foragers as a potential result of higher developmental temperatures will in turn reduce the proportion of in-hive bees, responsible for brood care. Developmental temperatures will then affect not only the foraging effort but also the population dynamics of the colony.

Based on these assumptions my thesis explores the impact of brood heating and pupal developmental temperatures on division of labour of the colony in the western honeybee (*Apis mellifera* L.):

- 1.) „A new device for continuous temperature measurement in brood cells of honeybees (*Apis mellifera*)“ addresses the subject of temperature measurement in honeybee colonies and presents a new kind of multi-sensor thermometer.
- 2.) „Gaps or caps in honeybees brood nests: Does it really make a difference?“ examines the question if honeybee workers take advantage of empty cells in the brood nest to improve the efficiency of heating.
- 3.) „Pupal developmental temperature and behavioral specialization of honeybee workers (*Apis mellifera* L.)“ delves into the influence of brood temperature on the performance of outside tasks by foragers.
- 4.) „Brood temperature, task division and colony survival in honeybees: a model“ evaluates the importance of these empirical findings for the organization and viability of the colony.

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CHAPTER 2

A new device for continuous temperature measurement in brood cells of honeybees (*Apis mellifera*)

Apidologie (2009) 40:577-584. DOI: 10.1051/apido/2009031

Received 7 October 2008 – Revised 6 March 2009 – Accepted 15 March 2009

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running title: Temperature measurement in honeybee cells

Abstract

Nest temperature in honeybees is a crucial factor for the brood development and influences the task specialization of adult workers. Accurate and reliable data on temperature distributions are hence of major interest to understand colony function. We present a new device for temperature measurements in brood cells of honeybee combs. The instrument allows for a continuous temperature recording at the bottom of 768 brood cells. In contrast to previous techniques, we can record the complete temperature history of individual developing larvae under natural conditions in the hive. The device consists of a dense grid of thermistors, connected to a computer for the recording and display of the temperature data. Software is provided to graphically display the temperature profile across the comb in false colors.

honeybee / brood comb / temperature measurement

1. Introduction

Temperature is an important factor affecting larval and pupal development of insects (Nylin and Gotthard, 1998). Increased temperatures typically result in higher growth rates, higher respiration rates and shorter development times and influence the adult body size (Büns and Ratte, 1991; Sibly and Atkinson, 1994, Petz et al., 2004). Also mortality rates are affected by temperature with extreme temperatures having lethal effects (Howe, 1967). Control of brood temperature is therefore considered as a most important evolutionary advantage of many eusocial insect colonies to optimize the rearing conditions of the brood. Specific behavioral

adaptations of the workers including active heating and cooling or transport of larvae to cooler or warmer nest regions ensure optimal temperature conditions for the brood (Steiner, 1929; Heinrich, 1993). The honeybee, *Apis mellifera*, has been shown to have a most precise temperature regulation in the brood nest and the brood temperature ranges within narrow limits between 32°C and 36°C with a mean of 34.5°C (Himmer, 1927; Kronenberg and Heller, 1982). This temperature homeostasis is on the one hand achieved by active heating of workers through clustering and/or the simultaneous activation of their thoracic muscles. On the other hand evaporation of water through fanning is used for cooling (Lindauer, 1954; Southwick, 1983; Harrison, 1987; Esch et al., 1991, Kleinhenz et al., 2003). Indeed the constant brood nest temperature is important because major deviations cause malformations of the emerging adults (Himmer, 1927; Groh et al., 2004). However, even the small temperature fluctuations in individual brood cells within the physiological limits regulated by the workers have substantial significance for the adult workers later in life. The brood temperature affects many traits of adult bees, including learning abilities, outdoor activities and the pace of temporal polyethism (Tautz et al., 2003; Groh et al., 2004). Since brood temperature interferes with the cognitive abilities of the bees and their task specialization, colony temperature is likely to be a major driver of colonial organization and allocation of workers to certain tasks. However, in order to quantify the effect of brood temperature on colony organization, it is essential to accurately measure the temperature profile in individual cells throughout the larval and pupal development and compare this with the behavioral phenotype of the adult worker. Although experiments have been conducted, using a large set of incubators each set to a different temperature, this approach does not allow for assessing the natural variance for each individual cell. Clearly it would be much more enlightening, could we obtain this data in situ in the colony to assess the natural temperature variance in brood cells. We developed a precise thermo-device which allows for monitoring the temperature profile across the brood comb at both a high spatial and temporal resolution as well as minimum disturbance for the colony.

2. Methods and results

2.1. General description

256 resistor sensors were arranged on a 15 x 15 cm area consecutively delivering temperature data from the bottom of the cells in a test comb (Fig. 1A). Each sensor touched the comb in the centre of three adjacent cells, so altogether temperature data from 768 brood cells could be recorded. The temperature sensors project through the front panel of a perspex box which

holds the electronic circuit board (Fig. 1B). A test comb is arranged in front of this box, so the sensors just reach the bottom of the cells in the test comb and are inaccessible to the bees. The sensor board is connected to a personal computer, which executes the addressing of the resistors and reads in the received data.

2.2. Sensor board

For the temperature measurements we used an array of 256 thermistors with a negative temperature coefficient (“NTC” resistors; SEMI 833 ET, Hygrosens[®] Instruments). The resistance of a NTC thermistor drops in a non-linear way when the temperature rises. These resistors are low cost items originally developed for the use in clinical thermometers. The small diameter (1.5 mm), a short reaction time (0.7 s), a high sensitivity, and a high long-term stability make them particularly useful for our purpose.

The 256 NTC-sensors were placed in a grid of 16 rows by 16 columns on a circuit board (Fig. 2). Each sensor was placed in the cell wall junction of three adjacent cells and did not insert into the cell lumen. This way an area of 768 cells could be monitored on the comb. The sensors were activated via four 8-channel multiplexers (MAX 4051, Maxim[®] Integrated Products). The addressing of the multiplexers was accomplished by a PC controlled I/O-board (PCI TTL-I/O 32, Quancom[®]). Standard diodes (1N4148) prevented the addressing of more than one sensor. The resulting analog signals were amplified (MAX 4166, Maxim[®] Integrated Products) converted to digital values (A/D-converter produced by Point Electronic, Halle, Germany) and stored in a text file.

2.3. Data record and processing

The sensors were consecutively addressed, with three measurements within three seconds for each sensor. The median of these three values was used for further analysis. The temperature in each cell was computed as the mean of the two nearest sensors, with the closer one weighted double. The data recording was conducted in an endless loop which could be continued for weeks and was graphically displayed by a software tool in Delphi/Pascal (source code available on request). This tool shows the graphic presentation of all monitored cells for any time step in false color (Fig. 3A) including parameters such as mean temperature, standard deviation, minimum and maximum temperature and number of cells in a given temperature range.

2.4. Empirical test of the instrument

We tested the instrument in a hive with the test comb and three additional frames and about 3000 workers bees. The colony was placed in the laboratory at room temperature (25°C) with a flight entrance to the outside. The queen was confined on the comb area over the sensor array with a queen excluder grid (workers can pass but not the queen) to ensure egg-laying on the test comb. Worker bees had access to the queen and the brood for feeding and tending, while the queen could not move to the other frames, until a sufficiently large brood nest was established. Temperature data were recorded as described above.

2.5. Results

We found a negative correlation between the absolute temperature and the temperature fluctuations in the brood nest. A higher temperature leads to a highly significantly reduced variance over time (Pearson test: $p = 0.0001$, $r = -0.64$, $N = 30$) (Fig. 5), indicating that temperatures are more constant in the centre of the brood nest, where the highest temperatures occur (Fig. 3, Fig. 4). As a consequence the negative correlation of mean brood cell temperatures with the distance from the brood nest centre is highly significant (Pearson test: $p = 0.0005$, $r = -0.60$, $N = 30$).

The mean temperature measurements in 30 brood cells during the three days of egg phase was 32.7°C, ranging from 30.5°C to 34.0°C. During the larval development, the mean temperature was 33.3°C (minimum larval cell temperature: 30.0°C, maximum temperature: 34.8°C) and for the pupal phase we measured a mean temperature of 33.2°C (minimum: 31.1°C, maximum 34.6°C). The coldest cells (mean temperatures < 30°C) as well as the highest temperature fluctuations (standard deviation greater than $\pm 1^\circ\text{C}$) were found in the broodless area (Fig. 3B).

2.6. Comparing front and back side temperatures

To detect the temperature differences between front and back side cell temperatures, we used two instruments, measuring the temperature distribution in an empty comb from both sides at the same time. The instruments were placed in an artificially generated temperature gradient without any bees. After a stable temperature distribution was reached, the temperatures on the warm side of the comb ranged from 29°C to 34°C, whereas the temperatures on the other side were at the average 1.4°C lower (Fig. 6). We thus underestimate the actual cell temperature by 1.4°C.

3. Discussion

First measurements of temperature in social insects were conducted with mercury thermometers (Newport, 1837; Himmer, 1927; Andrews, 1929), yielding only highest and lowest values with special maximum-minimum thermometers. The first continuous temperature measurements became possible with the use of thermocouples or thermistors. Yet both, thermocouples and thermistors were usually only applied in a small number, so that they did not show a global temperature pattern across a comb (e.g. Cameron, 1985 in *Bombus*; Southwick and Heldmaier, 1987; Fahrenholz et al., 1989 in *Apis*; Hozumi et al., 2005 in *Polybia*). More recently, infrared thermography has been used for temperature measurements in honeybees. Indeed this technique delivers a spatial temperature distribution and has been repeatedly used to monitor temperature data of individuals (Stabentheiner and Schmaranzer, 1987; Kastberger and Stachl, 2003; Kleinhenz et al., 2003). However, information from inside the colony can hardly be gathered, nor does this method allow to measure temperatures within individual cells of a comb.

In comparison with brood nest temperatures reported in older literature (about 34.5°C; Hess, 1926; Himmer, 1927; Dunham, 1931), the temperatures measured in our study were about 1.4°C lower which is exactly the difference obtained in our control experiment without bees, where we heat the air on one side of the comb and measure on the other. The average development temperature of 33.1°C is thus due to the construction of the instrument: bees only have access to the front side of the comb but not to the back side which holds the electronic apparatus. The insulative layer of worker bees on the back side of the comb was missing which causes the lower than expected temperatures. Since thermistors poking into the cell lumen would interfere with larval and pupal development, the actual temperatures inside the cells and in the developing larvae cannot be directly recorded. Nevertheless, temperature distributions and fluctuations over time can be accurately monitored by the thermistors on the back side of the combs, because the temperature difference of 1.4°C is linear over the expected range of temperatures observed in the hive (Fig. 6).

We found higher and more constant temperatures close to the centre of the comb. Temperatures in the periphery of the brood nest were not only lower, but showed a much stronger variance. As the brood temperature results mainly from the activity of adult bees, the temperature profile reflects the distribution of the workers on the comb. The high number of workers near the centre of the brood nest raised the temperature and reduced the temperature fluctuations. A lower temperature in the periphery of the brood nest was also found by

Rosenkranz and Engels (1994). For Carniolan honeybees, they measured mean temperatures in capped cells of 35.2°C to 35.4°C in the centre and of 33.5°C to 34.5°C in the peripheral areas.

The thermo-device we present here provides the possibility to constantly measure the temperature distribution under near natural conditions on the comb with a high spatial resolution. Difficulties due to lower temperatures on the back side of the test comb might be overcome by allowing the bees to enter both sides of the comb. Contrary to previous studies, where honeybee pupae had been raised in incubators to analyze the influence of brood temperature on the adults (Tautz et al., 2003; Groh et al., 2004), we now are able to record the complete temperature history of any individual from the egg stage to the emergence of the adult worker. This will not only open the way to understand the impact of temperature on the development of a honeybee, but also on the role it will play as an adult in the colony, and hence for overall colony organization of honeybees.

Summary

Nest temperature in honeybees is a crucial factor for brood development and is maintained between 32°C and 36°C. The workers can heat the hive by shivering their flight muscles or cool it down by fanning or evaporation of water. Although deviations from the mean brood temperatures are low, the developmental temperature affects many traits of the later adult bees, including learning abilities, dance performance and the pace of temporal polyethism. It is therefore of major interest, to obtain accurate data on the spatial and temporal temperature distributions in the brood nest for understanding colony function and division of labor among the workers.

We present a new device for temperature measurements in brood cells of honeybee combs. 256 temperature sensors were arranged on a 15 x 15 cm area consecutively delivering temperature data from 768 cells in a test comb (Fig. 1A). The temperature sensors projected through the front panel of a perspex box housing the electronic circuit board (Fig. 1B). Each sensor touched the test comb in the centre of three adjacent cells. The sensor board was connected to a personal computer, which executed the addressing of the resistors and the data logging. The recorded temperatures were graphically displayed by a software tool showing the mean and standard deviation of cell temperature over time, the minimum and maximum cell temperature, and number of cells in a given temperature range (Fig. 3A).

In an empirical test of the instrument, we recorded individual temperature profiles of the developing brood in the hive. We found higher and more constant temperatures close to the

centre of the brood nest in comparison to cells at the periphery (Fig. 4). The temperatures measured on the back side of the comb were at the average 1.4°C lower than on the front side, where the brood was developing. The instrument allows to record the complete temperature history of any individual from the egg stage to the emergence of the adult worker under near natural conditions.

Zusammenfassung

Die Nesttemperatur ist bei Honigbienen ein entscheidender Faktor für die Entwicklung der Brut. Sie schwankt zwischen 32°C und 36°C bei einem Mittel von etwa 34,5°C. Die Arbeiterinnen regulieren die Temperatur im Stock, indem sie durch Aktivierung ihrer Flugmuskulatur Wärme erzeugen oder durch Fächeln und Verdunstung von Wasser das Brutnest kühlen. Obwohl die Abweichungen von der mittleren Brutnesttemperatur gering sind, beeinflusst die Entwicklungstemperatur zahlreiche Eigenschaften der späteren adulten Tiere, darunter ihre Fähigkeiten zu lernen, ihre Entwicklungsgeschwindigkeit von Innendienst- zu Außendiensttätigkeiten sowie ihr Tanzverhalten. Um die Organisation der Kolonie und die Arbeitsteilung zwischen den Individuen besser zu verstehen, ist es daher von großem Interesse, die räumliche und zeitliche Temperaturverteilung im Brutnest aufnehmen zu können.

Wir stellen hier ein neues Gerät zur Temperaturmessung in den Brutzellen von Bienenwaben vor. 256 Temperatursensoren wurden auf einem 15 x 15 cm großen Raster angeordnet und liefern fortlaufend Temperaturwerte für 768 Zellen einer Testwabe (Abb. 1A). Die Messfühler ragen durch die Frontscheibe der Plexiglasbox, welche die Leiterplatte enthält (Abb. 1B) und berühren die Mittelwand der sich davor befindenden Testwabe. Das Ansteuern der Sensoren und die Temperaturdatenerfassung erfolgt über einen Personal Computer. Die aufgenommenen Temperaturen werden durch eine Software grafisch dargestellt, die auch wichtige Kenngrößen wie Durchschnittstemperaturen mit Standardabweichung über die Zeit, minimale und maximale Temperaturen und die Anzahl der Zellen in einem vorgegebenen Temperaturbereich ermittelt (Abb. 3A).

In einem empirischen Test des Gerätes haben wir im Bienenstock individuelle Temperaturprofile der sich entwickelnden Brut aufgenommen. Wir konnten höhere und konstantere Temperaturen im Zentrum des Brutnestes als in dessen Randbereichen feststellen (Abb. 4). Die auf der Rückseite der Wabe gemessenen Temperaturen waren dabei im Mittel um 1.4°C niedriger als die Temperaturen der Vorderseite, in der sich die Brut entwickelte. Das hier vorgestellte Gerät ermöglicht es zum ersten Mal unter fast natürlichen Umständen

innerhalb des Stockes, komplette Aufnahmen der Entwicklungstemperaturen vom Eistadium bis zum Schlupf auf der Ebene von Individuen durchzuführen.

Acknowledgement

For technical advices and help in programming we would like to thank Gunther Tschuch, Sven Black-Hand Ewald and Felix Lehmann. This study was funded by the Deutsche Forschungsgemeinschaft with a grant given to RFAM.

Figures and legends

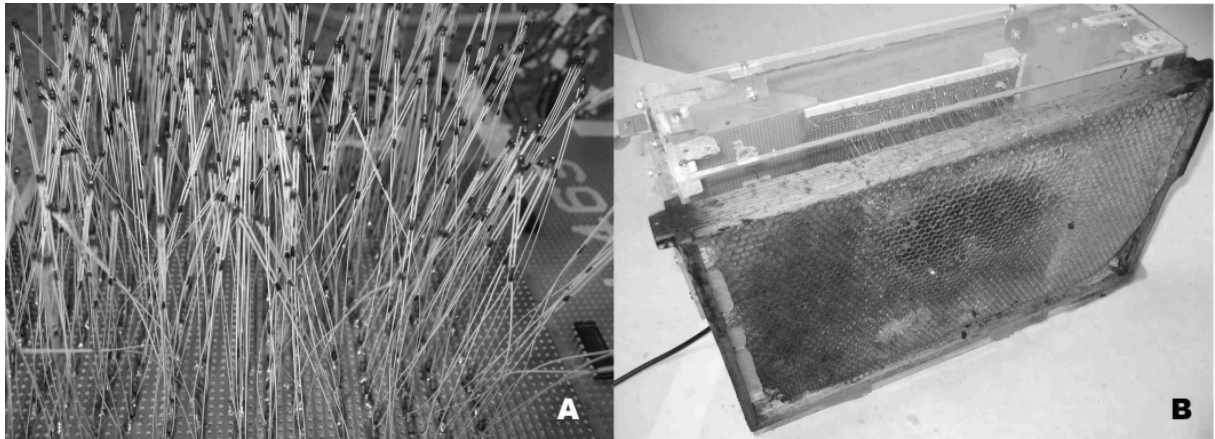


Figure 1. 256 resistor NTC resistor sensors were placed in a grid of 16 rows by 16 columns on a circuit board (A). The temperature sensors project through the front panel, just reaching the cell wall junctions of the test comb. The perspex box can easily be placed in any standard hive (B).

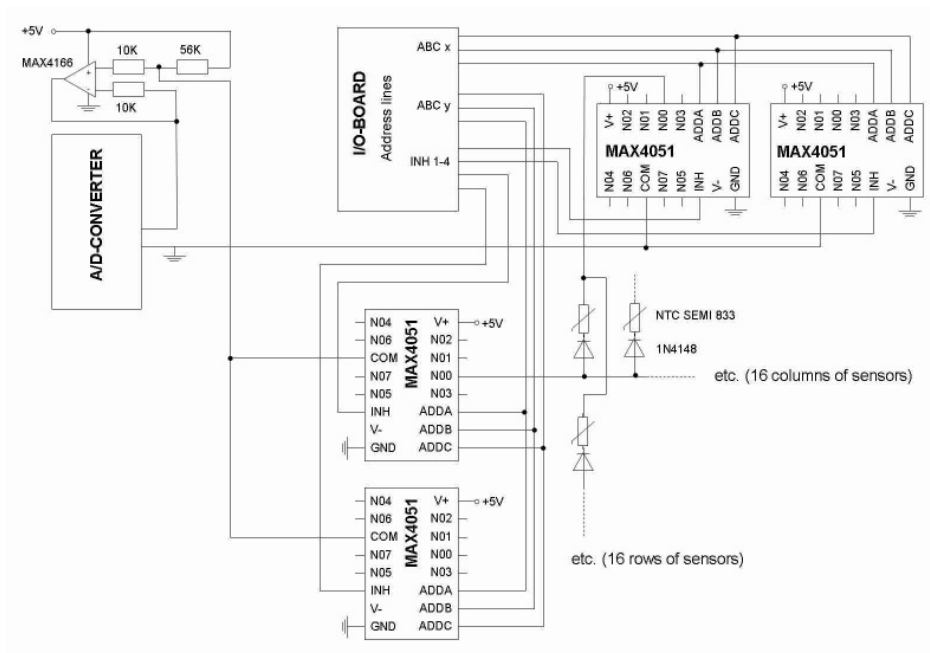


Figure 2. Simplified circuit diagram: 256 NTC sensors, each provided with a standard diode (1N4148) are placed on a 16x16 grid with rows and columns are chosen via four 8-channel multiplexers (MAX 4051). Addressing of the channels is accomplished by a PC controlled I/O-board (Quancom: PCI TTL-I/O 32), resulting signals are amplified (MAX 4166) and read in by an A/D-converter.

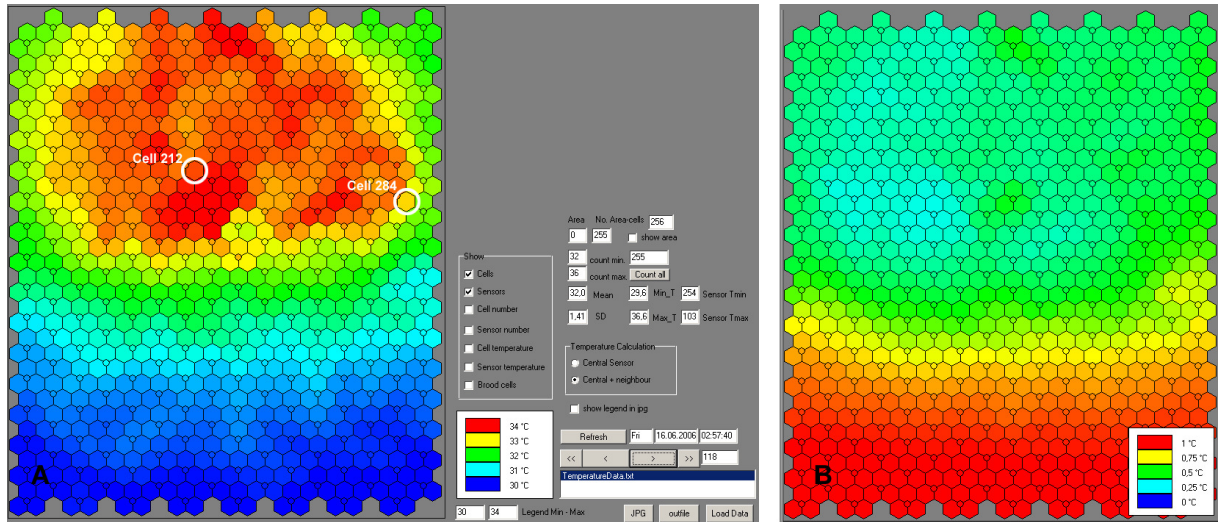


Figure 3A. Recorded temperature data are analyzed and displayed via a visualization software. Shown here is a snapshot of the temperature distribution on June 16, 2006, 2:57 a.m. with brood being present in the upper part of the comb (red and green area). For the cells No. 212 and No. 284 the temperature profile is given in Fig. 4. The software also provides fundamental statistical values (mean temperature, standard deviation, minimum and maximum temperature, number of cells in a given temperature range).

3B. The standard deviation of cell temperatures from 16.-24.06.2006. Temperature fluctuations are strongly reduced in the upper part of the comb, where the broodnest was located (blue and green area).

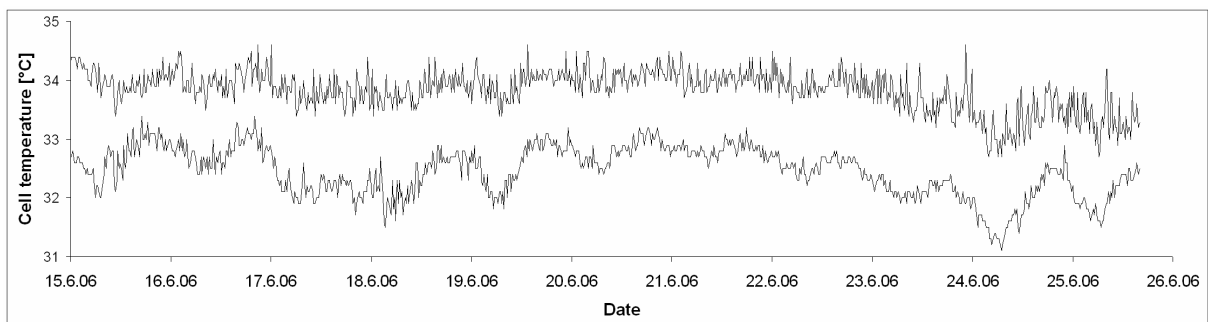


Figure 4. Two examples for individual temperature profiles of a warm and a cool broodcell during the pupal stage. The warm cell No. 212 was situated close to the centre of the broodnest, whereas the cooler cell No. 284 was located at the periphery (compare with Fig. 3A).

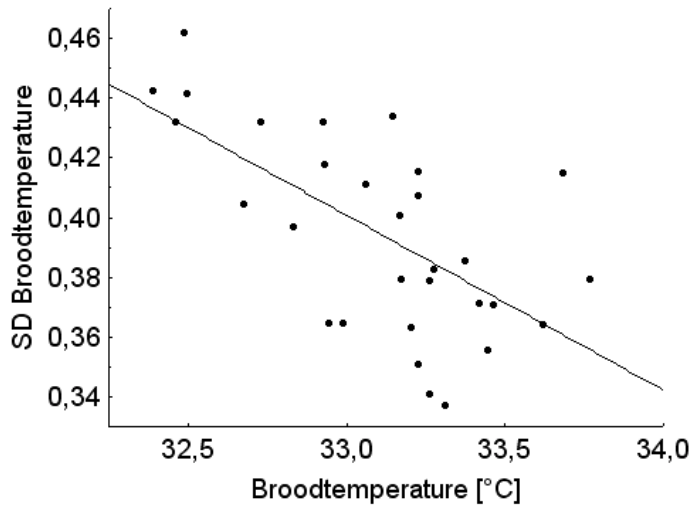


Figure 5. The standard deviation of the temperature plotted against mean developmental temperature of those cells, where definitely brood was present. (Pearson test: $p = 0.0001$, $r = -0.64$, $N = 30$).

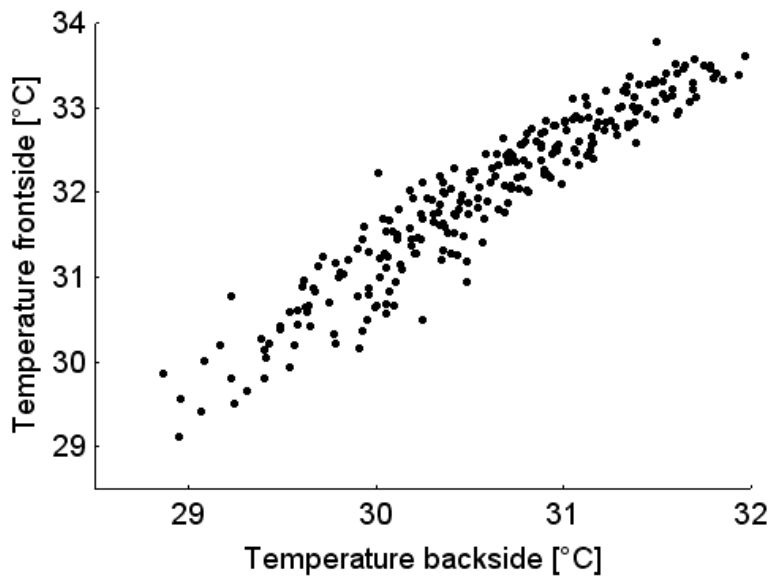


Figure 6. Comparison of front- and back side temperatures in an empty comb. We used two instruments placed in a temperature gradient to measure the temperatures in one comb simultaneously on both sides. Each data point represents the temperatures of a pair of two opposing sensors, averaged over two hours.

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CHAPTER 3

Gaps or caps in honeybees brood nests: Does it really make a difference?

submitted to *Naturwissenschaften*: 27 November 2009 – rejected: 28 December 2009
with opportunity to resubmit revised version

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Abstract

Honeybee (*Apis mellifera*) workers maintain brood nest temperatures at about 35°C by activation of their flight muscles. In the capped brood area there are always some cells scattered containing no brood. These empty cells are called “gaps”. Recently honeybee workers were observed to enter these gaps when heating the brood. A theoretical model predicted a significant increase in the thermoregulation efficiency if workers took advantage of these gaps since the heat produced is directly transferred to the adjacent brood cells. We tested this model by recording temperatures of 7x7cm brood pieces with and without gaps using multi-sensor thermometers and experimental groups of 150 honeybee workers. We neither found differences in the slope of the temperature increase as predicted by the computer model nor in the maximal temperatures. We conclude that honeybee workers may not intentionally use empty cells in the nest for brood heating and the loss of brood due to the gaps may trade off the potentially higher heating efficiency.

Keywords: Honeybee, *Apis mellifera*, Brood nest, Empty cells, Thermoregulation

Introduction

Honeybees (*Apis mellifera*) show a precise thermoregulation in the brood nest with temperatures of around 35°C to provide optimal conditions for the development of the brood (Himmer 1927; Jones et al. 2007). Workers can activate their flight muscles to increase their body temperatures if the brood needs to be heated or they evaporate water by fanning of their

wings to cool down the nest (Lindauer 1954; Esch 1960). Although honeybees typically form a compact brood nest on the centre of a comb, there are always some empty cells not containing any brood scattered across the comb (Woyke 1984). Empty cells in the brood nest can result from the erratic egg-laying behaviour of the queen as well as from the removal of unviable eggs and larvae by the workers and a proportion of 9% of empty cells is not unusual (Woyke 1984). These empty cells or „gaps“ might be seen as an inevitable deviation from an optimal case, where all cells of the brood nest are actually occupied by brood. However, Kleinhenz et al. (2003) suggested that bees use these empty cells for the thermoregulation of the brood, instead of heating via the brood cell's cap (Bujok et al. 2002). They distinguished between resting workers, sitting quite motionless in the cells and heating workers which are characterised by rapid respiratory movements of the abdomen and thoracic temperatures of about 40°C. Thoracic temperatures of cell visiting bees were higher when neighbouring cells contained brood. The authors suggested this behaviour to be more efficient than heating the brood via brood caps on the surface of the comb, as less heat is lost to the hive air. Based on these findings, Fehler et al. (2007) proposed a computer model to analyse the impact of this effect on thermoregulation for proportions of 0 – 50% of gaps in the brood nest. In this model bee agents (N=134) moved randomly over a brood nest (20x20 cells) and started heating when they encountered a brood cell with a temperature below a threshold of 33.5°C. Whenever empty cells were available, adjacent brood cells were heated from the side through the cell walls, otherwise the bees heated the brood cells from the top via the cell wax cappings. The model showed that combs with 20% empty cells provided the optimal conditions to minimize energy and the incubation time required to maintain the optimal brood nest temperature. However, it remains unclear if this behaviour for the thermoregulation actually matters at the colony level where several thousands of honeybees are available for temperature control of the brood. We therefore empirically tested the model of enhanced worker heating efficiency on brood combs with and without empty cells.

Material and Methods

We used experimental groups of 150 honeybee workers (*Apis mellifera*) confined on a test comb (18x18cm) with a piece of capped brood inserted in the centre of the comb. We recorded the temperature with an array of 256 resistors sensors as described in Becher and Moritz (2009). This instrument measures the temperature on the bottom of the brood cells in an area of 15x15cm.

Preparation of the test comb

We inserted a piece of capped brood with a size of 7x7cm (≈ 200 cells) into the centre of an empty comb without brood. The brood had an age of 9 to 14 days and contained no or only a few open cells. We prepared three types of brood nests: 1. brood with 10% empty cells, 2. brood with 10% empty cells, that were artificially re-capped so that worker could not enter the empty cells and 3. brood without empty cells.

For the preparation of the brood comb with 10% of gaps, we removed the cell wax cappings and the pupae in addition to the naturally empty cells to obtain a total of 20 uniformly distributed empty cells on the comb. To artificially re-cap these open cells, they were covered with a small circular piece of paper and then sealed by a droplet of bees wax.

Temperature measurement

All cells on the backside of the brood piece were emptied to allow the insertion of the thermo-sensor array and the temperature measurement in the target cells on the other side of the comb. 256 resistor sensors arranged on a 15x15cm area delivered consecutively the temperature data of all cells on the piece of brood and the surrounding host comb not containing brood. We used two instruments at the same time, one with a brood nest with gaps and one without gaps (Fig. 1).

Experimental procedure

Worker bees were collected from the same colony which provided the brood combs in the experiments. We brushed workers from a brood comb of the donor colony and let in-hive nurse bees with a negative phototactic behaviour run into a dark box, whereas older workers flew up into the air. To facilitate the handling of the bees, we cooled them at 6°C for about one hour, until they fell into chill coma, separated two groups with 150 bees each and caged them on the test combs in a 18x18cm area, with the brood nest being in the centre. We provided a tube with honey to each group and conducted the experiments under dark conditions. All experiments lasted for at least 17.5 hours to allow the honeybees to establish a constant broodnest temperature.

We restricted the temperature analysis to the piece of brood comb and hence „brood nest temperature“ refers to the mean temperature of the thermo-sensors covering the entire piece of brood comb and any particular time step. Statistical analyses were carried out using Statistica 6.0 and G*Power 3.0.10 (Faul et al. 2007).

Results

We found a strong temperature increase at the beginning of all experiments. After ca. 3 hours the curve flattens to reach its maximum after another hour. Then the temperature decreases again until it stabilizes after ca. 15 hours at an equilibrium temperature of about 28.5°C. As we measure the temperature not in the brood cells but on the backside of the comb, where no bees were present, the recorded temperatures are on average 1.4°C lower than the actual brood cell temperatures (Becher and Moritz 2009).

Naturally and artificially capped cells

There were no significant differences in the temperature of brood nests with only naturally and 10% artificially capped cells. The average maximum temperature of brood nests with only naturally capped cells was $29.44 \pm 0.80^\circ\text{C}$ and that of brood nest with 10% artificially capped cells $29.51 \pm 0.79^\circ\text{C}$ ($t = 0.15$, $P = 0.88$, $N = 8$, $Df = 8$). Since these minute differences were statistically not significant, we pooled both groups for the further analysis and refer to them as „capped“ cells.

Brood heating in brood nests with and without gaps

The mean temperatures of the brood nests with and without gaps are shown in Fig. 2. The strong temperature increase at the beginning was very similar for both treatments. We did not find a significant difference in the slope of both curves: The mean slope of the brood temperature within the first two hours was $0.99 \pm 0.54^\circ\text{C/h}$ (mean $\pm$ s.d.) for the capped treatment and $1.03 \pm 0.53^\circ\text{C}$ for the treatment with gaps ($t = -0.22$, $P = 0.41$ (one-tailed), $N = 46$, $Df = 44$). According to the model of Fehler et al. (2007), we would have expected a slope of 1.58°C/h for the gap treatment, as 37% of the incubation time should be saved. The difference of -0.55°C/h between the empirical slope and the theoretically predicted slope in the brood nest with gaps is highly significant ($t = -4.95$, $P < 0.0001$, $N = 23$, $Df = 22$).

Also for the maximum brood nest temperature we did not find a significant effect of the gap treatment. The average maximum temperature was $30.14 \pm 1.81^\circ\text{C}$ for the capped brood and $30.27 \pm 1.81^\circ\text{C}$ for the treatment with gaps ($t = -0.24$, $P = 0.40$ (one-tailed), $N = 46$, $Df = 44$).

Similarly, at the end of the experiments temperatures did not differ significantly between both treatments nor did they at any point of time. The average temperature after 17.5 hours was $28.45 \pm 2.39^\circ\text{C}$ in the capped treatment and $28.70 \pm 2.80^\circ\text{C}$ in the brood nests with gaps ($t = -0.33$, $P = 0.37$ (one-tailed), $N = 46$, $Df = 44$). The strongest differences (0.30°C) between both treatments occurred after 4h 17min but were also not statistically significant ($t = -0.37$, $P =$

0.36 (one tailed), $N = 46$, $Df = 44$). We conducted a power analysis to test if the lack of significance was merely a result of a too small sample size. With a sample size of 23 runs for each treatment, we achieve a sufficient power of 0.85 for an assumed effect size of $d = 0.8$ which suggests indeed that the effects of gaps at the colony level are very small if they can be detected at all.

Discussion

Fehler et al. (2007) claim that 10% gaps in the brood nest significantly reduce the incubation time per brood cell to maintain the correct temperature. At 28°C ambient temperature, the average incubation time in the model was predicted to be reduced by about 37% from 36.2 min in a brood comb with no gaps to 22.7 min in a brood comb with 10% gaps (derived from Fehler et al. 2007, Fig.1). Alas, the results of our experiments provide no evidence for such a clear benefit of empty cells in the brood nest. Since the temperature of the brood nest at the beginning of the experiments was well below the optimal brood nest temperature of 35°C, we see a strong effort of the bees to heat the brood with a steep slope of 1.0°C/h. However, the two treatments did not show significant differences in the heating phase and gaps in the brood nest did not provide a faster temperature increase. A power analysis showed that we would have detected the strong effect predicted by the model in our sample.

We also found no significant differences in the maximum temperature of the brood nest. The model of Fehler et al. does not predict higher temperatures in the presence of gaps under conditions where bees are able to maintain proper nest temperatures. But temperature differences might occur, if the bees do not manage to achieve optimal nest temperatures, because the ambient temperatures are too low, the insulation of the comb is weak or the number of bees relative to the brood nest size is too small. In this case the bees are permanently exposed to a stimulus to heat the brood. If gaps provide a benefit for the thermoregulation, higher temperatures should then be reached. Although brood nest temperatures in our experiments were always below the optimal temperature, we did not detect a significant increase in the maximum temperatures when gaps were present.

One might argue, that the removal of 10% of the pupae in the brood nests with gaps may have an influence on the thermoregulation, as the presence of brood provides a strong stimulus for the bees to heat (Koeniger 1978; Kronenberg and Heller 1982) and pheromones have been identified to which attract workers and stimulate thermoregulation behaviour. However, the temperature profiles in the experiments with artificially re-capped cells were not reduced at all in our experiments in comparison to those with naturally capped cells. The

artificially re-capped cells were empty and contained no pupae and hence did not emit a pheromonal signal which might release thermoregulation of workers. The mere removal of 10% of the brood had no detectable negative effect on the overall motivation of the bees to heat the brood.

Given that the heat transfer from an empty cell to the neighbouring sealed brood cells is more efficient than heating via the caps of brood cells, then obviously the workers did not take advantage of this mechanism in our study. Fehler et al. (2007) state that gaps which are present in the brood nest but *not* used for heating do not only bear no benefits in terms of thermoregulation but instead will increase the average incubation time by a factor of up to 1.2. We suggest that the position of a bee on the comb, if sitting on a brood cell's cap or in an empty cell, is largely independent of its thermoregulation activities. Workers enter frequently empty cells (van der Blom 1993; Johnson 2002) and a bee will probably start heating if the local temperature is below an individual threshold (Watmough and Camazine 1995; Beshers and Fewell 2001). Sometimes these empty cells will be in the brood nest where a low temperature will release heating behaviour of the bee in the cell. However, we found no evidence of an adaptive behaviour of honeybees intentionally entering cells to enhance their heating efficiency. The usage of gaps for heating might be an accidental event primarily based on those worker bees in search of empty cells (e.g. for sleeping). Once they are exposed to the brood pheromone they then participate in heating with extremely high body temperatures exceeding 42°C well beyond the optimal brood temperature. This might be an inevitable pattern in large groups of bees where there are always sufficient workers in search of empty cells. In the end however, the sheer number of bees on the comb surface will contribute the main heating energy. It should be remembered that gaps in the brood nest do have a considerable fitness cost to the colony and 10% gaps translate directly into 10% brood loss. So any increase in heating efficiency suffers a trade off in loss of workers. Our results suggest that gaps in the brood nest do not seem to matter that much in terms of thermoregulation whereas brood loss has been shown to have a significant impact on colony survival (Tarpy and Page 2002).

We therefore conclude that although the phenomenon has in principle a high potential to enhance honeybee worker efficiency, it is not used by the workers in an adaptive way providing another case where evolution has not (yet) shaped an optimal solution but got stuck with a well functioning mechanism.

Figures and legends

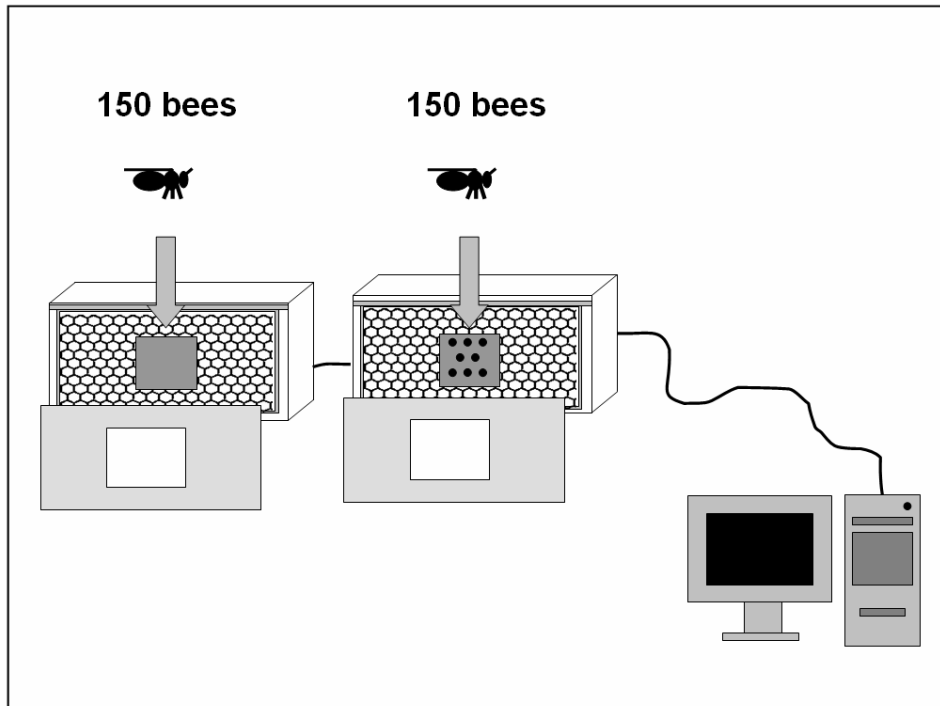


Fig. 1: 150 in-hive bees were caged on a piece of brood comb either with 10% or without empty cells. The temperature distribution on the brood was measured from the backside of the comb, using 256 thermosensors.

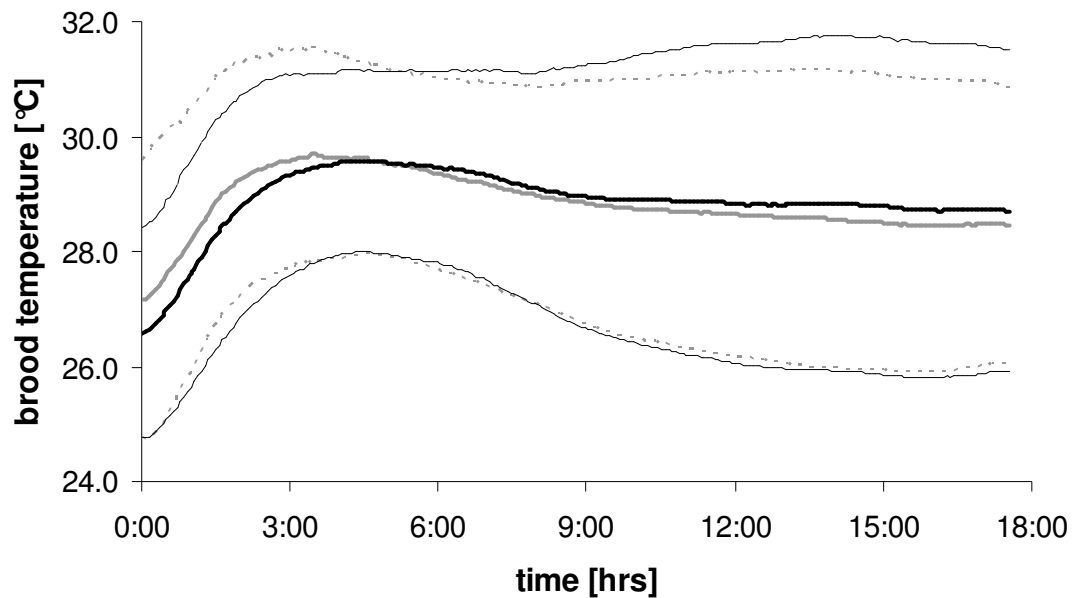


Fig. 2: Mean temperatures of a 7x7cm brood piece placed in a single test comb and generated by 150 bees. Black lines: brood nests with 10% gaps, grey lines: without gaps, dotted lines: $\pm$ SD (N=46).

Acknowledgement

We thank Marco P. Mesiano for helping to record the temperature data and the Deutsche Forschungsgemeinschaft (Mo373/19) for funding.

Declaration

The authors declare that they have no conflict of interest. All experiments performed in the present study comply with the current laws of Germany.

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CHAPTER 4

Pupal developmental temperature and behavioral specialization of honeybee workers (*Apis mellifera* L.)

Journal of Comparative Physiology A (2009) 195:673–679

DOI 10.1007/s00359-009-0442-7

Received 12 November 2008 - Revised 6 April 2009 - Accepted 9 April 2009

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Abstract

Honeybees (*Apis mellifera*) are able to regulate the brood nest temperatures within a narrow range between 32°C and 36°C. Yet this small variation in brood temperature is sufficient to cause significant differences in the behavior of adult bees. To study the consequences of variation in pupal developmental temperature we raised honeybee brood under controlled temperature conditions (32°C, 34.5°C, 36°C) and individually marked more than 4400 bees, after emergence. We analyzed dancing, undertaking behavior, the age of first foraging flight, and forager task specialization of these workers. Animals raised under higher temperatures showed an increased probability to dance, foraged earlier in life, and were more often engaged in undertaking. Since the temperature profile in the brood nest may be an emergent property of the whole colony, we discuss how pupal developmental temperature can affect the overall organization of division of labor among the individuals in a self-organized process.

Keywords

Honeybee - brood temperature - foraging - division of labor – self-organization

Abbreviations

AFF Age of first foraging flight
JH Juvenile hormone

Introduction

Mechanisms regulating division of labor are decisive for the effective functioning of social insect colonies. The caste system (sensu Wilson 1971), typical for all eusocial insects, results in the most obvious division of labor with the queen specializing in reproduction and sterile workers performing other tasks to maintain the colony. However, there is usually also specialization within the worker caste. In honeybees *Apis mellifera*, division of labor among workers is realized as a temporal polyethism, which has been studied in detail since the pioneering work of Rösch (1925). The individual development of a honeybee worker starts with in-hive tasks, including cell cleaning, comb building and brood tending, and ends with the outside tasks such as guarding and foraging (Winston 1987). These changes of behavioral patterns go along with fundamental changes in the physiology of workers including gland activity, juvenile hormone (JH) titers and levels of biogenic amines (Huang et al. 1994; Robinson and Vargo 1997; Schulz and Robinson 1999; Wagener-Hulme et al. 1999; Sullivan et al. 2000; Schulz et al. 2002; Deseyn and Billen 2005). In addition to a large suite of social interactions among individuals and reactions to intracolony as well as environmental conditions (Robinson 1992), also genetic variance has been identified to be an important factor for task specialization in honeybees. Workers of specific subfamilies preferentially participate in specific tasks, such as foraging for nectar, pollen (Robinson and Page 1989; Fewell and Page 2000) and water (Kryger et al. 2000). The genetic mechanisms regulating pollen and nectar foraging have been analyzed down to the gene level, suggesting that a few major loci can have strong effects on the forager's specialization (Page et al. 2000; Hunt et al. 2007). As the environment of a social insect is largely determined by the behavior of its nestmates, also indirect genetic effects will play a role in the behavioral differentiation of the individuals. This means, that the phenotype of an individual is influenced by the expression of genes in a conspecific individual (Wolf et al. 1998; Linksvayer 2006).

One example for genetically controlled threshold variance has recently been shown for temperature regulation in honeybees (Jones et al. 2004). Honeybee colonies maintain an accurate thermal homeostasis in the brood nest of the colony. Worker bees are able to regulate the brood nest temperature between 33°C and 36°C (Seeley 1995) by either heating through flight muscle activity, or cooling through ventilation and evaporation of water. The regulation of hive temperature primarily depends on the number of workers expressing heating behavior rather than the intensity of individual heating (Southwick 1982; Kronenberg and Heller 1982). The workers keep the temperature fluctuations in the brood nest sufficiently low to ensure appropriate conditions for larval and pupal development (Himmer 1927; Lindauer 1954;

Harrison 1987). Nevertheless, there is temperature variation in the brood nest which varies within a range of 3°C. Though this small variance does not obviously affect morphological traits in wings, stinger, proboscis or legs of workers (Himmer 1927; Groh et al. 2004), behavioral traits of adult workers as well as the synaptic organization of their brains have recently been shown to be substantially affected by pupal developmental temperature variation (Tautz et al. 2003; Groh et al. 2004; Jones et al. 2005). If pupal developmental temperature affects behavior and brain function, this might also interfere with the overall division of labor among the workers, and thus be a central and far reaching mechanism to fine-tune colony organization.

In this study, we analyze the influence of the nest temperature during the pupal phase on the behavioral repertoire of the adult bees. We particularly focus on the transition from in-hive to foraging tasks as the most drastic, and best studied change in worker behavior (Beshers et al. 2001) to reveal the potential impact of nest temperature on worker specialization.

Methods

Honeybee workers

Experiments were conducted in the summers of 2004 and 2005 in Halle/Saale (Germany). In both years, three brood combs of *A. mellifera carnica* with capped worker cells were transferred from one colony to three different incubators set to 32°C, 34.5°C and 36°C respectively each at 60% r.h. Larvae in uncapped cells left the comb after a few hours and were then removed from the rearing box. The pupae were reared in the incubators until emergence of the adult bees. The duration of controlled temperature treatment for individual pupae thus lasted for a minimum of six days to a maximum of 12 days, depending on the initial age of the pupae. Pupae on the same comb emerged within a period of not more than six days. Once a day, newly emerged worker bees (aged 0-24 h) from the incubated combs were individually labeled each with a numbered color tag (Opalith-Plättchen) and immediately released into the observation hives. One week later, a second set of three brood combs from the same colony was transferred to the incubators under the same conditions.

In 2004 we released 2374 individually labeled workers (965 workers raised at 32°C, 900 at 34.5°C, and 509 at 36°C) over a period of 12 days into three queenright foster colonies in observation hives with two combs and about 2000 unlabeled worker bees. All observation hives contained equal numbers of target bees of all temperature treatment groups. The observation colonies were placed inside the laboratory and connected to the outside by a trap system, which allowed to easily catch arriving or departing bees. In a replicate experiment in

the following year, we used the same setup, but introduced 753 workers raised at 32°C, 714 workers at 34.5°C and 564 workers at 36°C into two observation hives. All brood combs originated from the same donor colony in both years.

Observations

We drew a grid (40 quadrants, 8 x 7.5 cm) on each side of the observation hives and consecutively scanned all quadrants from left to right and top to bottom. Three daily observation scans were performed in parallel in all colonies by two observers per colony, each scan lasting about half an hour. In the experiments in 2004, we recorded various activities of inhive bees, including cell inspection, fanning, grooming, retinue behavior, trophallaxis, moving, and being inactive. Alas, since none of these traits yielded differences between the treatment groups we focused our subsequent studies in 2005 on the transition from inhive to outdoor worker and on activities of foragers. We recorded the behavior of all sighted marked bees on the combs, and on their way out of and into the hive and focused our behavioral analyses on dancing, undertaking, and foraging behavior. Bees were classified as dancers, when they were observed in dancing at least one time anywhere on the combs. We defined those workers as undertakers which engaged in removing dead bees from the hive. Labeled bees returning from a flight were caught in the trap during periods of high foraging activity. Bees carrying pollen pellets were classified as pollen foragers. Nectar foragers were identified by gently squeezing the abdomen, until a droplet of crop content was disgorged. We used qualitative glucose test-strips (Biophan G, Kallies Feinchemie AG, Germany) to assess the glucose concentration in the crop content on the basis of five reaction categories (0 to 4) to distinguish between water and nectar foragers. Foragers with a negative or the least positive reaction (0, 1) were classified as water foragers, all others (2 - 4) as nectar foragers. We continued these observations for 18 days.

Statistical analyses were conducted with analyses of variance and χ^2 -tests using the Statistica® and the R software packages. Normality of the data was tested with a Kolmogorov-Smirnow test.

Results

1) Proportion of dancers

There was no significant effect of the host colony environment on dancing ($\chi^2 = 5.9$; $p = 0.20$; $N = 1577$; $df = 4$) but we found a significant correlation between the proportion of dancers

and the treatment temperature (Spearman $R = 0.64$; $p = 0.01$; $N = 15$) (Fig 1). Higher developmental temperatures significantly increased the proportion of dancers observed in the five test colonies. Altogether, we found 14 dancing bees out of 549 bees observed in outdoor activities in the 32°C treatment group, 23 dancers out of 545 outdoor bees in the 34.5°C group, and 26 dancers out of 483 outdoor bees in the 36°C group.

2) *Age of first foraging*

In total, 1577 workers were observed in outdoor activities (2004: 547, 2005: 1030). 549 workers were from the 32°C temperature group (232 in 2004, 317 in 2005), 545 from the 34.5°C group (206 in 2004, 339 in 2005) and 483 from the 36°C group (109 in 2004, 374 in 2005). The age of first foraging could be determined for 1262 individuals. We assessed the influence of the host colonies on worker behavior with a factorial ANOVA using “age of first flight” as dependent variable and pupal developmental temperature, year and hive as categorical predictors.

We found significant differences for the age of first flight among the three temperature treatments ($F = 4.3$, $p = 0.0014$, $N = 1262$, $df = 2$), among the two test seasons in 2004 and 2005 ($F = 139.2$, $p < 0.001$, $N = 1262$, $df = 1$), and among the colonies within years (2004: $F = 11.1$, $p < 0.001$, $N = 241$, $df = 2$, 2005 ($F = 83.0$, $p < 0.001$, $N = 1021$, $df = 1$). The interaction between hive and pupal developmental temperature was not significant (ANOVA: in 2004: $F = 1.2$, $p = 0.33$, $N = 241$, $df = 4$; in 2005: $F = 1.1$, $p = 0.33$, $N = 1021$, $df = 2$). Thus, the effect of pupal developmental temperature on the age of first foraging did not differ between the host colonies.

Focussing on the age of first foraging within the colonies, we found a highly significant negative correlation of brood temperature and the age of the first observed foraging flight for both colonies in 2005 and also in 2004 there was a trend for a negative correlation in two out of three colonies (Tab. 1). In all colonies, the bees reared at 36°C had the earliest onset of foraging, whereas the bees reared at 32°C were the latest to start foraging in four of five colonies (Tab. 1). In a pooled analysis of the data of 2004 and 2005 using the deviation from the the mean age of first foraging in the corresponding year for each individual we found a highly significant correlation between treatment temperature and age of first foraging (Fig. 2 Spearman $R = -0.12$; $p = 0.00002$; $N = 1262$).

3) Undertaking

A worker was classified as an undertaker if it was observed removing a dead bee from the colony. Undertaking was an extremely rare behavior in all three observation colonies (only recorded in 2004). Only 20 cases of undertaking out of 7006 individual behavioral observations were recorded. The proportion of undertaker bees was not significantly different among the three host colonies tested in 2004 ($\chi^2 = 4.4$; $p = 0.11$, $N = 547$; $df = 2$). Pooling the data from the three colonies, we found that the frequency of undertakers in the 36°C group was about three to five times higher than in the other two groups (Tab. 2) resulting in a significant difference ($\chi^2 = 8.5$, $p = 0.014$; $N = 547$; $df = 2$).

4) Forager task specialization

We observed a weak preference of 36°C reared bees for water foraging, a slightly raised frequency of 34.5°C reared bees for pollen collection and the 32°C reared bees showed higher frequencies for nectar collection or returning empty from foraging trips (Tab. 3). The developmental temperature was negatively correlated with the proportion of nectar foragers in the five colonies (Spearman $R = -0.55$; $p = 0.03$; $N = 15$). No correlations were detectable for water foraging, pollen foraging and bees returning empty (water foragers: $R = 0.36$, $p = 0.19$, $N = 15$; pollen foragers: $R = -0.04$, $p = 0.89$, $N = 15$; empty bees: $R = 0.32$, $p = 0.24$, $N = 15$). To test, whether a non monotonous relation between developmental temperature and foraging preference might exist, we used the χ^2 -statistics. An overall χ^2 -test yielded no significant differences ($\chi^2 = 6.2$, $df = 6$, $p = 0.40$; $N = 368$). Based on hypotheses pointed out in the discussion, we compared the 36°C water foragers against the 32°C and 34.5°C water foragers and we compared the 34.5°C pollen foragers versus the 32°C and 36°C pollen foragers without significant results. However, we found a significantly increased number of nectar foragers and foragers returning empty in the 32°C temperature group compared to those of the 34.5°C and 36°C treatment group ($\chi^2 = 4.0$; $p < 0.05$; $N = 368$; $df = 1$).

Discussion

Active regulation of hive-temperature by adult worker bees is supposed to provide optimal conditions for brood rearing (Himmer 1927). However, in spite of this high capability to maintain colonial homeostasis there is clearly no perfectly uniform and even temperature distribution in the colony's brood nest (Levin and Collison 1990; Kraus et al. 1998). The temperature in the colony is often characterized by steep gradients depending on the size of the colony and the ambient temperature (Southwick and Heldmaier 1987; Fahrenholz et al.

1989). Bees in the periphery can be much cooler than in the centre of the nest. Moreover, the temperature distribution can be highly patchy, as workers are able to generate heat in empty cells scattered over the brood nest and thereby efficiently warm the brood in the adjacent cells (Kleinhenz et al. 2003). Irrespective how brood temperature varies, our findings suggest that even small deviations in the brood nest temperature may have global effects on the social organization of the colony.

Dance frequency

Worker bees reared at 32°C showed a reduced frequency of dancers compared to the other test-groups. These results support the findings of Tautz et al. (2003), who also regarded bees developed under 32°C as “bad dancers”, as they performed less dance circuits and had a increased variance in the duration of the waggle dance in comparison to the 36°C group.

Thus again, the specialization of adult workers depended on the temperature regime during their pupal phase. Variation in environmental conditions therefore caused variation of the behavioral performance and resulted in division of labor among the workers.

Age of first foraging

Brood rearing temperature also had significant effects on the transition from in-hive to outdoor workers. An increased pupal developmental temperature reduced the age of first foraging. Brood temperature therefore adds to the already known factors regulating the onset of foraging. Besides genotypic variability for age polyethism (Calderone and Page 1988; Giray and Robinson 1994; Pankiw and Page 2001), and behavioral plasticity due to social factors and colony age demography (Huang and Robinson 1992; Huang and Robinson 1996), also pheromonal effects have been suggested to influence the rate of behavioral development (Pankiw 2004). Workers start foraging at older ages if they are exposed to increased concentrations of queen mandibular gland pheromone (Pankiw et al. 1998) or to ethyl oleate, produced by foragers (Leoncini et al. 2004). Similar effects were observed when bees were treated with brood pheromone (Le Conte et al. 2001). In general, the physiological interplay of vitellogenin and juvenile hormone (JH) titers seems to be an essential element driving task specialization in honeybees (Amdam et al. 2004; Amdam et al. 2006).

Particularly the transition from in-hive worker bee to outdoor forager is well understood and closely associated with increased JH titers (Huang et al. 1994). JH has been suggested to act as “behavioral pacemaker” (Robinson and Vargo 1997; Sullivan et al. 2000) and is supposed to be responsible for the onset of foraging. Similar to JH, levels of the biogenic amine

octopamine influence the onset of foraging but probably acting on a shorter timescale (Schulz et al. 2002). Treatment of young bees with the biogenic amine octopamine resulted in precocious foragers (Schulz and Robinson 2001) and it may well be that pupal developmental temperatures interfere with JH metabolism. Huang and Robinson (1995) were able to show a close relationship between temperature and JH biosynthesis by moving colonies into a cold-room. Foragers had significantly reduced JH titers after this treatment. Accordingly, low pupal developmental temperatures might lead to reduced JH biosynthesis rates of the adult bees, cause a delayed behavioral development and hence a later onset of foraging.

Foraging preferences

If pupal developmental temperature interferes with JH metabolism and/or octopamine levels in the bee's brain, then foraging preferences of bees reared at higher temperatures should be similar to those treated with octopamine or the JH analogue methoprene. Pankiw and Page (2003) showed that treatment with octopamine and methoprene both reduced the sucrose response thresholds. They further examined the sucrose response threshold for the different forager groups and found that water foragers had the lowest thresholds, followed by pollen foragers. Nectar foragers had higher thresholds and workers returning empty had the highest response thresholds for sucrose (Pankiw and Page 2000). Thus the response thresholds of foragers increase in the following order: water < pollen < nectar < empty foragers. In light of these findings it may well be that our temperature treatment interfered with octopamine or JH titers in the developing pupae pathways, causing the observed differences in task specialization. We would then expect the foragers with highest developmental temperatures to preferably collect water, bees with medium developmental temperatures to forage pollen, and workers developed under cool conditions to forage for nectar or return empty from foraging trips. These predictions match our data with a negative correlation of developmental temperature and the proportion of nectar foragers, and with the highest frequency of nectar foragers and empty bees in the 32°C treatment group. The number of pollen foragers in the 34.5°C group and the number of water foragers in the 36°C group were higher than expected, however not significantly. Hence, the effect of pupal developmental temperature on foraging specialization was not very strong but significant for some aspects in our data set.

Other differentiation mechanisms including genetic variance within the colony due to the multiple matings of the queen may have a stronger effect on behavioral thresholds and on what a forager is collecting than the developmental temperature. Nevertheless, we conclude

that temperature during pupal development has quantifiable consequences for the behavior of adult workers and may influence task allocation on the colony level: Temperature dependent differences in the behavioral development from one to two days represent about 5% to 10% of an individual's total time spent for in-hive duties. Hence in a colony of 40000 workers, this effect would result in enhancing the in-hive workforce by 2000-4000 individuals at the expense of the foraging tasks.

It may therefore well affect the global structure of division of labor in the colony and social regulation as a whole, because it interferes with the crucial transition from in-hive to outdoor activities. Together with age polyethism and genetic variance, the thermal profile in the brood nest may therefore be a possible parameter when studying the regulation of division of labor in insect societies. As the colony grows so will the brood nest, and the number of in-hive bees heating the brood could affect the pace of behavioral development in the next generation of workers. This sets the stage for reinforcing feedback loops influencing the proportion of in-hive workers. A low number of heating bees will result in lower brood nest temperatures and might induce a delay in the behavioral development in the forthcoming worker generation – more in-hive bees available for heating the brood would then be the consequence. Hence, brood temperature might be one factor to fine-tune division of labor via self-organized patterns among the honeybee workers.

Acknowledgements

We thank the DFG for funding (RM) and K. Dahlke, T. Janik, C. Opitz, T. Schnelle, M. Ellis, C. Nossol, A. Schmidt and M. Thoss for helping with the behavioral observations and the anonymous reviewers for helpful comments on the manuscript.

All our experiments complied with the current German laws and also with the "Principles of animal care", publication No. 86-23, revised 1985 of the National Institute of Health

Figurs and legends

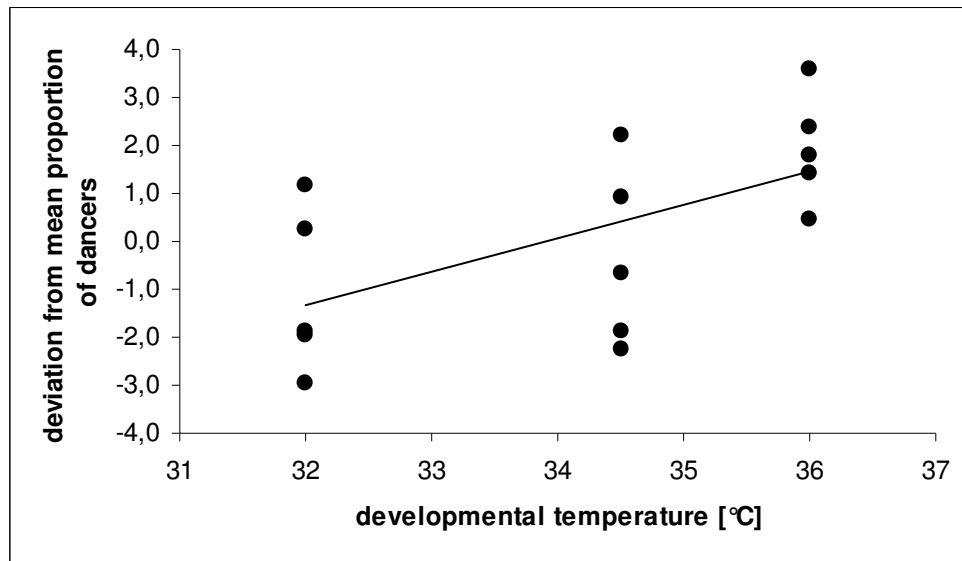


Figure 1 Relationship between dancing and the developmental temperature. Shown on the y-axis is the deviation from the mean frequency of dancers in each hive. We found a significant, positive correlation (Spearman $R = 0.64$; $p = 0.01$; $N = 15$).

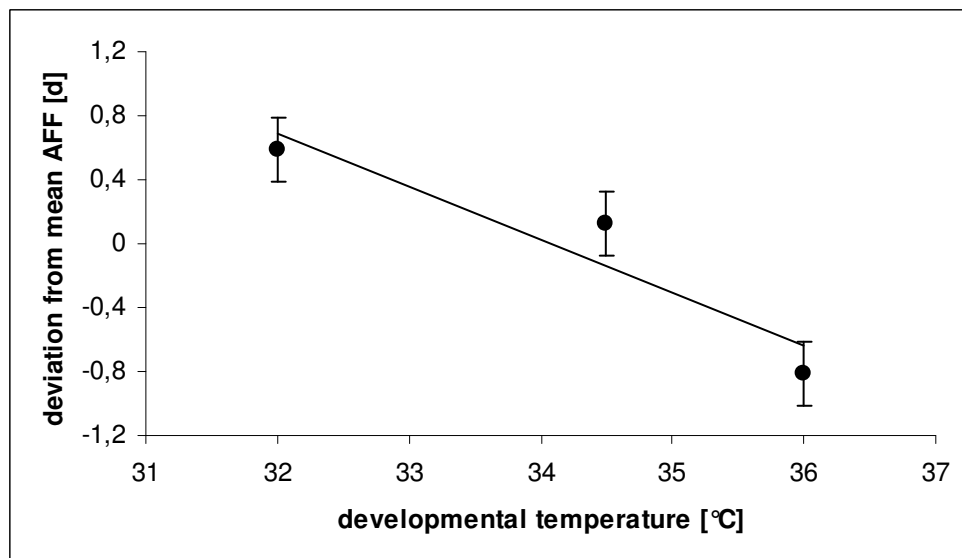


Figure 2 The effect of the brood temperature on the age of first flight (AFF) shown as the deviation from mean age of the first flight in days $\pm$ s.e.

Tab 1: Mean age of first flight (AFF)

	AFF		AFF		AFF		N	
Colony	32°C	N	34.5°C	N	36°C	N	total	p
1	8.6	42	9.2	36	7.8	4	82	0.561
2	13.1	38	12.2	47	12.1	9	94	0.299
3	12.1	32	11.5	28	7.6	5	65	0.072
4	16.9	143	16.8	150	15.4	158	451	0.001
5	14.8	172	14.2	186	13.6	212	570	0.003

Table 1 Mean age in days of first observed foraging flight (AFF) for all five colonies and number of individuals for each treatment group and colony. The p-values correspond to a Spearman rank order correlation. Experiments for colonies 1-3 were conducted in 2004, for colonies four and five in 2005.

Tab. 2: Frequency of undertakers

temperature (°C)	undertakers	other	outdoorundertakers
	N	bees N	%
32.0	7	225	3.0
34.5	4	202	1.9
36.0	9	100	8.3
total N	20	527	547

Table 2 The effect of the pupal developmental temperature on the frequency of undertakers in all outdoor bees.

Tab. 3: Forager task specialization

	32°C	32°C	34.5°C	34.5°C	36°C	36°C
	(observed)	(expected)	(observed)	(expected)	(observed)	(expected)
water	11	15.2	14	13.6	18	14.3
pollen	19	23.7	26	21.1	22	22.2
nectar	51	45.6	35	40.7	43	42.8
empty	49	45.6	41	40.7	39	42.8
N (individuals)	130		116		122	

Table 3 Absolute numbers of water, nectar, pollen foragers and empty bees in each treatment group and the expected values. Foraging loads correspond to foragers response thresholds for sucrose, which are influenced by the bees juvenile hormone titers. If developmental temperature interferes with the juvenile hormone metabolism, we expect a preference for the 36°C bees to collect water, for the 34.5°C bees to collect pollen and for the 32°C bees to collect nectar or to return empty from foraging trips. Testing the 36°C water foragers against the 32°C and 34.5°C water foragers we find no significant difference ($\chi^2 = 1.2$; $p = 0.26$; $N = 368$; $df = 1$). Also the frequency of 34.5°C pollen foragers is not significantly different from the pooled 32°C and 36°C pollen foragers ($\chi^2 = 1.6$; $p = 0.20$; $N = 368$; $df = 1$). However, there is a significant increase of nectar foragers and bees returning empty in the 32°C group compared to the 34.5°C and 36°C group ($\chi^2 = 4.0$; $p < 0.05$; $N = 368$; $df = 1$).

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CHAPTER 5

Brood temperature, task division and colony survival in honeybees: a model

Ecological Modelling (2009): published online DOI: 10.1016/j.ecolmodel.2009.11.016

Received 1 September 2009 - revised 6 November 2009 - accepted 17 November 2009

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Abstract

One of the mechanisms by which honeybees regulate division of labour among their colony members is age polyethism. Here the younger bees perform in-hive tasks such as heating and the older ones carry out tasks outside the hive such as foraging. Recently it has been shown that the higher developmental temperatures of the brood, which occur in the centre of the brood nest, reduce the age at which individuals start to forage once they are adult. It is unknown whether this effect has an impact on the survival of the colony. The aim of this paper is to study the consequences of the temperature gradient on the colony survival in a model on the basis of empirical data.

We created a deterministic simulation of a honeybee colony (*Apis mellifera*) which we tuned to our empirical data. In the model in-hive bees regulate the temperature of the brood nest by their heating activities. These temperatures determine the age of first foraging in the newly emerging bees and thus the number of in-hive bees present in the colony. The results of the model show that variation in the onset of foraging due to the different developmental temperatures has little impact on the population dynamics and on the absolute number of bees heating the nest unless we increase this effect by several times to unrealistic values, where individuals start foraging up to 10 days earlier or later. Rather than on variation in the onset of

foraging due to the temperature gradient it appears that the survival of the colony depends on a minimal number of bees available for heating at the beginning of the simulation.

Introduction

Eusocial insects are characterised by a highly sophisticated division of labour among the members of the colony (Robinson, 1992). In honeybees (*Apis mellifera*), a worker performs different tasks at different ages, starting with in-hive activities like cell cleaning and brood care and ending with foraging outside the hive (Rösch, 1925). This division of labour is called temporal polyethism. The behavioural development of worker bees may be accelerated or retarded due to environmental conditions and intranidal requirements caused for example by changes in the colony age structure (Robinson, 2002; Johnson, 2003).

This behaviour appears to be highly adaptive and plastic because even under extreme environmental variation it results in homeostatic conditions. The collective organization of homeostasis is best illustrated by the colony's ability to regulate the temperature in the hive (Jones and Oldroyd, 2007), which is essential for rearing larvae and pupae. Rearing requires brood temperatures within the narrow range of 32-36°C with a mean of 34.5°C (Hess, 1926; Himmer, 1927; Kronenberg and Heller, 1982). Deviations from this narrow range cause serious malformations in the adult bees. To cool the hive, workers start fanning, evaporate water by tongue lashing or spread droplets of water on the brood, a behaviour that also affects humidity and CO₂ concentration of the air (Lindauer, 1954; Lensky, 1964; Seeley, 1974; Human et al., 2006). Heat is generated metabolically by “shivering” of the flight muscles (Esch, 1960; Harrison, 1987; Kleinhenz et al., 2003). Furthermore, workers can regulate the colony temperature by clustering tightly together. Tightening of the cluster reduces thermal conductance and increases thermal insulation, whereas loosening of the cluster facilitates the cooling of the nest (Owens, 1971; Severson and Erickson, 1990; Stabentheiner et al., 2003). The more workers are available, the bigger the cluster can be, and the larger is the size of the heated region in the colony. As the colony size strongly increases in spring, the differences in the numbers of workers over the season will have a significant influence on the temperature of the brood nest.

The importance of temperature regulation is pervasive. Higher temperatures during their pupal development lead to an increased dancing activity, to better memory in adults (Tautz et al., 2003; Groh et al., 2004), and to a faster behavioural and physiological development, which results in precocious foraging (Becher et al., 2009). This in turn influences the number of bees that are present in the colony during daytime: colonies with higher brood nest

temperatures will have reduced numbers of in-hive bees because workers develop into foragers earlier. A reduced number of in-hive workers in turn will have negative effects on the size and temperature of the brood nest, as only a limited number of larvae can be fed and incubated by each nurse bee. Cooler developmental temperatures however will then extend the in-hive period of the emerging workers and hence increase the number of workers available for heating the nest. Thus, we hypothesise that the variance in the onset of foraging, caused by differences in the developmental temperature, strongly affects the population dynamics and the survival of the colony.

We study this interplay between brood nest size, brood nest temperature and the duration of the in-hive period with a deterministic model that combines the empirical temperature profiles in the brood nest with colony dynamics. We tuned the model with our own empirical data of the temperature distribution on brood combs and of the heat production of foragers and in-hive bees under various group sizes. In the first part of this article we describe the empirical results of temperature measurements. In the second part, we present the model and its results.

I. Empirical data

Methods

Natural temperature distribution on a brood comb

To analyse the temperature distribution on a brood comb we constructed a measurement device with 256 sensors. We recorded one sensor after the other every second, so each complete temperature record lasted 256 seconds (Becher and Moritz, 2009). The sensors were placed on a 15 x 15 cm area and touched the bottom of the cells at the backside of the test comb. The temperature measurement took place in a standard honeybee colony (*Apis mellifera*). The colony contained four frames with about 3000 workers and a laying queen. It was kept at room temperature (25°C) in the laboratory, with a flight entrance connecting to the outside. The queen readily laid eggs in the test comb, and size and position of the brood nest was verified at the end of the experiments. As workers had access only to one side of the comb, temperatures were about 1.5°C lower than under natural conditions.

Temperature gradient and number of heating bees

To determine, whether the temperature gradient, is influenced by the number of heating bees, we studied groups of various sizes (50 – 250 workers). In-hive bees were collected from the brood nest of a donor colony as follows.

We brushed bees in the daylight from a brood comb to the ground. Whereas older bees flew up, younger in-hive bees showing a negative phototactic behaviour were crawling in a dark box provided to the bees. Only those young bees were used for the experiments.

The in-hive bees were confined to the 15cm x 15 cm area of the empty test comb, where the temperature measurement took place. A square piece of capped brood containing either 100 or 200 cells was inserted into the centre of the comb. We recorded the temperature distribution for 20 hours. The temperature gradient was calculated as the temperature difference between the hottest and coldest sensor in the brood area, divided by the distance of these sensors, averaged over the last 30 time steps (2h 08min).

Contribution of foragers and in-hive bees to the brood temperature

To test for differences in the heat production by foragers and in-hive bees, we used a similar experimental setup as described for the analysis of the temperature gradient. Groups of 150 bees from a donor colony either collected at the flight entrance (foragers) or from the brood nest using negative phototactic behaviour (in-hive bees), were confined on the test comb of our temperature measurement instrument. Heat production was measured on a piece of capped brood (100 cells). Experiments took place at 25°C ambient temperature and lasted for ten hours. Bees were supplied with honey *ad libitum*.

Heat production of in-hive bees

To determine the capability of actual heat production of a single bee, we used the experimental setup described above to study various groups of in-hive bees on 200 capped brood cells on the test comb. We studied the group at room temperature (ca. 25°C) in the dark to reduce the disturbance of the bees. We recorded the ambient temperature as well as the position of the bees on the comb using an infrared camera. The heat, produced by single bee was calculated as

$$\text{Heat per bee}(t) = \frac{(\text{mean broodnest } T(t) - \text{ambient } T(t)) \cdot N \text{ broodcells}}{N \text{ heating bees}(t)} \quad (1)$$

Empirical results

Temperature distribution on a brood comb

Fig. 1 shows the temperatures of a transect through the central brood nest. We found an even temperature distribution in the core of the brood nest. Beyond this core area, the temperature linearly decreased with a slope of $0.45^{\circ}\text{C}/\text{cm}$. Brood cells were not only present in the well heated core area, but also at the cooler edge.

Temperature gradient and number of heating bees

We found no correlation between the size of the group and the slope of the temperature gradient (Spearman rank order: 2007: $R = 0.31$, $p = 0.45$, $N = 8$; 2008: $R = 0.15$, $p = 0.73$, $N = 8$) (Fig. 2). Thus, the slope of the temperature decrease at the edge of the brood nest was in our experimental setup independent of the number of bees.

Contribution of foragers and in-hive bees

Compared to foragers in-hive bees showed a significantly higher heat production resulting in in-hive temperatures above 31°C (Whitney-Mann U-test: $p = 0.04$, $N_{\text{in-hive}} = 4$, $N_{\text{Forager}} = 4$). Foragers maintained the brood nest temperature between 28°C and 29°C (Fig. 3).

Heat production of in-hive bees

We calculated that a single bee could increase the temperature in a single cell by 26.5°C . This temperature increase was reached within six hours and then held constant for another six hours (Fig. 4). We conclude that the heat production of a single bee allows her to raise the temperature of 2.65 cells by 10°C . These 10°C reflect the temperature difference in the model between the ambient temperature (25°C) and the optimal temperature (35°C). Thus a single heater bee in the model is able to maintain the optimal nest temperature for 2.65 brood cells

II. The Model

General Description of the Model

The model represents the dynamics of a colony on a single brood comb of an unlimited size. We assume three distinct life phases of every worker bee: the brood phase lasting 21 days, the in-hive phase of 15 days and the foraging phase of 10 days. The number of individuals at the first day of the brood phase equals the number of eggs being laid by the queen. During each

time step all individuals age one day and at the end of the foraging period, they die. The temperature distribution in the brood nest is determined by the total number of heating bees and by the number of brood cells. Brood nest temperatures are recorded in the model and determine the proportion of hot, medium tempered and cold bees at emergence. We give bees that develop in cells with a mean temperature below a certain threshold (so-called ‘cold’ bees) a prolonged in-hive period, those in cells with a temperature above this threshold (called ‘medium tempered bees’) a normal onset of foraging and 10% of the medium tempered bees (so-called ‘hot bees’) we induce to forage precociously (Tab. 1).

Initial colony situation

The comb is represented in the model as an unlimited, two-dimensional plane with brood on one side only. The colony starts without brood with $N_INITIAL$ WINTERBEES. The WINTERBEES represent those that overwintered and which are characterised by a very long lifespan. They do not develop further but they perform both the task of brood heating as well as foraging (not explicitly included into the model). These WINTERBEES die at a constant rate within 100 days .

$$\begin{aligned} \text{if } t \leq 100 \text{ then } N_WINTERBEES(t) &= N_INITIAL - \text{round}\left(\frac{t \cdot N_INITIAL}{100}\right) \\ \text{if } t > 100 \text{ then } N_WINTERBEES(t) &= 0 \end{aligned} \quad (2)$$

The temperature distribution

The temperature distribution on the comb depends on the number of heating bees (‘HEATERS’):

$$N_HEATERS(t) = N_WINTERBEES(t) + TOTAL_INHIVE_BEES(t) \quad (3)$$

We assume that the temperature in the core of the brood nest is uniform and constant at $T_OPTIMAL$ of 35°C. The maximum number of brood cells at $T_OPTIMAL$ (the so-called $MAX_OPTHEAT_CELLS$) depends on the number of bees that heat, and their maximal heating abilities:

$$MAX_OPTHEAT_CELLS(t) = N_HEATERS(t) \cdot MAX_HEATEDCELLS_PER_BEE \quad (4)$$

Beyond this area at $T_OPTIMAL$, the temperature decreases linearly following the parameter $TEMP_GRADIENT$ (°C/cm) as observed in the empirical data.

HEATERS reduce the actual number of heated brood cells to the minimum, which means, that only the brood nest and empty cells for the subsequent egg laying are heated properly.

Hence, as long as the brood nest temperature is not limited by the maximal heating abilities of the heating bees, the area with a temperature $\geq T_EDGE$ (32°C) contains $N_BROODCELLS$ brood cells and some empty cells. These empty cells are necessary for a continuous egg laying, as the queen lays eggs only in cells with a temperature of at least 32°C. The number of these empty cells equals the maximum number of eggs that can be laid by the queen on one day (= $MAX_EGGLAYING_RATE$). It is set to 1500 eggs per day. Given that there are enough $HEATERS$ to generate the required temperatures we calculate the actual number of cells with a temperature of $T_OPTIMAL$ (35°C) in a way that the above condition is fulfilled. The number of optimally heated cells is represented by the parameter $N_OPTHEAT_CELLS(t)$. This method ensures, that always some brood cells have temperatures below the optimum, even if the colony is large in relation to the brood nest.

Hot, cold, and medium tempered brood

We calculate the radius of the optimally tempered core region of the brood nest as follows:

$$RADIUS_OPT_T(t) = ((N_OPTHEAT_CELLS(t) \cdot CELLSIZE) / \pi)^{1/2} \quad (5)$$

The radius of medium tempered area, i.e. the area where the brood temperature is higher than the T_COLD threshold is then calculated as:

$$RADIUS_MEDIUM_T(t) = RADIUS_OPT_T(t) + \frac{T_OPTIMAL - T_COLD}{TEMP_GRADIENT} \quad (6)$$

with $TEMP_GRADIENT$ as the slope of the linear temperature decrease beyond the optimally heated area, set to 0.45°C/cm.

This allows us to calculate the number of the medium tempered and cold broodcells:

$$N_MEDIUM_T_CELLS(t) = \frac{\pi \cdot (RADIUS_MEDIUM_T(t))^2}{CELLSIZE} \quad (7)$$

$$N_COLD_T_CELLS(t) = N_BROODCELLS(t) - N_MEDIUM_T_CELLS(t) \quad (8)$$

Egg laying and brood nest

The number of new eggs laid per day is determined by the maximal egg laying rate (1500 eggs per day) and the number of empty brood cells in the potential brood nest, i.e. the area with a temperature $\geq T_EDGE$ (32°C).

$$EMPTY_CELLS(t) = \frac{\pi \cdot (RADIUS_T_EDGE(t))^2}{CELLSIZE} - N_BROODCELLS(t) \quad (9)$$

$RADIUS_T_EDGE(t)$ is the radius of the T_EDGE isotherm (32°C). Then the number of new laid eggs at timestep t is:

$NEW_EGGS(t) = EMPTY_CELLS(t)$
if $NEW_EGGS(t) > 1500$ **then** $NEW_EGGS(t) = 1500$
if $NEW_EGGS(t) < 0$ **then** $NEW_EGGS(t) = 0$ (10)

The number of brood cells is calculated as follows:

for $t=1$: $N_BROODCELLS(t) = NEW_EGGS(t)$
for $t>1$: $N_BROODCELLS(t) = N_BROODCELLS(t-1) + NEW_EGGS(t)$
 $- FROZEN_EGGS(t) - EMERGED_allT(t)$ (11)

$FROZEN_EGGS$ may occur, if the number of $HEATERS$ decreases due to the dying of $WINTERBEES$ or because too many $INHIVE_BEES$ developed into foragers. If the temperature at the edge of the brood nest falls below the critical threshold $T_FREEZING$ ($31^{\circ}C$), then brood cells will be lost due to freezing. $N_FROZEN_EGGS(t)$ is then calculated as:

$$N_FROZEN_EGGS(t) = \frac{\pi \cdot (RADIUS_BROODNEST(t)^2 - RADIUS_T_FREEZING(t)^2)}{CELLSIZE} \quad (12)$$

$RADIUS_BROODNEST(t)$ is the radius of the brood nest and $RADIUS_T_FREEZING(t)$ is the radius of the $T_FREEZING$ isotherm ($31^{\circ}C$) in timestep t . The number of frozen brood is subtracted from $N_BROOD(age, t)$, starting with the youngest age cohorts.

Brood development and Emergence

The number of brood at a given age is calculated as:

for $age = 1$: $N_BROOD(age, t) = NEW_EGGS(t)$
for $1 < age < 21$: $N_BROOD(age, t) = N_BROOD(age - 1, t - 1)$
for $age = 21$: $N_BROOD(age, t) = 0$ (13)

The number of newly emerged $INHIVE_BEES$ is computed from the number of brood in the oldest brood cohort:

$$EMERGED_allT(t) = N_BROOD(age = 20, t - 1) \quad (14)$$

The developmental temperature

To determine the proportion of $INHIVE_BEES$ developed under hot, medium, or cold conditions, the proportion of medium tempered and cold brood cells in each timestep is averaged over the complete brood development period, resulting in the parameter $PROP_MEDIUM_DEV_T$. As the model does not provide temperatures above the $T_OPTIMAL$ ($35^{\circ}C$), we derive the number of hot cells from the number of cells with

optimal temperature. We make 10% of the $N_MEDIUM_T_CELLS(t)$ into “hot” cells of 36°C.

$$PROP_MEDIUM_DEV_T(t) =$$

$$\frac{\sum_t^{t-21} N_MEDIUM_T_CELLS(t)}{\sum_t^{t-21} N_MEDIUM_T_CELLS(t) + \sum_t^{t-21} N_COLD_T_CELLS(t)}$$

(15)

The number of newly emerged medium tempered $INHIVE_BEES$ is then:

$$EMERGED_medium(t) = round(EMERGED_allT(t) \cdot PROP_MEDIUM_DEV_T(t) \cdot 0.9)$$

(16)

Only 90% of the $N_MEDIUM_T_CELLS(t)$ contribute to the new emerged medium tempered $INHIVE_BEES$. The remaining 10% of the $N_MEDIUM_T_CELLS(t)$ are assumed to be “hot” (36°C).

$$EMERGED_hot(t) = round(EMERGED_allT(t) \cdot PROP_MEDIUM_DEV_T(t) \cdot 0.1)$$

(17)

$$EMERGED_cold(t) = EMERGED_allT(t) - EMERGED_medium(t) - EMERGED_hot(t)$$

(18)

Behavioural development of the adult bees

In the absence of a temperature effect on the behavioural development, the number of $INHIVE_BEES$ is calculated as follows, with a maximum age of 15 days:

$$\text{for age}=1: N_INHIVE_BEES(age, t) = EMERGED_allT(t)$$

$$\text{for } 1 < \text{age} < 15: N_INHIVE_BEES(age, t) = N_INHIVE_BEES(age - 1, t - 1)$$

(19)

The total number of all $INHIVE_BEES$ is hence the summed number of $INHIVE_BEES$ over all age cohorts:

$$TOTAL_INHIVE_BEES(t) = \sum_{age=1}^{age=15} N_INHIVE_BEES(age, t) \quad (20)$$

The number of $FORAGERS$ is calculated on the basis of the oldest in-hive bees cohort:

$$\text{for age} < 15: N_FORAGERS(age, t) = 0$$

$$\text{for age}=15: N_FORAGERS(age, t) = N_INHIVE_BEES(age - 1, t - 1)$$

$$\text{for } 15 < \text{age} < 25: N_FORAGERS(age, t) = N_FORAGERS(age - 1, t - 1)$$

$$\text{for age} \geq 25: N_FORAGERS(age, t) = 0 \quad (21)$$

Influence of the temperature effect on population dynamics

If we include in the model the effect of brood temperature on the behavioural development, the in-hive period is no longer fixed at 15 days but depends on the mean brood temperature an individual was exposed to during its development. The parameter TEMP_EFFECT describes the strength of this temperature effect. A value of zero means no influence of brood temperature (i.e. the same duration of in-hive period for all bees, irrespective of their developmental temperature). A value of one reflects a one day shorting of the in-hive period for hot bees and a prolongation by one day for cold bees, a value of two reduces the in-hive period of hot bees by two days and prolongs it for cold bees by two days etc.

for age= -TEMP_EFFECT+1:

$$N_INHIVE(age, t) = N_INHIVE(age - 1, t - 1) + EMERGED_cold(t)$$

for age=1:

$$N_INHIVE(age, t) = N_INHIVE(age - 1, t - 1) + EMERGED_medium(t)$$

for age= TEMP_EFFECT+1:

$$N_INHIVE(age, t) = N_INHIVE_BEES(age - 1, t - 1) + EMERGED_hot(t)$$

for age<15 and age $\notin \{(-TEMP_EFFECT+1), 1, (TEMP_EFFECT+1)\}$:

$$N_INHIVE_BEES(age, t) = N_INHIVE_BEES(age - 1, t - 1)$$

for age < (-TEMP_EFFECT+1) or age $\geq$ 15:

$$N_INHIVE_BEES(age, t) = 0 \quad (22)$$

Model results

Colony dynamics

The colony dynamics show strong fluctuations in the number of INHIVE_BEES (Fig. 5). These fluctuations occur, because the queen stops egg-laying when no empty cells with a suitable temperature are available. The same pattern repeats itself in the foragers 10 days later. The linear decrease of the colony size in the beginning and after the maximal colony size is reached reflects the continuous loss of WINTERBEES. After 21 days, when the first adults emerge, the colony starts growing. The colony size reaches a steady state, when the number of new emerged INHIVE_BEES per day equals the maximal egg laying rate of the queen. When we increase the maximal egg laying rate of the queen (from 1000 to 2000 eggs per day), this results in proportionally increased colony sizes in the steady state.

Impact of initial colony size

The survival of a colony depends on the initial number of WINTERBEES (Fig. 6). The colony size will only increase, if enough new workers emerge and hence if the number of available brood cells is high enough. This directly depends on the initial number of WINTERBEES. Under the empirically based parameter set ($\text{TEMP_GRADIENT} = 0.45$, $\text{MAX_HEATEDCELLS_PER_BEE} = 2.65$) without temperature effect ($\text{TempEff} = 0$), we find that a minimal number of 3915 initial bees is required for colony survival. We call this value the “survival threshold”, the minimum number of initial bees to ensure colony survival. If this survival threshold is undershot by the initial number of WINTERBEES, the colony is doomed to die. The number of HEATERS in such a colony is too small to provide sufficient heat for a brood nest, that is large enough to maintain the colony size, even if there may temporarily be nearly 20,000 individuals present. Reducing the maximal egg laying rate to 1000 eggs/day decreases the survival threshold to 2283 bees whereas increasing it to 2000 eggs/day increases the survival threshold to 5640 bees.

Influence of the temperature effect

If we include the temperature effect in the simulation, leading to a prolonged in-hive period for cold bees (16d) and a shorter in-hive period for hot bees (14d), we find a reduction in the survival threshold of 27 individuals to 3888 initial bees. By increasing the impact of the temperature effect, we further reduce the minimal number of initial bees, needed for the colony survival (Fig. 7).

Structure of the parameter space

The survival threshold decreases when more cells are heated per HEATER and when the temperature decrease at the edge of the nest is low, so that temperature gradient is flat (Fig. 8). To assess the relation between costs for heating the brood and benefits by a reduction of the colonies survival threshold, we used the parameter „relative gain“, calculated as the percental decrease of the survival thresholds divided by the percental increase of the number of heated cells (respectively the slope of the temperature gradient) when moving through the parameter space (Fig. 9; Fig. 10). As long as the relative gain is above one, it should pay for the colony to increase the heating effort.

Discussion

Model assumptions

To implement the brood nest temperature in the model we used the data from literature (Himmer, 1927; Kronenberg and Heller, 1982) rather than our own slightly lower empirical data because bees were only able to heat on one side of the comb in our experiment. The values for the temperature gradient ($\text{TEMP_GRADIENT} = 0.45$), derived from the colony experiment is lower, than those, measured under the artificial conditions with small group sizes (Fig. 2). Since we estimated the temperature gradient for the experiments with small group sizes, as the temperature difference between the hottest and the coldest cell the value does not reflect the mean but the maximum temperature gradient. We therefore used the values derived from the colony measurements also because they are based on a more extensive data set (763 temperature records within eight days) recorded under the nearly natural conditions, reflecting the mean temperature gradient. Our estimate was very close to the temperature gradient derived from a diffusion model of a honeybee swarm by Myerscough (1993) with a temperature decrease of $0.46^{\circ}\text{C}/\text{cm}$ with 5000 bees at 25°C ambient temperature.

We found no correlation between the number of heating bees and the temperature gradient at the edge of the brood nest (Fig. 2), hence the colony size does not seem to influence the slope of the temperature decrease beyond the core brood nest.

As in-hive bees produced much higher brood temperatures than foragers (Fig. 3), the brood nest temperature in the model is determined by the `INHIVE_BEES` and the `WINTERBEES`, but not by the `FORAGERS`. Moreover, in reality foragers are usually located on the periphery of the combs or close to the flight entrance, but not near to the brood and hence are less involved in heating the brood.

The contribution of a single bee to the thermal profile of the brood nest was not influenced by the group size. We assume that the measured value of 26.5°C per bee represents the maximal heating capability of the bees, since the temperatures on the brood piece were always far below the optimal temperatures of 35°C .

Model results

Colony dynamics

Although we simulated extremely artificial conditions the results match the absolute size and dynamics of real honeybee colonies well. If we assume that the first time step in the model represents initial egg laying in mid February, then a colony starting with 6000 winter bees would peak in the mid of April ($\sim t = 60$), which is close to the actual swarming time of

natural colonies in late April and early May (Winston, 1980). The maximum colony size in the model is about 37,000 WORKERS. Imdorf et al. (1996) report of maximum colony sizes ranging from about 18,000 to about 35,000 workers, but clearly also much larger colonies with more than 60,000 workers can occur in apicultural operations (Farrar, 1937). Schmickl and Crailsheim (2007) present maximum colony sizes of 30,000 to 50,000 bees in their honeybee population model. While colony size naturally depends on a multitude of environmental and intracolony factors, the equilibrium colony size in the model depends mainly on the maximum egg laying rate of the queen. Winston et al. (1981) show the average colony dynamics of three Africanized and European honeybee colonies, starting with ca. 16,000 workers. After 20 days, when the first brood emerges the colonies have lost about 50% of the workers and after 65 days the colony sizes reach the maximum with 20,000 – 25,000 workers.

Initial colony size

According to our model a minimum colony size of 3915 WINTERBEES at the beginning of the simulation was required to ensure the survival of the colony. This resembles empirical data. For instance, beekeepers in Central Europe suggest minimal colony sizes in autumn of 5000 to 7500 workers. Colony sizes in spring are about 75% of the autumn colony sizes (Rosenkranz et al., 2008), which results in about 3750 – 5625 workers for small colonies. Note that these values are probably higher than the minimum colony sizes to avoid colony losses for the beekeepers. Winston (1980) presents data from swarming colonies, with minimum swarm sizes of 3765 individuals and of 3200 in Lee and Winston (1985). The increase of the survival thresholds when the maximal egg laying rate increases seems to be counterintuitive, but can be explained by amplified fluctuations in the number of in-hive bees. A sudden decrease in the number of HEATERS, when a large cohort of IN-HIVE BEES develops into FORAGERS can result in cooling of the brood with lethal consequences for the colony. In reality, a slow increase of the egg laying rate during spring is observed (Allen 1960). Our model suggests that this slow increase of the egg laying rate in empirical data may not only be due to constraints but may also be adaptive because it smoothens the colony growth.

Temperature effect

The shortened duration of the in-hive period due to high developmental temperature had a nearly negligible influence on the resulting survival thresholds under the empirical parameter

set. It reduced the minimum number of initial WINTERBEES necessary for colony survival by only 27 individuals or 0.4%. Therefore, it will have only limited impact on the organization of the overall colony structure. Reducing the proportion of hot bees, fixed in the model to 10% of the medium tempered bees, would gently decrease the survival threshold of the colony, as then more in-hive bees were available due to the later onset of foraging in the cold and medium tempered bees. Increasing the temperature effect more than eight fold leads to a strong variation of age of first foraging but does not decrease the survival threshold any further. This is caused by an overlapping of the generations of in-hive bees. For such large values of the temperature effect (i.e. ≥ 8), the in-hive period of bees developed under cold brood temperatures becomes longer than the duration of the development from egg to the adult bees. This strongly increases the number of in-hive bees present in the colony and guarantees a continuous egg laying.

Parameter space

The survival threshold decreases with increasing number of heated cells per HEATER and with a decreasing slope of the temperature gradient. A larger number of heated cells increases the core region of the brood nest whereas a flattened temperature gradient increases the 32°C to 35°C area of the brood nest. Hence, more eggs can be laid and more INHIVE_BEES will emerge to further increase the brood nest size. However, heat production is costly and the realized temperature distribution will be a result of a trade-off between the benefits of a stronger colony growth and the costs of higher energy expenditure. We did neither include heating costs nor energy income by foraging into the model, but we tried to assess the „relative gain“ of the colony. This is the percentage decrease of the survival threshold divided by the increase of the number of heated cells (respectively the slope of the temperature gradient) when moving through the parameter space. If the number of heated cells per HEATER is below 2.5, then the relative gain (for TEMP_GRADIENT = 0.45, Fig. 9) is clearly above one and hence it should be beneficial for the colony to further increase the heating efforts. The empirical value of 2.65 heated cells per bee the relative gain is close to one hence a further increase in the number of heated cells may seem not efficient.

Analysing the relative gain for 2.65 heated cells per HEATER, we should expect a temperature gradient of about 0.27°C/cm. This is lower than the empirical value of 0.45°C/cm.

Conclusions

Our model is based on very simple assumptions and does not intend to imitate the complex processes of a natural colony and its manifold interactions with the environment. Instead it focuses on the relation between colony size and temperature distribution in the brood nest. In real colonies, eggs are laid on both sides of the comb, and hence the bees can raise twice as much brood as in the model, with only little more heating effort. Usually the brood nest is distributed over several combs. If the same amount of brood is subdivided into several parts distributed over many combs, then the proportion of cells at the edge and hence the proportion of cold brood will be higher. This would possibly increase the influence of the temperature effect. On the other hand, the three-dimensional structure of a honeybee cluster on a real brood nest provides a higher insulation and an increased utilization of the produced heat resulting in more warm cells and reducing multiple edge effects. In any case, since the enhancing of the temperature effect by an order of magnitude well beyond the biological limits did not substantially change our results, it seems that the temperature effect can only have a limited impact on the organization of the division of labour in real colonies, even if the proportion of cold bees in real colonies would deviate from the proportions in our model. Instead, the absolute number of bees available for the heating and nursing processes seems to be the critical factor determining the thriving and survival of the colony.

Acknowledgement

We thank Julia Schröder, Martin Hinsch, Daan Reid and the other members of the Theoretical Biology Group in Groningen for helpful discussions, and all students involved in the record of empirical data (Nadine Hartmann, Judith Kreher, Juliane Mohr, Katja Schönefeld, Stefanie Stöckhardt, Anne Blaner, Christiane Hösel, Anne-Katrin Schwiderke, Alexandra Wölk). We further thank the Deutsche Forschungsgemeinschaft (RFAM) and the European Science Foundation (MAB) for funding.

Figures and Legends

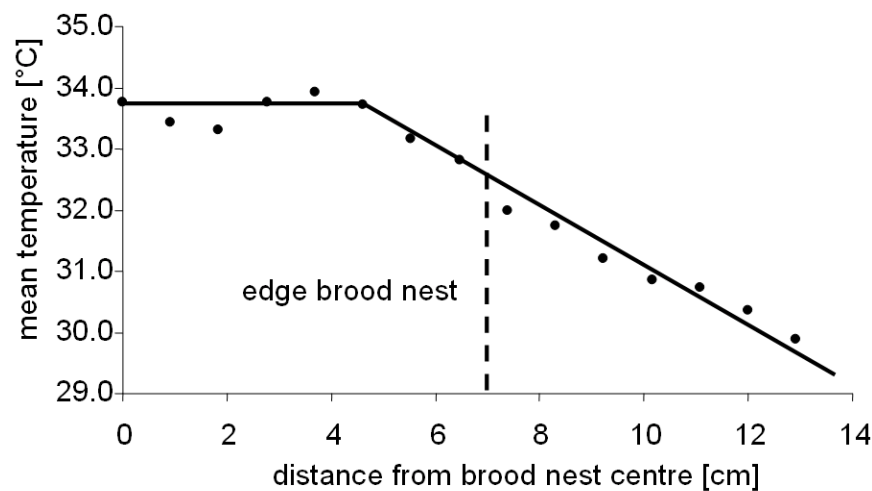


Fig. 1. Empirical temperatures along a transect through a brood nest area. Each data point contains the information of 763 temperature records (data collected between June 16th – 24th 2006 and measured in a common colony at 25°C).

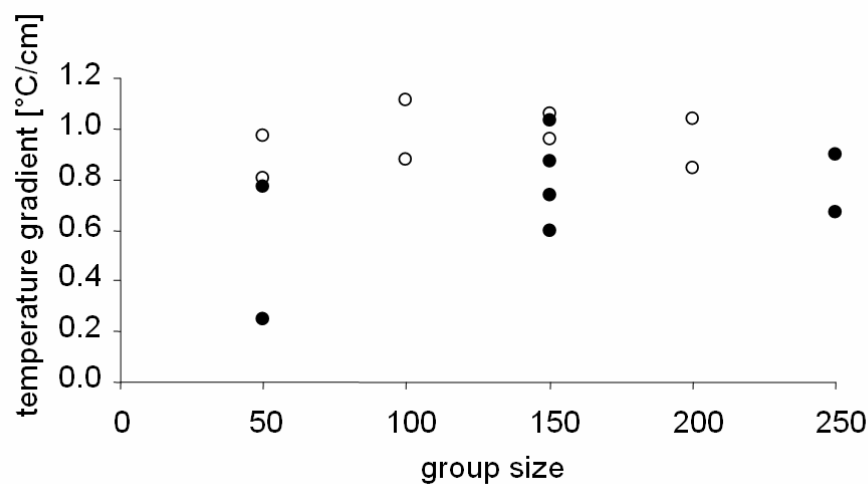


Fig. 2. Temperature gradients under experimental conditions of 100 (●) and 200 brood cells (○).

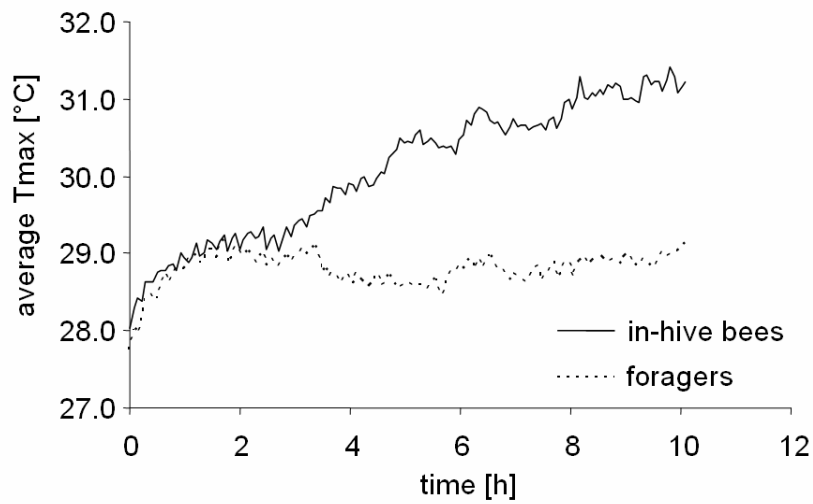


Fig. 3. Maximum temperatures on a piece of capped brood without gaps at ambient temperatures of 25°C produced by 150 test bees ($N_{\text{In-hive}} = 4$, $N_{\text{Forager}} = 4$).

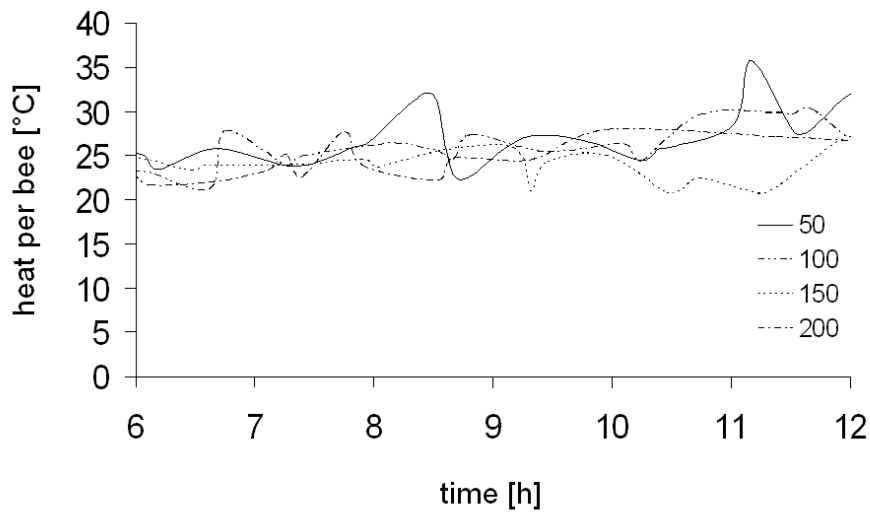


Fig. 4. Empirical heat production by a single worker on a piece of capped brood in groups of 50 to 200 bees. Each data series represents the average values of two replicates.

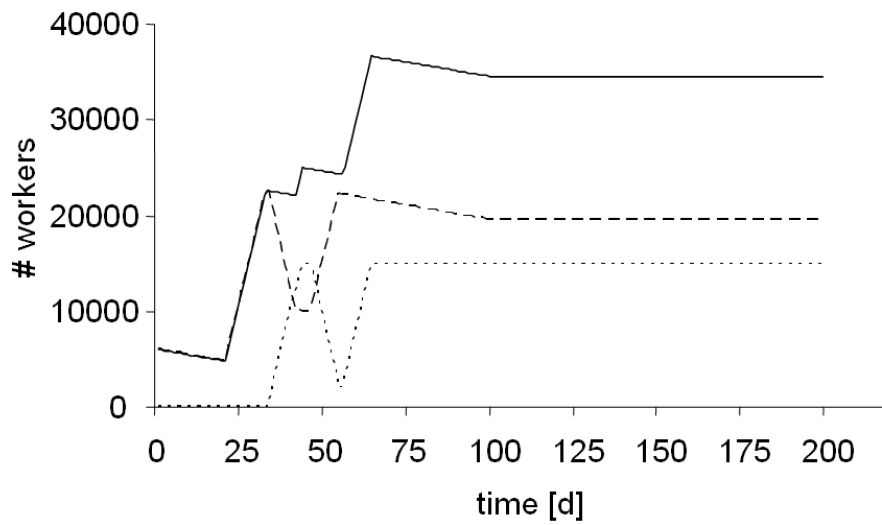


Fig. 5. Colony dynamics in the model starting with 6000 initial bees. The number of in-hive bees including initial bees (dashed line), the number of foragers (dotted line) and the total number of all workers (continuous line) are shown for the parameter set at default values ($TEMP_GRADIENT = 0.45$, $MAX_HEATEDCELLS_PER_BEE = 2.65$, $TempEff = 0$).

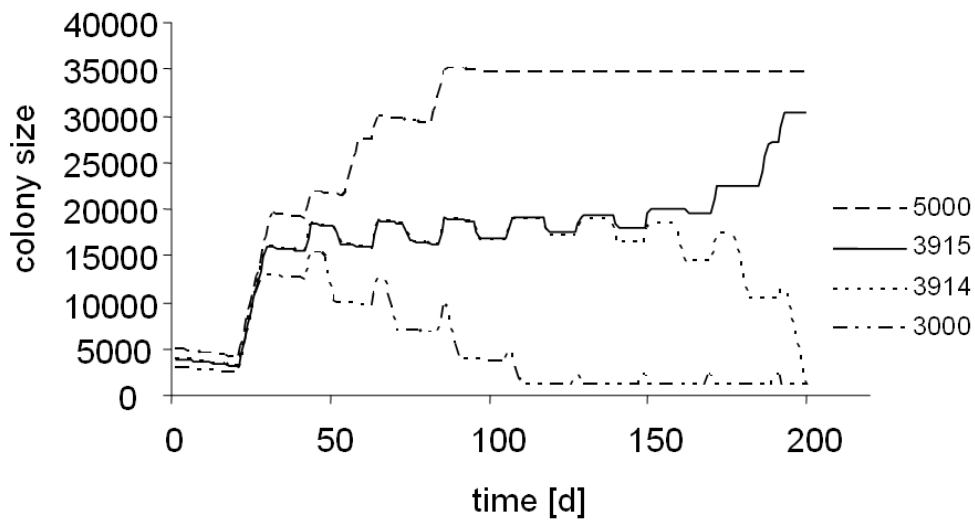


Fig. 6. The model dynamics of colony size (number of workers) for different numbers of initial bees (3000, 3914, 3915 and 5000) at default values ($TEMP_GRADIENT = 0.45$, $MAX_HEATEDCELLS_PER_BEE = 2.65$, $TempEff = 0$).

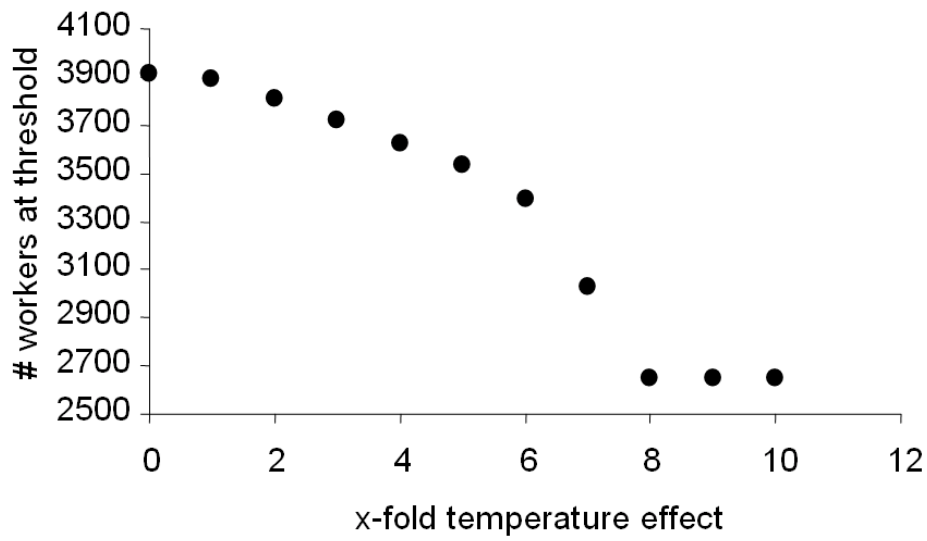


Fig. 7. Survival thresholds (i.e. minimal number of initial bees needed for colony survival) in the model in relation to the temperature effect (0 = no temperature effect, 1 = empirical temperature effect, >1: accordingly magnified temperature effect) (TEMP_GRADIENT = 0.45, MAX_HEATEDCELLS_PER_BEE= 2.65).

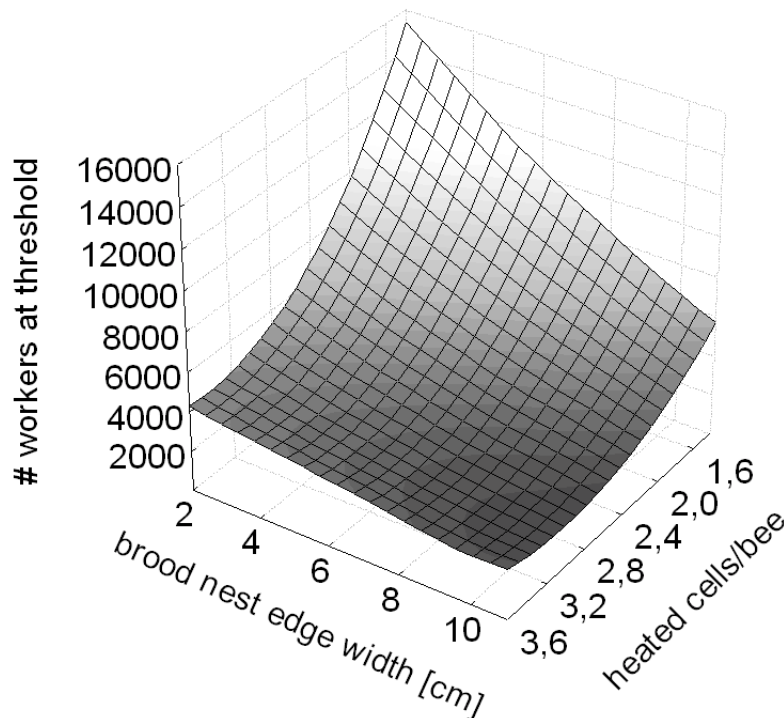


Fig. 8. Survival thresholds (i.e. minimal number of initial bees for colony survival) in relation to the width of the edge of the brood nest ($\leq 35^{\circ}\text{C}$) and the number of heated cells per bee (TempEff = 0).

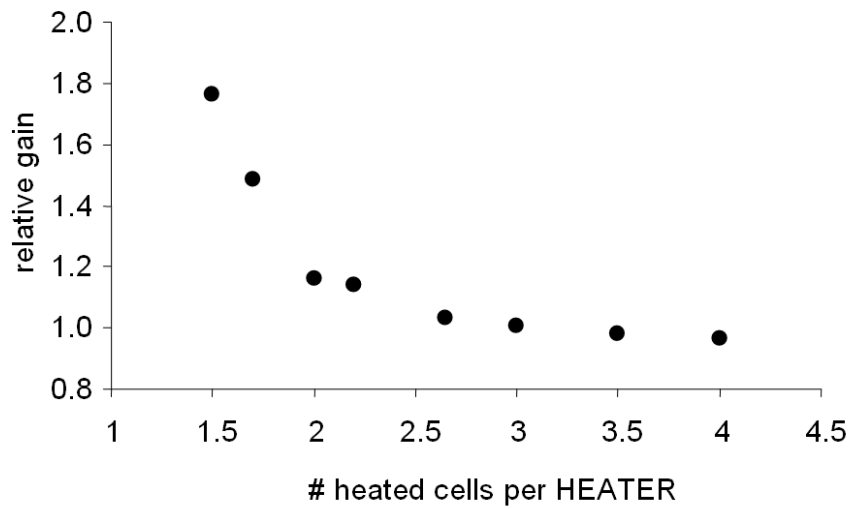


Fig. 9. The relative gain for different numbers of heated cells per heater. The relative gain is derived from benefits of enhanced survival in relation to the additional costs on increased heating per bee. Thus the relative decrease of number of bees at the survival threshold is divided by the relative increase in heating effort ($\text{TEMP_GRADIENT} = 0.45$).

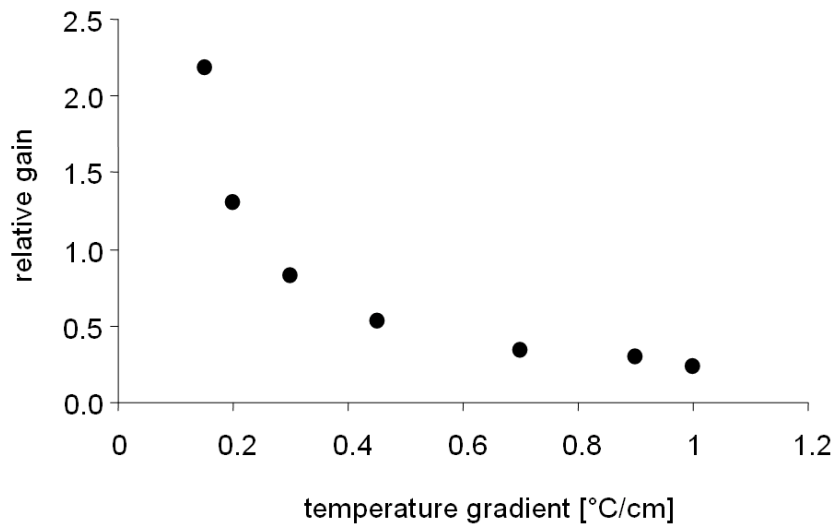


Fig. 10. The relative gain versus the temperature gradient. The relative gain is derived from benefits of enhanced survival in relation to the additional costs on a flattened temperature gradient. Thus the relative decrease of number of bees at the survival threshold is divided by the relative decrease of the temperature gradient ($\text{Heat per Bee} = 26.5^\circ\text{C}$).

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CHAPTER 6

Summary

Honeybee nest temperature is a potentially crucial factor for population dynamic and division of labour in the colony, since it influences not only the development of the brood but also the behavioural performance of the workers in their later adult life. It is hence of particular interest to obtain accurate and reliable data on the temporal and spatial temperature distribution in the brood nest. In order to achieve this purpose, we developed a new device for temperature measurement in honeybee combs. Contrary to established techniques of temperature record in honeybee colonies (i.e. using thermocouples or infrared thermography), the new method allows a continuous temperature measurement in close proximity to the brood under near natural conditions in the colony and with a high spatial and temporal resolution. The instrument consists of a grid of 256 thermistors with a negative temperature coefficient, which results in a reduced resistance if temperature rises. The sensors are consecutively addressed by a personal computer and deliver a temperature pattern of 768 cells. Recorded data are stored by the computer in a text file and further processed with a specially developed software tool. This program graphically displays the temperature distribution for any timestep in false colour and provides parameters such as mean temperature, standard deviation, minimum and maximum temperature in given area of the comb as well as the number of cells in a certain temperature range (Chapter 2).

With the help of this instrument we were able to test the impact of empty cells in the brood nest for thermoregulation. Although honeybees form a compact brood nest, there are always some empty cells („gaps“) in the capped brood area due to the egg-laying behaviour of the queen or the removal of unviable eggs and larvae by the workers. Recently, workers were observed entering these gaps for brood incubation, which was assumed to be a very efficient way of brood heating, saving up to 37% of the incubation time. We tested these predictions by using the multi-sensor thermometers and inserting pieces of capped brood (7x7cm) with and without gaps into empty test combs. The brood was heated by groups of 150 in-hive workers. However, we neither found differences in the slope of the temperature increase nor in the maximal temperatures. We conclude, that honeybee workers do not intentionally use empty cells in the nest for brood heating but enter them only occasionally and hence gaps seem not to increase the efficiency of thermoregulation (Chapter 3).

Developmental temperatures in honeybees have been shown to influence the olfactory learning ability, the dancing behaviour and the synaptic organisation in the brain of adult

workers. To further improve our understanding of the impact of brood temperature on division of labour among members of the colony, we raised honeybee pupae in incubators set to 32, 34.5 and 36°C, marked the emerging bees individually and released them in observation hives. We investigated dancing activities, undertaking behaviour, age of first foraging and forager task specialisation. We found an increased probability to dance and to engage in undertaking as well as an earlier onset of foraging when bees developed under higher temperatures.

These results confirm former findings that brood temperature influences the behavioural performance of adult bees. Brood temperature might hence potentially affect the overall organization of the colony. The onset of foraging defines the transition from an in-hive to an outdoor worker and allocation of workers among these major fields of activity is critical for survival of the colony. It determines the investment in brood care and colony homeostasis on the one hand and in energy input as precondition for colony growth and winter survival on the other hand. Developmental temperature might fine-tune the proportion of in-hive bees and foragers in the colony by affecting the individual pace of behavioural development (Chapter 4).

We tested this hypothesis with the help of a deterministic computer model. In the simulation, the number of in-hive bees determines the temperature distribution in the brood nest. Workers developed under cool temperatures (32°C) start foraging 1 day later than workers developed under medium temperatures (35°C), whereas bees developed under hot temperatures (36°C) show a one day shortened in-hive period, which reflects our empirical findings. For the parametrization of the model, we conducted several experiments, using the multi-sensor thermometers described in chapter 2 to determine the heating efforts of foragers and in-hive bees, the impact of a single worker on the thermoregulation of the brood nest and the temperature gradient in brood combs. Results of the model were analyzed over a large parameter space. However, the results of the simulation suggest that the temperature effect, i.e. the acceleration of the behavioural development with increasing developmental temperatures, has only little impact on the population dynamic and the survival of the colony. Instead, the number of bees at the beginning of the simulation runs mainly determines the survival of the colony (Chapter 5).

Conclusion: This study confirms that pupal developmental temperature affects individual traits of adult honeybee workers. Workers developed under higher temperatures show an earlier

onset of foraging as adults. However, this effect is only of minor importance for division of labour between in-hive duties and foraging on the colony level.

CHAPTER 7

Zusammenfassung

Die Temperatur im Brutnest der Honigbiene ist ein potentiell entscheidender Faktor für die Populationsdynamik und Arbeitsteilung im Bienenvolk, da sie nicht nur die Entwicklung der Brut sondern auch das Verhalten der Arbeiterinnen in ihrem späteren Leben als Adulte beeinflusst. Es ist daher von besonderem Interesse, genaue und zuverlässige Daten über die zeitliche und räumliche Temperaturverteilung im Brutnest zu erhalten. Zu diesem Zweck haben wir ein neuartiges Gerät für die Temperaturmessung in Bienenwaben entwickelt. Im Gegensatz zu etablierten Techniken der Temperaturaufnahme in Bienenvölkern (beispielsweise dem Einsatz von Thermoelementen oder Infrarotthermographie) erlaubt diese neue Methode eine kontinuierliche Temperaturmessung in unmittelbarer Nähe zur Brut und unter fast natürlichen Bedingungen innerhalb der Kolonie bei gleichzeitig hoher räumlicher wie zeitlicher Auflösung. Das Messgerät besteht aus einem Raster aus 256 Thermistoren mit negativem Temperaturkoeffizient, sogenannten Heißleitern, deren elektrischer Widerstand sinkt, wenn die Temperatur ansteigt. Die Sensoren werden nacheinander von einem Personal Computer angesteuert und liefern die Temperaturverteilung von 768 Zellen. Die aufgenommenen Daten werden in einer Textdatei abgespeichert und mittels einer speziell entwickelten Software weiterverarbeitet. Dieses Programm stellt die Temperaturverteilung zu jedem Zeitschritt graphisch dar und gibt Kenngrößen aus wie Durchschnittstemperatur, Standardabweichung, minimale und maximale Temperatur in einem gegebenen Bereich sowie die Anzahl der Zellen in einer bestimmten Temperaturspanne (siehe Kapitel 2).

Mit Hilfe dieses Messgerätes waren wir in der Lage, die Bedeutung von leeren Zellen im Brutnest für die Thermoregulation zu untersuchen. Obwohl Honigbienen ein kompaktes Brutnest anlegen, sind immer auch einzelne leere Zellen im verdeckelten Brutbereich vorhanden, die vom unregelmäßigen Eiablageverhalten der Königin oder dem Entfernen von Eiern oder Larven durch die Arbeiterinnen herrühren. Es wurde kürzlich beobachtet, dass Arbeiterinnen in diesen leeren Zellen die umliegende Brut geheizt haben, wobei angenommen wurde, dass durch dieses Verhalten auf sehr effiziente Weise Wärme auf die Brut übertragen würde und dadurch bis zu 37% weniger Zeit für die Thermoregulation aufgewendet werden müsste. Wir haben diese Vorhersagen mit dem beschriebenen Messgerät überprüft, indem wir verdeckelte Brutstücke (7x7cm) mit und ohne leere Zellen in eine Testwabe eingefügt haben. Die Brut wurde von jeweils 150 Arbeiterinnen geheizt. Wir konnten dabei weder Unterschiede im Temperaturanstieg zu Beginn der Messungen noch bei den

Höchsttemperaturen feststellen. Wir schließen daraus, dass Arbeiterinnen die Lücken im Brutnest nicht vorsätzlich sondern nur gelegentlich nutzen um darin zu heizen und diese leeren Zellen daher die Effizienz der Thermoregulation nicht verbessern (siehe Kapitel 3).

Es ist gezeigt worden, dass die Entwicklungstemperaturen bei Honigbienen das olfaktorische Lernen, das Tanzverhalten und die synaptische Organisation im Gehirn der adulten Arbeiterinnen beeinflussen. Um unser Verständnis der Bedeutung der Bruttemperatur auf die Arbeitsteilung zwischen den Mitgliedern einer Kolonie weiter zu verbessern, haben wir Honigbienen Puppen in Brutschränken bei 32, 34,5 und 36°C aufgezogen. Die geschlüpften Bienen wurden individuell markiert und in Beobachtungsstöcken freigelassen. Wir haben die Tanzaktivitäten, das Austragverhalten („undertaking“), das Alter des ersten Sammelfluges und Spezialisierungen für das Sammelgut untersucht. Dabei fanden wir bei Bienen, die sich unter höheren Temperaturen entwickelt hatten, eine erhöhte Wahrscheinlichkeit zu tanzen und Austragverhalten zu zeigen sowie einen früheren Beginn der Sammeltätigkeit.

Diese Ergebnisse bestätigen frühere Forschungsergebnisse, wonach die Bruttemperatur das Verhalten der adulten Bienen beeinflusst. Sie kann sich daher potentiell auf die Gesamtorganisation des Bienenvolkes auswirken. Der Beginn der Sammeltätigkeit stellt für eine Arbeiterin den Übergang von Innendienst- zu Außendiensttätigkeiten dar und die Verteilung von Arbeiterinnen zwischen diesen beiden Hauptbetätigungsfeldern ist von entscheidender Bedeutung für das Überleben eines Bienenvolkes. Es werden dadurch die Investitionen festgelegt, die auf der einen Seite in die Aufzucht des Nachwuchses und der Aufrechterhaltung des Volkes fließen und auf der anderen Seite in den Energieeintrag, als Voraussetzung für die Volksentwicklung und eine erfolgreiche Überwinterung. Die Entwicklungstemperatur könnte sich als wichtiger Faktor zur Feineinstellung des Anteils der Innendienstbienen und der Sammlerinnen herausstellen, indem sie an den individuellen Geschwindigkeiten in der Verhaltensentwicklung angreift (siehe Kapitel 4).

Mit einem deterministischen ComputermodeLL haben wir diese Hypothese überprüft. In der Simulation bestimmt die Anzahl der Innendienstbienen die Temperaturverteilung im Brutnest. Arbeiterinnen die sich unter kälteren Bedingungen (32°C) entwickelt haben beginnen ihre Sammeltätigkeit einen Tag später als Arbeiterinnen die sich unter mittleren Temperaturen (35°C) entwickelt haben, wohingegen Bienen, die sich unter höheren Temperaturen (36°C) entwickelt haben eine um einen Tag verkürzte Innendienstperiode zeigen, was unseren empirischen Befunden entspricht. Um das Modell zu parametrisieren haben wir verschiedene Experimente durchgeführt, bei denen wir mit Hilfe des in Kapitel 2 beschriebenen

Temperaturmessgerätes die Heizleistungen von Sammlerinnen und Innendienstbienen bestimmten, den Anteil einer einzelnen Biene an der Thermoregulation des Brutnestes sowie den Temperaturgradienten in der Brutwabe. Die Ergebnisse des Modells wurden über einen weiten Bereich des Parameterraumes analysiert. Die Ergebnisse legen allerdings nahe, dass der Temperatureffekt, also die Beschleunigung der Verhaltensentwicklung durch höhere Entwicklungstemperaturen, nur einen geringen Einfluss auf die Populationsdynamik und das Überleben des Bienenvolkes hat. Stattdessen bestimmte in erster Linie die Anzahl der Bienen zu Beginn eines Simulationslaufes das Überleben des Bienenvolkes.

Schlussfolgerung: Diese Arbeit bestätigt, dass Entwicklungstemperaturen während der Puppenphase individuelle Eigenschaften von Arbeiterinnen der Honigbiene beeinflussen. Arbeiterinnen die sich bei höheren Temperaturen entwickelt haben, zeigten als Adulte einen früheren Beginn der Sammeltätigkeit. Dieser Effekt ist allerdings für die Arbeitsteilung zwischen Innendienst- und Sammeltätigkeiten im Bienenvolk nur von untergeordneter Bedeutung.

Acknowledgements

I would like to thank Robin Moritz who gave me the opportunity to work in his lab and who supported me in every way.

I further thank Charlotte Hemelrijk for co-promoting my work and for the opportunity to visit the Theoretical Biology group in Groningen.

I thank all my co-authors for their input into the publications.

Particular thanks to Gunther Tschuch, Sven Ewald and Felix Lehmann who substantially helped me to construct the „Porcupine“.

My special thanks to Antje Jarosch, Benjamin Barth, Marina Pozzoli and Stephan Härtel who shared my office, to Hans-Hinrich Kaatz for many advices in beekeeping and bee handling, to Petra Leibe and Holger Scharpenberg for technical assistance, as well as to all other current and former members of the Molecular Ecology lab I had the pleasure to work with, especially to Nadine Al-Abadi, Dieter Behrens, Peter Bliss, Mogbel El-Niweiri, Silvio Erler, Vincent Dietemann, Franziska Hesche, Anett Huth-Schwarz, Qiang Huang, Rodolfo Jaffé, Andreas Katzerke, Jonathan Kidner, Denise Kleber, Bernhard Kraus, Berit Langer, Michael Lattorff, Peter Neumann, Martina Müller, Susann Parsche, Mario Popp, Mandy Rohde, Helge and Ellen Schlüns, Taher Shaibi, Sebastian Spiewok, Eckart Stolle, André Walter, Steffi Weinhold, Petra Weber, Constanze Westphal and Stephan Wolf.

I also wish to thank the Theoretical Biology group in Groningen for a warm welcome and fruitful discussions. Particular thanks to Hanno Hildenbrandt, Daan Reid, Martin Hinsch and Julia Schröder.

I am grateful to all the students who collected with great enthusiasm a vast amount of data:

Katja Dahlke, Tobias Janik, Carolin Opitz and Tanja Schnelle as well as Jana Junick, Kathleen Merx, Iljana Mögel, Steffi Prieskorn, Nadine Vogler and Doreen Wöllner (2004). Constanze Nossol, Michaela Thoß, Michael Ellis and Anja Schmidt as well as Ina Brauckhoff, T.-M. Ehnert, S. Erdmann, Cornelia Geßner and Melanie Wagner (2005). Antonia Hofmann, Eileen Winkler, Anne Hauptmann, Annekathrin Tews and Bianka Janack (2006). Kathleen Mönch, Nicole Stahl and Sonja Streicher as well as Nadine Hartmann, Judith Kreher, Juliane Mohr, Katja Schönefeld and Stefanie Stöckhardt (2007). Anna Mihan as well as Anne Blaner, Christiane Hösel, Anne-Katrin Schwiderke and Alexandra Wölk (2008) and Marco Mesiano (2009).

This work was financially supported by the Deutsche Forschungsgemeinschaft (RFAM) and the European Science Foundation (MAB).

My special thanks to my friends and family.

And last but not least I owe my gratitude to so many honeybees who selflessly devoted their lives to the greater glory of science.

APPENDIX

Curriculum vitae

Personal information

Name: Matthias Adolf Becher
Birth: 26.07.1974, Heidelberg, Germany
Nationality: German
Languages: German, English

Education

- 2003-2010: PhD at the Martin-Luther Universität Halle-Wittenberg, Germany. Dissertation thesis: The influence of developmental temperatures on division of labour in honeybee colonies
Supervised by Prof. Dr. Robin F.A. Moritz and Prof. Dr. Charlotte K. Hemelrijk
- 2002-2003: Diploma thesis: “Classification of the extinction risk of animal populations on the basis of population dynamic parameters” at the Julius-Maximilians Universität, Würzburg, Germany.
Supervised by Prof. Dr. H.-J. Poethke
- 1999-2003: Study of Biology (Zoology, Microbiology, Botany) at the Julius-Maximilians Universität, Würzburg, Germany
- 1996-1999: Basic studies in Biology at the Rupprechts-Karls-Universität, Heidelberg, Germany
- 1994-1995: Civilian service
- 1985-1994: Grammar School: Kurpfalz-Gymnasium Schriesheim, Germany

Publications and Editorial work

Becher MA, Scharpenberg H, Moritz RFA (2009) Pupal developmental temperature determines foraging specialisation of adult honeybee workers (*Apis mellifera* L.). J Comp Phys A 195:973-679 (DOI: 10.1007/s00359-009-0442-7)

Becher MA, Moritz RFA (2009) A new device for continuous temperature measurement in brood cells of honey bees (*Apis mellifera*). Apidologie 40:577-584 (DOI: 10.1051/apido/2009031)

Becher MA, Hildenbrandt H, Hemelrijk CK, Moritz RFA: Brood temperature, Task division and Colony survival in honeybees: a model. Ecological Modelling (DOI: 10.1016/j.ecolmodel.2009.11.016)

Becher MA, Moritz RFA: Gaps or caps in honey bees brood nests: Does it really make a difference? in prep.

Kaatz HH, **Becher M**, Moritz RFA (Eds.) (2005) Bees, ants and termites: applied and fundamental research. International Union for the Study of Social Insects (German speaking section), Halle

Declaration on the Author Contributions

1. A new device for continuous temperature measurement in brood cells of honeybees (*Apis mellifera*)

Apidologie (2009) 40:577-584. DOI: 10.1051/apido/2009031

Becher MA, Moritz RFA

I constructed the prototype of the device, developed the software and wrote the manuscript. RFA Moritz supervised the project and provided helpful technical suggestions.

2. Gaps or caps in honeybees brood nests: Does it really make a difference?

Becher MA, Moritz RFA

submitted to Naturwissenschaften, rejected (28. December 2009) with opportunity to resubmit

I performed the experiments, analysed the data and wrote the manuscript. RFA Moritz supervised the work and provided helpful discussions.

3. Pupal developmental temperature and behavioral specialization of honeybee workers (*Apis mellifera* L.)

Journal of Comparative Physiology A (2009) 195:673–679 DOI: 10.1007/s00359-009-0442-7

Becher MA, Scharpenberg H, Moritz RFA

I performed the experiments, analysed the results and wrote the manuscript. H Scharpenberg helped with the data collection in the first year. RFA Moritz supervised the work and provided helpful discussions.

4. Brood temperature, task division and colony survival in honeybees: A model

Ecological Modelling (2009) DOI: 10.1016/j.ecolmodel.2009.11.016

Matthias A. Becher, Hanno Hildenbrandt, Charlotte K. Hemelrijk, Robin F.A. Moritz

I designed, performed and analysed the empirical experiments. I further developed the model, performed the analysis and wrote the manuscript. H Hildenbrandt provided helpful suggestions for a first version of the model. CK Hemelrijk supervised a part of the project and edited the manuscript. RFA Moritz supervised the project and made valueable suggestions.

Erklärung

Hiermit erkläre ich, dass diese Arbeit von mir bisher weder der Naturwissenschaftlichen Fakultät I der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion eingereicht wurde. Ich erkläre, dass ich mich bisher noch nicht um den Doktorgrad beworben habe. Ferner erkläre ich, dass ich diese Arbeit selbständig und nur unter Zuhilfenahme der angegebenen Hilfsmittel und Literatur angefertigt habe.

Halle (Saale), den 6. Januar 2010

Matthias A. Becher