

RESEARCH ARTICLE

Hot bees in empty broodnest cells: heating from within

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Accepted August 22, 2003.

SUMMARY

Honeybee colonies maintain brood nest temperatures of 33–36°C. We investigated brood nest thermoregulation at the level of individual worker behaviour and the transfer of heat from workers to the brood. Worker bees contribute to the regulation of brood nest temperature by producing heat while sitting motionless on the caps of brood cells. We report here an additional, newly observed heating strategy where heating bees enter empty cells between sealed brood cells and remain there motionless for periods of up to 45 min.

Individually marked worker bees on the surface of sealed brood cells maintained thorax temperatures (T_{th}) between $32.2 \pm 1.0^\circ\text{C}$ and $38.1 \pm 2.5^\circ\text{C}$ (mean \pm S.D.; $N=20$ bees) with alternating warming and cooling periods. Most of the observed bees made one or several long-duration visits (>2 min) to empty cells within the sealed brood area. T_{th} at the time bees entered a cell [$T_{th(\text{entry})}$] was $34.1\text{--}42.5^\circ\text{C}$ ($N=40$). In 83% of these cell visits, $T_{th(\text{entry})}$ was higher (up to 5.9°C ; mean $2.5 \pm 1.5^\circ\text{C}$; $N=33$) than the mean T_{th} of the same bee. High values of $T_{th(\text{entry})}$ resulted from preceding heating activity on the comb surface and from warm-ups just prior to cell visits during which T_{th} increased by up to $+9.6^\circ\text{C}$.

Bees inside empty cells had mean T_{th} values of $32.7 \pm 0.1^\circ\text{C}$ (resting bees) to $40.6 \pm 0.7^\circ\text{C}$ (heat-producing bees) during long-duration cell visits without performing any visible work. Heating behaviour inside cells resembles heating behaviour on the brood cap surface in that the bees appear to be inactive, but repeated warmings and coolings occur and T_{th} does not fall below the optimum brood temperature.

Bees staying still inside empty cells for several minutes have previously been considered to be 'resting bees'. We find, however, that the heating bees can be distinguished from the resting bees not only by their higher body temperatures but also by the continuous, rapid respiratory movements of their abdomens. By contrast, abdominal pumping movements in resting bees are discontinuous and interrupted by long pauses.

Heat transfer to the brood from individual bees on the comb surface and from bees inside empty cells was simulated under controlled conditions. Heating on the comb surface causes a strong superficial warming of the brood cap by up to 3°C within 30 min. Heat transfer is 1.9–2.6 times more efficient when the thorax is in touch with the brood cap than when it is not. Heating inside empty cells raises the brood temperature of adjacent cells by up to 2.5°C within 30 min. Heat flow through the comb was detectable up to three brood cells away from the heated thorax.

KEY WORDS

honeybee

Apis mellifera carnica

thermoregulation

heat transfer

brood

comb

Introduction

Honeybee (*Apis mellifera*) colonies maintain stable brood nest temperatures of 33–36°C (Seeley, 1985) even when ambient temperatures reach extremes of -80°C to $+60^\circ\text{C}$ (Lindauer, 1954; Southwick, 1987). High ambient temperatures are counteracted by wing fanning and water intake for evaporative cooling (Hazelhoff, 1954; Lindauer, 1954). At low ambient temperatures, the bees crowd in the brood area (Kronenberg and Heller, 1982; Harrison, 1987) and produce heat by vibrating their thoracic muscles without moving the wings (Esch et al., 1991; Heinrich, 1993; Heinrich and Esch, 1994).

If the brood-rearing temperature deviates from the optimum 33–36°C range for longer periods, malformations and mortality of the brood increase (Himmer, 1927, 1932; Muzalewskij, 1933; Weiss, 1962). After emergence, the behavioural performance of worker bees is reduced if rearing temperature during pupal stage was in the lower range (Tautz et al., 2003).

Very little is known about how individual bees in the brood area contribute to the stability of brood nest temperature. Harrison (1987) found that most bees in a colony contribute to colonial heating in that they maintain a thorax temperature (T_{th}) above local ambient temperature. However, not all bees contribute equally, and variation of up to 12°C among individuals can be found (Harrison, 1987). Using chronically implanted thermocouples, which allow the combination of behavioural observations with continuous long-term temperature measurements of individual bees, Esch (1960) identified worker bees that specialized in the activity of brood nest warming. Such bees sit motionless on the surface of brood cells while maintaining a T_{th} above 35°C with intermittent warming and cooling and without performing any other work during

this time. Schmaranzer et al. (1988) have confirmed these findings with non-invasive methods. Endoscopic and thermographic observation of individual bees by Bujok et al. (2002) showed that brood-heating individuals often press their warm thoraces firmly onto the caps of sealed brood cells while staying motionless, thereby enhancing heat transfer to the brood by means of conduction. Heat transfer from such bees to the brood left a 'hot spot' in the thermographic image at the place where these bees had previously been sitting.

Our initial setup was designed to learn more about the long-term temperatures and behaviour of individual worker bees that are engaged in brood incubation. The results unveiled a remarkable, hitherto unknown behaviour of the honeybee and directed our interest to an elusive aspect of thermoregulation in its colonies: in the present work, we describe a series of observations showing that seemingly resting bees inside empty cells participate in the regulation of brood temperature and serve as a heat source for neighbouring sealed brood cells. Using an infrared-sensitive thermovision camera and a modified brood nest that allowed us to monitor all events inside empty cells between sealed brood cells, we investigated the behaviour and the thorax temperature of individual bees before and after visits to empty cells, during their stay inside empty cells and the participation of cell-visiting bees in broodnest warming. Different modes of heat transfer from worker bees to the brood were simulated under controlled conditions and allowed us to detect heat transfer from cell visitors to the adjacent brood even in a populous observation hive.

We find that many bees that are apparently resting inside empty cells in the brood comb are participating in the regulation of brood temperature by serving as a heat source for the neighbouring, sealed, brood cells. This hitherto unrecognised thermal activity of bees inside cells is remarkable because long-duration cell visitors were previously considered to be resting and also because heat production inside cells provides a way to transfer heat to the brood more efficiently than heating *via* the brood caps.

Materials and methods

Bees and hives

We used three different honeybee colonies (*Apis mellifera carnica* Pollm.) that were kept in observation hives at the bee research facility of the University of Würzburg. The observation hives stood indoors and the bees were allowed to leave the hive and laboratory through a plastic tunnel to fly and forage outdoors. To control the size and location of the brood nest, the common observation hives (setups A and D) contained only a single brood nest (~110–120 cm²) that was inserted into the centre of the upper comb. The queen was excluded from the brood area to prevent uncontrolled egg laying. The hives were thermally insulated, and part of the insulation was removed on one side to see the bees during the observation periods. The glass covering the exposed side was replaced by infrared transparent plastic film to enable thermographic determination of body temperatures. All observations were made early in the day when environmental temperatures were lower than 35°C and brood warming was necessary.

Measurement of thorax temperatures

Thorax surface temperatures (T_{th}) of worker bees were measured by remote sensing thermography. A real-time infrared-sensitive camera (Radiance PM 1/1.5.1b; waveband 3.5–5.6 μm ; accuracy $\pm 0.7^\circ\text{C}$; Raytheon-Amber, Goleta, CA, USA) allowed the simultaneous measurement of the temperatures of many bees without disturbing them. A 19 mm extension tube was used for close-up recordings of individual bees inside cells. Absolute temperatures were calculated by the camera's internal software using the emissivity value ($e=0.97$) of the bee's thorax (Stabentheiner and Schmaranzer, 1987) and were encoded as an eight-step greyscale in the image. Single infrared images were relayed to a computer and were analysed with camera-specific software (AmberTherm v1.28). The attenuation of thermal radiation by the plastic film was compensated during analysis using the T_{th} values of dead, artificially heated bees, which were measured in a different setup with and without the film in place.

Setups A and B: temperature and behaviour of worker bees

Setup A: thoracic temperature of bees on the brood comb surface prior to cell visits

This setup was designed for temperature measurements of bees on the surface of brood cells before and after cell visits. A common observation hive with two combs standing on top of each other (von Frisch, 1965) was used. During a period of 7 weeks prior to the observation, 3500 worker bees (reared at 35°C from brood combs in an incubator), all with individual colour marks on their abdomen (Kleinhenz and Tautz, 2003), were introduced into the colony. A 10 cm \times 11 cm piece of brood comb was set into the centre of the upper comb 9 days prior to the observation, allowing the larvae to develop to sealed stage. During the observation, room and hive temperatures (T_{room} and T_{hive} , respectively) were measured every 15 min with NiCr-Ni thermocouples connected to a digital twin thermometer (Voltcraft 502; Conrad electronic, Hirschau, Germany). Thermocouples were not placed in the brood area itself because they impeded the movement of the bees across the comb. T_{hive} outside the brood area (7.5 cm from the centre of the comb) was $30.4 \pm 0.3^\circ\text{C}$ at a T_{room} value of $22.3 \pm 0.3^\circ\text{C}$.

Observations were made from 05:30 h to 07:10 h (CEST). The brood area was videotaped (Panasonic AG-7350 and AG-5700) continuously during the observation period with the thermovision camera and a synchronized standard video system to identify individual bees by their colour codes. 20 bees were followed as long as they were visible in the brood area (4–91 min per individual; mean \pm S.D., 39.0 ± 26.5 min; total time 780 min). T_{th} was measured at least five times per minute and immediately before [$T_{th(entry)}$] and after [$T_{th(exit)}$] cell visits. Short cell inspections (<2 min) were not noted. Net temperature differences during a cell visit were calculated as $(\Delta T_{th})_{net} = T_{th(exit)} - T_{th(entry)}$. All T_{th} values were obtained from bees inside the brood area, although walking bees may also come across empty cells (scattered between the sealed brood cells) at the time when T_{th} was measured.

Setup B: thoracic temperature of bees inside empty cells

Since only the tip of the abdomen of bees inside comb cells is visible in common observation hives, it was necessary to use a different setup (Fig. 1): the queen, 1500 worker bees and three comb pieces (A, B and C; each 17 cm \times 4 cm) were obtained from a 10-frame hive. The comb pieces were cut out of a storage comb (piece A) and a mixed brood/storage comb (pieces B and C) and placed in a small observation hive with

the centre wall of the comb perpendicular to the front window (Fig. 1). This allowed the interior of the first cell in each row to be seen through the infrared-transparent front cover (Fig. 1). These cells are referred to as 'observation cells'. Bees were allowed 2 days to settle before observations started. Air temperature inside the hive between brood combs (T_{hive} ; see Fig. 1) and T_{room} were measured with thermistors (accuracy $\pm 0.1^\circ\text{C}$) connected to a digital data logger (Almemo 2290-8; Ahlborn, Holzkirchen; Germany) and were recorded every 2 s.

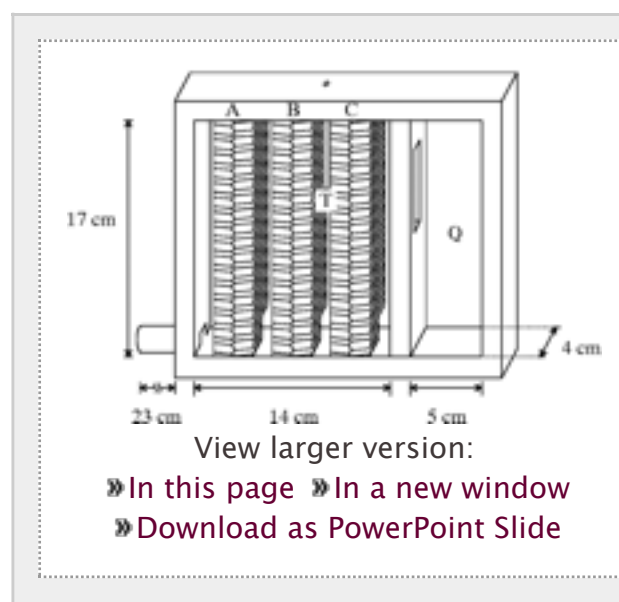


Fig. 1.

Observation hive with three comb pieces (A, B, C), allowing thermographic temperature measurements of bees inside observation cells. Comb cells in the background are indicated but could not be seen during the observations. The comb chamber was covered with infrared transparent film. The smaller chamber was half filled with sugar dough and contained the queen (Q) in a cage. Insulation covering the hive (4 cm polystyrene walls on all sides; not shown) was partially removed while the observations were being made. Hive temperature (T_{hive}) was measured with a thermistor (T) inside a plastic tube.

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Close-up thermographic recordings of three observation cells adjacent to the sealed brood (Fig. 2) provided us with data on individual bees during cell visits. Individual bees could be resolved until they left the camera's field of view (Fig. 2). During a 130 min observation period (10:30 h to 12:40 h CEST), the T_{hive} value was $33.2 \pm 0.6^\circ\text{C}$ at the T_{room} value of $23.1 \pm 0.3^\circ\text{C}$. T_{th} of worker bees inside the observation cells was measured every 15 s. Only cell visits of >2 min were considered.

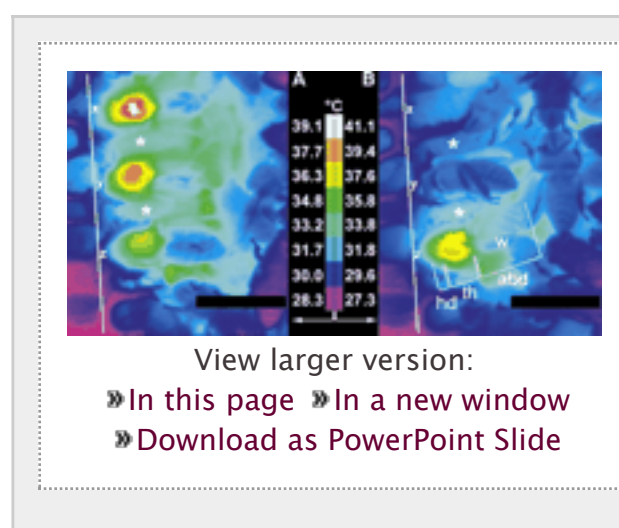


Fig. 2.

Close-up thermographic recordings of bees inside three empty cells adjacent to brood cells. A and B show a view of the same cells at different times. x, y and z each indicate the bottom of one of the observation cells. The white lines indicate the middle wall. (A) Three heating bees with different thorax temperatures (T_{th}); all of them are seen from their dorsal sides. (B) A resting bee in cell y and a heating bee in cell z (turned upside-down). Cell x is empty. Abbreviations: abd, abdomen; hd, head; th, thorax; w, wing. Asterisks mark the wax walls of two sealed pupae cells adjacent to the observation cells. The temperatures refer to the bee thorax.

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The respiratory pumping movements of the abdomen of bees lying still were recorded from the videotapes using computer software (The Observer 3.0; Noldus Information Technology, Wageningen, The Netherlands), which allowed registration of pumping movements up to 3 s^{-1} .

On three consecutive days, bees in a total of 84–104 observation cells in the central part of the combs were thermographed simultaneously to investigate the bee temperatures in different regions of the hive. Two types of observation cells were defined. Type 1: observation cells adjacent to and sharing at least one common cell wall with a sealed brood cell during the whole observation period. Type 2: observation cells adjacent to non-brood cells during the whole observation period. Observations were made daily from 7:30 h to 9:10 h (CEST) at ambient conditions (T_{room} and T_{hive}), as given in Table 1. Every 5 min during these 100 min observations, the T_{th} values of all bees in cells were measured from infrared images that were relayed to a computer. Data were analyzed using a one-tailed Kolmogoroff-Smirnoff test (Bortz et al., 2000). The T_{th} values of bees visiting type 1 observation cells (adjacent to brood cells) were tested separately for brood combs B and C and for each of the three observation days.

View this table: » In this window » In a new window	<p>Table 1.</p> <p><i>Thorax temperatures (T_{th}) of bees visiting empty cells adjacent to sealed brood cells (type 1) or non-brood cells (type 2)</i></p>
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Setups C and D: heat transfer from worker bees to the brood

How do bees inside cells and bees on the comb surface contribute to the temperature of brood cells? We used two different set-ups to answer this question. First (setup C), we simulated worker heating under controlled conditions to determine the extent and rates of warming in the adjacent brood cells. Second (setup D), we recorded brood cell temperatures in a common observation hive and analysed them with regard to the presence of cell visitors in the adjacent cells.

Setup C: simulation of worker heating

This setup was used to simulate worker heating with an artificially heated thorax and to measure its influence on the temperature of nearby brood cells. A brood comb was kept inside an incubator (B 5042; Heraeus, Hanau, Germany) and thermistors for measurement of T_{brood} were implanted in the bottom of three neighbouring sealed brood cells (Fig. 3). The thermistors were inserted from the back of the comb without damaging the caps of the investigated brood cells. Cells on the back of the comb that had been emptied to access the cell ground were later re-filled with a dead bee to substitute the brood and were closed with drops of beeswax. In some cases, we also measured the temperatures just beneath the brood caps (T_{cap}) near the comb surface (Fig. 3). To leave the pupae and brood caps intact, we inserted these thermistors through the caps of neighbouring cells and guided them to the investigated brood cells through small perforations in the common cell walls (Fig. 3).

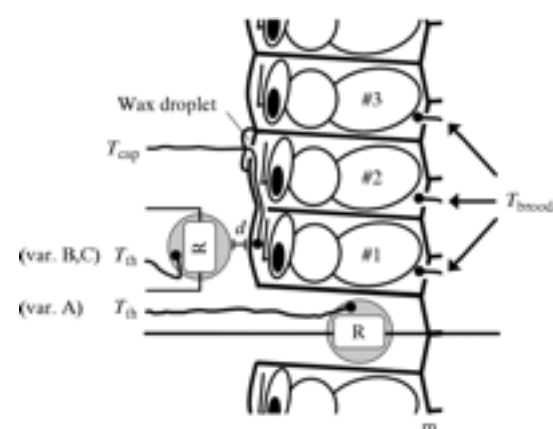


Fig. 3.

Simulation of worker heating with an artificially heated thorax (setup C). The cross-section shows one side of a comb. In different cycles, the thorax was placed inside an empty cell (variation A) or on the comb surface either touching the cap (variation B: $d=0$ mm) or at a distance of 1.0–1.5 mm from the brood cap (variation C). The locations of thermistors for measurement of thorax temperature (T_{th}), cap temperature (T_{cap}) and brood temperature (T_{brood}) are indicated. Other than shown, the T_{cap} thermistors were not introduced from brood cell #2 but from adjacent cells in the background that were not used for temperature measurements. Abbreviations: d , distance; m , middle wall of the comb; R, resistor inside an isolated bee thorax.

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The initial brood cell temperature (T_{brood}) before artificial heating started varied slightly on different days and in different combs but was kept in a narrow range from 32.8°C to 33.9°C in all cases. Regulatory oscillations of the air temperature inside the incubator (up to $\pm 0.40^\circ\text{C}$; period length 14–24 min) were buffered widely by the surrounding mass of comb and brood: when no simulation was conducted, T_{brood} in the comb's core was constant within $\pm 0.10^\circ\text{C}$ of the given level for periods up to 24 h.

We confined our simulation to the thorax, since it is the major heat source: in living honeybees, heat flow to the abdomen is greatly reduced by a countercurrent heat exchanger in the narrow petiole region, which conserves heat in the thorax (Heinrich, 1979, 1993; Heinrich and Esch, 1994) and keeps the abdominal temperature near ambient level (see Figs 2, 5; Stabentheiner and Schmaranzer, 1987; Stabentheiner and Hagmüller, 1991). A small resistor, about the size and mass ($M=29$ mg) of a bee's thorax, served as a heat source. The resistor was placed inside the isolated thorax of a dead bee to mimic its heat exchange properties. Internal T_{th} beneath the cuticle was measured with a thermistor and may be 1°C higher than the corresponding surface temperatures (Stabentheiner and Schmaranzer, 1987). During simulation, the thermistor values (T_{brood} , T_{cap} and T_{th}) were read at a resolution of 0.01°C and were stored automatically every second (Almemo 2290–8). Each heating cycle lasted 30 min, a time-span covering the mean duration of long cell visits (10–15 min) and most of the longest, non-interrupted cell visits that were found in the previous experiments (Tables 2, 3). Controlled heating of the thorax was achieved by applying constant voltage to the resistor. Using data from Tables 2 and 3, we simulated T_{th} from $35.2 \pm 0.4^\circ\text{C}$ to $41.7 \pm 0.9^\circ\text{C}$ (mean \pm S.D. during the whole heating cycle).

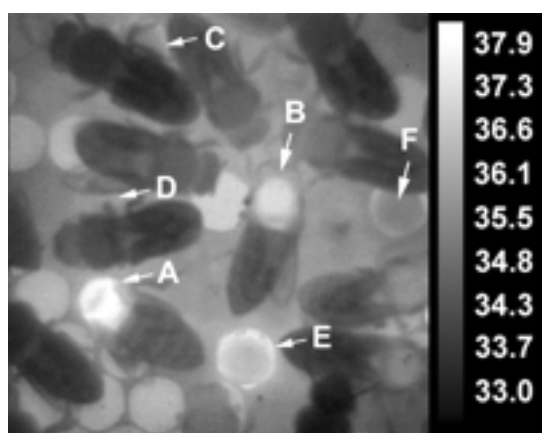


Fig. 5.

Thermogram of worker bees in the brood area, as seen in a common observation hive. The sealed area appears grey with no further details in this image; open cells are identifiable by the hexagonal structure of the cell rims. One bee with heated thorax [A; $T_{th(\text{entry})}=37.9^\circ\text{C}$] is about to enter an open cell adjacent to three sealed brood cells and pokes its head and the anterior part of its thorax into the cell (the dark structure on the heated thorax is the scutellum, seen from posterior–dorsal). Worker bee B has just left the open cell in the centre of the image [$T_{th(\text{exit})}=37.3^\circ\text{C}$]. The view on cell visitors C and D is partly hidden by bees on the comb surface. The onset of heat production during cell visits is roughly indicated when the cell interior and thorax (visible as a ring-like structure around the dark silhouette of the cool abdomen) start 'glowing' with increasing intensity. Note the different intensities caused by cell visitors E and F.

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Table 2.

Thorax temperatures (T_{th}) of individually marked bees on the surface of a capped brood cell, before and after visits to empty cells (setup A)

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Table 3.

Thorax temperatures (T_{th}) of worker bees inside three observation cells (setup B)

In different cycles, we varied the location of the thorax to simulate three basic behavioural patterns involving different modes of heat transfer (radiation and conduction) to the brood.

Variation A. The thorax was inside an empty cell adjacent to one of the brood cells that contained a thermistor. This arrangement simulated long-duration cell visits of heating bees as described in the present study (setups A and B). The thorax was placed near the bottom of the empty cell, since bees enter comb cells with their head and thorax first (Figs 2, 3).

Variation B. The thorax was located on the comb surface and its ventral side touched the brood cap. This behaviour can be observed in worker bees that are engaged in a special brood-heating activity (Bujok et al., 2002). Such bees stay motionless while producing heat without doing any other work during this time (Esch, 1960; Schmaranzer et al., 1988). Bujok et al. (2002) reported that such bees take a characteristic, crouched body posture for several minutes and contact the brood cap by pressing their heated thorax against it.

Variation C. As for variation B, but the thorax did not touch the brood cap. From endoscopic inspections of working and walking bees (Bujok et al., 2002) and observations at oblique angles in hives with transparent covers, we estimated a distance of 1.0–1.5 mm between the thorax and brood cap. Greater distances may occur but were found mainly in resting bees in the periphery of the comb (Kaiser, 1988; Kaiser et al., 1996) and were not simulated.

Setup D: brood cell temperatures and cell visitors within a colony

Can heat transfer from cell-visiting worker bees to the adjacent brood also be detected in populous colonies where many individuals contribute to the nest climate? As shown by the simulation experiment (results of setup C), the implantation of thermoprobes at the bottom of brood cells for measurements of T_{brood} is suitable to assess this question: due to their great distance to the probe (~ 11 – 14 mm), there is only slight interference from heating bees on the comb surface, whereas the thoraces of cell visitors are close (4–5 mm) and cause significantly higher warming rates (Table 4). Furthermore, fluctuations of local T_{hive} are buffered by the surrounding mass of brood and comb (see setup C).

View this table: » In this window » In a new window	Table 4. <i>Simulation of worker heating with an artificially heated thorax at varying locations (setup C, variations A, B and C)</i>
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A common observation hive with two combs standing on top of each other was established from a 20-frame colony 8 days before the observation started. To control the size and location of the brood area, we inserted a rectangular piece of brood (~ 11 cm \times 11 cm) from the original colony into the centre of the upper comb. Both sides of the hive were covered with a plate of Perspex (0.5 cm), each containing a 20 cm \times 10 cm 'window' made of plastic film in the centre of the upper comb, and were thermally insulated (~ 5 cm). On the observation side of the hive, part (20 cm \times 10 cm) of the insulation was removed during the observation to view the bees in the brood region through the plastic film and to determine their T_{th} by contactless thermography as described above.

Investigations on brood temperatures and cell visits were done in an area of $\sim 6\times 8$ comb cells (referred to as 'area of interest') containing empty cells and sealed brood. Five sealed brood cells, each of them surrounded by 4–6 empty cells, were equipped with temperature probes at the cell bottom for the measurement of T_{brood} . The probes (two thermistors and three thermocouples) were inserted from the back of the hive into the interior of the brood cells. This left the brood comb surface and brood caps on the observation side undamaged, and the movement of bees was not impeded by wires. The location of the probes was the same as in setup C (brood cell #1 in Fig. 3) to allow comparison with data obtained from the simulation.

A special construction on top of the hive (referred to as the 'cell-finder'; not shown) allowed us to select a certain brood cell on the observation side of the hive and to find the corresponding position on the opposite side of the comb. The cell-finder consisted mainly of a solid log with two parallel arms projecting downwards on both sides of the hive. Two thin steel pointers (diameter 1 mm; one pointer at the end of each arm) pointed inwards to the same position but on different sides of the comb. The whole construction was movable along the top of the hive and its height could be adjusted to point at any position within the brood area. The cell-finder was essential for orientation on two sides of a comb within a hive, because the bottom of each cell is formed by parts of three cells on the opposite side of the comb, and consecutive rows of cells are shifted half a cell diameter to match the hexagonal shape of the cells. After finding the corresponding position, one pointer was used to penetrate the plastic film on the back and to make a small hole in the bottom of the selected brood cell. The tip of the temperature probe was then implanted in the brood cell and the wires were fixed with tape. At the end of the experiment, the probes were pushed forward through the brood caps to confirm their position in the selected cell.

Data collection and analysis

During a 3-hour observation period (7:20 h to 10:20 hCEST), we investigated the temperatures of the five sealed brood cells and the visits of worker bees to the adjacent empty cells. For each visit, we noted its duration, $T_{\text{th(entr y)}}$ and $T_{\text{th(exit)}}$ from our real-video recordings and from the thermographic images. Short cell inspections (< 2 min) were not considered. For control purposes, the thorax temperatures of bees on the comb surface inside the area of interest were measured from the thermographic images every 2.5 min. The behaviour of the warmest bees ($T_{\text{th}} \geq 36^\circ\text{C}$) was observed ± 2 min from the time of measurement to see whether these bees were roaming around or standing motionless at a certain location for longer periods.

Thermistor values were recorded automatically every second (Almemo 2290–8). The thermocouples were connected to digital thermometers that provided no storage function. We recorded their displays continuously with a videocamera and later the values were read from the tapes every 15 s. Additional thermocouples measured T_{hive} in the brood area and T_{room} outside the hive.

The brood cell temperatures were analysed in two ways. First, we investigated whether the presence or absence of cell visitors had any influence on T_{brood} at all. Therefore, the temperature recordings of each brood cell were classified according to the number of long-duration cell visitors (N_{visitors}) currently occupying the empty cells adjacent to this brood cell. The first 1.5 min after a change of N_{visitors} were not included to allow the brood temperature to change to a noticeable extent (Table 4). The experimental conditions ($T_{\text{room}} = 26.0 \pm 0.7^\circ\text{C}$; partial removal of the insulation) ensured that brood warming rather than brood cooling was necessary to keep the brood temperature in the optimum range of 33–36°C. Since cell visitors may transfer heat to the brood and raise its temperature (results from setups A–C), we hypothesized that T_{brood} increases with N_{visitors} . Jonckheere's trend test (Bortz et al., 2000) was used to analyze the datasets for each brood cell.

Second, we investigated whether heat transfer from individual cell visitors to the brood is also detectable in this intact colony with many living bees. Results from the simulation experiment (setup C) proposed that cell visitors with high T_{th} ($> 36^\circ\text{C}$) increase the temperature of the adjacent brood cell at rates that are significantly higher (≥ 0.2 deg. min^{-1}) than the rates that are caused by bees on the comb surface, even if they press their thoraces on the comb and enhance heat transfer by means of conduction (Table 4). However, the T_{th} of cell visitors is not known in a

common observation hive and may vary during long-duration cell visits (setups A, B). Therefore, we focussed on those bees that already had high T_{th} when they entered a cell [$T_{th(entr)} \geq 36^\circ\text{C}$] and we analysed the changes of T_{brood} within the first 2 min after the start of these cell visits.

Results

Setup A: thoracic temperature of individual bees on the brood cap surface, prior to cell visits

T_{th} of all measured bees ($N=20$) was between 30.7°C and 43.0°C , with individual mean values from $32.2 \pm 1.0^\circ\text{C}$ to $38.1 \pm 2.5^\circ\text{C}$ (Table 2). The T_{th} of individuals varied over a range of 0.8 – 11.0°C (mean = $6.0 \pm 2.9^\circ\text{C}$; $N=20$) with intermittent warmings and coolings during their presence in the brood area.

16 of the observed bees made a total of 42 visits to empty cells surrounded by sealed brood cells. Visit duration ranged from 0.7 min to 32.9 min (mean 9.7 ± 7.8 min; total time 369 min; $N=38$ completely recorded cell visits).

Repeated measurements of T_{th} over a period of at least 0.5 min allowed the detection of changes in the thermal behaviour of individual bees. This was possible prior to 27 cell visits with observation times ranging from 0.9 min to 43.0 min (10.0 ± 9.4 min; $N=27$) before these bees entered a cell. In other cases, the bees were visible on the comb surface for only a few seconds between consecutive cell visits and allowed only single temperature measurements to be taken. Most bees raised their thoracic temperatures before entering a cell (Fig. 4) by values between $+1.1^\circ\text{C}$ and $+9.6^\circ\text{C}$ (mean $4.2 \pm 2.5^\circ\text{C}$; $N=22$ of 27 cell entries) during warming periods of 0.4 min to 4.9 min (mean 2.1 ± 1.1 min; $N=22$). Cell visits occurred when the bees were still increasing their temperature or shortly after reaching the highest T_{th} of a warming cycle, providing $T_{th(entr)}$ values up to 42.5°C (Table 2). In 83% of all cell visits, $T_{th(entr)}$ was higher (up to 5.9°C) than the mean T_{th} of the same bee during the whole observation period (Table 2).

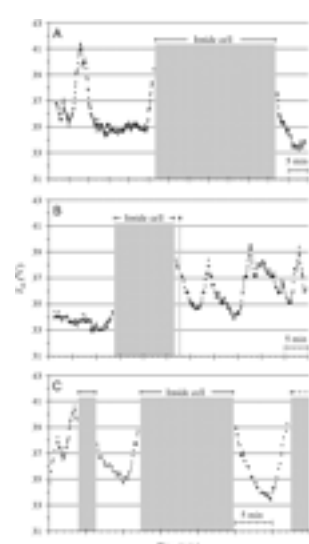


Fig. 4.

Thorax temperatures (T_{th}) of three different honeybees in the sealed brood area on the comb surface. (A) A 2 min warm-up ($+4.7^\circ\text{C}$) prior to a cell visit provides a $T_{th(entr)}$ of 39.4°C . (B) Heat production during a cell visit is revealed by a net warming, with $(\Delta T_{th})_{net} = +4.1^\circ\text{C}$. Heating was not resumed during a subsequent short-duration visit (0.7 min) to the same cell. (C) Repeated warmings prior to consecutive cell visits. Asterisks mark periods of 30 s or longer during which the bee's thorax was hidden by another bee and no temperature measurements could be made.

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Thorax temperatures at the end of cell visits [$T_{th(exit)}$] ranged from 34.3°C to 40.9°C (Table 2). Individual bees showed net temperature changes [$(\Delta T_{th})_{net}$] during cell visits over a range of -4.4°C to $+4.1^\circ\text{C}$ ($N=38$).

With this setup, T_{th} could not be recorded during cell visits. However, the onset of heat production during long-duration cell visits was suspected from the positive $(\Delta T_{th})_{net}$ values of some cell visitors and when the interior of the visited cell started 'glowing' conspicuously in the thermographic image with increasing and varying intensity (Fig. 5). Consequently, setup B was designed to provide more precise information about the T_{th} during cell visits.

Setup B: thoracic temperature of individual bees within the cells

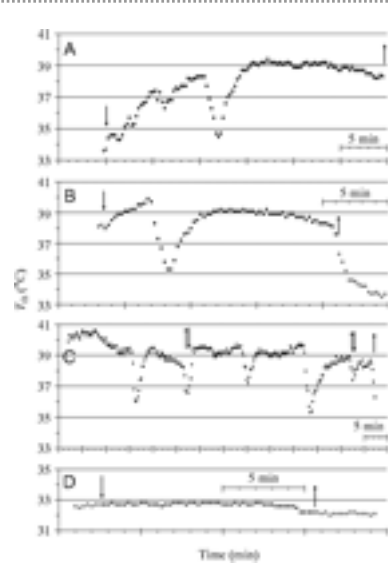
16 bees visited at least one of the three observation cells that were monitored with the close-up camera and remained within this cell for more than 2 min. Individual bees visited the same cell for periods ranging from 2.8 min to >63 min but with short interruptions when the bees exited and then re-entered the cells (Table 3). The longest stay without interruption was 33.8 min. The T_{th} values of the 16 bees ranged from 32.2°C to 41.7°C .

Three of the 16 bees showed vigorous body movements inside the cell, repeatedly moving forwards and backwards, turning around their longitudinal axes and applying their mandibles to the cell walls. These three individuals were considered to be 'working' (Table 3).

The remaining 13 bees lay still inside the observation cells, except for a few seconds after entering or before leaving the cell. Five of these 13 bees exhibited low and almost constant T_{th} values ranging from $32.7 \pm 0.1^\circ\text{C}$ to $33.4 \pm 0.3^\circ\text{C}$ and were regarded as resting ('resting bees' in Table 3; Fig. 6D; visitor to cell 'y' in Fig. 2B). Short bursts of pumping movements of their abdomens were interrupted by long pauses of up to 58 s (Fig. 7A,B).

Fig. 6.

Thorax temperatures (T_{th}) of different honeybees during long visits to empty cells adjacent to sealed brood cells. Bees that are lying still inside cells may be engaged in heat production (A–C) or resting (D). Heating bees with net temperature changes $(\Delta T_{th})_{net} = +3.9^\circ\text{C}$ (A) and -0.3°C (B). During a longer cell visit (C), repeated cooling and heating and short interruptions (arrows) occur. Symbols: ↓, bee enters cell; ↑, bee leaves cell. T_{th} of bee outside cell (open circles) and inside cell (filled circles) are plotted.



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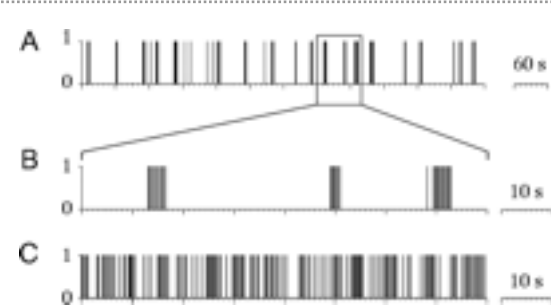


Fig. 7.

Diagram of respiratory pumping movements of the abdomen of bees inside cells. Pumping movements are drawn as spikes from 0 to 1 at the time of occurrence. (A) Resting bee with $T_{th}=33.4\pm 0.3^{\circ}\text{C}$. Thick lines are bursts of several pumping movements, which are shown at a higher temporal resolution in B. (C) Heating bee with $T_{th}=36.4\pm 0.3^{\circ}\text{C}$. Note that the time-scale of A is different from that of B and C.

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Eight of the 13 still bees maintained high (but not constant) T_{th} during most of the cell visit ('heat-producing bees' in Table 3; Fig. 6A-C; Fig. 2A). Individual T_{th} during cell visits varied in the range of 2.1°C to 6.6°C (mean $4.2\pm 1.6^{\circ}\text{C}$; $N=8$ bees) with intermittent cooling and heating periods. During the longest visits, T_{th} sometimes dropped several degrees but did not fall below 34.5°C before increasing again. $T_{th(\text{entry})}$ of these eight bees ranged from 34.4°C to 40.3°C (mean $38.3\pm 1.6^{\circ}\text{C}$; $N=11$ cell entries of eight bees). Three bees allowed longer temperature measurements before entering a cell and showed warm-ups (+2.9°C in 1.5 min and +3.3°C in 1.5 min) or high, rather stable T_{th} values ($39.4\pm 0.5^{\circ}\text{C}$ for 2 min). Net temperature differences [$(\Delta T_{th})_{\text{net}}$] at the beginning and at the end of cell visits ranged from -2.7°C to +3.9°C (Table 3; Fig. 6A-C).

Bees with high T_{th} showed rapid, almost continuous respiratory pumping movements of the abdomen (Fig. 7C), which frequently exceeded 3 s^{-1} in bees with the highest T_{th} ($>38^{\circ}\text{C}$).

Thoracic temperature of bees visiting cells outside the brood area

T_{th} values of bees visiting cells adjacent to storage or empty cells outside the brood area were lower than the mean T_{th} values of bees visiting cells adjacent to brood. The differences between the T_{th} values of these two groups of bees were, with one exception, statistically significant on all observation days (Table 1).

Setup C: simulation of worker heating

The presence of a heated thorax in an empty cell adjacent to or on the cap of a sealed brood cell caused clearly detectable warmings that were continuous throughout the heating cycle (Table 4). The extent and rate of warming depended on the T_{th} that was applied, on the location of the thorax (variations A, B and C) and on the location of the thermistors at the cell bottom (T_{brood}) or beneath the cap (T_{cap}). Details are presented below and in Table 4. Generally, warming rates inside the brood cell were highest at the beginning and declined during the simulation.

Variation A: location of the thorax inside an empty cell

Heating from inside an empty cell clearly raised the temperature of the surrounding brood up to three cells away from the heated thorax. Within a 30 min period, the temperature of brood cell #1 (Fig. 3) increased by values of up to +2.5°C at maximum warming rates of 0.5 deg. min^{-1} (Table 4). At the end of the heating cycles with T_{th} values of 35.2°C, 36.3°C, 38.6°C and 41.7°C, the T_{brood} of brood cell #2 had been raised by values of $0.32\pm 0.03^{\circ}\text{C}$, $0.41\pm 0.07^{\circ}\text{C}$, $0.75\pm 0.05^{\circ}\text{C}$ and $1.14\pm 0.04^{\circ}\text{C}$, respectively (data not shown in Table 4; final values at $t=30$ min, mean \pm S.D. from 3-4 cycles each), at warming rates of up to 0.1 deg. min^{-1} . At the same time, the temperature of brood cell #3 increased by values of $0.14\pm 0.02^{\circ}\text{C}$, $0.16\pm 0.04^{\circ}\text{C}$, $0.30\pm 0.03^{\circ}\text{C}$ and $0.45\pm 0.03^{\circ}\text{C}$ at warming rates of $<0.05\text{ deg. min}^{-1}$.

Variations B and C: location of the thorax on the comb surface

The presence of a heated thorax on the brood cap raised T_{cap} by up to $3.0\pm 0.5^{\circ}\text{C}$ within 30 min. The maximum warming rates (MWR) and ΔT_{cap} values indicate that heat transfer to the brood cell was 1.9-2.6 times more efficient when the thorax touched the brood cap (variation B) than when it was 1-1.5 mm away (variation C; Table 4). Differences between variations B and C were significant ($T_{th}=39.8^{\circ}\text{C}$; one-tailed Wilcoxon, Mann-Whitney U -test; Sachs, 1999; $P<0.05$ for each test of ΔT_{cap} at $t=2, 5, 10$ and 30 min and for the maximum warming rates).

The changes of T_{brood} at the cell bottom are important with regard to setup D because they determine the extent of 'interference' from worker bees on the comb surface. Warming rates of T_{brood} of up to $0.15\text{ deg. min}^{-1}$ may be caused by thoraces on the comb surface (T_{th} up to

40.3±0.7°C; Table 4) and by thoraces inside cells with $T_{th} < 36.3 \pm 0.4^\circ\text{C}$ (one-tailed Wilcoxon, Mann-Whitney U -test, $P > 0.05$, not significant).

Thoraces inside cells with $T_{th} \geq 36.3 \pm 0.4^\circ\text{C}$ caused significantly higher warming rates (MWR $0.19 \pm 0.01 \text{ deg. min}^{-1}$ to $0.52 \pm 0.03 \text{ deg. min}^{-1}$) than the warmest thoraces on the comb surface ($T_{th} = 40.3 \pm 0.7^\circ\text{C}$), even when the latter ones touched the brood cap and heat transfer was enhanced by means of conduction (one-tailed Wilcoxon, Mann-Whitney U -test, $P < 0.05$).

T_{brood} of the remote brood cells #2 and #3 (Fig. 3; final values at $t = 30 \text{ min}$; data not shown in Table 4) was raised by $0.50 \pm 0.09^\circ\text{C}$ and $0.38 \pm 0.13^\circ\text{C}$, respectively, when the thorax was in touch with the brood cap ($T_{th} = 40.3 \pm 0.7^\circ\text{C}$; $N = 5$) and by values of $0.33 \pm 0.10^\circ\text{C}$ and $0.22 \pm 0.07^\circ\text{C}$, respectively, when it did not touch it ($T_{th} = 40.1 \pm 0.8^\circ\text{C}$; $N = 7$). Warming rates were $\leq 0.05 \text{ deg. min}^{-1}$ in all cases.

Setup D: cell visits and temperatures of adjacent brood cells

During a 3 h observation period at $T_{room} = 26.0 \pm 0.7^\circ\text{C}$ and $T_{hive} = 32.3 \pm 0.6^\circ\text{C}$, the temperatures of the five investigated brood cells were maintained well in the optimum range for brood development at $33.6 \pm 0.6^\circ\text{C}$ (coolest cell) to $35.1 \pm 0.3^\circ\text{C}$ (warmest cell) (Table 5). T_{brood} never fell below 32.6°C (measured in the outermost cell) and was repeatedly raised up to 35.9°C . In different cells, warmings and coolings frequently occurred simultaneously (Fig. 8).

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Table 5.

Temperatures of five brood cells (indexed from a to e) in relation to the number of long-lasting cell visitors ($N_{visitors}$) in the adjacent cells (setup D)

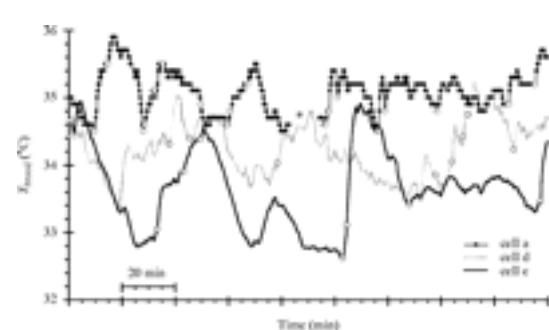


Fig. 8.

Interior temperatures of three different brood cells (T_{brood}) in an observation hive (setup D), measured simultaneously at the bottom of each cell. All cells were in the same region of the brood nest, 2–3 intermittent cells away from each other, and were adjacent to 4–6 empty cells. Note that cooling and warming may occur simultaneously in different cells. An open circle on the temperature curve marks the time when a bee with a thorax temperature (T_{th}) of $\geq 36^\circ\text{C}$ entered an empty cell adjacent to this brood cell for a long-duration cell visit ($\geq 2 \text{ min}$). See Results (setup D) and Discussion for cell visits that are not followed by a temperature change within 2 min and for temperature changes that are not preceded by such a cell visit. Temperatures of cells d and e were stored every second with a resolution of 0.01°C . The T_{brood} of cell a was read from video every 15 s with a resolution of 0.1°C (consecutive values are connected by a line for better visualisation of the curve). The asterisk marks a gap of 11 min in the data record of cell a.

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Bees on the comb surface

At any time during the observation, the comb surface in the 'area of interest' (6×8 cells) was covered by 6–16 bees (11.4 ± 2.1 bees) with T_{th} values ranging from 31.1°C to 42.8°C (830 values from 73 still images; mean T_{th} per image was $32.7 \pm 0.8^\circ\text{C}$ to $35.2 \pm 1.4^\circ\text{C}$). The behaviour of bees with $T_{th} \geq 36.0^\circ\text{C}$ ($N = 56$) was investigated more closely to check for possible influence on the warming rates of T_{brood} (Table 4, variations B and C). The majority of these bees were present only for a short time on the comb surface in the investigated area: 10 bees were crossing the area of interest while roaming around in the brood area without standing still at a certain location. Eight bees were present on the comb surface only for a few seconds during short interruptions of a cell visit (setups A, B) or between consecutive cell visits to different cells. 34 records of $T_{th} \geq 36.0^\circ\text{C}$ were obtained from bees that either had been visiting or were about to visit an empty cell within $\pm 2 \text{ min}$ of the time when we recorded their temperature and thus were no longer present on the comb surface. Six bees (including two bees that finally entered a cell) were standing motionless for at least 0.5 min in the area of interest, on or near the investigated brood cells (duration of immobility: 0.5–4.9 min; mean \pm S.D. $2.4 \pm 1.9 \text{ min}$; $N = 6$).

Cell visitors

A total of 98 long-duration cell visits with visit durations from 2.2 min to 46.6 min (mean $14.9 \pm 10.7 \text{ min}$; $N = 92$ completely recorded cell visits) to the empty cells adjacent to the brood were observed. During most of the time, 1–3 empty cells adjacent to a certain brood cell were occupied simultaneously by cell visitors (Table 5). The increase of T_{brood} with the number of cell visitors ($N_{visitors}$) was highly significant (Jonckheere's trend test, $P < 0.001$ for each of the five investigated cells).

The thorax temperatures of the cell visitors were consistent with those from the other experiments (setups A, B), indicating previous heating activity on the comb surface before entering the cell and heat production during the long-duration cell visits: $T_{th(entry)}$ values ranged from 33.0°C to 41.9°C , and $T_{th(exit)}$ values ranged from 33.3°C to 40.0°C . The $(\Delta T_{th})_{net}$ of these bees ranged from -4.9°C to $+5.3^\circ\text{C}$.

Changes of T_{brood} within two minutes of the disappearance of warm bees ($T_{th} \geq 36^\circ\text{C}$) into one of the adjacent empty cells were investigated in detail [$N = 73$ cell visits with $T_{th(entry)}$ from 36°C to 42°C]. In 81% of these visits, heat transfer from the cell-visiting bees to the brood was detectable as an increase in the warming rate of T_{brood} by at least $+0.2 \text{ deg. min}^{-1}$ shortly after the start of the cell visit (delay: $25.1 \pm 18.8 \text{ s}$; $N = 59$). The warming rates changed from $0.0 \pm 0.2 \text{ deg. min}^{-1}$ just prior to the cell visit to maximum values of $0.5 \pm 0.3 \text{ deg. min}^{-1}$ within the subsequent 2 min period ($N = 59$). The differences were statistically significant (Student's t -test, paired samples, $P < 0.001$). In the temperature recording (Fig. 8), these visits usually marked a change to stable $T_{brood} > T_{hive}$ from a previous cool-down cycle (i.e. warming rates of < 0 before the beginning of a cell visit) or they marked a change to a warm-up cycle from previously stable T_{brood} or from a previous cool-down cycle. The

highest warming rates were detected when two or three empty cells adjacent to the same brood cell were visited simultaneously or within a short time (<2 min) by two or three bees with $T_{th(entr)} \geq 36^\circ\text{C}$ or when the beginning of a cell visit coincided with a warm-up cycle that was caused by other bees (warming rate of >0 at the start of a cell visit), thereby increasing the warming rate to up to $1.5 \text{ deg. min}^{-1}$.

In 19% of the cell visits ($N=14$), no influence on T_{brood} or only a faint increase in the warming rate of the adjacent brood cell could be detected ($<0.2 \text{ deg. min}^{-1}$), suggesting that these bees temporarily stopped or reduced heat production after entering the cell. The brood temperatures at the beginning of these cell visits were already well in the optimum range for brood development ($T_{brood}=35.1 \pm 0.6^\circ\text{C}$; $N=14$), i.e. further warming was not essential at that time, and were significantly higher than in those cases where we subsequently noted an increase in the warming rate ($T_{brood}=34.4 \pm 0.6^\circ\text{C}$; $N=59$; two-tailed Wilcoxon, Mann-Whitney U -test, $P<0.01$).

Discussion

Heating and resting bees

During earlier investigations on division of labour in honeybee societies, bees were often seen to disappear into cells for periods of several minutes to over an hour and a half, apparently without performing any recognizable task (Lindauer, 1952; Sakagami, 1953). Cell-related activities like larval feeding or cell cleaning usually take only seconds or 2–3 min at the most (Lindauer, 1952). With reservations (Lindauer, 1952; Sakagami, 1953), longer visits were classified as 'idleness' or 'cell cleaning' behavioural categories, and later the neutral behavioural category 'in cell' was established for cell visits of more than 3 min duration and where no obvious body movements could be discerned (Kolmes, 1984).

Our observations show that some of the bees that undertake long-duration cell visits are engaged in heat production whereas others are indeed resting. Both heating and resting bees lie quietly inside cells but the heating bees maintain a T_{th} above T_{hive} whereas the resting bees do not. However, they can be distinguished from each other by their abdominal respiratory pumping movements, which are discontinuous with long breaks in resting and sleeping bees (Fig. 7A,B; Kaiser, 1988; Kaiser et al., 1996) but rapid and continuous in heating bees (Fig. 7C), implying high rates of respiration (Bailey, 1954; Fraenkel, 1932; Heinrich, 1972). Similar continuous and rapid respiratory movements have been described for various insects during warm-up before flight (Heinrich, 1993; Krogh and Zeuthen, 1941; Sotavalta, 1954) and for brood-incubating hornets (*Vespa crabro*; Ishay and Ruttner, 1971) and bumblebees (*Bombus* spp.), where conspicuous differences in the abdominal pumping movements of resting and heating individuals also occur (Heinrich, 1972). It should be stressed, however, that the abdominal movements must be observed for some minutes to detect long respiratory breaks that characterize the resting bees. Otherwise, a resting bee could be mistaken for a heating bee at the time when bursts of pumping movements occur (see Fig. 7B,C).

Net temperature changes during cell visits

Cell visitors may have high $T_{th(entr)}$ due to preceding heating activity on the brood cap surface immediately prior to a cell visit. If the bees that enter the cells do not continue to generate higher than normal body temperatures they would simply cool down to the ambient temperature of the brood, which would gain relatively little heat in the exchange.

The calculation of $(\Delta T_{th})_{net}$ values was helpful at an early stage of this work when the T_{th} of bees in a common observation hive were investigated. The onset of heat production of bees inside cells was clearly indicated when net warmings with $(\Delta T_{th})_{net} > 0$ (up to $+4.1^\circ\text{C}$; Figs 4B, 6A; Tables 2, 3) were found, although the amount of heat transferred to the brood comb cannot be calculated from this value. The occurrence of intermittent cooling and heating periods during long cell visits reduces the usefulness of the $(\Delta T_{th})_{net}$ values.

During detailed observation of individual bees inside empty cells (Table 3; Fig. 6), the T_{th} values of these bees were in a range of 9.5°C (32.2 – 41.7°C). Highest values of $|\Delta T_{th})_{net}|$ would be expected if $T_{th(entr)}$ is close to one extreme of this temperature range and $T_{th(exit)}$ is on the other extreme, i.e. if a bee changes its behaviour from resting to heating or *vice versa*. However, the $|\Delta T_{th})_{net}|$ values we measured were small (up to 3.9°C) in comparison with the total range of 9.5°C that might have been found under the experimental conditions. This was because the $T_{th(entr)}$ values were usually between these extremes due to preceding heating activity on the comb surface and because the T_{th} values of heating bees inside cells varied individually only within a range of 2.1 – 6.6°C and did not drop down to the level of resting bees.

Despite repeated cooling and warming during long cell visits, the $(\Delta T_{th})_{net}$ may not be detected at all if only $T_{th(entr)}$ and $T_{th(exit)}$ are known. This is clearly shown in Table 3, where T_{th} of two bees varied in a range of 5.3°C and 4.6°C during their cell visits, but $T_{th(entr)}$ and $T_{th(exit)}$ were similar and almost no net temperature changes were detected [$(\Delta T_{th})_{net}$ values between -0.4°C and $+0.3^\circ\text{C}$; see Table 3, y1 and z5; Fig. 6B]. Especially during long cell visits, intermittent heating activity of a bee may be masked if an apparent cooling occurs, i.e. $(\Delta T_{th})_{net} < 0$ (Table 3, y2, y5, z5, z7). Likewise, the detection of $(\Delta T_{th})_{net} > 0$ does not imply that heat production was continuous throughout the cell visit. This finding is relevant for our observation of bees on the comb surface in a common observation hive (Table 2 and setup D) where only $T_{th(entr)}$ and $T_{th(exit)}$ could be measured.

Heating behaviour inside cells

Why do bees with elevated T_{th} enter empty cells in the brood area and maintain high T_{th} during their long cell visits without performing any visible work? Resting or sleeping bees do not maintain high T_{th} values and their body temperatures are at ambient levels (Kaiser, 1988). The eight 'still' bees whose thoracic temperatures we measured inside cells were obviously not resting since they were engaged in heat production during most of the time. It is known that honeybee workers on the comb surface produce heat to warm their brood (Esch, 1960; Harrison 1987; Kronenberg and Heller, 1982; Schmaranzer et al., 1988; Bujok et al., 2002) and to keep it in a temperature range that is optimal for development (Himmer, 1927, 1932; Muzalewskij, 1933; Weiss, 1962). The observation cells in which we found heating bees were directly adjacent to sealed pupae cells, which leads us to propose that bees with high T_{th} are warming the pupae in the adjacent cells. Due to their high $T_{th(entr)}$ and the maintenance of high T_{th} during cell visits, these bees are a potential heat source for the neighbouring brood. This strategy provides a way to transfer heat from workers to the brood nest in addition to that achieved by workers on the brood cap surface. The transfer of

heat from worker bees to the brood during long-duration cell visits has to be expected due to the temperature gradient between the heated thorax and the surrounding cells and was clearly detectable during our simulation experiment and in an intact colony in an observation hive. A similar cell-visiting behaviour adjacent to sealed brood is known for wasps that rear their brood in paper nests with a similar cell arrangement (Ishay, 1972).

Heat transfer to adjacent brood

Heat transfer from individual cell visitors to the brood was simulated with setup C and could also be detected in most cell visits of bees with a $T_{th(entr)} \geq 36^\circ\text{C}$ in setup D. Not all prominent warmings of T_{brood} occurred within 2 min of the start of these cell visits. This is because long-duration cell visitors may stop and resume heating at any time during their stay inside a cell (setup B), and, during 70.4–88.1% of the observation time of setup D, there were two or more cell visitors simultaneously occupying empty cells that were adjacent to a certain brood cell (Table 5, $N_{visitors} \geq 2$). Although the T_{th} of long-duration cell visitors are not known in a common observation hive and cannot be estimated from their $T_{th(entr)}$ and $T_{th(exit)}$ values [see discussion of the $(\Delta T_{th})_{net}$ values], there is some indication that the prominent warmings of the investigated brood cells were caused mainly by bees inside empty cells rather than by bees on the comb surface. First, bees on the comb surface are further away from the T_{brood} probe and cause significantly lower warming rates at the cell bottom, even if they touch the brood caps with their thorax and enhance heat transfer by means of conduction (Table 4). The temperatures of most bees on the comb surface were in a range that was covered by the simulation parameters (T_{th} in Table 4). Although heat transfer from such bees could be detected with our methods, they may not account for the unusual high warming rates at the cell bottom (up to $+1.5 \text{ deg. min}^{-1}$). Second, most of the bees with $T_{th} \geq 36^\circ\text{C}$ were present for only a short time in the area of interest while roaming around. Interestingly, the majority of these warm bees were associated with cell visits ($N=42$ of 56 bees) and they were visible on the comb surface only during a short interruption or between two consecutive cell visits or they were about to enter or had recently left an empty cell adjacent to a brood cell. Third, due to its size, a brood cap may be covered only by one thorax of a bee on the comb surface. Additional heat transfer from bees on the caps of neighbouring brood cells occurs (setup C, variations B and C, brood cells #2 and #3) but may be negligible in this setup. This is not only because of the brief presence of warm bees with $T_{th} \geq 36^\circ\text{C}$ in the area of interest but also because the investigated brood cells were mainly surrounded by empty cells. By contrast, several bees inside empty cells that are adjacent to the same brood cell may produce heat simultaneously (Fig. 2A) and cause warming rates that are higher than those that were simulated with only one thorax as source of heat (Table 4, variation A, MWR up to $0.5 \text{ deg. min}^{-1}$). Finally, in a different analytical approach that considered the number of cell visitors at any time during the observation, the influence of $N_{visitors}$ on T_{brood} was found to be highly significant in all investigated brood cells (Table 5), confirming that cell visitors contribute to the temperature of the adjacent brood cells.

Efficiency of heat transfer

In the simulation (setup C), thoraces touching the brood cap raised T_{cap} by values of up to $+3.0^\circ\text{C}$ within 30 min whereas thoraces inside empty cells raised T_{brood} only by values of $+2.5^\circ\text{C}$ in the same time. Although different heat transfer properties of the brood caps and the cell walls cannot be excluded, this difference is thought to be mainly due to the different distances of the thoraces to the thermoprobes: the T_{cap} probe was located just beneath the brood cap, $\sim 1 \text{ mm}$ away from the thorax that was on the cap, whereas the T_{brood} probe was placed at the cell bottom and in the centre of the brood cell, $\sim 4\text{--}5 \text{ mm}$ away from the thorax that was inside the empty cell (Fig. 3).

The size of the thorax allows it to cover only one brood cap completely or up to three brood caps partially. Conductive heat transfer is possible only from the ventral side of the thorax that is in touch with the brood caps, whereas the lateral and dorsal thorax surfaces are exposed to the hive air. Thermal radiation from these surfaces is obviously not transferred to specific brood cells but may contribute to a less specific warming of the air between two combs, of the neighbouring comb's surface or of nearby bees. In comparison to the heating behaviour on the surface of brood combs, the use of empty cells for brood nest warming appears to be advantageous for two reasons. First, in colonies that do not produce abnormal offspring, empty cells make up 8–12% of cells within the brood nest (Woyke, 1984). Due to the hexagonal arrangement of comb cells, a heating bee inside an empty cell in the brood area can be fully surrounded by up to six brood cells, and heat that is emitted from the ventral, dorsal and lateral thorax surfaces may be transferred to six pupae in the adjacent cells. Second, heat loss to the abdomen and to the hive air is minimized because the heat source (i.e. the thorax) is deep in the comb (almost down to the middle wall) and heat flow to the cooler abdomen is reduced by a countercurrent heat exchanger in the narrow petiole region (see Fig. 2; Heinrich, 1993; Heinrich and Esch, 1994).

Worker bees that are pressing their thoraces against the brood caps (Bujok et al., 2002) enhance heat transfer by factors of 1.9–2.6 in comparison with motionless bees of the same T_{th} that do not contact the brood cap (Table 4). The strong superficial warming of the brood cell by up to $+3.0 \pm 0.5^\circ\text{C}$ (setup C, T_{cap} in variation B) compares well to the maximum value of 3.2°C for 'hot spots' that were detected inside a populous observation hive and with different methods by Bujok et al. (2002). Bees displaying this brood incubation behaviour usually touch the brood caps with the tips of their antennae (Bujok et al., 2002), where thermoreceptors and other sensilla are located (Heran, 1952; Lacher, 1964), suggesting that sensory feedback is used to adjust the temperature of specific brood cells. This assumption is supported by our recordings of brood cell temperatures (Fig. 8), which were repeatedly raised to 35.9°C but not to higher temperatures that are detrimental to the brood (Himmer, 1927, 1932; Muzalewskij, 1933; Weiss, 1962; Tautz et al., 2003). By doing this, the bees also counteracted the cooling of the brood below the lower limit for optimum brood development. Although most of the bees in a colony contribute to the hive's microclimate due to their body temperature, precise regulation of brood temperature is obviously done on a smaller scale by individuals that display specialized brood incubation behaviour on the comb surface (Esch, 1960; Schmaranzer et al., 1988; Bujok et al., 2002) and inside empty cells (present study) in the absence of any other activity, thereby efficiently transferring heat to the sealed brood cells in their vicinity. Further studies need to show what exactly elicits this behaviour at a certain location in the brood area.

ACKNOWLEDGEMENTS

We thank D. C. Sandeman for valuable comments on the manuscript, R. Stelzer for reading the thermocouple values from video and H. Demmel for providing the bees and hives. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 554 and GK 200).

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