

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

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Abstract – Nest temperature in honeybees is a crucial factor for brood development since it 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, as well as 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 an 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 small temperature fluctuations in individual brood cells within the physiological limits regulated by the workers have substantial significance for 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; Becher et al., 2009).

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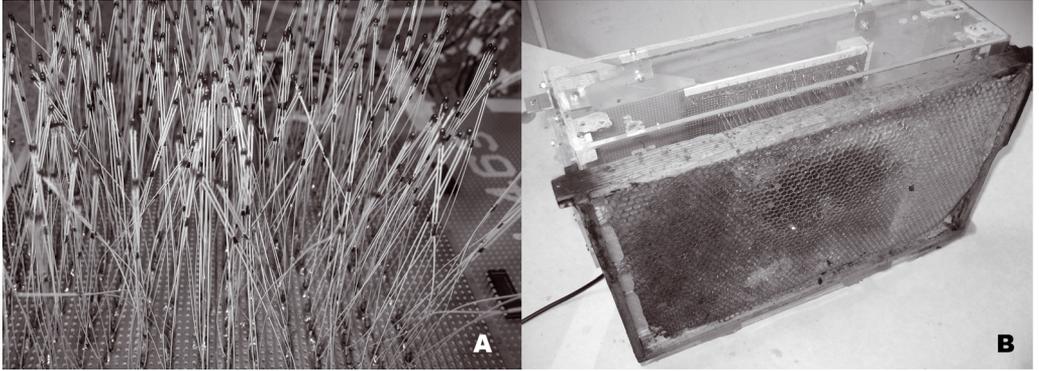


Figure 1. 256 resistor NTC resistor sensors were placed in a grid of 16 rows by 16 columns on a circuit board (A). The temperature sensors project through the front panel, just reaching the cell wall junctions of the test comb. The perspex box can easily be placed in any standard hive (B).

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 if we could 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 while providing minimal disturbance to the colony.

2. METHODS AND RESULTS

2.1. General description

256 resistor sensors were arranged on a 15×15 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 nonlinearly 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

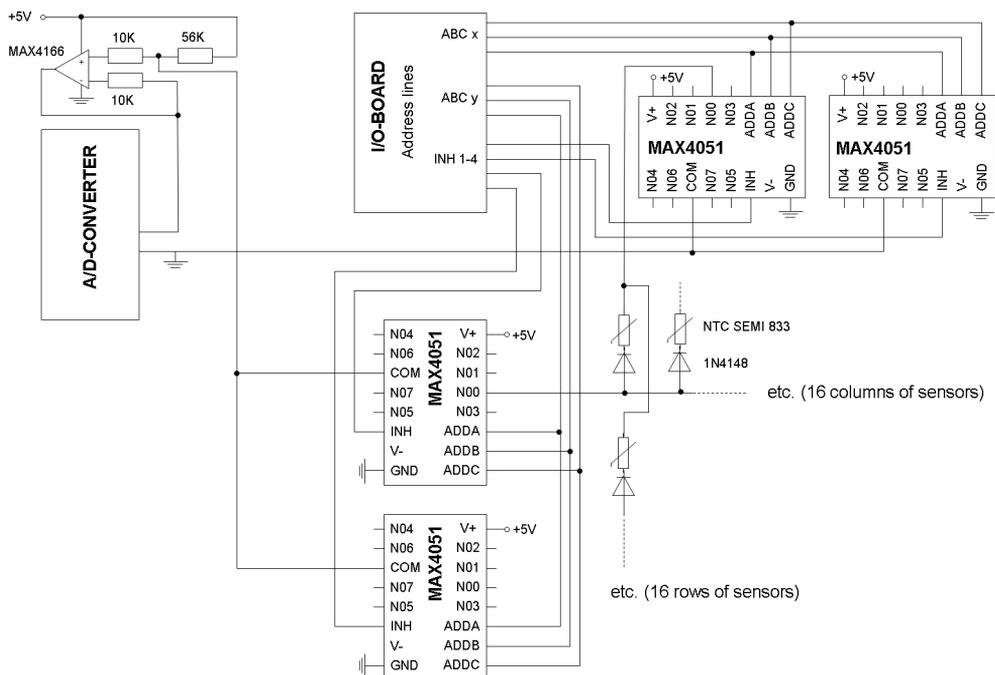


Figure 2. Simplified circuit diagram: 256 NTC sensors, each provided with a standard diode (1N4148) are placed on a 16×16 grid with rows and columns are chosen via four 8-channel multiplexers (MAX 4051). Addressing of the channels is accomplished by a PC controlled I/O-board (Quancom: PCI TTL-I/O 32),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

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 that 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. The software tool together with a data file is provided as electronic-only material on the Apidologie website (<http://www.apidologie.org>).

2.4. Empirical test of the instrument

We tested the instrument in a hive with the test comb, three additional frames and about 3000 workers bees. The colony was placed in

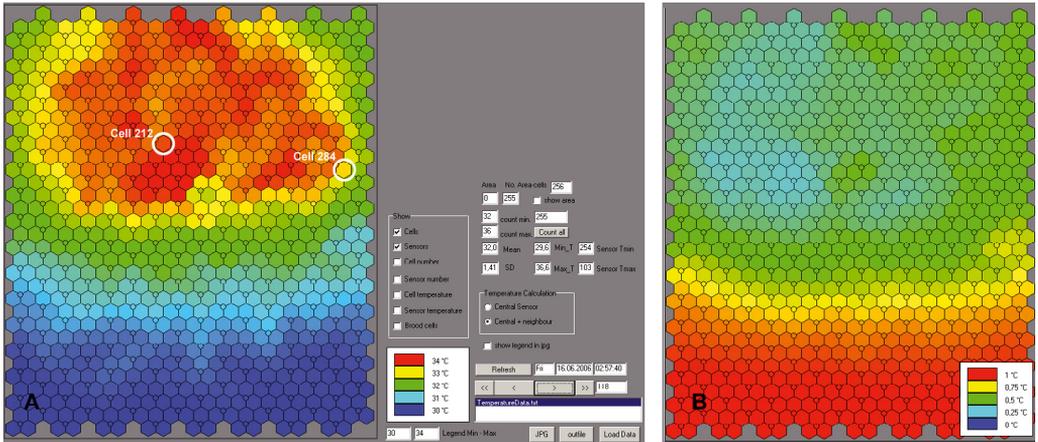


Figure 3. (A) Recorded temperature data are analyzed and displayed via a visualization software. Shown here is a snapshot of the temperature distribution on June 16, 2006, 2:57 a.m. with brood being present in the upper part of the comb (red and green area). For the cells No. 212 and No. 284 the temperature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

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 (through which 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 the egg phase was 32.7 °C, ranging from 30.5 °C to 34.0 °C. During 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 ± 1 °C) were found in the broodless area (Fig. 3B).

2.6. Comparing front and back side temperatures

To detect the temperature differences between the front and back sides of cells, 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

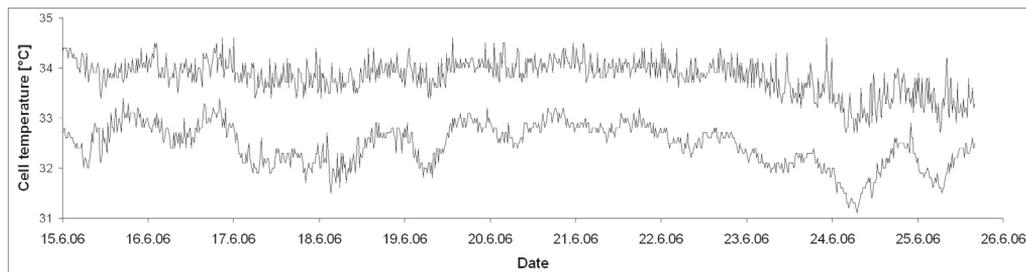


Figure 4. Two examples for individual temperature profiles of a warm and a cool broodcell during the pupal stage. The warm cell No. 212 was situated close to the centre of the broodnest, whereas the cooler cell No. 284 was located at the periphery (compare with Fig. 3A).

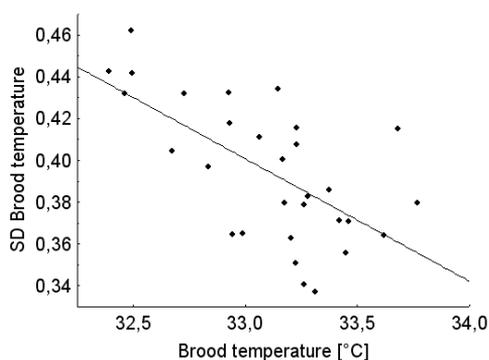


Figure 5. The standard deviation of the temperature plotted against mean developmental temperature of those cells, where definitely brood was present. (Pearson test: $P = 0.0001$, $r = -0.64$, $N = 30$).

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 were usually only applied in a

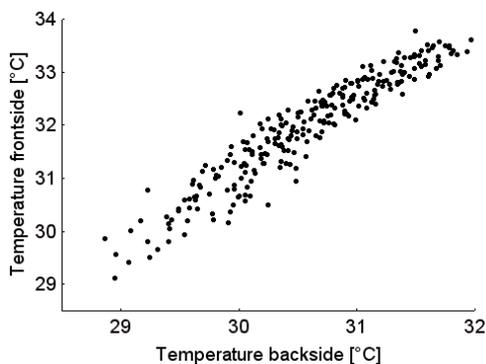


Figure 6. Comparison of front- and back side temperatures in an empty comb. We used two instruments placed in a temperature gradient to measure the temperatures in one comb simultaneously on both sides. Each data point represents the temperatures of a pair of two opposing sensors, averaged over two hours.

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 heated the air on one side of the comb and measured 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 they showed a much stronger variance. As 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 the role it will play as an adult in the colony and hence for overall colony organization of honeybees.

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Nouveau dispositif pour mesurer en continu la température dans les cellules de couvain de l'Abeille domestique (*Apis mellifera*).

Apis mellifera / couvain / température / mesure

Zusammenfassung – Eine neuartige Methode der kontinuierlichen Temperaturmessung in den Brutzellen von Honigbienen (*Apis mellifera*). 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 × 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.

Honigbiene / Brutwabe / Temperaturmessung

REFERENCES

- Andrews E.A. (1929) Populations of Ant Mounds, *Q. Rev. Biol.* 4, 248–257.
- Becher M.A., Scharpenberg H., Moritz R.F.A. (2009) Pupal developmental temperature and behavioral specialization of honeybee workers (*Apis mellifera* L.), *J. Comp. Physiol. A*, DOI:10.1007/s00359-009-0442-7.
- Büns M., Ratte H.T. (1991) The combined effects of temperature and food consumption on body weight, egg production and developmental time in *Chaoborus crystallinus* De Geer (Diptera: Chaoboridae), *Oecologia* 88, 470–476.
- Cameron S.A. (1985) Brood care by male bumble bees, *Proc. Natl Acad. Sci. USA* 82, 6371–6373.
- Dunham W.E. (1931) Hive temperatures for each hour of a day, *Ohio J. Sci.* 31, 181–188.
- Esch H., Goller F., Heinrich B. (1991) How do bees shiver? *Naturwissenschaften* 78, 325–328.
- Fahrenholz L., Lamprecht I., Schrick B. (1989) Thermal investigations of a honey bee colony, thermoregulation of the hive during summer and winter and heat production of members of different bee castes, *J. Comp. Physiol. B* 159, 551–560.
- Groh C., Tautz J., Rössler W. (2004) Synaptic organization in the adult honey bee brain is influenced by brood-temperature control during pupal development, *Proc. Natl Acad. Sci. USA* 101, 4268–4273.
- Harrison J.M. (1987) Roles of individual honeybee workers and drones in colonial thermogenesis, *J. Exp. Biol.* 129, 53–61.
- Heinrich B. (1993) *The Hot-Blooded Insects*, Harvard University Press, Mass, p. 606.
- Himmer A. (1927) Ein Beitrag zur Kenntnis des Wärmehaushaltes im Nestbau sozialer Hautflügler, *Z. Vgl. Physiol.* 5, 375–389.
- Hess W.R. (1926) Die Temperaturregulierung im Bienenvolk, *Z. Vgl. Physiol.* 4, 465–487.
- Howe R.W. (1967) Temperature Effects on Embryonic Development in Insects, *Annu. Rev. Entomol.* 12, 15–42.
- Hozumi S., Yamane S., Miyano S., Mateus S., Zucchi R. (2005) Diel changes of temperature in the nests of two Polybia species, *P. paulista* and *P. occidentalis* (Hymenoptera, Vespidae) in the subtropical climate, *J. Ethol.* 23, 153–159.
- Kastberger G., Stachl R. (2003) Infrared imaging technology and biological applications, *Behav. Res. Methods, Instrum. Comput.* 35, 429–439.
- Kleinhenz M., Bujok B., Fuchs S., Tautz J. (2003) Hot bees in empty broodnest cells: heating from within, *J. Exp. Biol.* 206, 4217–4231.
- Kronenberg F., Heller H.C. (1982) Colonial thermoregulation in honey bees (*Apis mellifera*), *J. Comp. Physiol. B* 148, 65–76.
- Lindauer M. (1954) Temperaturregulierung und Wasserhaushalt im Bienenstaat, *J. Comp. Physiol.* A 36, 391–432.
- Newport G. (1837) On the Temperature of Insects, and Its Connexion with the Functions of Respiration and Circulation in This Class of Invertebrated Animals, *Philos. T. Roy. Soc. B* 127, 259–338.
- Nylin S., Gotthard K. (1998) Plasticity in Life-History Traits, *Annu. Rev. Entomol.* 43, 63–83.
- Omholt S.W., Amdam G.V. (2004) Epigenetic Regulation of Aging in Honeybee Workers, *Sci. Aging Knowl. Environ.* 26, pe28.
- Petz M., Stabentheiner A., Crailsheim C. (2004) Respiration of individual honeybee larvae in relation to age and ambient temperature, *J. Comp. Physiol. B* 174, 511–518.
- Rosenkranz P., Engels W. (1994) Genetic and environmental influences on the duration of preimaginal worker development in eastern (*Apis cerana*) and western (*Apis mellifera*) honey bees in relation to Varroaosis, *Rev. Bras. Genet.* 17, 383–391.

- Scherba G. (1962) Mound Temperatures of the Ant *Formica Ulkei* Emery, Am. Midl. Naturalist 67, 373–385.
- Sibly R.M., Atkinson D. (1994) How rearing temperature affects optimal adult size in ectotherms, Funct. Ecol. 8, 486–493.
- Southwick E.E. (1983) The honey bee cluster as a homeothermic superorganism, Comp. Biochem. Physiol. A 75, 641–645.
- Southwick E.E., Heldmaier G. (1987) Temperature control in honey-bee colonies, Bioscience 37, 395–399.
- Stabentheiner A., Schmaranzer S. (1987) Thermographic determination of body temperatures in honey bees and hornets: calibration and applications, Thermology 2, 563–572.
- Steiner A. (1929) Temperaturuntersuchungen in Ameisennestern mit Erdkuppeln, im Nest von *Formica exsecta* Nyl. und in Nestern unter Steinen, Z. Vgl. Physiol. 9, 1–66.
- Tautz J., Maier S., Groh C., Rössler W., Brockmann A. (2003) Behavioral performance in adult honey bees is influenced by the temperature experienced during their pupal development, Proc. Natl Acad. Sci. USA 100, 7343–7347.