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 CO2 production, water vapor loss, wingbeat frequency and body segment temperatures during flight at varying air temperatures (Ta). 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 Ta 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 Ta values. As Ta increased from 26 to 35 °C, increases in evaporative water loss were relatively small compared with the decrease in carbon dioxide emission. As Ta values increased from 26 to 35°C, the factorial decreases in metabolic heat production and the elevation of thorax temperature above Ta 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 Ta values, but a strong negative water balance at Ta values above 31 °C. During the first minute of flight in the respirometry chamber, wingbeat frequency was independent of Ta. However, by the fourth minute, there was a significant negative relationship between Ta 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 (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 (Ta) 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, 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 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, 1971a; Casey, 1976) do not vary metabolic rate with $T_{ab}$. 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, 1971b, 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 and 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 largely 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. (1996a, 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 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 thermoregulation in C. pallida, as is the case for A. mellifera (Harrison et al. 1996a, 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 13:00h 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) 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 ($R_{ab}$):

$$R_{ab} = (T_{ab} - T_a)/(T_{th} - T_a)$$  \hspace{1cm} (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 ($R_{h}$):

$$R_h = (T_h - T_a)/(T_{th} - T_a)$$  \hspace{1cm} (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, CO2-free air for 1 min at 401 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, CO2-free, 90 ml min⁻¹ airstream drawn sequentially over a Panametrics (Waltham, MA, USA) Al₂O₃ water sensor (for details, see Hadley et al. 1982) and an Anarad (Santa Barbara, CA, USA) AR 400 infrared CO₂ analyzer. The CO₂ analyzer, accurate to ±1 p.p.m. in the range 0–5000 p.p.m., was calibrated with a certified span gas and CO₂-free air. The outputs of the CO₂ 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 H₂O l⁻¹ in the range 0–5 mg H₂O l⁻¹. The standard curve relating voltage to water vapor density was nonlinear; a third-order polynomial regression provided an r² value of 0.995.

The airstream drawn over the CO₂ 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 CO₂ ($F_{CO₂}$) and water vapor density ($V_{H₂O, mg l⁻¹}$) 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 CO₂ or water vapor concentration over 4 min for an empty chamber, indicating that there were no significant leaks in the respirometry chamber. For CO₂ 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 ($\dot{V}_{H₂O}$) (mg H₂O g⁻¹ h⁻¹) was calculated as:

$$\dot{V}_{H₂O} = (L - L_b)(V_{H₂O} - V_{H₂O})M_b^{-1} I^{-1}, \tag{3}$$

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 g cm⁻³) and $V_{H₂O}$ is the water vapor density at the end of the trial. Values of $V_{H₂O}$ were multiplied by the latent heat of evaporation, 2.451 mg⁻¹ H₂O, to obtain mass-specific rates of evaporative heat loss (reported as mW g⁻¹).

The mass-specific rate of carbon dioxide production ($\dot{V}_{CO₂}$) (ml CO₂ g⁻¹ h⁻¹) was calculated as:

$$\dot{V}_{CO₂} = 1000(L - L_b)(F_{CO₂} - F_{CO₂})M_b^{-1} I^{-1}, \tag{4}$$
where $F_{\text{CO}_2}$ 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 $V_{\text{CO}_2}$. To obtain rates of metabolic heat production (reported as mW g$^{-1}$), $V_{\text{CO}_2}$ was multiplied by the energy yield per amount of CO$_2$ formed, 21.4 J mol$^{-1}$ 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:

$$B = aV_{\text{CO}_2} - \dot{V}_{\text{H}_2\text{O}},$$

where $B$ is water balance and the multiplier $a$ (0.813 mg H$_2$O mol$^{-1}$ CO$_2$) of $V_{\text{CO}_2}$ is the stoichiometric relationship of metabolic water formed per amount of CO$_2$ produced during the oxidation of hexose sugars (Withers, 1989), and the fraction of power input liberated as heat during flight.

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 $-5^\circ$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 ($\pm 0.2^\circ$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 ($M_h$, in g) using the relationship: $S=12M_h^{0.667}$ (Edney, 1977). Cuticular permeability ($\mu$g H$_2$O cm$^{-2}$ h$^{-1}$ mmHg$^{-1}$; 1 mmHg=0.133 kPa) was determined at 30, 35 and 42 $^\circ$C ($N=8$ for each treatment).

Values are presented as means $\pm$ S.E.M.

**Results**

**Body segment temperatures**

Values of $T_{th}$, $T_h$ and $T_{ab}$ for C. pallida males during flight 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$\pm$2 $^\circ$C (mean $\pm$ S.E.M., $N=30$) between $T_a$ values of 21 and 35 $^\circ$C, and a low, but significant, slope of 0.149 for the regression 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 $T_{th}=0.219T_a+34.307$, $N=30$, $r^2=0.24$, $P<0.01$; 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 $T_{th} = T_{th}$, where $T_{th}$ is segment (head or abdomen) temperature.
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, CO₂ production and water loss, since focusing analyses on this best-performing subset of bees simplified the task of interpreting the observed thermal variation in $V_{CO₂}$ (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 $V_{CO₂}$, $V_{H₂O}$ 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).

**Fig. 3.** Effect of air temperature ($T_a$) on wingbeat frequency (WBF) during the first and fourth minutes of flight by $C. pallida$ 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.

$T_a$ 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 CO₂ production ($V_{CO₂}$) were
significantly affected by $T_a$ (Fig. 4B), but not by body mass ($r^2=0.14$) or flight duration ($r^2=0.04$). $\dot{V}_{\text{CO}_2}$ was positively correlated with mean WBF (Fig. 5). Rates of evaporative water loss ($\dot{V}_{\text{H}_2\text{O}}$) were not significantly affected by body mass ($r^2=0.01$) or flight duration ($r^2=0.06$), although the relationship between $\dot{V}_{\text{H}_2\text{O}}$ and $T_a$ (Fig. 6) was nearly significant ($P=0.053$, $r^2=0.30$). When $\dot{V}_{\text{CO}_2}$ and $\dot{V}_{\text{H}_2\text{O}}$ were converted to units of heat flux (mW g$^{-1}$), the relationship between metabolic heat production $H_P$ and $T_a$ was:

$$H_P = -28.06T_a + 1463.4,$$  \hspace{1cm} (6)

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

$$H_L = 3.53T_a - 56.34.$$ \hspace{1cm} (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 $T_{\text{th}}$ values than variation in evaporation.
Thermal balance in flying bees

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 $T_h$ 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 $T_{th}$ 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 $T_h$ because heat transfer from the thorax to the head is increased as $T_h$ 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, 1995a).

**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. 1996a,b) and while entering/exiting colonies (Spangler, 1992). The dragonflies *A. junius* and *Epitheca cyanosura* (May, 1995b) 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 $T_h$ 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 $T_h$ values may have direct inhibitory effects on the flight muscles. Since the changes in $T_h$ 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 $T_h$ values increase from 39 to 45 °C (Coelho, 1991), while vertical force production of tethered dragonflies (*Libellula pulchella*) decreases by approximately 66 % as $T_h$ 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 $T_h$ 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$_2$O 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 $T_h$ 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 $V_{CO_2}$. 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 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. 1996a,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, 1971a; 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. 1996a,b) and dragonflies (May, 1995), indicate that variation in metabolic heat production can be the major mechanism of thermoregulation during flight in endothermic insects.

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References


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