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Calorimetric investigations on metabolic rates and thermoregulation of sleeping honeybees (*Apis mellifera carnica*)

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Abstract

Heat production rates of sleeping honeybees were determined at ambient temperatures between 20 and 35 °C. They increased with temperature from 4.7 mW g^{-1} (S.D. 2.2 mW g^{-1}) at 20 °C ($n = 18$) up to a value of 12.3 mW g^{-1} (S.D. 7.6 mW g^{-1} $n = 12$) at 35 °C. This indicates that honeybees behave ectothermicly during sleep. The preferred ambient temperatures for sleep were investigated in a temperature choice experiment. The highest number of sleeping bees were found at 28 °C. Evaluation of sleep behaviour in an observation hive revealed that bees prefer the same ambient temperature of about 28 °C under natural conditions. Honeybees save energy during sleep with an ectothermic behaviour, but do not reduce their metabolic rates as much as possible by choosing places in the beehive with the lowest temperature. Instead, they prefer places with moderate intermediate temperatures, probably in order to promote regenerative processes during sleep. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: *Apis mellifera*; Honeybee; Sleep; Calorimetry; Thermoregulation

1. Introduction

The phenomenon of sleep has puzzled scientists for ages. Sleep cannot be found only in mammals, but also in other vertebrate groups such as turtles and birds and even in invertebrates [1]. The most prominent insect, in which sleep-like states can be found is the honeybee, *Apis mellifera* [2].

Recently, some new attempts have been made to explain the function of sleep. Sleep may allow the resetting of physiological systems and to facilitate restoration [3]. It also maintains the integrity of the immune system and its modulation [4,5]. Moreover, there is some evidence that growth hormones are being released during sleep [6]. Sleep is important for the improvement of memory capabilities of the brain [7–9] and restoration of the neural functions and properties [10,11].

In this work, we follow an attempt of Berger and Phillips [12], in which the need for sleep is being explained with the necessity to conserve energy during inactive phases. Honeybees are good model organisms to test this hypothesis, as they can switch between different modes of individual thermoregulation [13] and, thus, energy expenditure. While performing daily

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activities like foraging for food, honeybees maintain a constant body temperature, which is actively regulated. Honeybees can elevate their body temperature through simultaneous contraction of flight muscle antagonists, which means that they shiver [14]. In a physiological sense, honeybees are endothermic animals,[1] a process with a high demand for energy. Nevertheless, during rest honeybees can be thermoregulatorly inactive or ectothermic, and their body temperature equals the ambient temperature.

Here, we try to test the energy conservation hypothesis for sleeping animals. If sleep really conserves energy during the night, honeybees should behave ectothermicly. As the metabolism of ectothermic organisms follows the ambient temperature regime, one may assume that honeybees should not stay in the warmest parts of the hive at night but should select lower temperatures to reduce their metabolic rate. To test this, we investigated the heat production rates of sleeping honeybees and their preferred ambient temperatures during sleep in a laboratory assay and a hive.

2. Experimental

2.1. Calorimetry

Heat production rates of sleeping honeybees were determined at 20, 25, 30 and 35 °C by means of an isoperibolic batch microcalorimeter (Calvet, Setaram, Lyons, France) connected to a chart recorder (Linseis L 2005, Selb, Germany). The calorimeter had two samples and two reference vessels with a volume of 100 ml each. The sensitivities were 51.15 and 55.14 $\mu V\ mW^{-1}$, respectively. All bees were caught at the hive entrances before 5.00 p.m., brought to the laboratory, weighed with a mechanical balance (type 414/13, Sauter, Ebingen, Germany) and inserted into the calorimeter vessel. The inside of the vessel was completely dark. During the experiment, the bees had access to a small piece of food (approximately 0.2 g bee bread, a mixture of pollen and honey that is frequently used by beekeepers for additional feeding of bees for various purposes). All calorimetric measurements of the heat production rates were started before 6.00 p.m. and ended after 22–23 h at 4.00–5.00 p.m. on the following day when the bees were weighed again. Before and after the experiment, the calorimeter baseline was recorded with the sample vessel charged with food only. The average heat production rates of sleeping bees were determined by electronic integration (Digikon, Kontron, Munich, Germany) of the power–time (P–t) curves. The state of sleep was indicated in the P–t curves as phase without fluctuations in the heat production (i.e. no locomotion of the bees).

2.2. Temperature choice experiment

The preferred ambient temperature of sleeping bees was determined in a temperature gradient chamber. The chamber consisted of an elongated plastic box (length 65 cm, width 18 cm, height 10 cm) with a perspex lid and a metal bottom plate (Fig. 1). The bottom plate was connected to an electrical heater on one end of the chamber and a water cooling pipe on the other end. The temperature inside the chamber was determined by means of five serially arranged mercury thermometers. The gradient was between 20 °C on one and 35 °C on the other end of the chamber, and was thus within the range of temperatures which could be expected inside the bee hives at night. Bees were inserted singly in the temperature gradient chamber and their behaviour was observed under the red light (as bees are unable to detect this part of the spectrum).

2.3. Observation of sleeping bees in the hive

For monitoring the sleeping honeybees inside the hive, we used a standard bee observation hive that can be seen in Fig. 2. Temperature sensors were inserted in the wax combs and spaced in four intervals of 10 cm at places where bees tend to sleep on the combs according to previous observations. The sensors were connected to data loggers (Hobo Temp, Onset Comp. Corp., Pocasset, MA, USA). Fifty-two bees were marked individually with small plastic plates on their thorax and their behaviour in the hive at night was protocolled in intervals of 30 min from 10.30 p.m. to 6.00 a.m. at 12 and 13 September 1999. In order to

[1] The reader should be aware that we use the term "endotherm" in a biological sense. It denotes a process, in which an organism actively elevates its body temperature, which is of course a process were heat is being produced and not consumed, as the physicochemical use of the word "endotherm" would implicate.

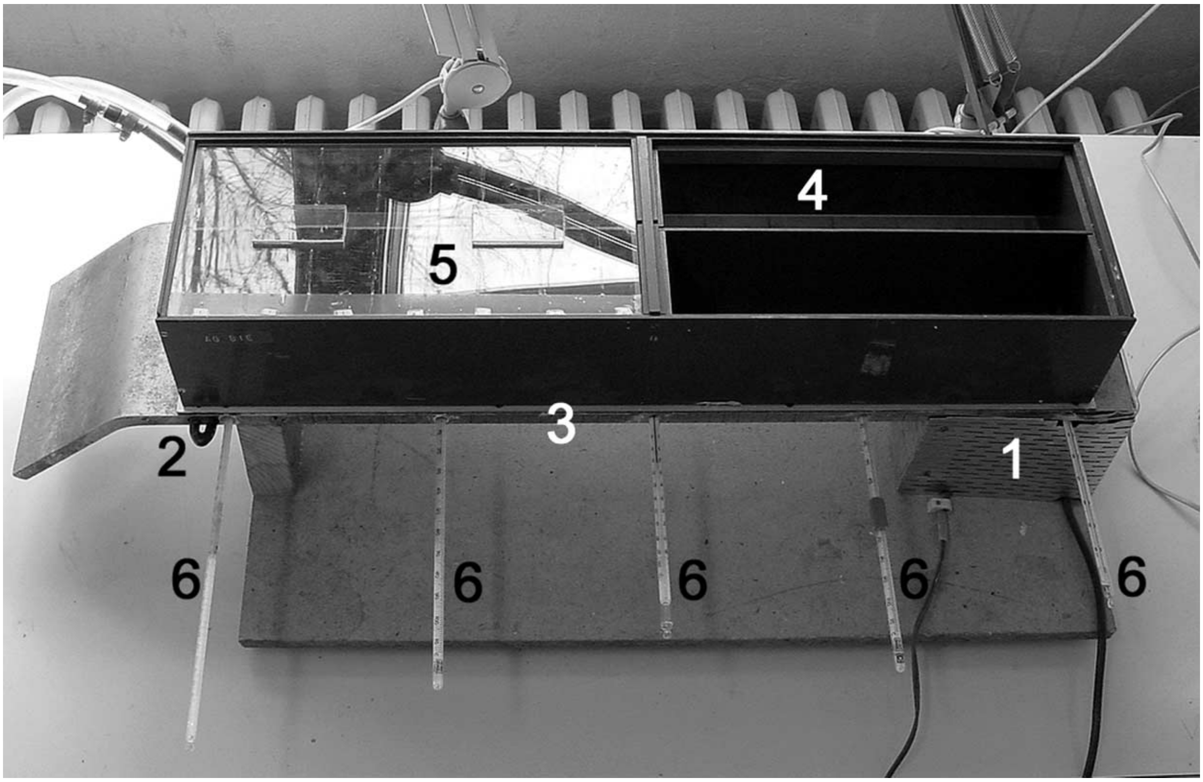

Fig. 1. Experimental set-up of the temperature choice chamber: (1) electrical heater; (2) water cooling pipe; (3) metal bottom plate; (4) test chamber; (5) perspex lid of test chamber; (6) mercury thermometers.

identify the bees in darkness, a dark room red light lamp was used.

3. Results

3.1. Heat production rates of sleeping honeybees

From a total of 94 calorimetric experiments, 57 could be evaluated. In 34 experiments, the bees did not sleep and in the remaining three, the bees died from unknown reasons. Sleep and non-sleep states of bees could be clearly differentiated in the *P–t* curves. Typically, the curve showed strong fluctuations due to locomotoric activities of the bees inside the vessel (Fig. 3). Between 9.00 and 10.00 p.m., the bees began to rest and, after some 30 min, eventually to sleep. Then, *P–t* curves became very smooth with no or only minor fluctuations. The bees slept for 5–6 h. The wake-up could be clearly seen, as the bees began to move, which again caused fluctuations in the *P–t* curves.

The heat production rates of sleeping bees were positively correlated with ambient temperature (Fig. 4). Thus, bees had the lowest heat production rates with 4.7 mW g^{-1} (S.D. 2.2 mW g^{-1}) at 20 °C ($n = 18$). The heat production rates at 25 °C were not statistically different from the values at 20 °C (Mann–Witney *U*-test, $\alpha = 0.05$) and amounted to 5.1 mW g^{-1} (S.D. 2.3 mW g^{-1}; $n = 13$). A statistically significant difference could only be observed between the heat production rates at 25 and 30 °C, but the heat production rates at 30 °C with 10.5 mW g^{-1} (S.D. 4.2 mW g^{-1}; $n = 14$) again were not significantly different from those at 35 °C with 12.3 mW g^{-1} (S.D. 7.6 mW g^{-1}; $n = 12$). All bees did not loose weight during the experiment and had a very uniform body mass of 100 mg (S.D. 10 mg; $n = 57$).

Fig. 2. Bee observation hive. In principle, a wooden frame, covered on both sides with glass plates, is containing a bee colony on two wax combs: (1) wooden frame; (2) glass plates; (3) entry and exit tunnel.

3.2. Temperature choice experiment

A total of 19 bees have been investigated under laboratory conditions in a temperature gradient chamber for their preferred ambient temperature during sleep at night (Fig. 5). All investigated bees fell in a state of sleep. The majority of bees slept at a preferred ambient temperature of 28 °C ($n = 10$). Three and four bees slept at 27 and 29 °C, respectively. Only one bee chose 26 °C as ambient temperature for sleeping.

3.3. Observation of sleeping bees in the hive

From a total of 52 marked bees, 15 could be detected in the observation hive for both monitoring nights, which allowed a sufficient number of observations. The whereabouts of the sleeping bees were not

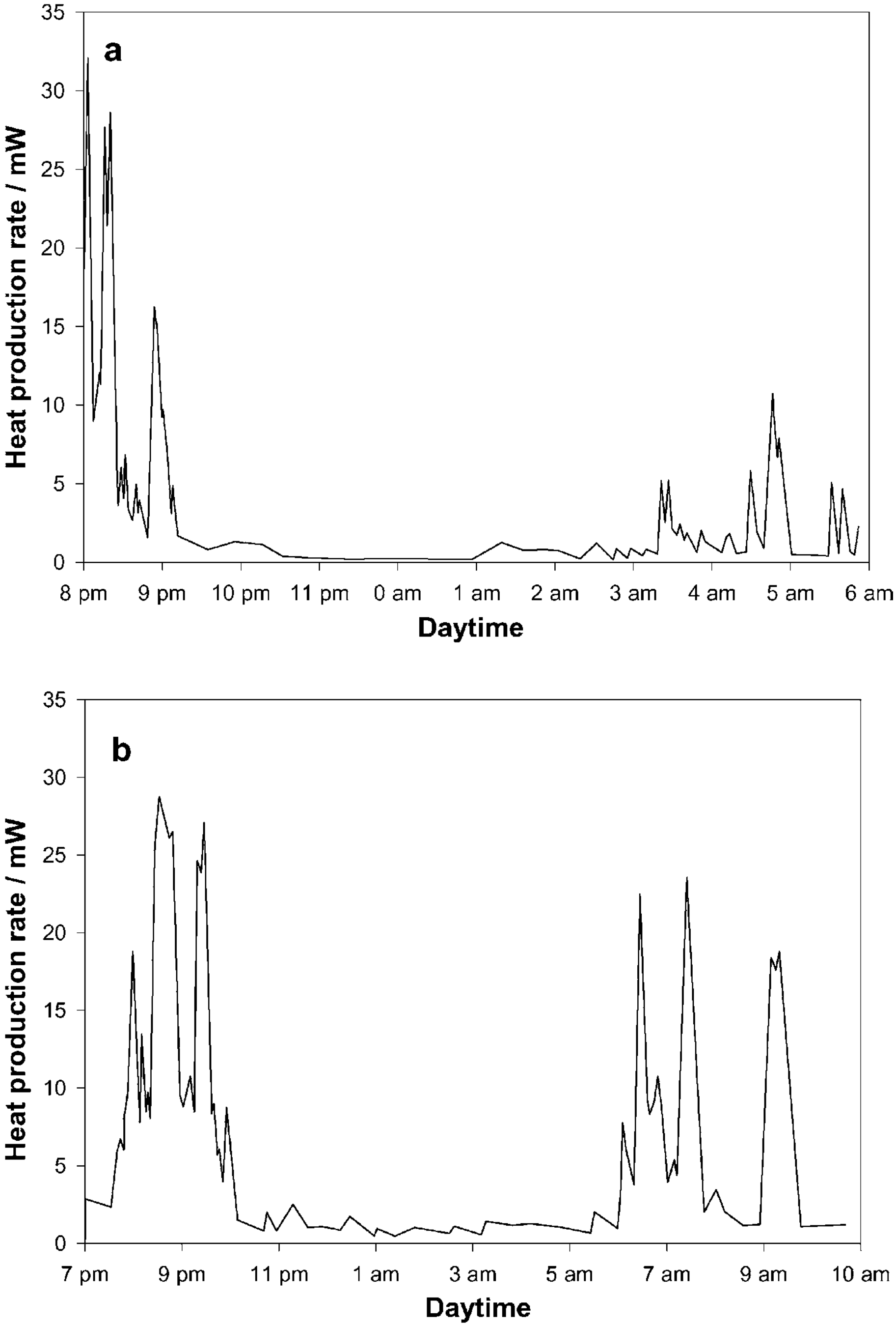

Fig. 3. *P–t* curves of the two experiments with sleeping bees at 20 °C: (a) after strong movements in the calorimeter vessel, the bee eventually falls asleep at 9.15 p.m. and wakes up at 3.20 a.m.; (b) a longer sleeping phase can be observed from 10.15 p.m. to 6.00 a.m., preceded and followed by strong locomotoric activities of the bee.

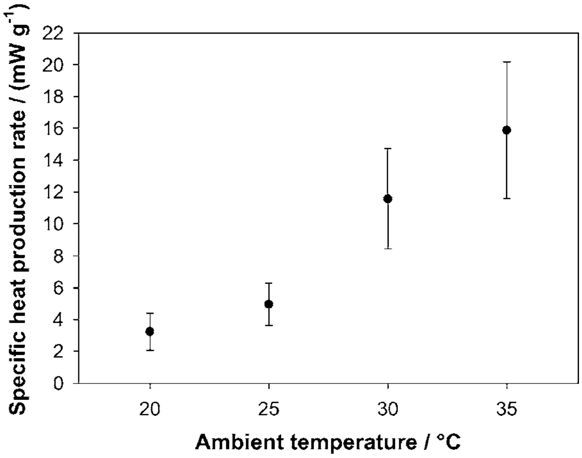

Fig. 4. Specific heat production rates of sleeping bees at different ambient temperatures. Error bars indicate S.D. Number of experiments: $n = 18$ at 20 °C; $n = 13$ at 25 °C; $n = 14$ at 30 °C; $n = 12$ at 35 °C.

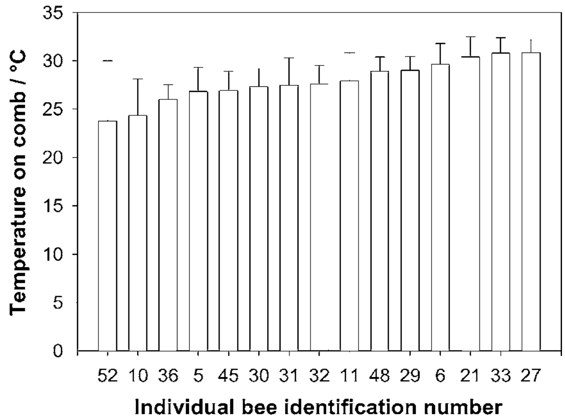

Fig. 6. Preferred ambient temperatures of sleeping bees on a wax comb in an observation hive. Each bee was individually marked and observed from 10.30 p.m. to 6.00 a.m. in following two nights. The whereabouts of the bees were protocolled in 30 min intervals. The mean of all observations on each bee is given, error bars indicate S.D.

constant during the night. From time to time, the bees woke up and moved some centimeters, to fall in sleep again. Thus, each bee experienced slightly different ambient temperatures during one night. The lowest average ambient temperature was preferred by bee no. 52 with 23.8 °C, whereas the highest ambient temperature was with 30.8 °C protocolled for bee no. 27 (Fig. 6). Taken the total average of all observations, the preferred ambient temperature for sleep was 27.9 °C.

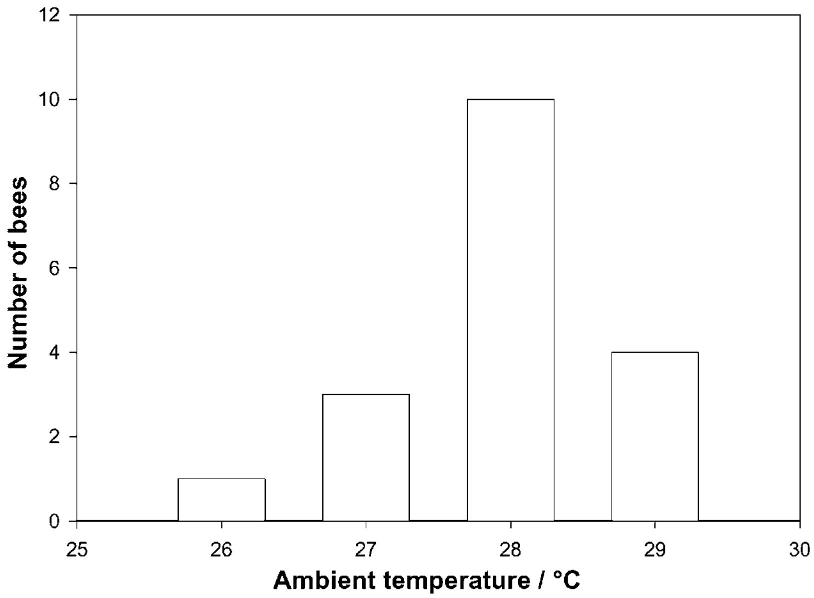

Fig. 5. Preferred ambient temperature of sleeping bees in a temperature choice experiment ($n = 19$).

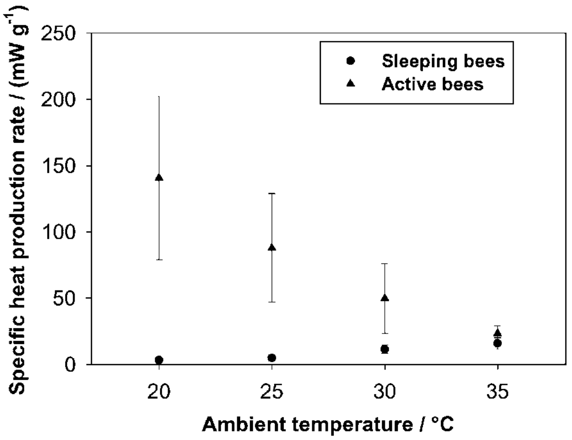

Fig. 7. Specific heat production rates of sleeping honeybees determined by means of direct calorimetry compared to specific heat production rates of active bees during the day obtained by the same method. Data of active bees were taken from the literature [16].

4. Discussion

During the night, the heat production rates of honeybees followed an ectothermic pattern, which means a deviation from their thermoregulatoric behaviour during daytime (Fig. 7). Bees obviously behave ectothermicly to save energy. Nevertheless, the bees did not choose the lowest ambient temperature, neither in the hive nor in the temperature choice assay. The preferred ambient temperature was nearly identical in both the experiments, with 28 °C in the temperature choice experiment and an average temperature of 27.9 °C in the hive. In the calorimetric experiments, the bees also survived well and slept at cooler temperatures. This leads us to the conclusion, that a preferred ambient temperature of 28 °C is not essential for the survival of the bees. At 30 °C, a temperature very near to the preferred ambient temperature of 28 °C, sleeping bees have a metabolic rate of 11.6 mW g^{-1}, compared to active bees at day which have an about four-fold higher metabolic rate of 49.8 mW g^{-1} [15,16]. Even at relatively high ambient temperatures, the energy savings are significant, but our results indicate that bees do not merely sleep in order to conserve energy. At 28 °C, the metabolic rates of sleeping bees are moderately high to facilitate restorative processes in the body and in the nervous system of honeybees. Thus, the reduced thermoregulatoric behaviour of sleeping honeybees seems to be a compromise between energy conservation and feasibility of regenerative processes.

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