MECHANISMS OF THERMAL BALANCE IN FLYING *CENTRIS PALLIDA* (HYMENOPTERA: ANTHOPHORIDAE)

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Summary

Thermoregulation of the thorax is critical for bees and other endothermic insects to achieve high rates of flight muscle power production. However, the mechanisms allowing insects to regulate thorax temperatures during flight are not well understood. To test whether variations in metabolic heat production, evaporation or heat transfer from the thorax to the abdomen contribute to the maintenance of stable body temperatures during flight in the bee *Centris pallida*, we measured CO_2 production, water vapor loss, wingbeat frequency and body segment temperatures during flight at varying air temperatures (T_a). While hovering in the field and while flying in the respirometer, *C. pallida* males maintain extremely stable, elevated thorax temperatures (45±2 °C; mean ± S.E.M.). Measurements of head, thorax and abdomen temperatures as a function of T_a during hovering flight in the field indicated that *C. pallida* males were not actively increasing heat transfer from the thorax to the head or abdomen at high T_a values. As T_a increased from 26 to 35 °C, increases in evaporative water loss were relatively small compared with the decrease in carbon dioxide emission. As T_a values increased from 26 to 35 °C, the factorial decreases in metabolic heat production and the elevation of thorax temperature above T_a were closely matched (35 %), suggesting that variation in metabolic heat production is the major mechanism of thermoregulation in flying *C. pallida*. The thermal effects on rates of water loss and metabolic water production resulted in a strong positive water balance at cooler T_a values, but a strong negative water balance at T_a values above 31 °C. During the first minute of flight in the respirometry chamber, wingbeat frequency was independent of T_a. However, by the fourth minute, there was a significant negative relationship between T_a and wingbeat frequency, which was similar to the thermal relationship observed for wingbeat frequency in the field. These data suggest that, either through homeostatic regulation or resulting secondarily from thermal effects on flight motor properties, variation in metabolic heat production may occur *via* altered wingbeat kinematics.

Key words: flight energetics, thermoregulation, water loss, bee, *Centris pallida*.

Introduction

For many insects, regulation of thoracic temperatures within narrow limits is critical for optimal flight muscle performance (Coelho, 1991; Heinrich, 1993; Marden, 1995). However, maintaining thoracic temperatures (T_{th}) within these limits poses a serious challenge for flying insects that are active across widely varying thermal conditions. This is especially true for bees (Hymenoptera: Apoidea) living in desert environments, where high air temperatures (T_a) and intense solar radiation combined with elevated rates of metabolism and ventilation increase the risk of overheating as well as desiccation. Although endothermy and thermoregulation in bees have been studied for decades (see reviews by Heinrich, 1981, 1993), the physiological mechanisms by which bees and other insects thermoregulate during flight remain unclear. In the present study, we examine potential thermoregulatory mechanisms

during flight in the anthophorid bee *Centris pallida*, which inhabits the deserts of the southwestern United States.

During flight, T_{th} is a function of the metabolic heat produced in the flight muscles and heat exchange between the insect and environment, which occurs by evaporation, radiation and convection. In recent reviews, regulation of T_{th} in flying bees has been attributed to variation in evaporative or convective heat loss (Casey, 1989; Heinrich, 1993; Heinrich and Esch, 1994). Variation in metabolic heat production for thermoregulatory purposes has been largely discounted on the basis of the assumption that mechanical power output and metabolic rates during flight are determined solely by aerodynamic requirements (Casey, 1989; Heinrich, 1993; Heinrich and Esch, 1994). These conclusions are drawn primarily from studies showing that flying bumblebees

(Heinrich, 1975) and sphinx moths (Heinrich, 1971*a*; Casey, 1976) do not vary metabolic rate with T_a. Instead, these insects regulate T_{th} by transferring variable amounts of the heat generated in the thorax to the abdomen, where it is lost *via* convection (Heinrich, 1971*b*, 1976).

Variation in evaporative heat loss has been considered the primary mechanism of T_{th} regulation in flying honeybee (*Apis mellifera*) workers on the basis of the observations that recently fed honeybees regurgitate when their heads are heated to 40–45 °C (Esch, 1976; Heinrich, 1980*a,b*; Louw and Hadley, 1985) and that the fraction of foragers carrying nectar droplets externally in their mandibles upon returning to the hive increases at high T_a values (Cooper *et al.* 1985). However, only one study to date (Nicolson and Louw, 1982) has measured evaporative water loss as a function of T_a in freely flying bees. In their study of the carpenter bee *Xylocopa capitata*, the authors concluded that evaporation does not contribute significantly to thermoregulation, since the increase in water loss with T_a was linearly related to vapor pressure deficit and the T_{th} values of tethered, flying *X. capitata* at low and high humidities did not differ.

Recent studies by Harrison *et al.* (1996*a,b*) have demonstrated that flying honeybees significantly reduce metabolic rate and wingbeat frequency (WBF) as T_a increases, supporting the hypothesis that, for some bees, variation in metabolic heat production may contribute to thermoregulation during flight. An alternative hypothesis that the decrease in metabolic rate and WBF with increasing T_a is a response to thermally dependent aerodynamic requirements is poorly supported, since air density decreases and kinematic viscosity increases as T_a rises (Lide, 1991), thus predicting a slight increase in aerodynamic power requirements (Ellington, 1984). During hovering flight, changes in metabolic heat production may be accomplished by varying mechanical power production and/or mechanochemical efficiency. Changes in power output can be mediated by varying a number of kinematic variables, including WBF, stroke amplitude, body angle and stroke plane angle (Ellington, 1984). Wingbeat frequency is an especially important kinematic variable affecting power output, since power output is approximately proportional to the cube of WBF (Ellington, 1984; Dickinson and Lighton, 1995) when other kinematic variables are held constant.

In this investigation, we measured thermal variation in CO_2 production, water vapor loss and WBF of *C. pallida* males flying in a respirometry chamber. We also measured body segment temperatures as a function of T_a for *C. pallida* males flying in the field. These data allowed us to address three potential mechanisms of thermoregulation during flight in *C. pallida*: intersegmental heat transfer, varying evaporative heat loss and varying metabolic heat production. We chose *C. pallida* males as a model system because of their exceptional thermoregulatory ability; males effectively regulate T_{th} at 44–46 °C between T_a values of 25 and 40 °C (Chappell, 1984). For a few weeks between April and June in the Sonoran Desert, *C. pallida* males, in search of virgin females, aggregate at nest-emergence sites and engage in near-continuous hovering flight

from sunrise into the midday hours. At the nest-emergence site, males either patrol just above the ground in search of emergent females or hover at nearby shrubs and trees, awaiting virgin females that have eluded patrolling males (Alcock *et al.* 1977). Hovering territorial males decrease WBF by approximately 20 % (from 210 to 170 Hz) as T_a increases from 21 to 35 °C (Roberts and Harrison, 1998), suggesting that variation in metabolic heat production may be an important mechanism contributing to thermostability in *C. pallida*, as is the case for *A. mellifera* (Harrison *et al.* 1996*a,b*).

Materials and methods

Body temperatures in the field: analysis of inter-segmental heat transfer

Thorax, head and abdomen temperatures (T_{th}, T_h and T_{ab}, respectively) were measured for *Centris pallida* males hovering at a nest aggregation site near Apache Junction, AZ, USA, between 08:00 and 13:00 h on 20–25 April 1994. Individual bees were netted and quickly restrained by gently flattening the net on a piece of low-density foam. Within 10 s, temperature measurements were taken with a Physitemp MT 29/1B microprobe thermocouple (diameter 0.33 mm, time constant 0.025 s) connected to a Physitemp BAT-12 thermometer. Shade T_a was then measured at the location where the flying bee was netted. The bees were placed individually in small vials and frozen over dry ice. They were then returned to the laboratory and weighed to the nearest 0.0001 g on a Mettler AE240 analytical balance.

We used the measurements of T_{th}, T_h and T_{ab} immediately following flight in the field to test the hypothesis that *C. pallida* males thermoregulate T_{th} *via* differential transfer of thoracic heat to the head or abdomen. Variation in heat transfer between the thorax and head or abdomen was analyzed according to a model detailed by Baird (1986), May (1991, 1995*a*) and Stavenga *et al.* (1993). The model assumes (a) that heat production occurs only in the thorax, (b) that heat transfer between the thorax and the head or abdomen is driven by the temperature differential between the thorax and the head or abdomen, (c) that heat transfer between each body segment and the environment is driven by the temperature differential between the body segment and the environment and (d) that heat transfer coefficients of conductance, convection and radiation of the integument do not vary with T_a.

Variation in the heat transfer between the thorax and head or abdomen was inferred from the ratio of the head or abdominal temperature excess (T_h-T_a or $T_{ab}-T_a$, respectively) to the thoracic temperature excess ($T_{th}-T_a$). If heat transfer from the thorax to the abdomen does not vary with T_a, then the abdominal temperature excess ratio (R_{ab}):

$$R_{ab} = (T_{ab} - T_a)/(T_{th} - T_a) \qquad (1)$$

should be independent of T_a. Similarly, if heat transfer from the thorax to the head does not vary with T_a, then the head temperature excess ratio (R_h):

$$R_h = (T_h - T_a)/(T_{th} - T_a) \qquad (2)$$

should be independent of T_a. However, if heat transfer from the thorax to the head or abdomen is increased at high T_a, as would be expected if the head or abdomen were being used to dissipate excess heat generated in the thorax, then R_h or R_{ab} should increase with T_a. Conversely, if heat transfer from the thorax to the head or abdomen is increased at low T_a values, as would be expected if T_h or T_{ab} were being regulated at a value above T_a, then R_h or R_{ab} should decrease with T_a.

Flight performance during respirometry: flight duration and wingbeat frequency

Wing movements were recorded acoustically throughout each respirometry trial with a Realistic SCP-29 cassette recorder and a small microphone cemented into the lid of the respirometry chamber (see below). The acoustically recorded wing movements were then digitized and visualized using the SoundEdit (Farallon Computing Inc., Emeryville, CA, USA) sound-analysis program for Macintosh. For each bee, a mean WBF was determined for each minute of the respirometry trial from eight wingbeat sequences selected from the first 20 s of each minute. Each sequence contained 6–10 clearly distinguishable, uninterrupted wingbeats. WBF was determined to the nearest 0.02 Hz by dividing the number of wingbeats in the sequence by the duration of the sequence (measured to the nearest 0.0001 s).

Bees in chambers often exhibit frequent, brief periods of non-flight behavior. In many endothermic bees, including *C. pallida* (Chappell, 1984), metabolic rate during non-flight activities can be extremely variable and temperature-dependent. Thus, a knowledge of the percentage of time spent in flight and in non-flight activities during closed-system respirometry trials is necessary for interpreting thermal variation in metabolic rate and flight performance. Therefore, we quantified flight duration within each minute of the respirometry trial to the nearest 0.1 s by summing the durations of flight bouts, which were identified from the digitized audio recordings as periods when wingbeats were produced.

Respirometric measurement of carbon dioxide and water production

Metabolic and evaporative water loss rates for flying *C. pallida* were determined using closed-system respirometry. Variation in T_a throughout the study (26–35 °C) was a function of varying ambient thermal conditions at the on-site laboratory, an outdoor shaded room. Immediately (<1 min) after individual hovering males were netted in the field, they were taken into the on-site laboratory and placed in a 550 ml glass metabolic chamber, which was then flushed with dry, CO_2-free air for 1 min at 40 l min^{-1}. The chamber was sealed, and bees flew within the chamber for 4 min (with flight behavior maintained by gently shaking the chamber). The flight of *C. pallida* in the chamber was characterized by hovering interrupted by brief periods of non-flight, when bees landed on the chamber floor, and frequent bouts of rapid darting flight towards the walls, which appeared to be escape attempts. A 50 ml gas sample was then taken using a 50 ml glass gas-tight syringe (Scientific Glass

Engineering, Ringwood, Australia) and injected into a dry, CO_2-free, 90 ml min^{-1} airstream drawn sequentially over a Panametrics (Waltham, MA, USA) Al_2O_3 water sensor (for details, see Hadley *et al.* 1982) and an Anarad (Santa Barbara, CA, USA) AR 400 infrared CO_2 analyzer. The CO_2 analyzer, accurate to ±1 p.p.m. in the range 0–5000 p.p.m., was calibrated with a certified span gas and CO_2-free air. The outputs of the CO_2 analyzer and the water sensor were digitized and monitored on-line using a Sable Systems (Las Vegas, NV, USA) data-acquisition system and a PC computer. The water sensor was calibrated with air of known vapor densities, which was generated by a flow of saturated air (at 50 ml min^{-1}) through a large water-jacketed, temperature-controlled glass condensing column. The temperature of the 4:1 ethylene glycol:water mixture pumped through the condensing column jacket was varied using a temperature-controlled circulating water bath. The vapor density of excurrent air from the condensing column was determined from the temperature inside the column and the known relationship between saturation vapor pressure and T_a (Lide, 1991). The water sensor was accurate to ±3.5×10^{-3} mg H_2O l^{-1} in the range 0–5 mg H_2O l^{-1}. The standard curve relating voltage to water vapor density was nonlinear; a third-order polynomial regression provided an r^2 value of 0.995.

The airstream drawn over the CO_2 analyzer and the water sensor was controlled by an Ametek (Pittsburgh, PA, USA) R-1 rotameter-type flow controller that had been calibrated with a soapfilm flowmeter. Initial fractional concentrations of CO_2 ($F_{I CO_2}$) and water vapor density ($V_{I H_2O}$, mg l^{-1}) in the chamber were determined from trials where gas samples were taken from the chamber (containing a bee) immediately after flushing. There were no detectable changes in either CO_2 or water vapor concentration over 4 min for an empty chamber, indicating that there were no significant leaks in the respirometry chamber. For CO_2 and water vapor, signal-to-background ratios were greater than 35:1 and 20:1, respectively. Chamber T_a was monitored continuously throughout each trial with a thermocouple inserted through the lid and connected to a Physitemp BAT-12 thermometer. After the respirometry trials, bees were individually sealed in small vials and frozen over dry ice. They were then returned to the laboratory and weighed as described above.

The mass-specific rate of water vapor loss ($\dot{V}_{H_2O}$) (mg H_2O g^{-1} h^{-1}) was calculated as:

$$\dot{V}_{H_2O} = (L - L_b)(V_{E H_2O} - V_{I H_2O})M_b^{-1}t^{-1}, \qquad (3)$$

where M_b is body mass, t is time in the chamber, L is the volume of the respirometry chamber (l), L_b is the volume of the bee (determined by multiplying the bee's mass by 1.15×10^{-3} l g^{-1}) and $V_{E H_2O}$ is the water vapor density at the end of the trial. Values of $\dot{V}_{H_2O}$ were multiplied by the latent heat of evaporation, 2.45 J mg^{-1} H_2O, to obtain mass-specific rates of evaporative heat loss (reported as mW g^{-1}).

The mass-specific rate of carbon dioxide production ($\dot{V}_{CO_2}$) (ml CO_2 g^{-1} h^{-1}) was calculated as:

$$\dot{V}_{CO_2} = 1000(L - L_b)(F_{E CO_2} - F_{I CO_2})M_b^{-1}t^{-1}, \qquad (4)$$

where F_{ECO_2} is the fractional concentration of CO_2 at the end of the trial. Water vapor production during the trial accounted for less than 0.4 % of the chamber volume and was therefore not included in the calculation of $\dot{V}_{CO_2}$. To obtain rates of metabolic heat production (reported as mW g^{-1}), $\dot{V}_{CO_2}$ was multiplied by the energy yield per amount of CO_2 formed, 21.4 J ml^{-1} CO_2, assuming simple carbohydrate catabolism (Beenakkers *et al.* 1984; Bertsch, 1984; Rothe and Nachtigall, 1989), and the fraction of power input liberated as heat during flight, 0.96. The fraction of power input liberated as heat during flight was estimated using body mass-specific rates of flight metabolism (726 mW g^{-1}; Casey *et al.* 1985) and mechanical power output (28 mW g^{-1}; Dudley, 1995) from male *Euglossa* spp. (Apidae) bees with body mass and WBF similar to those of *C. pallida* males.

Water balance (B, mg H$_2$O g^{-1} h^{-1}) was calculated using the following equation:

$$B = a\dot{V}_{CO_2} - \dot{V}_{H_2O}, \qquad (5)$$

where B is water balance and the multiplier a (0.813 mg H$_2$O ml^{-1} CO_2) of $\dot{V}_{CO_2}$ is the stoichiometric relationship of metabolic water formed per amount of CO_2 produced during the oxidation of hexose sugars (Withers, 1992), which constitute the fuel utilized by bees during flight (Suarez *et al.* 1996).

It was impossible to extract the bee and measure body temperatures in a timely manner after withdrawing and injecting the gas sample. Therefore, to ensure that bees flying in the respirometry chamber were thermoregulating T_{th}, T_{th} values were obtained for a second group of bees flown individually for 4 min in the chamber. After 4 min of flight, the bees were shaken into a small plastic bag and restrained by gently flattening the bag on a piece of low-density foam. Within 10 s, T_{th} was measured as described above.

Cuticular permeability

The effect of temperature on *C. pallida* cuticular water permeability was determined gravimetrically using dead bees. Freshly caught *C. pallida* males were killed by freezing at −5 °C for 1 h, and then sealed at the mouth and anus with paraffin wax. They were then weighed to the nearest 0.0001 g and placed in a stream of dry air (21 min^{-1}) in a temperature-controlled chamber (±0.2 °C). After a 1 h pretreatment to remove any adsorbed water from the cuticle, the bees were reweighed and returned to the chamber. Three hours later, the bees were weighed again. Surface area (S, in cm^2) was estimated from live mass (M_b, in g) using the relationship: $S=12M_b^{0.667}$ (Edney, 1977). Cuticular permeability (μg H$_2$O cm^{-2} h^{-1} mmHg^{-1}; 1 mmHg=0.133 kPa) was determined at 30, 35 and 42 °C ($N=8$ for each treatment).

Values are presented as means ± S.E.M.

Results
Body segment temperatures

Values of T_{th}, T_h and T_{ab} for *C. pallida* males during flight
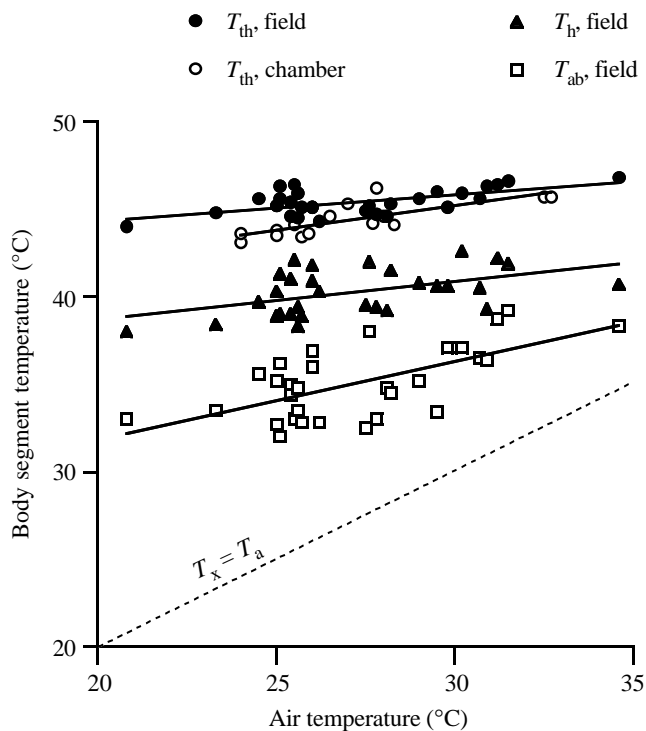

Fig. 1. Effect of air temperature (T_a) on thoracic temperature (T_{th}), head temperature (T_h) and abdominal temperature (T_{ab}) of *Centris pallida* males hovering in the field and the effect of T_a on T_{th} of *C. pallida* males flying in the respirometry chamber. Least-squares linear regressions: field $T_{th}=0.149T_a+41.336$, $N=30$, $r^2=0.37$, $P<0.001$; field $T_h=0.219T_a+34.307$, $N=30$, $r^2=0.24$, $P<0.01$; field $T_{ab}=0.447T_a+22.895$, $N=30$, $r^2=0.41$, $P<0.001$; chamber $T_{th}=0.281T_a+36.768$, $N=15$, $r^2=0.58$, $P<0.001$. The broken line shows $T_a=T_x$, where T_x is segment (head or abdomen) temperature.

in the field are plotted as a function of T_a in Fig. 1. The thoracic temperature was quite stable during flight in the field, with T_{th} values maintained at 45±2 °C (mean ± S.E.M., $N=30$) between T_a values of 21 and 35 °C, and a low, but significant, slope of 0.149 for the regression of T_{th} on T_a. The slope of T_{th} *versus* T_a for bees flying in the respirometry chamber (Fig. 1) was not significantly different from that for bees hovering in the field (analysis of covariance, ANCOVA, $F_{1,41}=3.41$, $P=0.07$). However, the intercept was significantly lower for bees in the chamber (ANCOVA, $F_{1,41}=36.48$, $P<0.001$), probably because bees in the field experienced a much higher short-wave (solar) radiative heat load than bees in the chamber. On average, T_{th} in the respirometry chamber was approximately 0.8 °C lower than T_{th} in the field. The stability of T_h (the slope of the regression of T_h on $T_a=0.219$) for field-netted bees suggests that the head may also be thermoregulated during flight. This conclusion is supported by the significant negative relationship between R_h and T_a (Fig. 2), which indicates an increase in heat transfer from the thorax to the head as T_a decreases. There was no significant correlation between R_{ab} and T_a (Fig. 2), indicating that the abdomen is neither actively thermoregulated nor used to dissipate excess thoracic heat at high T_a values.
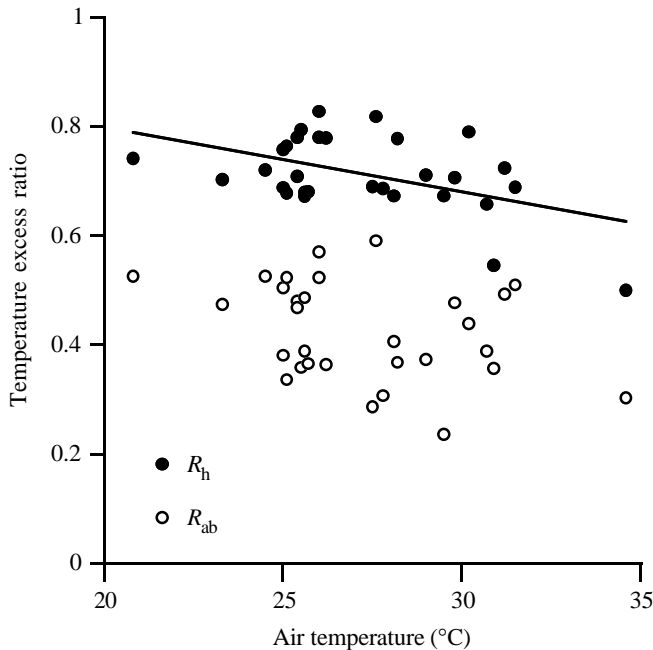
Fig. 2. Effect of air temperature (T_a) on head temperature excess ratio (R_h) and abdomen temperature excess ratio (R_{ab}) of *Centris pallida* males hovering in the field. Least-squares linear regressions: $R_h = -0.012T_a + 1.035$, $r^2 = 0.23$, $P < 0.01$; $R_{ab} = -0.011T_a + 0.714$, $r^2 = 0.11$, $P = 0.07$ (not significant).
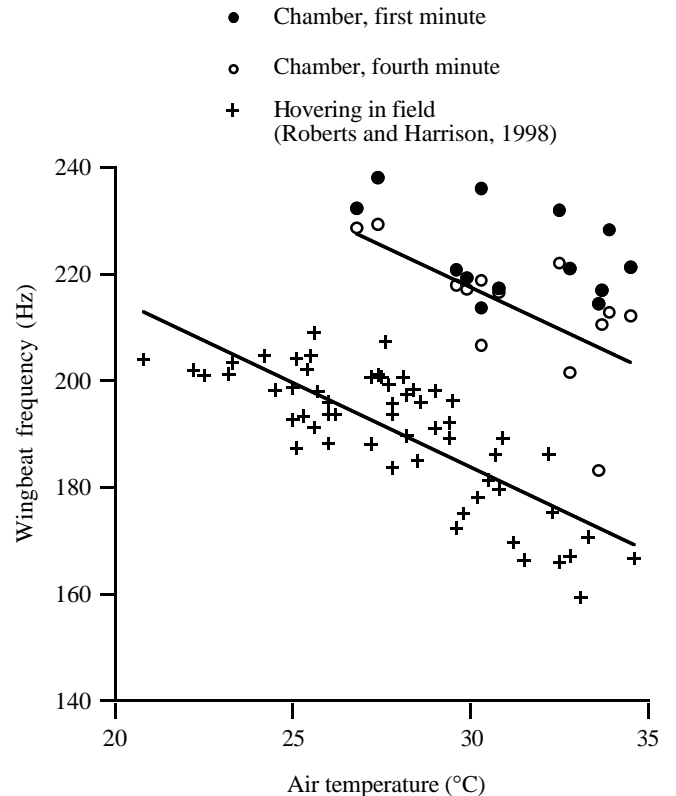

Fig. 3. Effect of air temperature (T_a) on wingbeat frequency (WBF) during the first and fourth minutes of flight by *Centris pallida* in the respirometry chamber. Least-squares linear regressions: $WBF_{1min} = -1.48T_a + 270.27$, $N = 13$, $r^2 = 0.19$, $P = 0.13$ (not significant); $WBF_{4min} = -3.14T_a + 311.72$, $N = 13$, $r^2 = 0.419$, $P = 0.017$. Also shown are data from Roberts and Harrison (1998) for *C. pallida* males hovering in the field.

Flight performance in the respirometer: flight duration and wingbeat frequency

Only the bees that flew for at least 75 % of the respirometry trial (13 out of 30 bees) were included in the analyses of WBF, CO_2 production and water loss, since focusing analyses on this best-performing subset of bees simplified the task of interpreting the observed thermal variation in $\dot{V}_{CO_2}$ (see below). The mean percentage of time spent in flight for these bees was 80.2 ± 1.3 % (mean ± S.E.M., range 75–88 %, $N = 13$) and was not significantly correlated with either T_a ($r^2 = 0.06$) or body mass ($r^2 = 0.01$). The mean body mass for all *C. pallida* males used in this study was 131.4 ± 3.1 mg (range 88–191 mg, $N = 64$), while the mean body mass of the 13 bees used in the analysis of $\dot{V}_{CO_2}$, $\dot{V}_{H_2O}$ and WBF was 146.4 ± 6.5 mg (range 105–191 mg). The percentage of time spent in flight within each minute of the respirometry trial did not vary significantly over the 4 min respirometry period (repeated-measures ANCOVA, $F_{3,33} = 0.39$, $P = 0.76$) and was not significantly affected by T_a (repeated-measures ANCOVA, $F_{1,11} = 0.64$, $P = 0.44$), suggesting that bee motivation did not decline over time in the respirometer or at higher T_a values.

Wingbeat frequency during flight in the respirometry chamber was significantly affected by T_a (repeated-measures ANCOVA, $F_{1,11} = 5.70$, $P = 0.036$), but not by time in the respirometer (repeated-measures ANCOVA, $F_{3,33} = 2.41$, $P = 0.08$). However, there was a significant interaction between time and T_a (repeated-measures ANCOVA, $F_{3,33} = 3.20$, $P < 0.035$), indicating that the relationship between WBF and

T_a was dependent on when WBF was measured during the respirometry trial. During the first minute of the respirometry trials, there was no significant effect of T_a on WBF. However, by the fourth minute of the respirometry trials, there was a significant negative relationship between WBF and T_a (Fig. 3). For individuals that reduced WBF over time (generally bees measured at higher T_a values), there was no clear pattern of temporal variation in WBF. Some bees exhibited a linear decrease in WBF over time, others stabilized WBF after reducing it in the first minute or two of the respirometry trial, and others reduced WBF only during the last minute of the respirometry trial. Wingbeat frequency, averaged across the 4 min respirometry period, decreased significantly as T_a increased (Fig. 4A). The mean WBF during flight in the chamber decreased significantly with increasing body mass ($WBF = -0.215M_b + 250.08$, $N = 13$, $r^2 = 0.33$, $P = 0.039$). There was no significant correlation between body mass and T_a ($r^2 = 0.03$).

Carbon dioxide production, water vapor loss, water balance and cuticular permeability

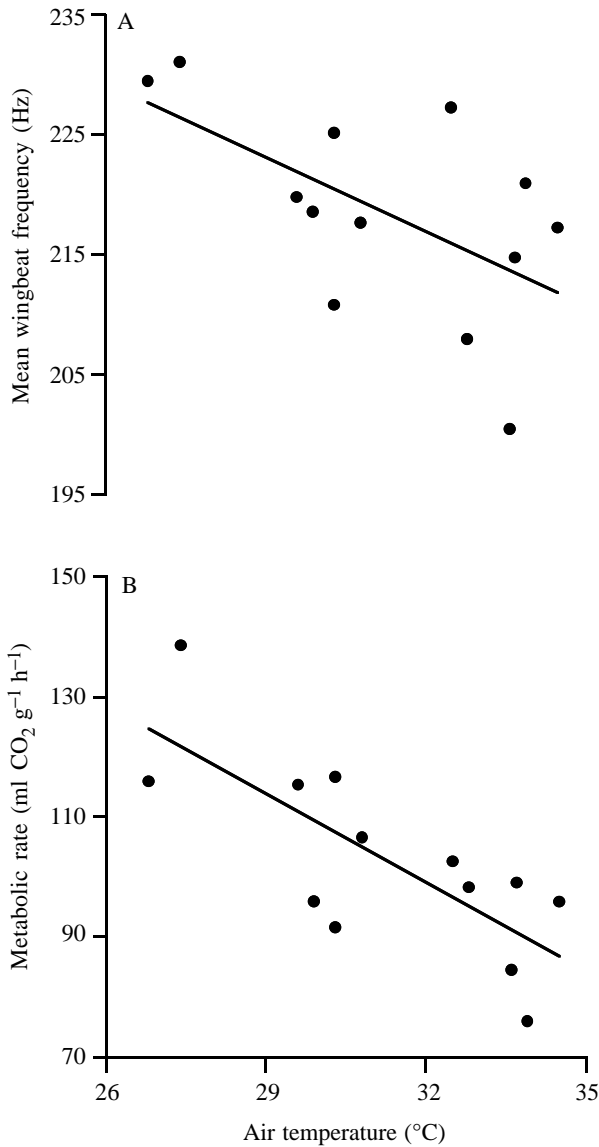Mass-specific rates of CO_2 production ($\dot{V}_{CO_2}$) were

Fig. 4. (A) Wingbeat frequency (WBF) (averaged over the entire respirometry trial) *versus* air temperature (T_a) for flying *Centris pallida* males. Least-squares linear regression: $WBF=-2.06T_a+282.86$, $r^2=0.34$, $P=0.036$. (B) Mass-specific rate of CO_2 production ($\dot{V}_{CO_2}$) *versus* T_a for flying *C. pallida* males. Least-squares linear regression: $\dot{V}_{CO_2}=-4.92T_a+256.44$, $N=13$, $r^2=0.57$, $P<0.001$.
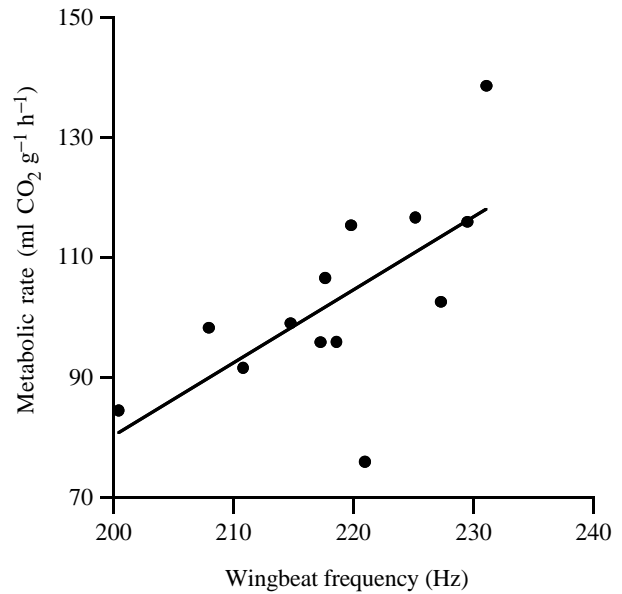

Fig. 5. Mass-specific rate of CO_2 production ($\dot{V}_{CO_2}$) *versus* wingbeat frequency (WBF) for flying *Centris pallida* males. Least-squares linear regression: $\dot{V}_{CO_2}=1.21WBF-162.52$, $N=13$, $r^2=0.43$, $P=0.014$.
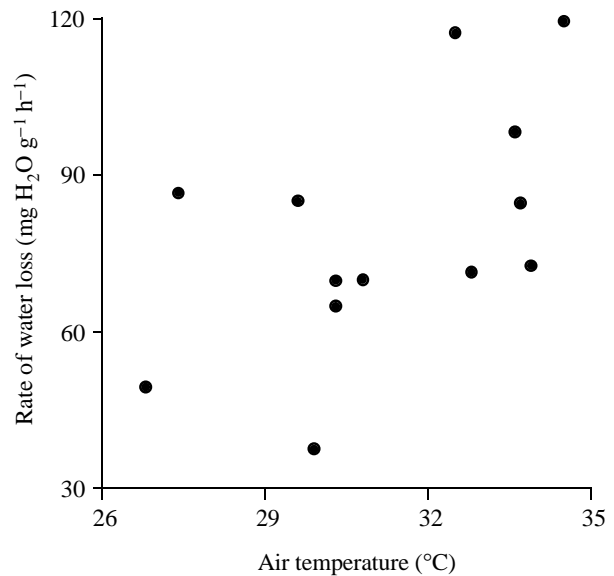

Fig. 6. Mass-specific rate of water loss ($\dot{V}_{H_2O}$) *versus* air temperature (T_a) for flying *Centris pallida* males. Least-squares linear regression: $\dot{V}_{H_2O}=5.18T_a-82.78$, $N=13$, $r^2=0.30$, $P=0.053$ (not significant).

significantly affected by T_a (Fig. 4B), but not by body mass ($r^2=0.14$) or flight duration ($r^2=0.04$). $\dot{V}_{CO_2}$ was positively correlated with mean WBF (Fig. 5). Rates of evaporative water loss ($\dot{V}_{H_2O}$) were not significantly affected by body mass ($r^2=0.01$) or flight duration ($r^2=0.06$), although the relationship between $\dot{V}_{H_2O}$ and T_a (Fig. 6) was nearly significant ($P=0.053$, $r^2=0.30$). When $\dot{V}_{CO_2}$ and $\dot{V}_{H_2O}$ were converted to units of heat flux ($mW\,g^{-1}$), the relationship between metabolic heat production H_P and T_a was:

$$H_P = -28.06T_a+1463.4, \qquad (6)$$

and the relationship between evaporative heat loss H_L and T_a was:

$$H_L = 3.53T_a - 56.34. \qquad (7)$$

Notice that, as T_a increased, the decrease in metabolic heat production was nearly eight times greater than the increase in evaporative heat loss, indicating that, for bees in the respirometry chamber, variation in metabolic heat production was much more important to maintaining stable, elevated T_{th} values than variation in evaporation.
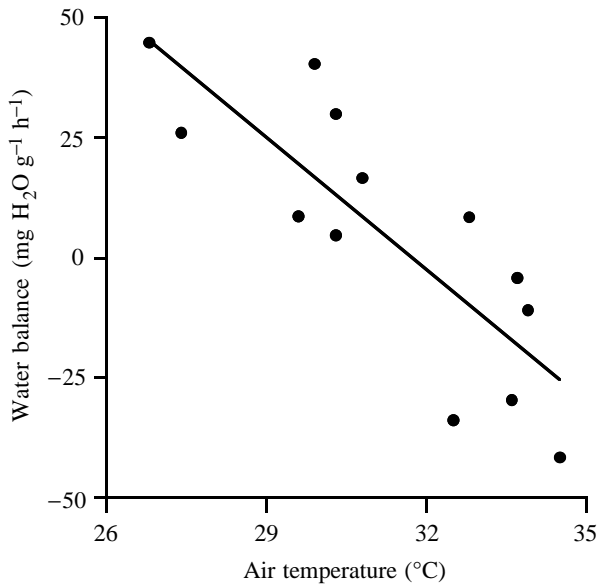
Fig. 7. Water balance *B versus* air temperature (T_a) for flying *Centris pallida* males. Least-squares linear regression: $B=-9.18T_a+291.25$, $N=13$, $r^2=0.67$, $P<0.01$.
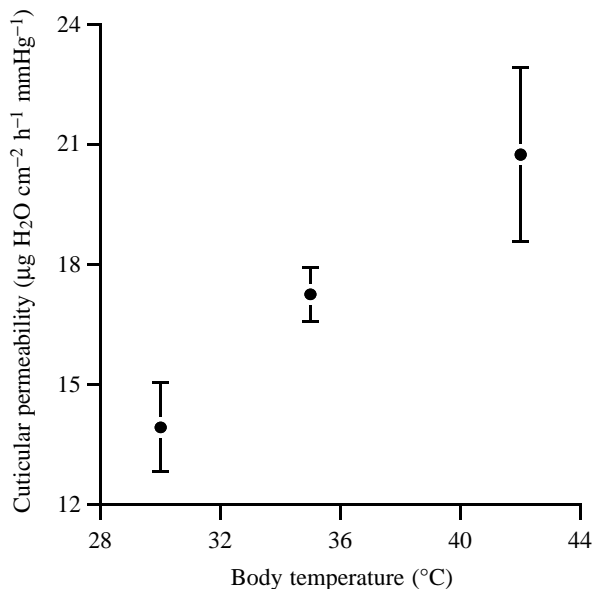

Fig. 8. Cuticular permeability *versus* air temperature (T_a) for freshly killed, mouth-and-anus-sealed *Centris pallida* males. Values are means ± S.E.M., $N=8$. 1 mmHg=0.133 kPa.

Water balance was significantly affected by T_a (Fig. 7) but not by body mass ($r^2=0.03$) or flight duration ($r^2=0.09$). Bees were in positive water balance at T_a values below 31 °C and in negative water balance at higher T_a values. The increase in water vapor loss and the decrease in metabolic water production each account for approximately half of the thermal variation in water balance. There was a significant positive effect of temperature on the cuticular water permeability of

sealed, freshly killed *C. pallida* males (ANOVA, $F_{2,22}=5.42$, $P=0.013$; Fig. 8).

Discussion

Centris pallida males exhibit some of the most precise thoracic thermoregulation observed for flying insects. By what mechanisms do they meet this impressive physiological challenge? Measurements of body segment temperatures in the field indicate that thermoregulation of the thorax by hovering *C. pallida* males is not due to increasing heat transfer from the thorax to the head or abdomen at high T_a values. *Centris pallida* males decrease metabolic rate and tend to increase evaporative water loss as T_a rises. However, the variation in metabolic heat production must be substantially more important to thermoregulation, since (a) the decrease in metabolic rate between T_a values of 26 and 35 °C was almost eight times greater than the increase in evaporative heat loss and (b) the factorial decrease in T_{th} excess ($T_{th}-T_a$) of 0.36 as T_a rose from 26 to 35 °C closely matched the factorial decrease in metabolic heat production (0.35). Because trials were always conducted in shaded conditions and there was minimal forward flight in the chamber, variations in radiative heat gain or convection are unlikely candidates for explaining thermoregulation in the chamber. Thus, variation in metabolic heat production must be the major mechanism of thoracic thermoregulation exhibited by *C. pallida* males flying in the respirometry chamber. However, for the reasons discussed below, there are some uncertainties in extrapolating this conclusion to *C. pallida* flying in the field and to the condition of perfect hovering.

Body temperatures and inter-segmental heat transfer

Even when compared with other large endothermic insects, flying *C. pallida* males are remarkable in their ability to maintain stable, elevated T_{th} and T_h across a wide range of T_a values. In large (>50 mg) bees studied to date (three families, 10 genera, 17 species), T_{th} during flight is usually maintained above 34 °C, with the slope of the regression of T_{th} on T_a typically ranging from 0.2 to 0.6 (reviewed by Roberts and Harrison, 1998). While hovering in the field, male *C. pallida* maintained a T_{th} value of 45–46 °C, with a slope of the regression of T_{th} on T_a of only 0.15. Between T_a values of 21 and 35 °C, male *C. pallida* hovering in the field lowered their T_{th} excess by approximately half, from 24 to 11 °C. The impressive regulation of T_{th} in flying *C. pallida* males is not accomplished by varying heat transfer between the thorax and the head or abdomen, as neither R_h nor R_{ab} is positively related to T_a (Fig. 2).

The significant negative relationship between R_h and T_a and the low slope (0.219) of the regression of T_h on T_a suggest that *C. pallida* males also regulate T_h during flight. If heat transfer between the head and thorax were unregulated and R_h remained constant at the value observed at $T_a=35$ °C, then T_h at $T_a=21$ °C should be 4 °C lower than the observed value of T_h. Regulation of an elevated T_h *via* modulated heat transfer

from the thorax compounds the problem of regulating T_{th} because heat transfer from the thorax to the head is increased as T_a falls. However, the increased heat transfer from the thorax to the head during flight at low T_a values is probably offset by the increase in metabolic heat production within the thorax.

The function of an elevated and well-regulated T_h in *C. pallida* males is not known. However, *C. pallida* males rely on vision to navigate and perceive conspecifics and on olfaction to locate emergent females and to discriminate between mated and virgin females (Alcock *et al.* 1976). Regulation of an elevated T_h may function to increase visual and chemosensory perception as well as to enhance other sensory, endocrine and neuronal processes. Regulation of T_h may be widespread among insects, as a significant negative relationship between R_h and T_a during flight has also been reported for bees in the genus *Xylocopa* (Baird, 1986; Heinrich and Buchmann, 1986) and for the dragonfly *Anax junius* (May, 1995*a*).

Wingbeat frequency

The observation that WBF decreases at higher T_a values during flight in respirometry chambers and during hovering flight in the field (Fig. 3) suggests that flying *C. pallida* males may modulate wingbeat kinematics in order to vary heat production for thermoregulatory purposes. Similarly, a negative relationship between WBF and T_a has been documented within foraging bumblebee individuals (*Bombus pratorum* and *B. pascuorum*; Unwin and Corbet, 1984), in male *C. caesalpinae* patrolling over nest-emergence sites (Spangler and Buchmann, 1991) and in honeybee foragers flying in metabolic chambers (Harrison *et al.* 1996*a,b*) and while entering/exiting colonies (Spangler, 1992). The dragonflies *A. junius* and *Epitheca cynosura* (May, 1995*b*) and the hummingbirds *Amazilia fimbriata* (Berger and Hart, 1972) and *Archilochus colubris* (Chai *et al.* 1997) also reduce WBF with increasing T_a, suggesting that this kinematic response may be widespread among fliers.

Understanding the physiological significance of the WBF *versus* T_a relationship is difficult without additional information. One possibility is that the lower WBFs at high T_a values indicate that hovering *C. pallida* males are lowering metabolic heat production by generating less mechanical power. However, mechanical power output is influenced by several kinematic factors (Ellington, 1984), and mechanical power output could remain independent of T_a *via* compensatory changes in other kinematic variables. For example, during hovering flight in the hummingbird *A. colubris*, wingbeat frequency decreases from 51 to 45 Hz and stroke amplitude increases from 128 to 149 ° as T_a rises from 5 to 25 °C, yielding rates of mechanical power output that are independent of T_a (Chai *et al.* 1997).

Another possible explanation is that the decrease in WBF and metabolic rate with increasing T_a is not an active thermoregulatory response in insects, but instead represents an epiphenomenon that occurs secondarily due to thermal effects on flight motor properties. For instance, small elevations in T_{th}

may increase elastic storage of energy by the cuticle or muscle, requiring muscles to produce less aerodynamic power throughout the wingstroke and allowing the insect to operate at higher efficiencies at higher T_a values. Additionally, elevated T_{th} values may have direct inhibitory effects on the flight muscles. Since the changes in T_{th} with T_a are so small in flying *C. pallida* males (<2 °C over the T_a range studied here), such strong thermal effects on the flight motor seem unlikely. However, this may not be impossible, since strong thermal effects in other endothermic insects can occur at muscle temperatures similar to those of *C. pallida*. For example, vertical force production of tethered honeybees decreases by 45 % as T_{th} values increase from 39 to 45 °C (Coelho, 1991), while vertical force production of tethered dragonflies (*Libellula pulchella*) decreases by approximately 66 % as T_{th} values increase from 43 to 48 °C (Marden, 1995).

Evaporative water loss

For *C. pallida* males flying across a moderate range of T_a values (26–35 °C), variation in evaporative heat loss apparently has only a minor role in the regulation of stable T_{th} values. Although water vapor loss approximately doubled between T_a values of 26 and 35 °C, equating to an elevation in evaporative heat loss of $32 \, mW \, g^{-1}$, this increase was only 12 % of the decrease in metabolic heat production. Evaporative cooling sufficient for thermoregulation under desert conditions requires water loss rates of over 100 % of body water per hour (Louw and Hadley, 1985; Cooper *et al.* 1985; Hadley *et al.* 1989; Hadley, 1994); the hourly water loss of *C. pallida* flying at high T_a values is approximately 10 % of their body water. *Centris pallida* males hover at trees and shrubs, patrol for emergent females and chase conspecific males nearly continuously throughout the morning and early afternoon, foraging only briefly between long bouts of flight (Alcock *et al.* 1976, 1977; Chappell, 1984). Individuals experiencing water loss rates of the magnitude required to thermoregulate solely by evaporative cooling would quickly succumb to dehydration stress.

The cuticular water permeabilities measured for *C. pallida* are low and similar to those of other xeric insects (Hadley, 1994), suggesting that these bees have evolved to minimize cuticular water loss. For bees in the chamber, the mechanism by which water loss tends to increase at high T_a values is unclear. On the basis of the cuticular water permeabilities determined from freshly killed bees, cuticular water loss of a flying *C. pallida* male should approximately double, from 13.0 to $24 \, mg \, H_2O \, g^{-1} \, h^{-1}$ as T_a increases from 26 to 35 °C. Assuming that cuticular permeability is the same in freshly killed and living bees and that total water loss is the sum of respiratory and cuticular water loss, respiratory water loss increases by approximately 81 % over this same thermal range, from 42 to $76 \, mg \, g^{-1} \, h^{-1}$. However, if saturated air is expired through the thoracic spiracles during flight (Bailey, 1954) and ventilatory airflow remains constant with temperature, then the 2 °C increase in T_{th} as T_a rises from 26 to 35 °C should result in only a 14 % increase in expiratory vapor density and

respiratory water loss. Thus, the mechanisms by which water loss varies in flying bees deserve further study.

The strong relationship between water balance and T_a indicates that the thermal environment may greatly influence the water intake and renal function of active bees. At cooler T_a values, *C. pallida* were in positive water balance, requiring either excretion of excess water or risking a decrease in hemolymph osmotic concentration. Copious production of hypo-osmotic urine has been reported for carpenter bees (Nicolson and Louw, 1982; Willmer, 1988; Nicolson, 1990) and bumblebees (Bertsch, 1984) flying at cool T_a values. At high T_a values (above 31 °C), the water balance of flying *C. pallida* became strongly negative, requiring in nature either an increased water intake or the risk of an increase in hemolymph osmotic concentration. Willmer (1986) reported that the desert-inhabiting solitary bee *Chalicodoma sicula* experiences increased hemolymph osmotic concentrations while collecting the sand required for building nest cells, but not while foraging for nectar. Willmer (1986) also reported a positive relationship between water intake and T_a for foraging *C. sicula*. Although the conclusions regarding water balance of *C. pallida* are based upon measurements in dry air, they can reasonably be applied to individuals in the Sonoran Desert, where atmospheric dewpoints regularly fall below −5 °C (S. P. Roberts, personal observation).

Variation in metabolic heat production as a mechanism of thermoregulation in flight

To study how *C. pallida* maintains such stable T_{th} values while flying in the field, we used closed-system respirometry, flying bees for 4 min and measuring $\dot{V}_{CO_2}$ and $\dot{V}_{H_2O}$ over that period. The main advantage of this method is that it allowed us to measure $\dot{V}_{H_2O}$ accurately and thus to compare the relative variation in both metabolic heat production and evaporative heat loss, a topic for which there are minimal data in flying animals. However, when compared with a flow-through respirometry protocol, the temporal resolution provided by closed-system respirometry is poor. Since bees in the chamber were not in continuous flight, engaged in several bouts of darting escape flight and often varied WBF, the poor temporal resolution of the respirometry introduces some uncertainty to the interpretation of the $\dot{V}_{CO_2}$ data. We note that such uncertainties may also apply to previously published studies employing closed-system respirometry to evaluate flight thermoregulation in bees. Also, our impression during the respirometry trials was that the bees were flying nearly continuously; it was only after careful analysis of the audio recordings that it became clear that the wings were not activated for a significant percentage of the time. For this reason, we strongly recommend that future research using closed-system respirometry to evaluate bee flight should incorporate a continuous audio or video record of flight behavior.

For the bees observed in our respirometry trials, the decrease in $\dot{V}_{CO_2}$ as T_a increased from 26 to 35 °C cannot be explained by variation in metabolic rate during non-flight periods, even under very conservative assumptions about thermal variation in non-flight metabolic rate. If metabolic rates during flight and non-flight periods were equal (129 ml CO_2 g^{-1} h^{-1}) at 26 °C and non-flight metabolic rate at 35 °C was equal to the minimum value measured for *C. pallida* males (4 ml CO_2 g^{-1} h^{-1}; Chappell, 1984), then there should have been at most a 19 % decrease in $\dot{V}_{CO_2}$ as T_a increased from 26 to 35 °C (the actual decrease in $\dot{V}_{CO_2}$ was 35 %). However, it is very unlikely that metabolic rates could have fallen as low as 4 ml CO_2 g^{-1} h^{-1} during the non-flight periods at 35 °C, as non-flight periods typically lasted only seconds while the chamber was shaken in order to induce flight. The metabolic rates recorded by Chappell (1984) for active, non-flying *C. pallida* were approximately 50 ml CO_2 g^{-1} h^{-1}. If we take this value as the non-flight metabolic rate at a T_a of 35 °C, then there should have been at most a 12 % decrease in $\dot{V}_{CO_2}$ as T_a increased from 26 to 35 °C. Therefore, assuming that non-flying *C. pallida* males at low T_a values cannot increase their metabolic rates above those during flight, it is mathematically impossible for the variation in non-flight metabolic rate to account for the decrease in $\dot{V}_{CO_2}$ that we observed across T_a. Finally, the fact that there was a significant positive relationship between $\dot{V}_{CO_2}$ and WBF (Fig. 5) argues strongly that the thermal variation in $\dot{V}_{CO_2}$ was due to variation in flight metabolic rate.

Is the decrease in $\dot{V}_{CO_2}$ at high T_a values simply a result of deterioration of flight behavior at high T_a values? This possibility is suggested by the larger decrease in WBF for bees at high T_a values. Two points argue against this interpretation. First, flight duration in the chambers did not vary with T_a. Second, WBF decreases with increasing T_a during hovering in both the laboratory and the field (Fig. 3). However, the fact that WBF in the chamber was higher than WBF in the field indicates that flight behaviors in these settings were not identical, so we cannot completely discount the possibility that flight motivation and performance were thermally dependent.

The observation that the WBFs of bees flying at warmer T_a values did not reach a plateau during the 4 min respirometry trial suggests that flying bees were not in a physiological steady state at high T_a values. To our knowledge, this is the first study to examine how WBF during free flight in a chamber varies with T_a and time over a period of several minutes. Further investigations are necessary to examine this question in more detail. Most useful would be a study using video analysis of all aspects of wing kinematics in combination with flow respirometry of high temporal resolution. The fact that WBFs during respirometry at high T_a values did not reach equilibrium values (with WBFs apparently continuing to decline) suggests that our experimental protocol probably underestimated thermal effects on $\dot{V}_{CO_2}$ and WBF.

In addition to having higher WBFs than bees hovering in the field, bees flying in the chamber frequently exhibited erratic darting flights. Thus, there is some uncertainty regarding the extent to which we can extrapolate our data to perfectly hovering bees. The high WBF and brief bouts of darting, escape behavior indicate that flight in the chamber was more 'intense' than in the field. It is possible that thermal variation

in the frequency of darting flights may have accounted for some of the thermal variation in $\dot{V}_{CO_2}$. Although we have no data on the duration or frequency of darting flights, results from previous studies of forward and 'agitated' flight in other bee species suggest that there may be little energetic difference between hovering and darting flight. In bumblebees, flight speed up to $4.5\,\mathrm{m\,s^{-1}}$ does not affect metabolic rate (Ellington *et al.* 1990), while in honeybees, the metabolic rate during agitated flight (in which 'bees fly rapidly and erratically about the respirometry chamber') and undisturbed hovering flight are very similar (Harrison *et al.* 1996*a*,*b*).

While the ability to vary metabolic rate and heat production has been observed during load-carrying in bumblebees (Heinrich, 1975) and honeybees (Wolf *et al.* 1989), it has not been widely regarded as a possible mechanism of thermoregulation in flying insects (Heinrich, 1993; Heinrich and Esch, 1994), since it is generally believed that the power necessary for flight is solely dependent on aerodynamic requirements (Casey, 1989; Heinrich, 1993; Heinrich and Esch, 1994). Although studies of bumblebees (Heinrich, 1975), carpenter bees (Nicolson and Louw, 1982) and sphinx moths (Heinrich, 1971*a*; Casey, 1976) report independence of metabolic rate from T_a during free flight, our results, as well as recent findings for honeybees (Harrison *et al.* 1996*a*,*b*) and dragonflies (May, 1995*b*), indicate that variation in metabolic heat production can be the major mechanism of thermoregulation during flight in endothermic insects.

References

ALCOCK, J., JONES, C. E. AND BUCHMANN, S. L. (1976). Location before emergence of the female bee, *Centris pallida*, by its male (Hymenoptera: Anthophoridae). *J. Zool., Lond.* **179**, 189–199.

ALCOCK, J., JONES, C. E. AND BUCHMANN, S. L. (1977). Male mating strategies in the bee *Centris pallida* Fox (Hymenoptera: Anthophoridae). *Am. Nat.* **111**, 145–155.

BAILEY, L. (1954). The respiratory currents in the tracheal system of the adult honey-bee. *J. exp. Biol.* **31**, 589–593.

BAIRD, J. M. (1986). A field study of thermoregulation in the carpenter bee, *Xylocopa virginica virginica* (Hymenoptera: Anthophoridae). *Physiol. Zool.* **59**, 157–167.

BEENAKKERS, A. M. T., VAN DER HORST, D. J. AND VAN MARREWWIJK, W. J. A. (1984). Insect flight metabolism. *Insect Biochem.* **14**, 243–260.

BERGER, M. AND HART, J. S. (1972). Die Atmung beim Kolibri *Amazilia fimbriata* wahrend des Schwirrfluges bei verschiedenen Umgebungstemperaturen. *J. comp. Physiol.* **81**, 363–380.

BERTSCH, A. (1984). Foraging in male bumblebees (*Bombus lucorum* L.): Maximizing energy or minimizing water load? *Oecologia* **62**, 325–336.

CASEY, T. M. (1976). Flight energetics of sphinx moths: heat production and heat loss in *Hyles lineata* during free flight. *J. exp. Biol.* **64**, 545–560.

CASEY, T. M. (1989). Oxygen consumption during flight. In *Insect Flight* (ed. G. J. Goldsworthy and C. H. Wheeler), pp. 257–272. Boca Raton, FL: CRC Press.

CASEY, T. M., MAY, M. L. AND MORGAN, K. R. (1985). Flight energetics of euglossine bees in relation to morphology and wingstroke frequency. *J. exp. Biol.* **116**, 271–289.

CHAI, P., CHEN, J. S. C. AND DUDLEY, R. (1997). Transient hovering performance of hummingbirds under conditions of maximal loading. *J. exp. Biol.* **200**, 921–929.

CHAPPELL, M. A. (1984). Temperature regulation and energetics of the solitary bee *Centris pallida* during foraging and intermale mate competition. *Physiol. Zool.* **57**, 215–225.

COELHO, J. R. (1991). The effect of thorax temperature on force production during tethered flight in honeybee (*Apis mellifera*) drones, workers and queens. *Physiol. Zool.* **64**, 823–835.

COOPER, P. D., SCHAFFER, W. M. AND BUCHMANN, S. L. (1985). Temperature regulation of honeybees (*Apis mellifera*) foraging in the Sonoran Desert. *J. exp. Biol.* **114**, 1–15.

DICKINSON, M. H. AND LIGHTON, J. R. B. (1995). Muscle efficiency and elastic storage in the flight motor of *Drosophila*. *Science* **268**, 87–90.

DUDLEY, R. (1995). Extraordinary flight performance of orchid bees (Apidae: Euglossini) hovering in heliox (80 % He/20 % O2). *J. exp. Biol.* **198**, 1065–1070.

EDNEY, E. B. (1977). *Water Balance in Land Arthropods*. Berlin: Springer.

ELLINGTON, C. P. (1984). The aerodynamics of hovering insect flight. *Phil. Trans. R. Soc. Lond. B* **305**, 1–181.

ELLINGTON, C. P., MACHIN, K. E. AND CASEY, T. M. (1990). Oxygen consumption of bumblebees in forward flight. *Nature* **347**, 472–473.

ESCH, H. (1976). Body temperature and flight performance of honey bees in a servomechanically controlled wind tunnel. *J. comp. Physiol.* **109**, 254–277.

HADLEY, N. F. (1994). *Water Relations of Terrestrial Arthropods*. San Diego: Academic Press.

HADLEY, N. F., STUART, J. L. AND QUINLAN, M. C. (1982). An airflow system for measuring total transpiration and cuticular permeability in arthropods: studies on the centipede *Scolependra polymorpha*. *Physiol. Zool.* **55**, 393–404.

HADLEY, N. F., TOOLSON, E. C. AND QUINLAN, M. C. (1989). Regional differences in cuticular permeability in the desert cicada *Diceroprocta apache*: implications for evaporative cooling. *J. exp. Biol.* **141**, 219–230.

HARRISON, J. F., FEWELL, J. H., ROBERTS, S. P. AND HALL, H. G. (1996*a*). Achievement of thermal stability by varying metabolic heat production in flying honeybees. *Science* **274**, 88–90.

HARRISON, J. F., NIELSEN, D. I. AND PAGE, R. E. (1996*b*). Malate dehydrogenase phenotype, temperature and colony effects on flight metabolic rate in the honey-bee, *Apis mellifera*. *Funct. Ecol.* **10**, 81–88.

HEINRICH, B. (1971*a*). Temperature regulation of the sphinx moth,

Manduca sexta. I. Flight energetics and body temperature during free and tethered flight. *J. exp. Biol.* **54**, 141–152.

HEINRICH, B. (1971*b*). Temperature regulation of the sphinx moth, *Manduca sexta*. II. Regulation of heat loss by control of blood circulation. *J. exp. Biol.* **54**, 153–166.

HEINRICH, B. (1975). Thermoregulation in bumblebees. II. Energetics of warm-up and free flight. *J. comp. Physiol.* **96**, 155–166.

HEINRICH, B. (1976). Heat exchange in relation to bloodflow between the thorax and abdomen in bumblebees. *J. exp. Biol.* **64**, 561–585.

HEINRICH, B. (1980*a*). Mechanisms of body temperature regulation in honeybees, *Apis mellifera*. I. Regulation of head temperatures. *J. exp. Biol.* **85**, 61–72.

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

HEINRICH, B. (1981). (ed.) *Insect Thermoregulation*. New York: Wiley.

HEINRICH, B. (1993). *The Hot-blooded Insects*. Cambridge: Harvard University Press.

HEINRICH, B. AND BUCHMANN, S. L. (1986). Thermoregulatory physiology of the carpenter bee, *Xylocopa varipuncta*. *J. comp. Physiol.* **156**, 557–562.

HEINRICH, B. AND ESCH, H. (1994). Thermoregulation in bees. *Am. Sci.* **82**, 164–170.

LIDE, D. R. (1991). (ed.) *Handbook of Chemistry and Physics*. Boca Raton, FL: CRC Press.

LOUW, G. N. AND HADLEY, N. F. (1985). Water economy of the honeybee: A stoichiometric accounting. *J. exp. Zool.* **235**, 147–150.

MARDEN, J. H. (1995). Large-scale changes in thermal sensitivity of flight performance during adult maturation in a dragonfly. *J. exp. Biol.* **198**, 2095–2102.

MAY, M. L. (1991). Thermal adaptations of dragonflies, revisited. *Adv. Odonatol.* **5**, 71–78.

MAY, M. L. (1995*a*). Simultaneous control of head and thoracic temperature by the green darner dragonfly *Anax junius* (Odonata: Aeshnidae). *J. exp. Biol.* **198**, 2373–2384.

MAY, M. L. (1995*b*). Dependence of flight behavior and heat production on air temperature in the green darner dragonfly *Anax junius* (Odonata: Aeshnidae). *J. exp. Biol.* **198**, 2385–2392.

NICOLSON, S. W. (1990). Osmoregulation in a nectar-feeding insect, the carpenter bee *Xylocopa capitata*: water excess and ion conservation. *Physiol. Ent.* **15**, 433–440.

NICOLSON, S. W. AND LOUW, G. N. (1982). Simultaneous measurement of evaporative water loss, oxygen consumption and thoracic temperature during flight in a carpenter bee. *J. exp. Zool.* **222**, 287–296.

ROBERTS, S. P. AND HARRISON, J. F. (1998). Mechanisms of thermoregulation in flying bees. *Am. Zool.* (in press).

ROTHE, U. AND NACHTIGALL, W. (1989). Flight of the honeybee. IV. Respiratory quotients and metabolic rates during sitting, walking and flying. *J. comp. Physiol.* B **158**, 739–749.

SPANGLER, H. G. (1992). The influence of temperature on the wingbeat frequencies of free-flying honey bees, *Apis mellifera* L. (Hymenoptera: Apidae). *Bee Sci.* **2**, 181–186.

SPANGLER, H. G. AND BUCHMANN, S. L. (1991). Effects of temperature on wingbeat frequency in the solitary bee *Centris caesalpinae* (Anthophoridae: Hymenoptera). *J. Kansas Ent. Soc.* **64**, 107–109.

STAVENGA, D. G., SCHWERING, P. B. W. AND TINBERGEN, J. (1993). A three-compartment model describing temperature changes in tethered flying blowflies. *J. exp. Biol.* **185**, 325–333.

SUAREZ, R. K., LIGHTON, J. R. B., JOOS, B., ROBERTS, S. P. AND HARRISON, J. F. (1996). Energy metabolism, enzymatic flux capacities and metabolic flux rates in flying honeybees. *Proc. natn. Acad. Sci. U.S.A.* **93**, 12616–12620.

UNWIN, D. M. AND CORBET, S. A. (1984). Wingbeat frequency, temperature and body size in flies and bees. *Physiol. Ent.* **9**, 115–121.

WILLMER, P. G. (1986). Foraging patterns and water balance: problems of optimization for a xerophilic bee, *Chalicodoma sicula*. *J. Anim. Ecol.* **55**, 941–962.

WILLMER, P. G. (1988). The role of insect water balance in pollination ecology: *Xylocopa* and *Calotropis*. *Oecologia* **76**, 430–438.

WITHERS, P. C. (1992). *Comparative Animal Physiology*. Fort Worth, TX: Saunders College Publishing.

WOLF, T. J., SCHMIDT-HEMPEL, P., ELLINGTON, C. P. AND STEVENSON, R. D. (1989). Physiological correlates of foraging efforts in honeybees: oxygen consumption and nectar load. *Funct. Ecol.* **3**, 417–424.