

Endothermic heat production in honeybee winter clusters

Anton Stabentheiner*, Helga Pressl, Thomas Papst, Norbert Hrasnigg and Karl Crailsheim

Institut für Zoologie, Universität Graz, Universitätsplatz 2, A-8010 Graz, Austria

*Author for correspondence (e-mail: anton.stabentheiner@uni-graz.at)

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Summary

In order to survive cold northern winters, honeybees crowd tightly together in a winter cluster. Present models of winter cluster thermoregulation consider the insulation by the tightly packed mantle bees as the decisive factor for survival at low temperatures, mostly ignoring the possibility of endothermic heat production. We provide here direct evidence of endothermic heat production by ‘shivering’ thermogenesis. The abundance of endothermic bees is highest in the core and decreases towards the

surface. This shows that core bees play an active role in thermal control of winter clusters. We conclude that regulation of both the insulation by the mantle bees and endothermic heat production by the inner bees is necessary to achieve thermal stability in a winter cluster.

Key words: honeybee, *Apis*, winter cluster, heat production, endothermy, thermoregulation, thermography.

Introduction

Honeybees settle in a wide range of climates, from the tropics to the cool-temperate zones. Some races of the Western honeybee (*Apis mellifera* L.) are able to survive cold northern winters as a whole colony (Heinrich, 1993; Southwick, 1985, 1988; Moritz and Southwick, 1992). This is possible because at low temperatures they crowd tightly together in a winter cluster. There is agreement among scientists that the insulation by the tightly packed outer bees of the cluster, which reduces heat loss drastically, is a decisive factor in survival at low temperatures. However, in the absence of measurements of the body temperature inside natural winter clusters, there are diverging hypotheses of how the heat production inside the cluster, which is necessary to compensate for the heat loss, is accomplished (Simpson, 1961).

The ‘heat entrapment model’ favours the hypothesis that thermal stability and a high core temperature (approximately 20–35°C) of broodless winter clusters is achieved predominantly *via* regulation of the heat loss by the mantle bees. The cluster is supposed to maintain a high core temperature by the metabolism of resting or slowly moving bees alone, without additional active heat production by ‘shivering’ with the flight muscles (Lemke and Lamprecht, 1990; Heinrich, 1993; Myerscough, 1993; Watmough and Camazine, 1995). ‘Shivering’ heat production by the inner bees is, if at all, assumed only at temperatures below approximately –10°C (Heinrich, 1993), where the metabolism of winter clusters begins to increase steeply as the ambient temperature decreases (Southwick, 1988; Moritz and Southwick, 1992).

The more general ‘superorganism model’ agrees with this hypothesis concerning the important role of the insulating mantle bees but, in addition, assumes that there are always

some bees inside the cluster that actively produce heat by ‘shivering’ thermogenesis, the amount depending on ambient temperature (Moritz and Southwick, 1992; Omholt and Lonvik, 1990; Fahrenholz et al., 1989). We present here direct evidence of shivering thermogenesis in the core of winter clusters.

Materials and methods

Two winter clusters of *Apis mellifera carnica* Pollmann (approximately 7000–9000 bees) were placed on four combs in a cage with the side walls open to allow access to the cluster surface. The cage, which was set up on a loggia with access to the outside, could be opened between the two central combs on low-friction hinges. As opening of the cage would have disturbed the bees, two infrared-transmissive plastic foils were inserted between the two central combs. This way, the bees remained very quiet when the cage was opened because they were not separated from each other, and cooling of the cluster interior was minimized. Four small windows (each 2.5 cm × 3 cm) in the edges of the foils allowed the bees to communicate and to move between the two parts of the cluster. To ensure a broodless cluster, the queen was caged on one of the central combs. The body surface temperature of the bees was measured at night (23:00 h–01:00 h) or in the early morning (05:00 h–06:00 h) by means of infrared thermography (AGA 782 SW and ThermaCam SC2000; FLIR Inc., Danderyd, Sweden; Stabentheiner and Schmaranzer, 1987). Body surface temperature was calibrated using the cuticular emissivity of the honeybee (0.97) and an AGA 1010 reference radiator (Schmaranzer, 1983; Stabentheiner and Schmaranzer,

1987; Schmaranzer and Stabentheiner, 1988). With the AGA 782 SW, measurements were made with close-up instrumentation along 38 mm broad vertical scans from bottom to top (Pressl, 1999). A scan lasted 10–15 s. With the ThermaCam SC2000, which has a higher spatial resolution than the AGA 782 SW, all bees on the central comb of a different cluster could be measured simultaneously and immediately after opening (Fig. 1).

In another experiment, a broodless winter cluster was thermographed day and night (1 picture s^{-1}) from the side of the narrow ends of the combs (ThermaCam SC2000). To avoid any disturbance of the cluster, all recording equipment was placed in an adjacent laboratory, and recordings were

evaluated only from night-time and two days after the experimental loggia had been entered for the last time.

Results

Body temperatures were lowest in the outer bees and increased towards the core (Figs 1, 2; Table 1). In the bees on the outer surface of the flat side of the combs, which were exposed to the highest thermal strain, mean thorax surface temperature (T_{thorax}) was 12.1°C . Only three out of 134 bees (<3%) had a T_{thorax} below 9°C . By contrast, abdominal temperature (T_{abdomen}) was frequently below 9°C . On the central comb, T_{thorax} increased from 16.9°C in the outermost

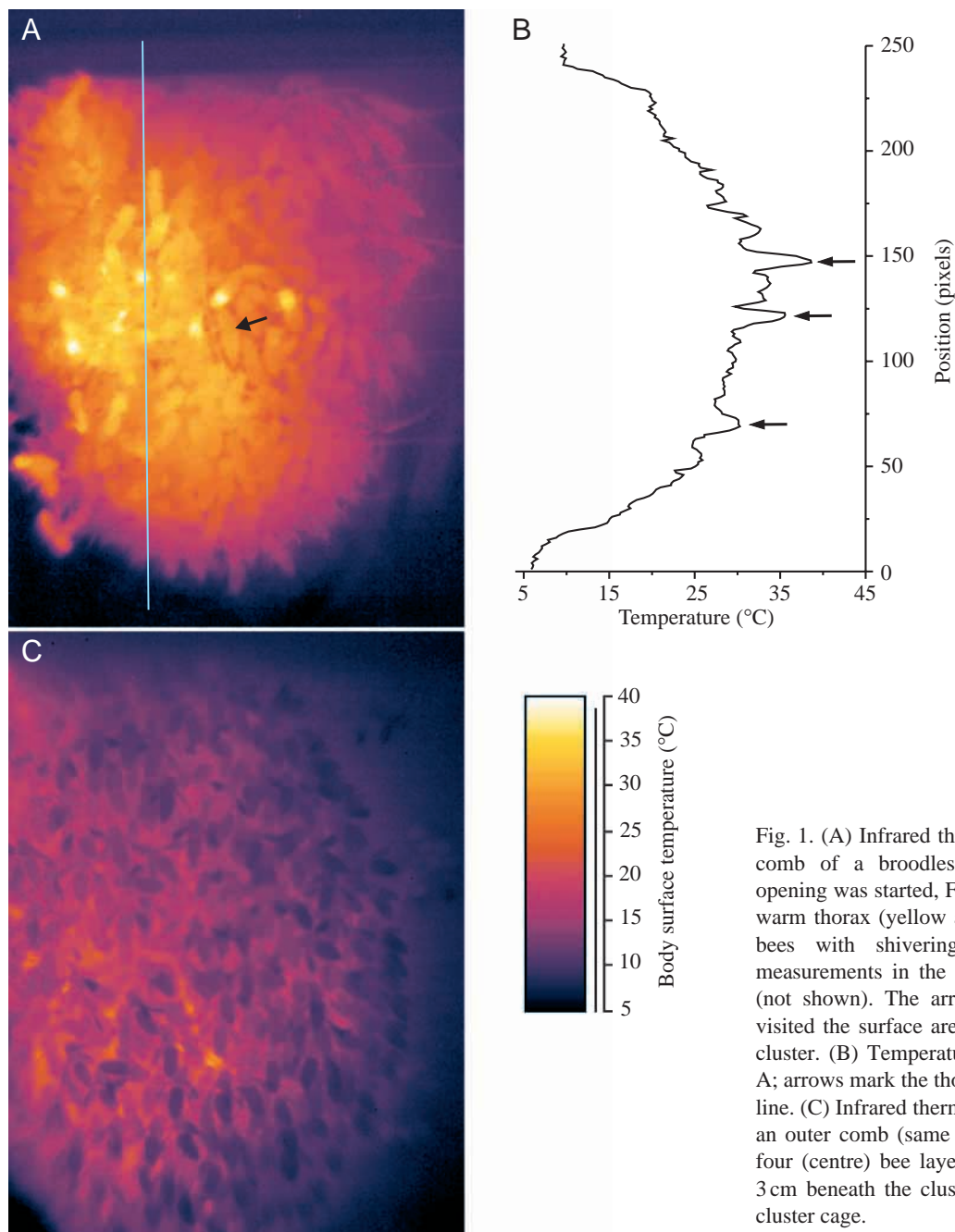


Fig. 1. (A) Infrared thermogram of the bees on the central comb of a broodless winter cluster (3.5 s after cage opening was started, FLIR ThermaCam SC2000). Note the warm thorax (yellow and white spots) of the endothermic bees with shivering thermogenesis. Two additional measurements in the same cluster confirmed this finding (not shown). The arrow points to the queen. Bees that visited the surface are visible at the lower left end of the cluster. (B) Temperature profile along the line marked in A; arrows mark the thoraces of endothermic bees hit by the line. (C) Infrared thermogram of the bees on the flat side of an outer comb (same cluster as in A); one (periphery) to four (centre) bee layers. Ambient temperature was 5.5°C 3 cm beneath the cluster and 3.7°C 1 m outside the open cluster cage.

ring of bees to 22.8°C in the bees between the outer ring and the core, and to 30.4°C in the core bees (Table 1). Bees were counted as being in the core when T_{abdomen} was not more than

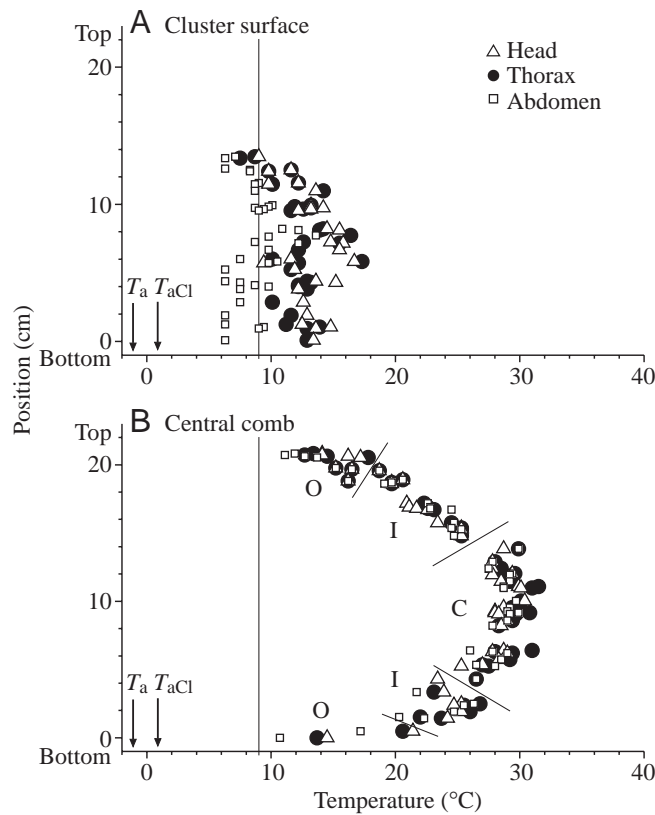


Fig. 2. Body surface temperatures of bees in a winter cluster along vertical scans on the surface of the flat side of an outer comb (A) and on the central comb (B). Lines at 9°C mark chill coma temperatures (Free and Spencer-Booth, 1960; Esch, 1988; Goller and Esch, 1990). T_a , air temperature 1 m outside the cluster cage; T_{aCl} , air temperature 3 cm beside the cluster surface; O, outermost bee layer; I, intermediate bees; C, core bees.

3°C below the lowest T_{abdomen} in the centre. Ambient temperature was 0.9°C to 6.3°C within 3 cm of the clusters and -1.1°C to 3.7°C 1 m outside the cage, the difference amounting to 0.6–3.1°C. For comparison, the temperature difference between the outermost air space inside the hive and the air temperature outside the hive amounted to 3.6–6.1°C with the Plexiglas side walls of the cage closed. In standard colonies, it amounts to approximately 8–9°C (H. Kovac, personal communication).

Fig. 1 provides direct evidence of endothermic heat production by bees inside a winter cluster. In addition to many bees with no or only little endothermy, a considerable number of bees with actively heated thoraces was observed. Intense endothermy was directly visible because of the hot thorax (Fig. 1), but weak endothermy could not be judged easily because of the steep temperature gradients inside the clusters (Figs 1, 2). Therefore, we counted only bees with T_{thorax} at least 0.2°C higher than T_{head} and T_{abdomen} as definitely endothermic ($T_{\text{head}} < T_{\text{thorax}} > T_{\text{abdomen}}$). Most endothermic bees were located in the core (approximately 15–16% of all bees on the central comb), and their abundance decreased towards the surface (Figs 1, 3). This relationship was reversed in those bees that were assumed to primarily follow the local temperature gradient, with no or only minute endothermic heat production ($T_{\text{head}} > T_{\text{thorax}} > T_{\text{abdomen}}$; Fig. 3). Among the surface bees on the flat side of the combs, which experienced the most extreme thermal strain, 56% belonged to this latter class (Fig. 3A). Bees sitting ‘the wrong way round’ in the temperature gradient (i.e. with the head pointing outwards to the lower temperature) were seldom on the cluster surface and in the outer ring of bees on the central comb but occurred at a higher frequency inside the cluster (Fig. 3).

Because of the steep and variable temperature gradients inside a winter cluster (Figs 1, 2), the amount of endothermy cannot be estimated directly from the differences between T_{thorax} and ambient or abdominal temperature. However,

Table 1. Mean body surface temperatures of honeybees in broodless winter clusters

Part of cluster	Temperature (°C)			N	N _m
	Head	Thorax	Abdomen		
Surface	12.6±2.06 (8.7–20.0)	12.1±1.92 (7.5–18.3)	9.6±2.14 (6.2–16.9)	98/134/144	3
Central comb					
Outermost bee layer	17.1±2.77 (11.2–24.7)	16.9±2.72 (11.2–24.3)	15.2±2.88 (10.7–22.9)	65/65/65	4
Intermediate bees	22.5±3.80 (15.2–31.5)	22.8±3.71 (15.2–31.7)	21.8±3.05 (15.2–27.8)	136/136/135	4
Core bees	29.1±2.81 (23.2–36.2)	30.4±3.22 (23.1–39.6)	29.0±2.24 (21.7–33.9)	110/114/114	4

Temperature values are means ± s.d. (range given in parentheses).

N, number of values (head/thorax/abdomen); N_m, number of measurements. Ambient air temperature was 0.9°C to 6.3°C 3 cm beside the clusters, and -1.1°C to 3.7°C 1 m away.

Body parts significantly different between different parts of the cluster ($P < 0.0001$, *t*-test).

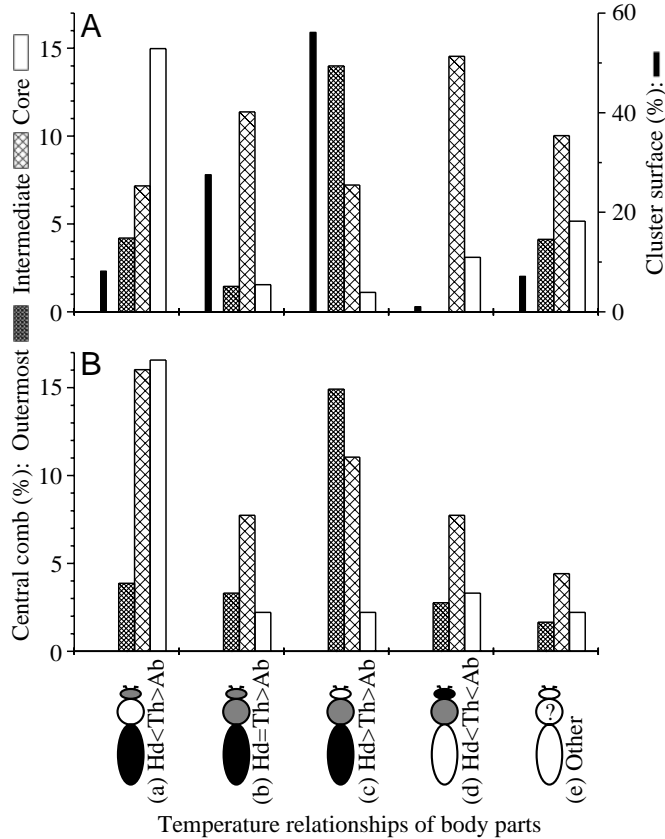


Fig. 3. Frequencies of bees with different temperature relationships between body parts in winter clusters on the central comb and on the cluster surface on the flat side of an outer comb. Hd, head; Th, thorax; Ab, abdomen. For cluster parts, compare Fig. 2. Body parts were considered as different if their temperatures differed by at least 0.2°C. Distributions significantly different within (A) and (B) at $P < 0.001$, except intermediate and core in B ($P < 0.1$; Bonferroni χ^2 statistics). (A) Sum of three measurements made with close-up instrumentation along 38 mm broad vertical scans (AGA 782 SW) within 10–15 s after the start of cage opening. For the central comb, frequencies were projected to the whole comb assuming a circular distribution of the bees (604 bees=100%; 132 bees measured). For the cluster surface, 78 bees=100%. (B) Simultaneous measurement of all bees on the central comb of a different cluster (Fig. 1A) 3.5 s after the start of cage opening (181 bees=100%).

comparison of the bees that were classified to primarily follow the local temperature gradient (Class 'c' in Fig. 3) with the bees classified as endothermic (Class 'a' in Fig. 3) shows that, although in Class 'c' the difference of $T_{\text{thorax}} - T_{\text{abdomen}}$ decreased towards the core as expected, it remained nearly the same in Class 'a' despite the decreasing temperature gradients (Fig. 4). The relative proportion of bees with a $T_{\text{thorax}} - T_{\text{abdomen}}$ of $> 2^\circ\text{C}$ was lower in Class 'a' than in Class 'c' (30% versus 65%, respectively) in the outermost bee layer, slightly higher in the intermediate bees (36% versus 22%) but clearly higher (40% versus 14%) in the core (relationship between Classes 'a' and 'c' was significantly different among all three parts of the cluster at $P < 0.05$,

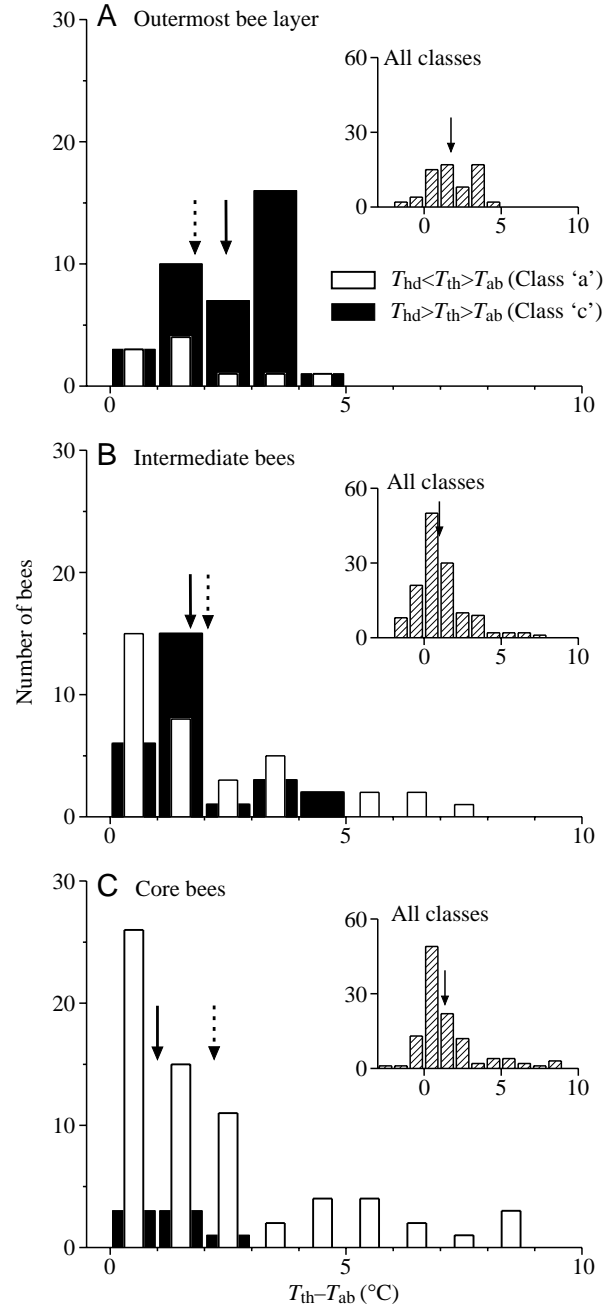


Fig. 4. Frequency distributions of the temperature difference between thorax (T_{th}) and abdomen (T_{ab}) in different parts on the central comb of broodless winter clusters in endothermic bees (Class 'a') and in bees with no or only minute endothermy (Class 'c'; compare Fig. 3; four measurements). Broken arrows represent the means of Class 'a': 1.8 ± 1.26 ($N=10$) in (A), 2.1 ± 1.95 ($N=36$) in (B) and 2.2 ± 1.17 ($N=68$) in (C). Unbroken arrows represent the means of Class 'c': 2.5 ± 0.99 ($N=37$) in (A), 1.7 ± 1.14 ($N=27$) in (B) and 1.0 ± 0.70 ($N=7$) in (C). Insets show distributions with all classes included; means were 1.7 ± 1.41 ($N=65$) in (A), 1.0 ± 1.63 ($N=135$) in (B) and 1.4 ± 2.04 ($N=114$) in (C). Medians in no part of the clusters were significantly different between Class 'a' and Class 'c'; medians were significantly different between the outermost bee layer and the intermediate and core bees in Class 'c' and when all classes were pooled (insets) ($P < 0.005$, U -test). T_{hd} , head temperature.

Bonferroni χ^2 test; compare Fig. 4). The outermost bees minimized endothermy. Strongly endothermic bees that remained stationary, as were found in the core, were not observed on the cluster surface nor in the outer ring of bees on the central comb (Figs 1–3).

Additional (and conclusive) evidence that there is always a certain number of endothermic bees inside a broodless winter cluster at air temperatures around the freezing point was provided by the experiment where the cluster surface was thermographed continuously for many hours. Evaluation of 21 h of recordings from night-time (21:00 h–08:00 h) showed that bees appeared on the cluster surface at a rate of 6–80 bees h^{-1} . All of them were endothermic, with a mean T_{head} of $21.6 \pm 3.58^\circ\text{C}$, a T_{thorax} of $27.5 \pm 4.09^\circ\text{C}$ and a T_{abdomen} of $20.9 \pm 3.62^\circ\text{C}$ ($N=1183$ values, 781 bees; compare Fig. 1). Of these bees, 90% re-entered the cluster within 10 s, and the rest re-entered within 3 min. We conclude that visiting the surface by endothermic bees is part of the natural behaviour in winter clusters.

Discussion

The present investigation showed that endothermic heat production occurs regularly in winter clusters. This supports a report by Esch (1960) who monitored the body temperature of bees of a winter cluster in an observation hive. Bees with thermocouples attached to thorax and abdomen ('on the line') repeatedly showed heating phases. In the present investigation, heating as an immediate reaction to cage opening was not observed. In the measurement shown in Fig. 1, it cannot even theoretically account for the high thorax temperatures in the intensely endothermic core bees because, within 3.5 s of the start of cage opening, heating at a rate of $7\text{--}8^\circ\text{C min}^{-1}$ (Heinrich, 1981; Stabentheiner et al., 2002) would only account for a thoracic temperature increase of $0.4\text{--}0.5^\circ\text{C}$.

We conclude that current models of winter cluster thermoregulation (Lemke and Lamprecht, 1990; Myerscough, 1993; Watmough and Camazine, 1995) that neglect the role of active (endothermic) heat production are incomplete. The following points have to be incorporated in these models (see below).

Some of a winter cluster's bees are endothermic, and most of these bees are located inside the cluster although these bees are not in immediate danger of freezing or falling into chill coma. From efficiency considerations, one would expect the bees to make the most of the heat if it is produced in the core. In fact, most endothermic bees were located in the core (approximately 15% of all bees on the central comb), and their abundance decreased towards the surface (Figs 1–3) and not the other way round. However, not only the probability but also the intensity of endothermy increased with increasing local temperature in the cluster (towards the core; Figs 1, 4). The heat of the endothermic core bees is of benefit for the thermal comfort of themselves *and* their sisters on the cluster periphery. This supports a main assumption of the superorganism model of winter cluster thermoregulation which

says that the core bees play an active role in thermal control (Moritz and Southwick, 1992).

Resting metabolism – although higher in the core – is not alone the source of the heat that is necessary to compensate for the heat loss (Fig. 1), because there must be some convection in the cluster (either cyclic or permanent) that allows the cluster to get rid of the CO_2 produced (Heinrich, 1981; Van Nerum and Buelens, 1997) by both the endothermic bees through shivering thermogenesis and the ectothermic bees according to their resting metabolism.

Southwick (1983, 1988) and Southwick and Heldmaier (1987) showed that the oxygen consumption of winter clusters increases as the ambient temperature decreases. The increase is moderate between approximately $+10^\circ\text{C}$ and -5°C and is steep below approximately -5°C to -10°C . In swarm clusters, the steep increase has already started at $+10^\circ\text{C}$ (Heinrich, 1981). Oxygen consumption also increases with decreasing cluster size (Southwick, 1985). Therefore, the frequency and intensity of endothermic heat production have to be assumed to increase with decreasing ambient temperature and cluster size. On the other hand, we suggest that at higher ambient temperatures large (swarm) clusters that have come to rest (e.g. at night) may be able to largely reduce endothermy (Heinrich, 1981).

If the thorax cools below the chill coma temperature of $9\text{--}11^\circ\text{C}$, honeybees are no longer able to activate their flight muscles for heating (Free and Spencer-Booth, 1960; Esch, 1988; Goller and Esch, 1990) and eventually fall off the cluster. Watmough and Camazine (1995) assumed that the outer bees react with thoracic heating to avoid chill coma. Efficiency considerations, however, suggest that the surface bees should avoid endothermy because any heat from the surface bees is immediately lost to the surrounding air. Our data show that endothermy seldom occurs in the outer bees and, if it does occur, is only weak (Figs 1–3). Intense endothermy of surface bees was observed only in an emergency case when only one layer of bees was left on the flat side on an outer comb. Before the bees walked to the adjacent beeway between the combs they heated up their thoraces. On the opposite cluster surface, where up to four layers of bees were sitting, intense endothermy was not observed. There, the body temperatures resembled the situation shown in Fig. 1C (our unpublished observations).

It should be pointed out that the occurrence of endothermy inside winter clusters does not contradict the hypothesis of self-organized cluster thermoregulation (Heinrich, 1981, 1993; Moritz and Southwick, 1992; Watmough and Camazine, 1995). It is well conceivable that the endothermic bees decide individually to start shivering thermogenesis or to stay ectothermic. However, in order to optimize energetic investment, the core bees should know about the thermal needs of the bees sitting closer to the surface. Future investigations will have to solve the question of whether or not the endothermic bees that visit the surface play a role in the regulation of heat production in winter clusters.

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References

- Esch, H.** (1960). Über die Körpertemperaturen und den Wärmehaushalt von *Apis mellifica*. *Z. Vergl. Physiol.* **43**, 305-335.
- Esch, H.** (1988). The effects of temperature on flight muscle potentials in honeybees and cuculiinid winter moths. *J. Exp. Biol.* **135**, 109-117.
- Fahrenholz, L., Lamprecht, I. and Schricker, 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.
- Free, J. B. and Spencer-Booth, Y.** (1960). Chill-coma and cold death temperatures of *Apis mellifera*. *Entomol. Exp. Appl.* **3**, 222-230.
- Goller, F. and Esch, H.** (1990). Comparative study of chill coma temperatures and muscle potentials in insect flight muscles. *J. Exp. Biol.* **150**, 221-231.
- Heinrich, B.** (1981). The mechanisms and energetics of honeybee swarm temperature regulation. *J. Exp. Biol.* **91**, 25-55.
- Heinrich, B.** (1993). *The Hot-Blooded Insects, Strategies and Mechanisms of Thermoregulation*. Berlin, Heidelberg, New York: Springer.
- Lemke, M. and Lamprecht, I.** (1990). A model of heat production and thermoregulation in winter clusters of honeybees using differential heat conduction equations. *J. Theor. Biol.* **142**, 261-273.
- Moritz, R. F. A. and Southwick, E. E.** (1992). *Bees as Superorganisms: an Evolutionary Reality*. Berlin, Heidelberg, New York: Springer.
- Myerscough, M.** (1993). A simple model for temperature regulation in honeybee swarms. *J. Theor. Biol.* **162**, 381-393.
- Omholt, S. and Lonvik, K.** (1990). Heat production in the winter cluster of the honeybee, *Apis mellifera*. A theoretical study. *J. Theor. Biol.* **120**, 447-456.
- Pressl, H.** (1999). *Temperaturregulation bei Winterbienen*. Graz: Diploma Thesis at the Institut für Zoologie, Universität Graz, Austria.
- Schmaranzer, S.** (1983). Thermovision bei trinkenden und tanzenden Honigbienen (*Apis mellifera carnica*). *Verh. Dtsch. Zool. Ges.* **76**, 319.
- Schmaranzer, S. and Stabentheiner, A.** (1988). Variability of the thermal behavior of honeybees on a feeding place. *J. Comp. Physiol. B* **158**, 135-141.
- Simpson, J.** (1961). Nest climate regulation in honey bee colonies. *Science* **133**, 1327-1332.
- Southwick, E. E.** (1983). The honey bee cluster as a homeothermic superorganism. *Comp. Biochem. Physiol. A* **75**, 641-645.
- Southwick, E. E.** (1985). Allometric relations, metabolism and heat conductance in clusters of honey bees at cool temperatures. *J. Comp. Physiol. B* **156**, 143-149.
- Southwick, E. E.** (1988). Thermoregulation in honey-bee colonies. In *Africanized Honey Bees and Bee Mites* (ed. G. Needham, R. E. Page, M. Delfinado-Baker and C. E. Bowman), pp. 223-236. New York: Wiley.
- Southwick, E. E. and Heldmaier, G.** (1987). Temperature control in honey bee colonies. *Bio Sci.* **37**, 395-399.
- Stabentheiner, A., Kovac, H. and Schmaranzer, S.** (2002). Honeybee nestmate recognition: the thermal behaviour of guards and their examinees. *J. Exp. Biol.* **205**, 2637-2642.
- Stabentheiner, A. and Schmaranzer, S.** (1987). Thermographic determination of body temperatures in honey bees and hornets: calibration and applications. *Thermology* **2**, 563-572.
- Van Nerum, K. and Buelens, H.** (1997). Hypoxia-controlled winter metabolism in honeybees. *Comp. Biochem. Physiol. A* **117**, 445-455.
- Watmough, J. and Camazine, S.** (1995). Self-organized thermoregulation of honeybee clusters. *J. Theor. Biol.* **176**, 391-402.