ROLES OF INDIVIDUAL HONEYBEE WORKERS AND DRONES IN COLONIAL THERMOGENESIS

By JON M. HARRISON

*Department of Environmental, Population, and Organismic Biology,
University of Colorado, Boulder, CO 80309–0334, USA*

SUMMARY

1. The individual roles of honeybee workers and drones in heat regulation were investigated using single combs of bees and brood (about 1000 individuals) placed in boxes at 15°C. After 1 h and before cluster formation, I measured the elevation of bee thoracic surface temperature (T_{ths}) above local ambient temperature (T_a). Bees were then left overnight at 15°C.

2. During the preclustering period, the density of bees over the brood slowly increased. In the clusters left overnight, bees in the innermost layer were significantly younger than bees in the outermost layer. One-day-old bees and drones were always located in the innermost cluster layer.

3. 89% of all workers measured had $T_{ths} - T_a \geqslant 2$°C, indicating that most workers contribute to colonial thermogenesis. Average $T_{ths} - T_a$ was 4·1°C. Drones measured had the same average $T_{ths} - T_a$ as unmarked workers. $T_{ths} - T_a$ did not differ among bees 2 days of age and older. Location on or off the brood did not affect $T_{ths} - T_a$.

4. Cooling constants of dead bees placed near the comb in the box averaged $1·036 \, \text{min}^{-1}$ and were independent of location on the comb. Calculated average thoracic conductance was $0·829 \, \text{cal} \, \text{g}^{-1} \, °\text{C}^{-1} \, \text{min}^{-1}$.

5. Average calculated heat production per worker was $0·095 \, \text{cal} \, \text{min}^{-1}$, less than 15% of the maximal oxygen consumption of 4-day-old bees. Calculations indicate that the larger drones contribute more heat per bee than do the workers.

6. Brood warming prior to clustering is due to relatively uniform heat production by individual bees and the attraction of bees to the brood area. This results in a higher heat production per unit volume of air over the brood than away from the brood.

INTRODUCTION

The nest-warming capacity of honeybee (*Apis mellifera*) colonies has helped allow this tropical insect to expand its range into cold-temperate environments. Although the behavioural and physiological responses of honey-bee colonies to cold are well characterized (Simpson, 1961; Seeley & Heinrich, 1981; Kronenberg & Heller, 1982; Southwick, 1985), the division of the labour of thermogenesis among individual bees has received little attention. In this study, I examine the thermogenic

roles of individual workers and drones in a colony of brood-rearing honeybees, *A. mellifera*.

Colonies with brood regulate brood areas at a temperature between 30 and 35 °C (Seeley & Heinrich, 1981). Survival and normal growth of honeybee brood requires brood nest temperatures between 30 and 36 °C (Himmer, 1927, 1932; Jay, 1963). In colonies with brood, environmental temperatures below 30 °C stimulate an increase in colonial metabolic rate, and temperatures below 15 °C induce clustering centred on brood areas (Corkins & Gilbert, 1932; Free & Simpson, 1963; Southwick & Mugaas, 1971; Koeniger, 1978; Kronenberg & Heller, 1982; Ritter, 1982; Southwick, 1985).

It is not known how the increase in colonial metabolic rate is divided among individual bees. In winter clusters, it has been reported that only bees in the cluster centre activate their flight muscles for heat production (Esch, 1960). However, in swarms, resting metabolism suffices for heat production and the thermoregulatory pattern is consistent with a simple model where bees individually thermoregulate towards a preferred body temperature (Heinrich, 1980, 1985). A number of factors may influence whether an individual bee contributes to colonial heat production in a summer, brood-rearing colony. Young bees generally feed and care for larvae and are usually located near the colony centre (Seeley, 1982). However, workers increase thermogenic and thermoregulatory capacities with age and foragers have the greatest thermogenic capacities (Himmer, 1925; Allen, 1959; Nijland & Hepburn, 1985; Harrison, 1986). Himmer reported that worker temperature in a colony increases with age (Himmer, 1932). Drones are also present in summer, brood-rearing colonies. Drones have poorer thermoregulatory capacities than workers (Cahill & Lustick, 1976), and are generally thought to contribute little to colony work (Wilson, 1971). However, bumble-bee males have recently been shown to incubate pupae (Cameron, 1985).

In this study, I investigated the effect of age, foraging behaviour, location on or off the brood area, and sex on bee contribution to colonial heat production. I also examined the effect of age and sex on bee location within summer clusters.

MATERIALS AND METHODS

The honeybee colony used in this study was started from a commercial 2 lb package (McCary Apiary; Buckatunna, MS) on 1 May 1983, and maintained at the University of Colorado, Boulder. Beginning on 15 June, 100 newly emerged bees were added to the colony each day until 13 August. Introduced bees were obtained from several donor colonies and were colour marked as described previously (Harrison, 1986). No differential mortality, measured using a dead bee trap (Gary, 1960), occurred among introduction days. The effect of age on foraging behaviour was characterized on 13, 14 and 15 August using a technique described previously (Harrison, 1986).

Thermoregulatory experiments were conducted with the four brood-containing frames from this colony during 9–12 August. Frames containing brood (both pupae and larvae) and approximately 1000 bees were removed from the colony at

12.00 h ± 0·25 h (range) and placed within a wooden single frame box. At 16.00 h ± 0·25 h the frame was transferred to a 15 °C ± 0·5 °C temperature-controlled room. The box containing the frame was edged with clear Mylar cut with an 8×4 array of flaps which were taped shut. The flaps could be opened to allow access to the frame and bees. Throughout the time the frame was kept at 15 °C, brood surface temperatures were maintained above 30 °C by the bees. After 1–1·5 h, the bees began to cluster. I studied the division of thermogenesis in the frames of bees during the 30 min prior to clustering. Temperature measurements were made of bees located behind the windows, and age and location of the bee were recorded. A bee touching an adjacent bee could be heated passively by its neighbour. Therefore, only bees not touching other bees were measured.

The thoracic surface temperatures of individual bees were measured with a Bailey MT-4 micro temperature probe which was slipped in through the windows in the Mylar edging to touch the dorsal surface of the bee. The microprobe used had a time constant of 0·25 s. When freshly killed bees were heated internally with a wire resistor, thoracic surface temperature was always within 0·5 °C of internal temperature, indicating that surface temperature accurately reflected bee thoracic temperature. Internal thoracic temperature was measured with an implanted 0·25 mm copper-constantan thermocouple connected to a Wescor thermocouple thermometer. Bees feeding larvae and bees regurgitating food to one another were measured without sign of disturbance. Only measurements during which there were no behavioural signs that the bee was disturbed were used.

Although ambient temperature was 15 °C, air temperature within the box containing the frame of bees varied relative to the location of the brood. Air temperature 1 cm above the comb in the frame centre averaged 28 °C; while near the frame edge air temperature averaged 21 °C 1 cm above the comb. For this reason, bee thoracic surface temperature (T_{ths}) was expressed relative to local air temperature 1 cm above the bee (T_a). This temperature was measured with a second temperature probe taped at a fixed distance (1 cm) above the first probe. As $T_{ths} - T_a$ could be affected by movement of the bee within the frame, only bees observed to remain in a given area for >30 s were measured. Only bees with T_{ths} above T_a will contribute to colonial heat production.

The four frames described above were maintained overnight (16 h) at 15 °C. In all cases, the bees were tightly clustered over the brood the next morning, with temperatures at the cluster centres averaging 33 °C. I recorded the age of marked bees in the outer layer of the cluster. Clusters were then dispersed by directing a heat lamp at the cluster. The clusters were 4–5 layers thick. The age and sex of marked bees directly over the brood (in the innermost layer) were recorded.

To evaluate the local thermal environment of different parts of the frame, I measured cooling curves of bees freshly killed with carbon dioxide at various frame locations under conditions similar to those described above. Wire mesh cylinders (1 cm radius, 0·3 cm mesh) were installed between the Mylar edging and the comb surface. These cylinders allowed measurement of cooling curves without interference from patrolling bees. Preliminary experiments demonstrated that cooling

curves of dead bees measured inside a cylinder were identical to cooling curves of
bees not enclosed within a cylinder. Cooling curves were measured at four sites over
the brood near the frame centre, and at four sites away from the brood near the frame
edge. Cooling curves were measured by implanting a 0·25 mm copper-constantan
thermocouple into the thorax of a freshly killed bee. The bee was connected by
0·5 cm of thermocouple wire to a small (0·2 cm diameter) wooden splint.. The bee
was heated with a microscope lamp until internal thoracic temperature (T_{th})
measured 40°C, and then the bee was quickly placed within a cylinder 0·5 cm over
the comb. Local ambient temperature (T_a) was measured with a second thermo-
couple located 1 cm above the bee and $T_{th} - T_a$ was continuously recorded on a
Linear Instruments chart-recorder. Cooling constants of the thorax (min^{-1}) were
converted to thoracic conductance (C, cal g^{-1} °C^{-1} min^{-1}) assuming a specific heat of
0·8 cal g^{-1} °C^{-1}.

RESULTS

Prior to cooling, bees appeared to be evenly distributed over the frame. During the
preclustering period, bees on and off the brood area walked, cleaned cells, exchanged
food, groomed or stood quietly. Individual bees moved on and off the brood area;
however, the density of bees located over the brood area slowly increased until the
cluster was formed after 1–1·5 h at 15°C.

The effect of age and sex on bee location within clusters

Data from the four trials were not pooled as average bee age varied among trials
(Table 1). Bees in the inner layer were significantly younger than bees in the outer
layer of clusters in three of the four trials, and were close to being significantly
younger in the fourth trial (t-test, $P = 0·058$, Table 1). One-day-old bees were all
located at the centre of the cluster (four trials, 49 bees), as were drones (four trials,
52 bees).

Thoracic surface temperature elevations of workers and drones

The elevation of T_{ths} above T_a was quite variable among workers (Fig. 1). Of the
unmarked bees 89 % of those probed had $T_{ths} \geqslant 2$°C above T_a, indicating that most
bees contribute to colonial heating; average $T_{ths} - T_a$ was 3·6°C (s.e. = 0·17,
$N = 145$). When marked and unmarked bees are included in the calculation, T_{ths}

Table 1. *Comparison of the age (in days) of bees on the inside layer and on the
outside layer of clusters kept overnight at 15°C*

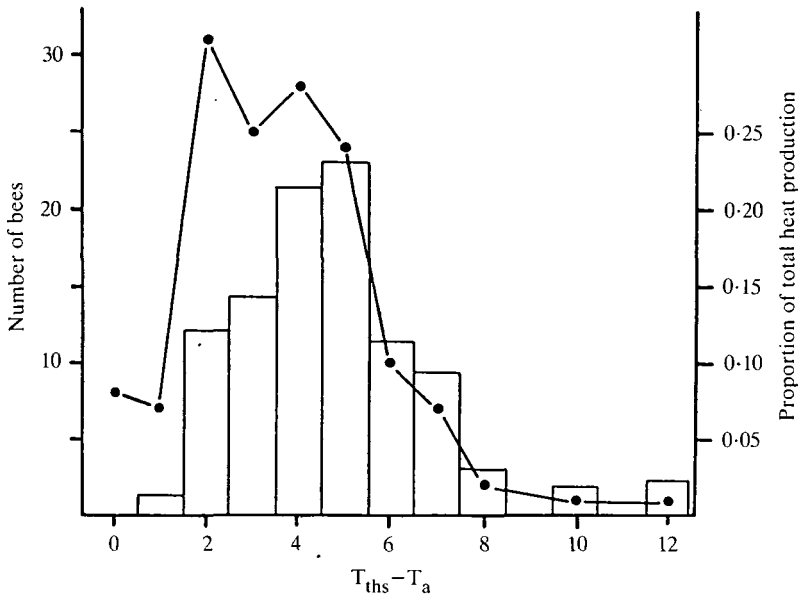|	Inside bees			Outside bees				
Trial	x̄	S.E.	N	x̄	S.E.	N	t-value	
1	6·4	1·17	38	13·3	1·14	42	4·21	$P < 0·01$
2	5·2	1·23	39	16·2	1·31	52	5·08	$P < 0·01$
3	9·9	1·53	46	14·7	2·03	28	1·90	$P = 0·058$
4	9·1	1·47	30	16·7	2·40	19	2·96	$P < 0·01$

Fig. 1. Temperature elevation of the thorax ($T_{ths}-T_a$, °C, solid circles, $N = 145$) for unmarked workers approximately 1 h after exposure of combs of bees to 15°C, and the relative contribution of each category (°C ±0·5°C categories) (histograms) to total colonial heat production.

averaged 4·1°C above T_a. T_{ths} of bees over the brood averaged 7·0°C warmer than bees off the brood area, but $T_{ths}-T_a$ did not vary with location (Table 2).

As the age of drones was not known, drone temperatures were compared to those of unmarked workers. $T_{ths}-T_a$ for drones ($\bar{x} = 3·48$, S.E. $= 0·31$, $N = 41$) did not differ from that observed for unmarked workers ($\bar{x} = 3·61$, S.E. $= 0·17$, $N = 145$; t-test, $P = 0·594$).

The effect of age on worker foraging behaviour and $T_{ths}-T_a$

One-day-old bees had significantly lower $T_{ths}-T_a$ than older bees (Scheffe's *a posteriori* test, 0·05 level), and in many 1-day-old bees T_{ths} equalled T_a (Fig. 2).

Table 2. *Effect of location on or off the brood area on thoracic surface temperatures (T_{ths}) and the elevation of thoracic surface temperature above local ambient temperature ($T_{ths}-T_a$) for marked and unmarked bees*

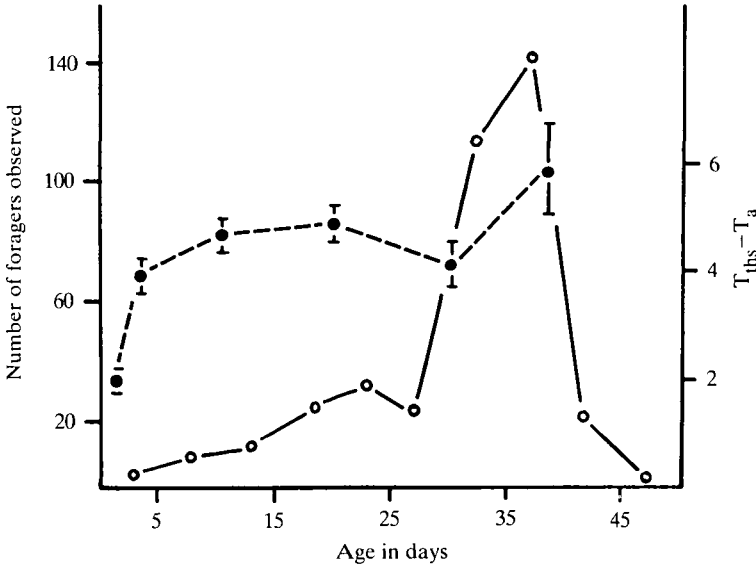|	On brood area	Off brood area	Probability (t-test)
T_{ths} (°C)	$N = 113$	$N = 92$	
$\bar{x}$	32·2	25·0	$P < 0·001$
S.E.	0·2	0·3	
$T_{ths}-T_a$ (°C)			
$\bar{x}$	4·2	4·0	$P = 0·5$
S.E.	0·2	0·2	

Fig. 2. The effects of age on worker foraging behaviour (open circles) and on the temperature elevation of the thorax, $T_{ths}-T_a$ (°C, closed circles ±S.E.), approximately 1 h after exposure of combs of bees to 15°C.

However, $T_{ths}-T_a$ did not differ among all bees 2 days of age and older (Scheffe's test, 0·05 level). Peak foraging behaviour occurred in workers that were 30–37 days old (Fig. 2). $T_{ths}-T_a$ of bees of foraging age did not differ from younger bees (Fig. 2).

Effect of location on or off the brood on thoracic cooling

Temperatures of dead bees equalled T_a when equilibrated, indicating that T_a accurately reflected operative environmental temperature. Thoracic cooling curves of dead bees were linear when plotted on a semilogarithmic plot. All linear regressions for the cooling curves were highly significant (all $r^2 > 0·98$) and the slopes were not significantly different among sites (on the brood: $\bar{x} = 0·447$, S.E. $= 0·036$, $N = 13$, average $T_a = 25·1$°C; off the brood: $\bar{x} = 0·453$, S.E. $= 0·022$, $N = 12$, average $T_a = 19·9$°C; t-test, $P = 0·894$). When all data are pooled, the cooling constant averaged $1·036$ min^{-1}, and the calculated thoracic conductance, C, was $0·829$ cal g^{-1}°C^{-1} min^{-1}.

Individual worker thermogenesis

Heat production of bees is estimated from:

$$M = C \times (T_{ths} - T_a) \times \text{thorax mass}.$$

Heat production per bee did not vary with location as neither C nor $T_{ths}-T_a$ varied with location (thorax mass averages 28 mg and is constant with age in this population of honey-bees; Harrison, 1986). Average heat production per worker, M,

was $0{\cdot}095\,\mathrm{cal\,min^{-1}}$ ($4{\cdot}1\,^{\circ}\mathrm{C} \times 0{\cdot}829\,\mathrm{cal\,g^{-1}\,^{\circ}C^{-1}\,min^{-1}} \times 0{\cdot}028\,\mathrm{g\,bee^{-1}}$). This corresponds to an oxygen consumption ($\dot{V}_{O_2}$) of $40{\cdot}0\,\mathrm{ml\,O_2\,g\text{-}thorax^{-1}\,h^{-1}}$ [assuming that the respiratory quotient (RQ) = 1].

DISCUSSION

In honey-bee colonies containing brood, most of the individual bees contribute to colonial thermogenesis (Fig. 1). The age-related transition to foraging of worker bees is not associated with a significant increase in heat production on the comb (Fig. 2). All workers 2 days of age and older contribute equally to colony heat production under these conditions (Fig. 2). This is possible because of the relatively low cost of thermogenesis. The thorax-specific $\dot{V}_{O_2}$ necessary for the average elevation of T_{ths} above T_a ($4{\cdot}1\,^{\circ}\mathrm{C}$), is $40\,\mathrm{ml\,O_2\,g\text{-}thorax^{-1}\,h^{-1}}$, only 15 % of the thorax-specific maximal oxygen consumption rate of 4-day-old bees (Harrison, 1986). The calculated average metabolic rate of $58\,\mathrm{W\,kg^{-1}}$ (assuming 115 mg average bee mass) is within one standard deviation of the average daytime metabolic rate determined for frames of honeybees at $15\,^{\circ}\mathrm{C}$, as determined by Kronenberg & Heller (1982). This supports the validity of these indirect measures of honeybee heat production.

Despite a $7\,^{\circ}\mathrm{C}$ average difference in T_{ths} between workers on and off the brood, average heat production per bee is not affected by worker location. This result must be interpreted with caution, as it is unlikely that conductances will be identical in different parts of a honeybee hive as they are on the single frame. Thoracic cooling constants of dead bees on the frame average 58 % higher than those measured under standard conditions (Heinrich, 1980). Cooling constants ($\mathrm{min^{-1}}$) are measured with respect to T_a, which equals box wall temperature under standard conditions. For bees on the cold-exposed frame, box wall temperatures are cooler than T_a, substantially increasing the rate of heat loss relative to T_a.

An important question is whether thermoregulation may be achieved simply with resting heat production rates as occurs in swarms (Heinrich, 1981). CO_2 production of honeybees engaged in moderate activity such as slow walking and cleaning appendages averages $10{\cdot}1\,\mathrm{ml\,CO_2\,g^{-1}\,h^{-1}}$ (Louw & Hadley, 1985). Assuming a Q_{10} of 2 and RQ = 1 (Southwick, 1985), $\dot{V}_{O_2}$ at $21\,^{\circ}\mathrm{C}$ will be $22\,\mathrm{ml\,O_2\,g\text{-}thorax^{-1}\,h^{-1}}$, and resting metabolism will provide roughly 90 % (on the brood) and 55 % (off the brood) of the heat production necessary for the $4{\cdot}1\,^{\circ}\mathrm{C}$ elevation of T_{ths} over T_a. However, in Louw & Hadley's study, muscle activity was not measured. Bumblebees monitored to exclude the possibility of flight muscle activity have a resting $\dot{V}_{O_2}$ of $2\,\mathrm{ml\,O_2\,g\text{-}thorax^{-1}\,h^{-1}}$ at $21\,^{\circ}\mathrm{C}$ (Kammer & Heinrich, 1974). If metabolism is similar in honeybees, then metabolism without flight muscle activity will contribute only 10 % (on the brood) and 5 % (off the brood) of the heat necessary to achieve the average $T_{ths}-T_a$. Thoracic temperatures of individual bees implanted with thermocouples and exposed to cooling frame temperatures exhibited intermittent rises and falls (Kronenberg & Heller, 1982). This supports the hypothesis that shivering thermogenesis by individual bees is necessary for thermoregulation in cold-exposed frames of bees prior to clustering.

Drone cooling constants may be estimated from the allometric equation for bees reported by May (1976; drone thoracic mass averages 85 mg, J. Coehlo, personal communication). If drone cooling constants are increased 58% when frames are exposed to 15°C as found for workers, drone thoracic conductance will be $0.488 \, cal \, g^{-1} \, °C^{-1} \, min^{-1}$, and the average heat production of a drone will be $0.145 \, cal \, bee^{-1}$. Accordingly, each drone will contribute 1.5 times as much heat as a worker to colonial thermogenesis, mainly because drones are larger.

It is unclear why drones should invariably be located within the centre of the clusters. Drones are inferior to workers in thermoregulation at temperatures below 25°C (Cahill & Lustick, 1976), so perhaps their location within the cluster centre, where temperatures are higher, allows their continued contribution to colonial thermogenesis. Alternatively, drone presence in cluster centres may increase sperm viability. Honeybee sperm decreases in viability *in vitro* below 15°C (J. Harbo, personal communication). Males of the eusocial Hymenoptera rarely contribute to colony welfare (Wilson, 1971). This study and the observations by Cameron (1985) on bumble-bees demonstrate that drone contribution to colony heat production represents an exception to the general lack of altruism by male Hymenoptera. However, the energetic cost of this behaviour is low.

Individual bees seek out environmental temperatures near 35°C (Heran, 1952). The pattern of thermoregulation demonstrated here is not compatible with a simple model where individuals attempt to maintain their own body temperature near the preferred temperature for individual honeybees, as has been hypothesized for swarms and clusters within the hive (Heinrich, 1985). Bees on the frame periphery allow their thoracic temperatures to drop to an average of 25°C. However, the attraction of bees to the brood area may be partly due to behavioural thermoregulation. These experiments suggest that honeybee brood warming before clustering is due to relatively uniform heat production by individual bees combined with the attraction of bees to the brood area.

REFERENCES

ALLEN, D. M. (1959). Respiration rates of worker honeybees of different ages at different temperatures. *J. exp. Biol.* **36**, 92–101.

CAHILL, K. & LUSTICK, S. (1976). Oxygen consumption and thermoregulation in *Apis mellifera* workers and drones. *Comp. Biochem. Physiol.* **55A**, 355–357.

CAMERON, S. A. (1985). Brood care by male bumble bees. *Proc. natn. Acad. Sci. U.S.A.* **82**, 6371–6373.

CORKINS, C. L. & GILBERT, C. S. (1932). The metabolism of honeybees in winter cluster. *Bull. Wyo. agric. Exp. Stn* **187**, 1–30.

Esch, H. (1960). Über die Körpertemperaturen und den Wärmehaushalt von *Apis mellifera*. *Z. vergl. Physiol.* **43**, 305–335.

Free, J. B. & Simpson, J. (1963). The respiratory metabolism of honeybee colonies at low temperatures. *Entomologia. exp. appl.* **6**, 234–238.

Gary, N. E. (1960). A trap to quantitatively recover dead and abnormal honeybees from the hive. *J. econ. Ent.* **53**, 782–785.

Harrison, J. M. (1986). Caste-specific changes in honeybee flight capacity. *Physiol. Zool.* **59**, 175–187.

Heinrich, B. (1980). Mechanisms of body-temperature regulation in honeybees, *Apis mellifera*. II. Regulation of thoracic temperature at high air temperatures. *J. exp. Biol.* **85**, 73–87.

Heinrich, B. (1981). The mechanisms and energetics of honeybee swarm temperature regulation. *J. exp. Biol.* **91**, 25–55.

Heinrich, B. (1985). The social physiology of temperature regulation in honeybees. In *Experimental Behavioral Ecology and Sociobiology* (ed. B. Holldobler & M. Lindauer), pp. 393–406. Stuttgart, New York: Gustav Fischer-Verlag.

Heran, H. (1952). Untersuchungen über den Temperatursinn der Honigbiene (*Apis millifica*) unter besonderer Berücksichtigung der Wahrnehmung strahlender Wärme. *Z. vergl. Physiol.* **34**, 179–206.

Himmer, A. (1925). Körpertemperaturmessungen an Bienen und anderen Insekten. *Erlanger. Jb. Bienenk.* **3**, 44–115.

Himmer, A. (1927). Ein Beitrag zur Kenntnis des Wärmehaushalts in Nestbau sozialer Hautflügler. *Z. vergl. Physiol.* **5**, 375–389.

Himmer, A. (1932). Die Temperaturverhältnisse bei den sozialen Hymenopteren. *Biol. Rev.* **7**, 224–253.

Jay, S. C. (1963). The development of honeybees in their cells. *J. apicult. Res.* **2**, 117–134.

Kammer, A. E. & Heinrich, B. (1974). Metabolic rates related to muscle activity in bumblebees. *J. exp. Biol.* **61**, 219–227.

Koeniger, N. (1978). Das Warmen der Brut bei der Honigbiene (*Apis mellifera* L.). *Apidologie* **9**, 305–320.

Kronenberg, F. & Heller, H. C. (1982). Colonial thermoregulation in honey bees (*Apis mellifera*). *J. comp. Physiol.* **148**, 65–76.

Louw, G. N. & Hadley, N. F. (1985). Water economy of the honeybee: a stoichiometric accounting. *J. exp. Zool.* **235**, 147–150.

May, M. L. (1976). Warming rates as a function of body size in periodic endotherms. *J. comp. Physiol.* **111**, 55–70.

Nijland, M. J. M. & Hepburn, H. R. (1985). Ontogeny of a circadian rhythm in the cluster temperature of honeybees. *S. Afr. J. Sci.* **81**, 100–101.

Ritter, W. (1982). Experimenteller Beitrag zur Thermoregulation des Bienenvolks (*Apis mellifera* L.). *Apidologie* **13**, 169–195.

Seeley, T. D. (1982). Adaptive significance of the age polyethism schedule in honeybee colonies. *Behav. Ecol. Sociobiol.* **11**, 287–293.

Seeley, T. D. & Heinrich, B. (1981). Regulation of temperature in the nests of social insects. In *Insect Thermoregulation* (ed. B. Heinrich), pp. 159–234. New York, Toronto: John Wiley & Sons, Inc.

Simpson, J. (1961). Nest climate regulation in honeybee colonies. *Science* **133**, 1327–1333.

Southwick, E. E. (1985). Allometric relations, metabolism and heat conductance in clusters of honey bees at cool temperatures. *J. comp. Physiol.* **156**, 143–149.

Southwick, E. E. & Mugaas, J. N. (1971). A hypothetical homeotherm: the honeybee hive. *Comp. Biochem. Physiol.* **40A**, 935–944.

Wilson, E. O. (1971). *The Insect Societies*. Cambridge: Harvard University Press.