

METABOLIC RATE AND THERMAL STABILITY DURING HONEYBEE FORAGING AT DIFFERENT REWARD RATES

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Summary

During honeybee foraging, the stabilization of thoracic temperature (T_{th}) at elevated values is necessary to meet the power requirements of flight at different air temperatures (T_a). To understand how the bee achieves thermal stability at different reward rates, the metabolic rates of undisturbed foraging bees were measured at different T_a values and different sucrose solution flow rates. Metabolic heat production, calculated from the rate of carbon dioxide production, decreased linearly from 49.7 to 23.4 mW as T_a increased from 19 to 29 °C (sucrose flow rate 1.75 $\mu\text{l min}^{-1}$, 50 % w/w). In contrast, crop load and inspection rate remained constant. Metabolic rate

displayed a linear relationship with both T_a and the logarithm of the flow rate of sucrose solution (range analyzed 0.44–13.1 $\mu\text{l min}^{-1}$, 50 % w/w). Metabolic rate decreased by 3.13 ± 0.52 mW (mean \pm S.E.M., $N=37$) for every 1 °C increase in T_a and increased by 4.36 ± 1.13 mW for a doubling in flow rate. These changes in metabolic power output might be used to achieve thermal stability during foraging. It is suggested that the foraging bee might increase its T_{th} in accordance with the reward rate.

Key words: *Apis mellifera ligustica*, honeybee, foraging, motivation, thermoregulation, flight energetics.

Introduction

Like many other endothermic insects, honeybees regulate their thoracic temperature (T_{th}) relatively well during flight over a substantial range of air temperatures (T_a) (Coelho, 1991; Heinrich, 1993). Thermal stability is necessary to keep the thoracic muscles within the range of temperatures adequate to produce the power output required for flight (Esch, 1976; Coelho, 1991). During warm-up, thermal stability is achieved by regulating shivering thermogenesis, a tetanic contraction of the flight muscles against a skeletal stop (Goller and Esch, 1991; Esch et al., 1991). This mechanism is thought not to be available during flight and, until recently, it was thought that metabolic rate was determined by aerodynamic needs rather than by thermoregulatory ones. Moreover, early measurements (Heinrich, 1980b) showed that the metabolic rate of hovering bees did not change when T_a increased by 20 °C. The commonly accepted mechanism of thermal stabilization was the control of heat loss by evaporation (Heinrich, 1980a; Heinrich, 1993; Heinrich and Esch, 1994). However, this point of view has started to change: extensive measurements have demonstrated a decrease in the metabolic rate of 'agitated' bees in continuous flight with increasing T_a (Harrison et al., 1996). These data were criticized on a number of grounds, in particular the possibility that the honeybees were not in steady-state flight (Heinrich and Esch, 1997; Stevenson and Woods, 1997). However, Roberts and Harrison (Roberts and Harrison, 1999) showed that flying bees

were at thermal equilibrium and confirmed the variation in the metabolic rate. After determining the heat-exchange pathways, they found that thermal stability is achieved by varying metabolic rate at T_a values between 21 and 33 °C. At T_a values between 33 and 45 °C, variations in metabolic heat production and evaporative heat loss are equally important in preventing overheating during flight (Roberts and Harrison, 1999).

During foraging at decreasing T_a , an increase in metabolic power output is therefore likely to occur. However, estimates of the energetic costs of free flight using laboratory data might not be appropriate under many natural conditions (Wolf et al., 1999). Moreover, in contrast to the results obtained during 'agitated' flying, the metabolic rate during tethered flight increases with ambient temperature (Hrassnigg and Crailsheim, 1999), showing that the relationship between metabolic rate and air temperature is dependent on experimental conditions. Results obtained from caged or tethered bees cannot be extrapolated to foraging bees; direct measurements are needed. Esch et al. (Esch et al., 1994), while studying how the bee measures distance, made the first direct measurements at different ambient temperatures of foraging metabolic rates. They found a reduction in the metabolic rate when T_a exceeded 30 °C. Their measurements were based on non-flying foraging bees: the bees freely entered and left the metabolic chamber but could not fly inside it. There have

been no direct measurements of the metabolic rate of free-flying foraging bees at different T_a .

By placing a food-source simulator within a respirometry chamber, the metabolic rate of undisturbed freely foraging bees has recently been measured (Balderrama et al., 1992; Moffatt and Núñez, 1997; Moffatt, 2000). In these studies, the metabolic rate was found to depend on the reward rate obtained at the food source. This increase in metabolic rate with increasing ambient temperature was interpreted in terms of a hypothetical motivational state of the animal (Roces, 1993; Núñez and Giurfa, 1996). The motivational state is modulated by the reward found at the food source (Roces and Núñez, 1993; Moffatt and Núñez, 1997). There is experimental evidence to suggest that a 'motivated' animal increases its locomotory activity (Roces, 1993), its metabolic rate (Moffatt and Núñez, 1997; Moffatt, 2000) and its response to a visual stimulus (Farina and Josens, 1994).

In the present study, the effect of T_a and reward rate on the metabolic rate of honeybees foraging steadily on an artificial food source providing a controlled flow rate of sucrose solution was investigated.

Materials and methods

Experiments were carried out at the apiary of the Experimental Field Station of the Universidad de Buenos Aires (34°33'S, 58°26'W), Argentina, between March and May 1997. The bees used (*Apis mellifera ligustica* Spinola) belonged to a hive placed 50 m from the laboratory. During the measurements, ambient temperature fluctuated between 14 and 32 °C. The temperature of the flying chamber varied between 19 and 30 °C.

Experimental device

Bees were trained to collect sucrose solution inside an acrylic respirometry chamber big enough to allow short flights inside (Fig. 1). The experimental device was connected to the outside of the building through a window. To prevent sunlight entering, the front of the experimental device, the laboratory window glass, was replaced with a thick cardboard sheet with a small opening (20 cm high, 10 cm wide). Individual bees entered the respirometry chamber through a lateral entrance tunnel with an automatic door.

During each visit, the experimental bee collected, in successive inspections, the sucrose solution provided at each feeder, flying from one feeder to the next. An automatic sucrose pump provided an equal flow rate of sucrose solution to each of seven feeders; a plastic cannula connected each feeder with the microburette of the pump. Infrared barriers were positioned above the access point to the sucrose solution, at the bottom of each feeder tube (Fig. 1, detail in dashed box), to record every inspection. The infrared barriers detected the bees when they reached the bottom of the feeder tubes, so the bees triggered them while they fed (Fig. 1, detail of the bee feeding). Incomplete inspections, in which the bees did not enter the tube and therefore did not feed (Núñez, 1967; Giurfa and Núñez, 1992; Giurfa, 1996), were not recorded by the detectors.

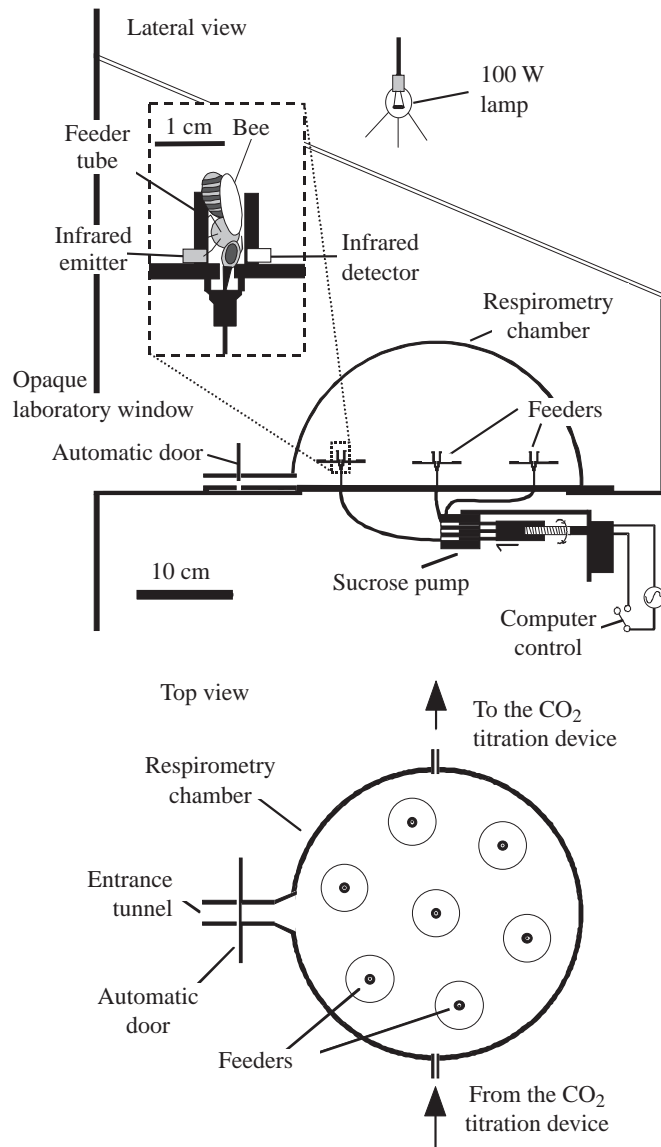


Fig. 1. Experimental device. An automatic food source is accommodated within a respirometry chamber of volume 7 l. The bee enters and exits the chamber through an automatic door situated in a lateral tunnel and triggered by the presence of the bee. A sucrose pump provides an equal flow rate of sucrose solution to each of seven feeders. The dashed box encloses an amplified diagram of the feeder with a bee in the feeding position. An infrared barrier (emitter+detector) is situated on the bottom of the feeder tubes to detect the presence of the bee. The air in the chamber is pumped through a titration device to measure its CO₂ concentration.

Infrared barriers were also positioned at each end of the entrance tunnel to control the automatic door and to record the start and end of the visit. The operation of the automatic door ensured that the bee determined when to finish the visit without any interference from the experimenter. The sucrose pump was turned on when the bee arrived to the food source and reached the first infrared barrier of the entrance tunnel, and it was

turned off when the bee left the chamber and again reached the same infrared barrier.

The chamber air was circulated by pumps at 0.51 min^{-1} through a titration device to measure CO_2 concentration. CO_2 concentration was measured by determining the amount of time the air current needed to titrate 112 nequiv of $\text{Ba}(\text{OH})_2$. The titration endpoint was determined by measuring the absorbance at 590 nm of the titration solution, which contains the pH indicator Thymol Blue. The CO_2 titration device was calibrated using air currents of different CO_2 concentration. The response time, accuracy and sensitivity of the respirometry device (i.e. respirometry chamber + CO_2 titration device) was determined by the injection of CO_2 at different rates. The accuracy and sensitivity of CO_2 determinations were $\pm 7.15\%$ and $\pm 2.02\%$, respectively. The respirometry device has been described in detail previously (Moffatt, 2000).

Experimental procedure

Every morning, the food source (i.e. the seven feeders combined) offered sucrose solution (50% w/w) at $1.75 \mu\text{l min}^{-1}$ to a group of 1–4 foraging bees. Between 08:00 h and 10:00 h, all bees except one (which was marked while feeding by painting a coloured spot on its thorax or abdomen) were captured and caged. The marked experimental bee was used for 1–4 days. Captured bees were released every evening after the measurements with the experimental bee had been finished. The metabolic rate and behavioural responses of the experimental bee were measured for constant ($1.75 \mu\text{l min}^{-1}$) or for variable reward flow rates. In the latter case, one of the following flow rates (0.44, 0.87, 1.75, 3.49, 6.55 and $13.1 \mu\text{l min}^{-1}$) was offered for 2–4 successive visits, after which a new flow rate was used. Ascending or descending reward programs, in which the flow rate was gradually increased or decreased, were offered. Sucrose concentration was kept constant at 50% (w/w). Whenever possible, all the flow rates were offered to an experimental bee in a single day. Measurements started at 08:00–11:00 h and finished at 16:00–18:00 h.

Variables measured

Metabolic rate (mW) was measured for each visit by dividing the increment in the CO_2 concentration of the chamber by the time elapsed inside the chamber and multiplying by the chamber volume. This value was multiplied by the energy yield of $21.4 \text{ mJ } \mu\text{l}^{-1} \text{ CO}_2$ assuming simple carbohydrate catabolism (Rothe and Nachtigall, 1989).

Crop load (μl) was obtained by multiplying the duration of the visit (i.e. the time for which the sucrose pump was switched on) by the flow rate provided. As the maximum ingestion rate ($60 \mu\text{l min}^{-1}$; Núñez, 1966) is much greater than the flow rates offered, the bee was presumed to have collected all the sucrose provided. Previous studies have shown that this assumption is correct (Balderrama et al., 1992).

Inspection rate (min^{-1}) was the number of complete inspections performed per minute of visit. This was measured automatically by the infrared barrier located in the bottom of

each feeder tube (Fig. 1, detail). Rejections (Giurfa and Núñez, 1992) were not counted.

Feeding time (%) was the percentage of the visit that the bee spent inside the feeder tubes triggering the infrared barriers (Fig. 1, detail).

Air temperature ($^{\circ}\text{C}$) was measured by a thermometer situated on the internal surface of the respirometry chamber.

Statistical analyses

With the constant reward rate, measurements were obtained from 103 visits made by 14 bees over 18 days; with variable reward rates, 76 visits by eight bees on 16 days were analyzed. As repeated measurements were made on individual bees, one-way analyses of covariance (ANCOVAs) (Winer, 1971) with random effects were performed to factor out the variation among bees, taking inter-individual variations as the random factor and temperature and in one case the logarithm of the flow rate as the covariates. Two ANCOVAs of fixed effects were performed to separate the contribution of the time of day and of the reward rate from that of T_a and to analyze whether there were strong interactions between these factors and T_a . Every ANCOVA was made only if no significance was found in the corresponding parallelism test. In all cases, mean values \pm S.E.M. are given.

Results

Changes in metabolic rate: hourly modulation or effect of T_a ?

When a constant sucrose flow rate was offered, metabolic rate was strongly correlated with T_a (Fig. 2A). However, T_a changed with the time of day. A correlation between metabolic rate and T_a might arise if the bee were to have an endogenous program (Moore et al., 1989) of higher metabolic rate in the morning and lower metabolic rate in the afternoon. To eliminate this possibility, the relationship between metabolic rate and T_a was analyzed for different times of day. To avoid pseudoreplication (see Hurlbert, 1984), for measurements obtained from the same bee, at the same T_a , on the same day and at the same time of day, one mean value was calculated. The statistical analysis showed that metabolic rate was related to air temperature and not to time of day: measurements taken at the same time of day correlated with T_a (Fig. 2A; ANCOVA of fixed effects, covariate T_a : $t_{43} = -7.9$, $P < 0.0001$), while there were no differences between measurements taken at the same temperature but at different times of day (Fig. 2A; factor time of day: $F_{4,43} = 0.17$, not significant).

Measurements were made repeatedly on individual bees, so a statistical analysis was chosen to take into account the lack of independence of the data. A standard repeated-measures analysis was not possible because not all the bees were exposed to the same temperatures. However, because a linear relationship between metabolic rate and T_a was expected, an ANCOVA of random effects was performed. In this way, differences that arose because of individual variability could be separated from those that were due to an effect of T_a . The results of the ANCOVA showed that there was a significant correlation between metabolic rate and temperature [covariate

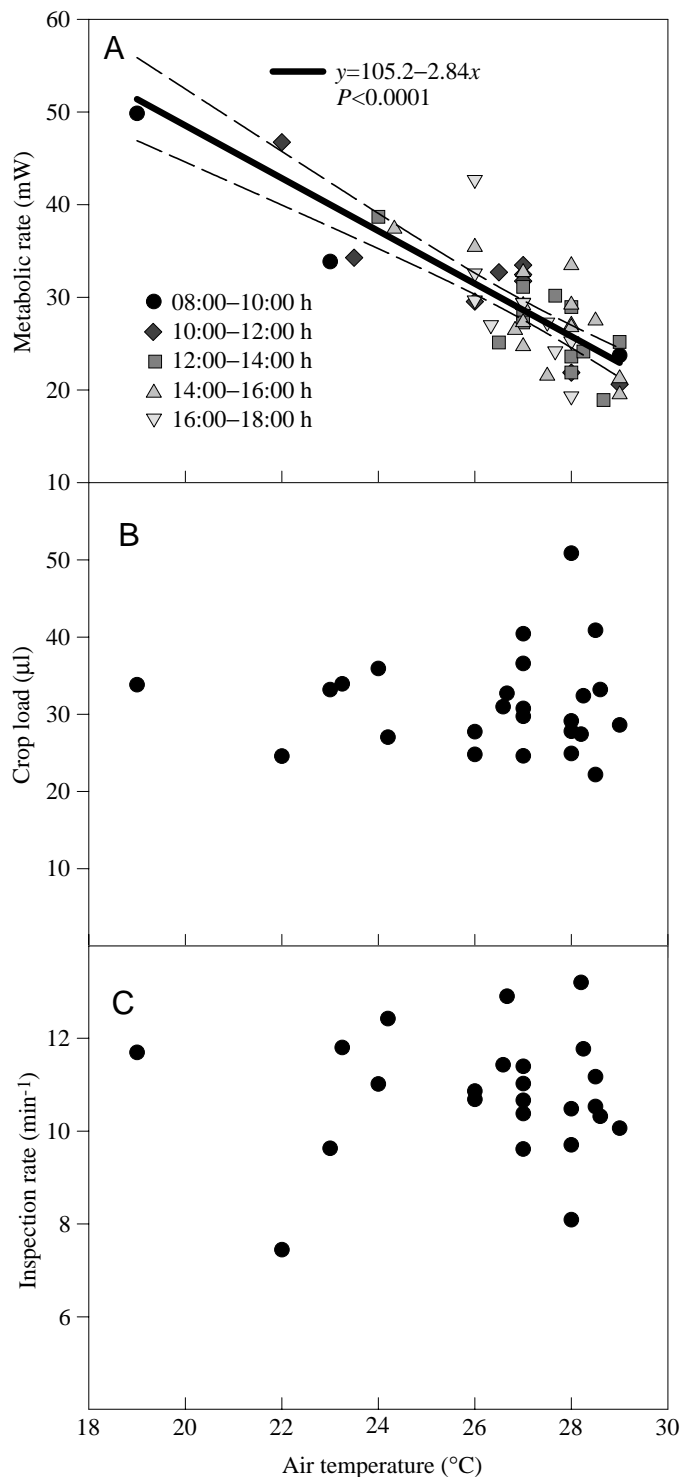


Fig. 2. Metabolic rate (A), crop load (B) and inspection rate (C) as a function of air temperature. Data in A are identified by the time of day of the visit. Sucrose (50% w/w) flow rate was $1.75 \mu\text{l min}^{-1}$. Each point represents the mean of a set of visits to the experimental device performed by the same bee, on the same day and at the same air temperature. In A, the dashed curves enclose the 95% confidence limits of the least-squares regression line. Data are from 103 visits made by 14 bees on 18 days. Neither crop load nor inspection rate was significantly correlated with air temperature.

T_a : $r^2=0.88$; slope of the regression, $b=-2.49 \pm 0.27 \text{ mW}$ (mean \pm S.E.M.), $t_{13}=-9.09$, $P<0.0001$] and that there were significant differences among bees in the metabolic rate attained at the same temperature (factor bees: $F_{5,10}=3.49$, $P<0.05$), but there were no differences among bees in the slope of the relationship (test of parallelism: $F_{4,7}=2.07$, not significant). In this analysis, to further prevent pseudoreplication, one mean value was taken for the same bee and air temperature (note the decrease in the number of degrees of freedom for the t -value). Fig. 2 contains relatively few data at low T_a ; nevertheless, the same correlation was found for visits at T_a values above 24°C (covariate T_a : $r^2=0.85$; $t_8=-6.74$, $P<0.0005$).

Does the behaviour of the bee change with T_a ?

Despite the large changes in metabolic rate with T_a , neither the crop load (Fig. 2B; ANCOVA of random effects: factor individual bees: $F_{5,10}=5.11$, $P<0.05$; covariate T_a : $r^2=0.26$, $t_{10}=-1.87$, not significant; parallelism test $F_{5,5}=1.35$, not significant) nor the inspection rate (Fig. 2C; ANCOVA of random effects: factor individual bees: $F_{5,10}=3.02$, $P=0.06$; covariate T_a : $r^2=0.02$, $t_{10}=0.47$, not significant; parallelism test: $F_{5,5}=2.2$, not significant) showed significant changes with T_a . The constant crop load indicates that the flying bees had a constant mass for the flying muscles to support. The absence of changes in the inspection rate implies that the frequency of flights between feeders did not change either.

Unfortunately, it was not possible to measure directly the time the bee spent flying. The infrared detectors only allowed the feeding time and the non-feeding time to be quantified. The latter included the time taken to come out of the feeder tube and to take off, the time taken to fly to the next feeder and the time between landing and reaching the bottom of the feeder tube, but it also included brief periods that the bees sometimes spend walking on the chamber walls and floor and the time to reject a feeder. Feeding time did not correlate with T_a ($t_{13}=0.21$, not significant) and averaged $50 \pm 1\%$.

Combined effects of T_a and reward rate

Metabolic rate was also measured during programs of ascending and descending reward rates. For the same flow rate ($1.75 \mu\text{l min}^{-1}$), the metabolic rate was the same regardless the reward program (i.e. ascending, constant or descending), as shown by an ANCOVA of fixed effects (factor program: $F_{2,40}=0.59$, not significant). In all cases, there was a significant correlation between metabolic rate and T_a (covariate T_a : $t_{40}=-8.6$, $P<0.0001$), and there were no differences among reward programs in the slope of the relationship between metabolic rate and T_a (test of parallelism: $F_{2,38}=2.09$, not significant).

Each time the sucrose flow rate was doubled, the metabolic rate increased by $4.36 \pm 1.13 \text{ mW}$ (Fig. 3; ANCOVA of random effects, covariate \log_2 of the flow rate: $t_{28}=10.88$, $P<0.001$). Moreover, at each flow rate, it was found that the metabolic rate correlated with T_a (Fig. 3, regression lines; covariate T_a : $b=-3.13 \pm 0.52 \text{ mW } ^\circ\text{C}^{-1}$, $t_{28}=-5.9$, $P<0.001$). Again, significant differences were found among individual

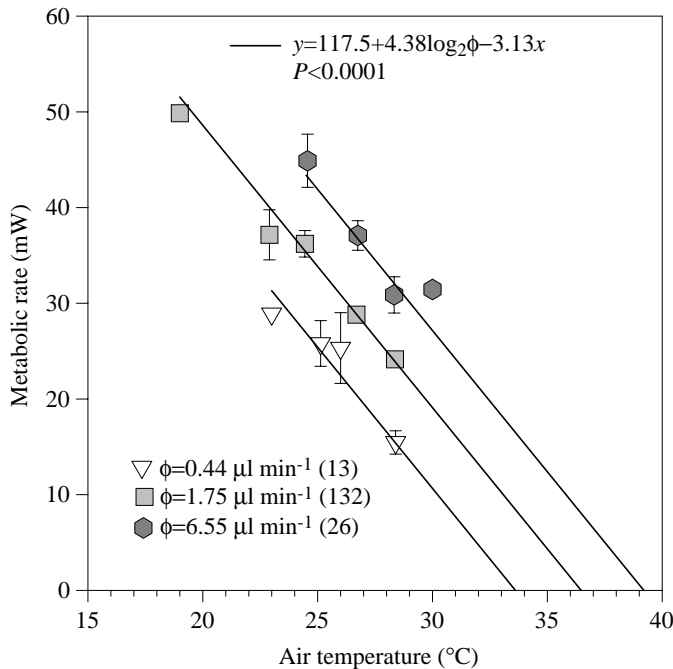


Fig. 3. Metabolic rate plotted as a function of air temperature at different flow rates (ϕ) of 50% (w/w) sucrose solution. Values are means \pm S.E.M. Values in parentheses indicate the number of visits. Lines indicate fit to ANCOVA multiple regression. Note the extrapolations to the abscissa: they are in the range of physiological thoracic temperature. Data are from 171 visits made by 16 bees on 33 days.

bees in the absolute values (factor bee: $F_{7,28}=3.23$, $P<0.05$), but not in the slopes (test of parallelism, $F_{14,14}=0.62$, not significant). Note in Fig. 3 the extrapolations of the ANCOVA regression lines: they cross the abscissa in the range of physiological T_{th} .

Discussion

The present study shows that the metabolic rates of foraging honeybees vary inversely with ambient temperature and depend positively on the reward rate offered. Both flower nectar content and ambient temperature are likely to vary widely in nature: the present results show that these variations have strong effects on flight energetics.

Routes of heat exchange and the mechanism of thermoregulation

To maintain a constant body temperature, the metabolic heat gain must balance the sum of convective heat exchange, evaporative heat loss and radiative heat exchange (Casey, 1988). Changes in the metabolic rate will occur if there are changes in the sum of the other routes of heat exchange as a consequence of changes in air temperature.

Convective losses are linearly dependent on the temperature difference between the body and the air and non-linearly dependent on the wind speed (more precisely on the range of

velocities of the air layer that surrounds the body). If the animal does not thermoregulate, body and air temperatures will be similar, but when the animal actively thermoregulates, body temperature will be independent of air temperature such that the temperature difference increases linearly with decreasing air temperature. The resulting increase in passive heat loss must be balanced in some way to keep the body temperature constant. If there is no parallel decrease in the evaporative heat loss or increase in the radiative heat gain, there must be an increase in metabolic heat production. In this latter case, an inverse relationship between metabolic rate and air temperature will be found.

Evaporative heat losses increase more than linearly with air temperature (Roberts and Harrison, 1999). In this way, they are important in the prevention of overheating at high temperatures (Heinrich, 1980a; Heinrich, 1980b; Heinrich, 1993); below 30°C, they are relatively small and of little significance for heat regulation (their further decrease at lower temperatures is much smaller than the increase in the convective heat loss; Roberts and Harrison, 1999).

Could the increased nectar intake be associated with an increase in evaporative heat loss? If this were the case, the increase in metabolic rate with reward rate could be produced by an increase in evaporative heat loss. The idea is simple: a fraction of the water from the ingested nectar is evaporated, and the metabolic rate increases to compensate for the increased heat loss. However, a closer inspection of the processing of the ingested solution reveals that this possibility is very unlikely. The first contact of the bee with the sucrose solution is during ingestion. Evaporation of a water drop extruded onto the mouthparts is a known mechanism for preventing overheating at elevated air temperatures (Heinrich, 1980a). However, in the present experiments, the bee introduced its proboscis into a capillary tube to drink the solution, so the mouthparts were protected from convective air currents and the amount of water evaporated from the mouthparts was likely to be small during ingestion. Ingested nectar is stored in the crop, and bees carry greater crop loads at higher reward rates (Núñez, 1966; Moffatt, 2000). Is a bee carrying a greater crop load susceptible to a greater evaporative heat loss? Again, this possibility is unlikely: the crop is impermeable to water.

A fraction of the sucrose solution stored in the crop is used for the forager's own metabolism, while the remainder is transferred to other nest-mates after returning to the hive. At higher reward rates, an increased metabolic rate is associated with an increased production of water. Although the evaporation of this water cannot explain the increment in the metabolic rate (this would be a circular argumentation), it is easy to determine how much heat the bee could lose in this way. Every 1 μ l of 50% (w/w) sucrose consumed by the bee provides 10.2 J and produces 483 μ l of CO₂ and 1 mg of H₂O (0.39 mg from oxidative metabolism and 0.61 mg from the solution itself). For a neutral water balance, the water produced must be eliminated. Water is eliminated as vapour through the spiracles and body wall and as liquid in the urine. The associated evaporative heat loss is 2.45 J mg⁻¹ H₂O, whereas the associated

heat loss from the elimination of urine is the amount of heat required to warm the urine, which is less than 1% of the evaporative heat loss. Therefore, even in the very unlikely case that all the water produced is evaporated, the associated heat loss will only account for 24% ($=2.45\text{ J}/10.2\text{ J}$) of the metabolic heat gain.

Radiative heat exchange ranges from huge heat gains in direct sunlight to slight heat losses in shadow. Solar radiation under clear skies often exceeds 1000 W m^{-2} on a plane perpendicular to the solar beam (Coulson, 1975) or approximately 50 mW for the bee, assuming a body surface area of 0.5 cm^2 (Roberts and Harrison, 1999), a value close to its maximum metabolic rate (Balderrama et al., 1992). In shadow, radiative heat losses are similar in magnitude to the convective heat losses in still air and they are also dependent on the difference between the body temperature and the air temperature. The active avoidance of foraging on shaded flowers is an important mechanism of thermoregulation, especially at low temperatures.

Another way to retain heat may be a reduction in the convection coefficient, i.e. the constant of proportionality between convective heat losses and the temperature difference. Such a reduction could be achieved by reducing the flight time. Because of its dependence on wind speed, the convective coefficient is greater during flight than when the bee is not flying. At T_a values lower than 15°C , a reduction in convective heat loss might be achieved by intermittent warm-up following intermittent flight (Heinrich, 1979). At these low temperatures, the maximal metabolic power cannot balance the convective losses (Harrison et al., 1996). Whether the bee can also modulate the convection coefficient by altering its wingbeat kinematics is not clear. It might be possible if such changes were to generate differences in the velocity of the air layer surrounding the thorax.

Mechanisms of regulation of metabolic rate

The indirect flight muscles are responsible for most of the energy consumed during flight, as suggested by their size (Snodgrass, 1956) and the elevated temperature of the thorax (Heinrich, 1979; Schmaranzer and Stabentheiner, 1988). In a non-flying bee, the indirect flight muscles are activated during thermogenesis (Esch et al., 1991); but during flying, they have dual role, thermogenesis and flight. Therefore, changes observed in energy consumption during flight should be related to both these roles.

Roberts and Harrison (Roberts and Harrison, 1999) proposed three mechanisms for the observed reduction in metabolic power output with increasing T_a : (i) a decrease in the mechanical power output; (ii) an increase in the efficiency of heat conversion; and (iii) inhibitory effects of high T_{th} on flight muscle performance.

Decreases in the mechanical power requirements might be achieved by decreasing wingbeat frequency and/or stroke amplitude or by altering the frequency of energetically demanding flight behaviors such take-off, turning, acceleration and landing. The first possibility is supported by observations

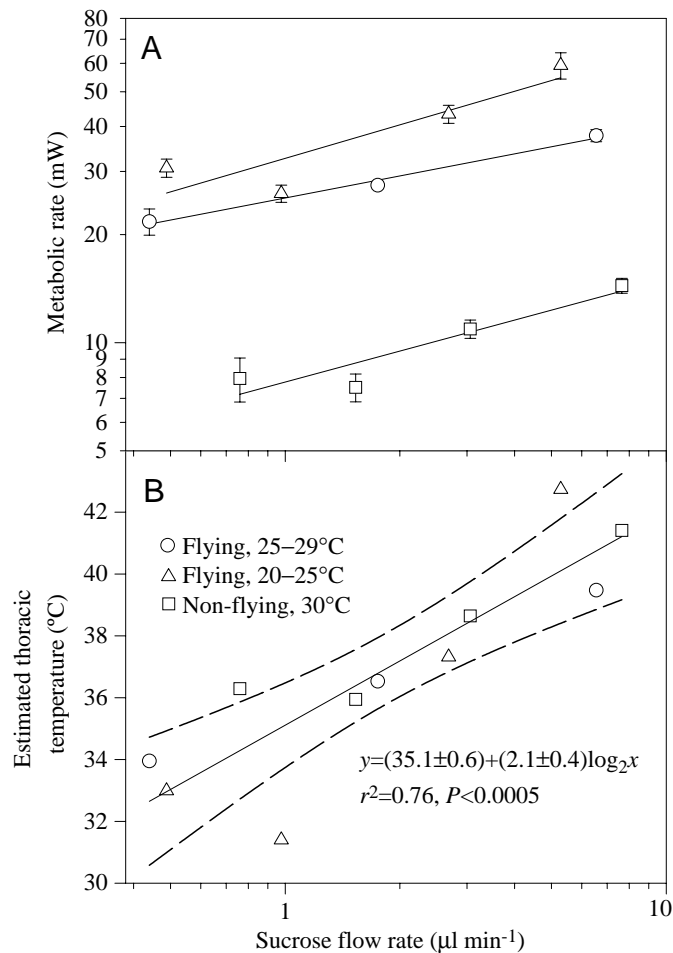


Fig. 4. Metabolic rates (A) and estimated thoracic temperatures (T_{th}) (B) as a function of the flow rate of 50% (w/w) sucrose solution. Each series corresponds to a different experimental condition: open circles, flying at $25\text{--}29^\circ\text{C}$ (present data); open triangles, flying at $20\text{--}25^\circ\text{C}$ (Balderrama et al., 1992); open squares, non-flying at 30°C (Moffatt and Núñez, 1997). Note the logarithmic scales for sucrose flow rate and metabolic rate, but the linear scale for T_{th} . Values are means \pm S.E.M., $N=13\text{--}132$. T_{th} values were estimated from the estimated thermal conductance (C), the measured metabolic rate (MR) and the T_a values using the Newton cooling equation [$MR=C(T_{th}-T_a)$]. Dashed curves enclose the 95% confidence limits of the least-squares linear regression.

indicating a decrease in the wingbeat frequency with increasing T_a (Spangler, 1992; Harrison et al., 1996). However, this mechanism is limited by the fact that the bees must still generate enough lift to remain airborne. The second possibility might be supported by changes in the inspection rate or in the sucrose load carried; however, no such changes were found in present study.

Approximately 4% of the metabolic rate is converted to mechanical work during flight (Roberts and Harrison, 1999). This fraction is estimated to be constant. It is possible, however, that the bee could alter the efficiency of the conversion of energy input to mechanical work. Such changes

could explain how the bee could halve its metabolic output and still generate enough lift for flight.

An inhibitory effect of high temperatures might explain the results obtained during 'agitated' flight by Roberts and Harrison (Roberts and Harrison, 1999). Thoracic temperatures in those conditions were within the range where the mechanical power output decreases with increasing T_{th} (Coelho, 1991). This inhibitory effect, however, cannot explain the present results.

In the present study, the bees spent less than half their time flying, so I cannot exclude the possibility that they increased their metabolic output by increasing shivering thermogenesis during the periods when they did not fly. Further experiments are needed to clarify this point.

Metabolic rate and reward

Little attention has been paid to the possibility that the metabolic rate increases with the reward rate obtained at the food source. To my knowledge, the honeybee is the only animal in which this possibility has been investigated experimentally. Carbon dioxide production (Balderrama et al., 1992; Moffatt, 2000), oxygen consumption (Moffatt and Núñez, 1997) and thoracic temperature, determined both by telethermography (Schmaranzer and Stabentheiner, 1988) and by inserting a thermoprobe (Waddington, 1990), have all been found to increase with the reward rate offered at the food source. Is this increase a direct consequence of the perception of the increased reward rate or a mere byproduct of the foraging dynamics at greater reward rates? In particular, the increase might be a consequence of carrying an increased load. This possibility was recently analyzed for non-flight foraging (Moffatt and Núñez, 1997) and for the same conditions as in the present study (Moffatt, 2000). It was found that for the same loads the metabolic rate increased with the reward rate, indicating a direct effect of the reward.

Thoracic temperature as a function of the sucrose solution flow rate

There are no data available on the relationship between T_{th} and the reward rate. However, although not as reliable as actual measurements, estimates of T_{th} at different flow rates are valuable. Fig. 4A summarizes the available data on the relationship between sucrose solution flow rate and metabolic rate. Data from different experimental situations are shown: flying at 25–29 °C (present study), flying at 20–25 °C (Balderrama et al., 1992) and foraging on a single food source without flying (Moffatt and Núñez, 1997). Metabolic rate depended both on the experimental situation and on the reward rate. T_{th} was estimated using thermal conductance values of 2.9 mW °C⁻¹ for foraging flight (Roberts and Harrison, 1999) and 1.24 mW °C⁻¹ for non-flight foraging (Goller and Esch, 1991). Evaporative heat losses were estimated from the results of Roberts and Harrison (Roberts and Harrison, 1999). The estimated thoracic temperature shows a clear linear increase with the logarithm of the reward rate, with appreciable agreement among the different experimental situations

(Fig. 4B). The estimated increase in T_{th} for a doubling in flow rate is 2.1 °C (Fig. 4B), which is similar to that measured after doubling the sucrose concentration (1.5 °C; Schmaranzer and Stabentheiner, 1988).

Although there is no direct proof that the bee attained different T_{th} values at the different reward rates, several lines of evidence suggest that this is the case: (i) T_{th} of foraging bees arriving, drinking and taking off from a pneumatic feeder depends on the sucrose concentration offered (Schmaranzer and Stabentheiner, 1988); (ii) T_{th} of foraging bees dancing at the hive has a similar dependence (Stabentheiner and Haggmüller, 1991; Stabentheiner et al., 1995; Stabentheiner, 1996); (iii) metabolic rate increases with reward rate (Balderrama et al., 1992; Moffatt and Núñez, 1997; Moffatt, 2000); and (iv) the disparity among the metabolic rates obtained in different experimental conditions (Fig. 4A) is explained by differences in the thermal conductance and air temperature and disappears if the corresponding T_{th} values are calculated (Fig. 4B).

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