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A TEMPERATURE-INDUCED SWITCH FROM DIFFUSIVE TO CONVECTIVE VENTILATION IN THE HONEYBEE

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Summary

It is known that many insects emit CO_2 in widely spaced 'bursts' or discontinuous ventilation events, usually characterized by active abdominal ventilation. We describe the discontinuous CO_2 emission characteristics of the honeybee (*Apis mellifera ligustica* Spinola), and utilize its 'chill coma' temperature threshold (12°C) to effect transitions from continuous, diffusive to discontinuous, convective ventilation regimes. Increasing temperature abruptly switched the dynamics of ventilation from diffusive and continuous (≤11°C) to convective and discontinuous (>12°C). The ventilation cycle frequency was 7.84 mHz and CO_2 output per ventilation event (burst phase) was 1.56 μl: neither variable was temperature-dependent in the range 12–15°C. The rate of CO_2 emission did not change significantly in the range 7–15°C, possibly owing to increased membrane leakiness at lower temperatures. At 15°C, honeybee metabolic rate (2.69 $W kg^{-1}$, mean mass 0.094 g) is similar to that of other similarly sized insects capable of significant endothermy.

Introduction

Honeybees (*Apis mellifera*) are in some respects atypical insects. In particular, they are seldom near thermal equilibrium with their environment. Highly social, they maintain high and stable colony temperatures by active coordinated thermoregulation (Kronenberg and Heller, 1982; Southwick, 1982), while individual foragers similarly maintain high flight muscle temperatures by active endothermy (see Jungmann *et al.* 1989). Muscular performance at a thoracic temperature below about 28°C is much impaired (Jungmann *et al.* 1989), while below about 12°C a chill coma or generalized muscular paralysis develops (Allen, 1959). Hence, an individual, cold, heterothermic honeybee is a physiological as

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well as a social orphan, deprived of the thermal context to which its muscular and enzyme systems are adapted.

Such a decontextualized state can be used to explore some interesting questions. Because convective ventilation in resting insects (see Kestler, 1985, for a review) is dependent on pumping movements produced by abdominal musculature (Lighton, 1988*a*; Corbet, 1988), and/or oscillations in hemolymph pressure which are muscular in origin (Slama, 1988), do these biophysical underpinnings of convective ventilation preclude the adoption of the simpler, 'classic' diffusion ventilation strategy (Krogh, 1941) in an insect that normally ventilates discontinuously? We investigated the existence and implications of this putative temperature-linked change in ventilation strategy by examining the ventilation characteristics of honeybees and utilizing the chill coma to switch their capacity for active ventilation on or off.

Materials and methods

Honeybees (*Apis mellifera ligustica*) were captured while foraging at bottlebrush inflorescences at Warren Hall, University of California at Los Angeles. On capture each bee was immobilized by chilling for 30 min at 4°C, and a small copper–constantan thoracic thermocouple was affixed with wax in direct contact with its scutum. Thoracic thermocouple placement was external to avoid disruption of air-sacs. In still air, external and internal thoracic temperatures are equivalent (Esch, 1960).

Ventilation and metabolic rate (MR) were measured by flowthrough CO_2 respirometry with temperature control and correction for analyzer baseline drift (Lighton, 1988*b*, 1990). Measurements were made at 1°C intervals from 7 to 15°C, with 1 h equilibration at each temperature. Respiratory quotient (RQ) was measured using closed-system gas analysis (Lighton, 1988*b*). Continuous monitoring of activity (Lighton, 1988*b*) and thoracic temperature allowed us to exclude measurements made on active or significantly endothermic bees. DATACAN IV software was used for data acquisition, control and analysis (Sable Systems, 1015 Gayley Avenue, Los Angeles, CA 90024). Mean values are given ±S.E.M.

Results

Metabolic rate and respiratory quotient

The rate of CO_2 production was $0.4098\pm0.0268\,ml\,g^{-1}\,h^{-1}$ ($N=10$ honeybees, mean live mass 0.0944 ± 0.0108 g, mean dry mass 0.0245 ± 0.0018 g), and did not vary significantly over the temperature range 7–15°C. RQ over the temperature range 5–20°C, measured by a closed-system technique with simultaneous determination of O_2 and CO_2 exchange, was 0.864 ± 0.066, and was also not temperature dependent ($N=5$–7 bees at each of nine temperatures; $P>0.3$). An RQ of 0.864 yields an energy equivalent of $23.6\,J\,cm^{-3}\,CO_2$, allowing calculation of standard

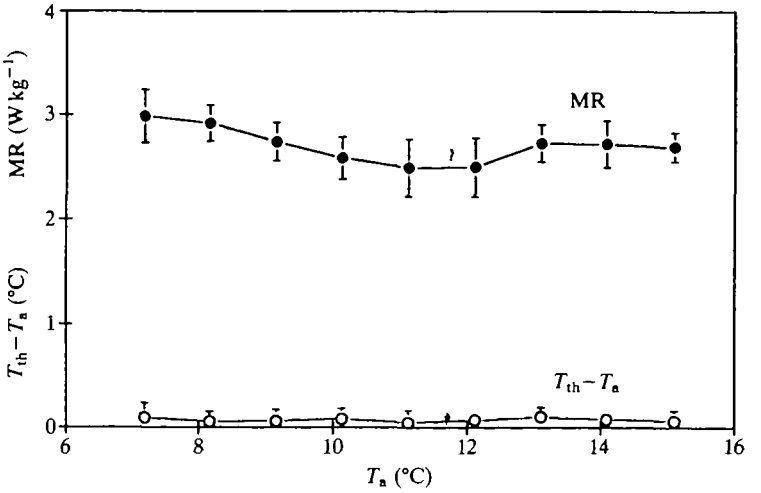

Fig. 1. Metabolic rate (MR in W kg^{-1}) and elevation of thoracic temperature (T_{th}) above ambient temperature (T_a) ($T_{th}-T_a$ in °C) as a function of ambient temperature. N=10 honeybees as in Fig. 2. Error bars denote ±1 S.E.

MR (SMR) from CO_2 emission rates. SMR was 2.69±0.17 W kg^{-1} (Fig. 1). The elevation of thoracic temperature above ambient was constant at 0.075±0.018°C (Fig. 1).

Ventilation

Honeybee ventilation was profoundly affected by temperature, with two distinct regimes operating above and below 12°C (Figs 2, 3). Below 12°C, ventilation was continuous and presumably largely or entirely diffusion-based. At or above 12°C, however, a distinct discontinuous ventilation cycle (DVC) appeared. This changeover point was well-defined and reproducible, whether temperature was ramped up or down. The DVCs were stereotyped and strongly periodic (Fig. 3). 'Bursts' of CO_2, corresponding to the V phase of the DVC (see Kestler, 1985, for a review) occurred at a characteristic frequency of 7.84±0.76 mHz (N=291 DVCs in 10 bees). Photoelectric monitoring of activity revealed small pulsations of the abdomen that coincided with the CO_2 emissions; these pulsations were also observed visually in bees not undergoing respirometry. We did not detect any V phases below 12°C in any of our recordings. Burst frequency did not depend significantly on ambient temperature (P>0.3). Each burst was accompanied by visible abdominal pumping movements and corresponded to a total emission of 1.56±0.21 μl of CO_2; again, no temperature-dependence was found. Between these periodic emissions of CO_2, little external CO_2 emission took place (Fig. 3). We did not quantify ventilation at higher temperatures because almost all our honeybees were active and intermittently endothermic above 15°C.

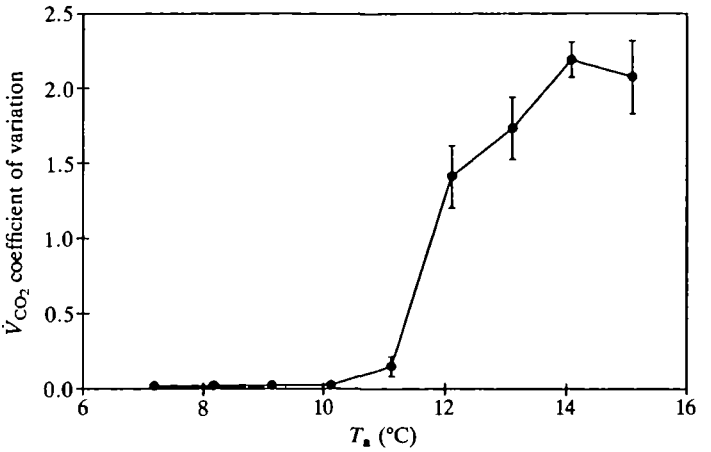

Fig. 2. The change from continuous, diffusive to discontinuous, convective ventilation, as shown by the coefficient of variation (CV) of CO_2 emission rate in 10 honeybees (mean live mass 0.0944±0.0108 g, mean dry mass 0.0245±0.0018 g) as their ambient temperature was increased from 7 to 15 °C in 1 °C steps. 1 h equilibration was allowed at each temperature prior to measurement. CV is the standard deviation of CO_2 emission rate over 20 min, sampled every 2 s, divided by its mean over the same period. Error bars denote ±1 S.E.

Discussion

Individual metabolic rate

Perhaps the most surprising aspect of our SMR data is their near-constancy over the ambient temperature range 7–15 °C. This observed constancy of SMR over temperature is not a product of endothermic responses, which markedly increase MR as temperature drops (see Kronenberg and Heller, 1982). Similarly low variation of SMR over a restricted temperature range, although unusual, has been documented in other insects (see Hoffmann, 1984) and its presence over the temperature range of our study can be inferred from other published accounts of honeybee metabolism (Rothe and Nachtigall, 1989).

It is perhaps significant that the lowest MR occurs at 11–12 °C, suggesting that some factor may act to elevate MR below the chill coma temperature. Both the chill coma and the slightly increasing energetic requirements of the honeybee below 12 °C (Fig. 1) may be caused by progressive disruption of ionic homeostasis with decreasing temperature. For example, changes in cell membrane fluidity at low temperatures may reduce the efficiency of the honeybee Na^+/K^+-ATPase or other critical transmembrane transport proteins. This would inhibit muscle and nerve function, as observed, and require increased ATP production (and hence increased oxidative metabolism, as seen in Fig. 1) in order to maintain intracellular ionic homeostasis.

The SMR of individual honeybees has never been measured satisfactorily, as a number of authors have stated (Rothe and Nachtigall, 1989, and references therein). This is because of the intense activity that bees typically exhibit as

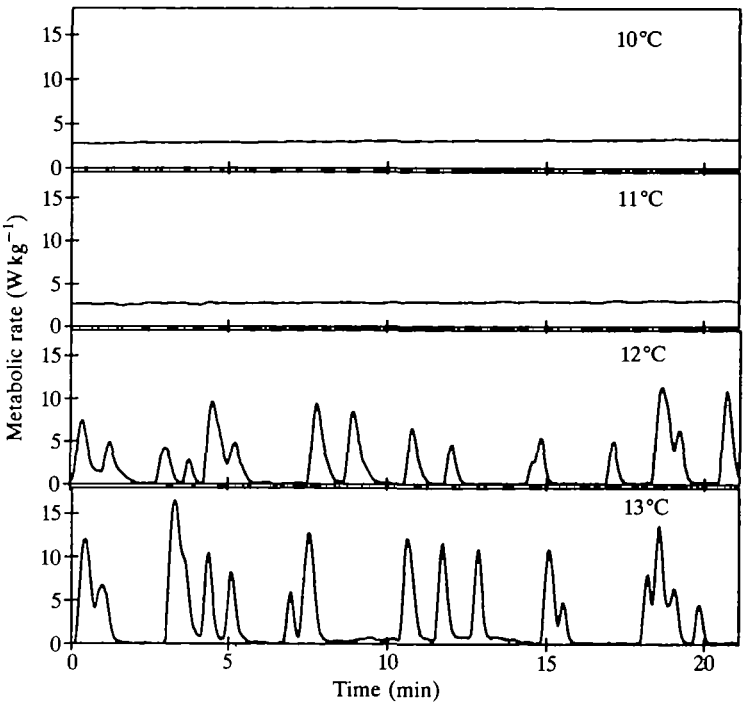

Fig. 3. Typical change of ventilation patterns near 12°C in a honeybee, live mass 0.0840 g, dry mass 0.0234g. Metabolic rate (MR in $W\,kg^{-1}$) is expressed on a live mass basis. Note that actual MR is constant. It appears to fluctuate above 11°C because it is estimated from CO_2 emission rate, which varies over the discontinuous ventilation cycle.

individuals, and their capacity for endothermy. SMRs as high as $70\,W\,kg^{-1}$ have been measured (Withers, 1981) – about 10-fold higher than predicted for insects of similar mass (Bartholomew and Casey, 1977). In contrast, our data, which controlled for activity and endothermy, show that the SMR of individual bees in a normal ventilation state at 15°C, above the chill coma temperature, does not significantly differ from that of other insects of equivalent mass and with the capacity for endothermy during flight (Bartholomew and Casey, 1977; assuming Q_{10}=2.0). As a general principle, comparative data on insect metabolic rate are best obtained from insects that are known to be ventilating in a state characteristic of motionlessness and that are known not to be producing significant excess metabolic heat, as here.

Discontinuous ventilation

The discontinuous CO_2 emission of our sample of honeybees above 12°C is similar to that displayed by the ant *Camponotus vicinus* (Lighton, 1988*b*), though at a much higher frequency (3.5 times at 15°C). No F (fluttering-spiracle) phase is

unambiguously visible. CO_2 emission is therefore concentrated in the V (ventilation) phase, with a small amount released during the C (closed-spiracle) phase, as with *C. vicinus*. The extent to which a diminution of the importance of the F phase is widespread among small insects is still uncertain. Extrapolating from the as yet very limited database, a tendency for xeric insects to release proportionately more CO_2 in the F phase than do mesic insects is becoming apparent (Lighton, 1990, and in preparation).

It should be stressed that the V phase in our honeybees, when it occurred, was invariably accompanied by externally visible abdominal pulsations. Although such pulsations were not unambiguously visible in *C. vicinus* (Lighton, 1988*b*), it is probable that low-magnitude abdominal pulsations did in fact occur, and that the V phase in that species was characterized by convective rather than diffusive ventilation, as here (see also Slama, 1988; Corbet, 1988). More sophisticated movement detection systems, or visual observation under magnification during discontinuous ventilation, should resolve this question.

The switch from passive ventilation below 12°C to active ventilation above that temperature was very marked and reproducible. The honeybees suffered no apparent ill effects from prolonged passive, diffusion-driven ventilation. They could be kept below 12°C for several hours and recovered rapidly and fully when returned to room temperature. From this we infer that purely diffusive ventilation did not induce damaging hypoxia or hypercapnia, and that honeybees can switch from active, convection-based to passive, diffusion-based ventilation at low temperatures without limiting their rates of O_2 and CO_2 exchange.

The only comparable metabolic and ventilation data on honeybees are those of Rothe and Nachtigall (1989). They did not explore honeybee ventilation in detail, but it is apparent that at least some of their bees may have ventilated discontinuously (Rothe and Nachtigall, 1989; Fig. 1E). The CO_2 exchange patterns that they observed differ significantly from those that we report here, but their honeybees were at a much higher ambient temperature (26.5°C). In our hands, data on discontinuous ventilation could not be obtained reliably above 15°C because of activity and endothermy, both of which we monitored. In the presence of either variable, CO_2 emission rates rose steeply, showing few well-defined V phases, all but eliminating the C phases, and fluctuating in accordance with the degree of activity or endothermy. It is therefore possible that some of the CO_2 emission cyclicity observed by Rothe and Nachtigall (1989) was derived from bouts of activity or endothermy, which would explain the long periodicity and large emission volumes they observed.

Our RQ data are somewhat lower, and MR data higher, than the values obtained by Rothe and Nachtigall (1989) in their sample of completely quiescent *A. mellifera carnica* honeybees. This could be a difference between the two subspecies or races. If different subspecies or races of honeybees differ in MR and ventilation parameters at low temperatures, such variation may provide useful insights into their ecophysiology. If differences exist and are robust, they could be of some use in rapidly distinguishing between morphologically similar but

behaviorally and ecologically different races (e.g. European *versus* Africanized honeybees in the Americas).

The conventional model of small-insect ventilation, in which O_2 is obtained and CO_2 released by passive diffusion through open spiracles, therefore does apply to honeybees, but only when they are in a state of muscular paralysis. Raised above their chill coma temperature, increased spiracular control accompanied by active abdominal ventilation movements allow honeybees to switch from a continuous, passive to a markedly discontinuous, convective ventilation regime. Therefore, even if a given insect is found to employ purely diffusive ventilation, it may well have the capacity to ventilate discontinuously and convectively under appropriate circumstances.

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