

## RESEARCH ARTICLE

# A desert bee thermoregulates with an abdominal convector during flight

Meredith G. Johnson\*, Jordan R. Glass and Jon F. Harrison

## ABSTRACT

Flying endothermic insects thermoregulate, likely to improve flight performance. Males of the Sonoran Desert bee, *Centris caesalpiniae*, seek females at aggregations beginning at sunrise and cease flight near midday when the air temperature peaks. To identify the thermoregulatory mechanisms for *C. caesalpiniae* males, we measured tagma temperature, wingbeat frequency, water loss rate, metabolic rate and tagma mass of flying bees across shaded air temperatures of 19–38°C. Surface area, wet mass and dry mass declined with air temperature, suggesting that individual bees do not persist for the entire morning. The largest bees may be associated with cool, early mornings because they are best able to warm themselves and/or because they run the risk of overheating in the hot afternoons. Thorax temperature was high (38–45°C) and moderately well regulated, while head and abdomen temperatures were cooler and less controlled. The abdominal temperature excess ratio increased as air temperature rose, indicating active heat transfer from the pubescent thorax to the relatively bare abdomen with warming. Mass-specific metabolic rate increased with time, and air and thorax temperatures, but wingbeat frequency did not vary. Mass-specific water loss rate increased with air temperature, but this was a minor mechanism of thermoregulation. Using a heat budget model, we showed that whole-body convective conductance more than doubled through the morning, providing strong evidence that the primary mechanism of regulating thorax temperature during flight for these bees is increased use of the abdomen as a convector at higher air temperatures.

**KEY WORDS:** Heat budget, Thermal biology, Thermoregulation, Biophysical ecology, Solitary bees

## INTRODUCTION

Differences in organismal thermoregulatory mechanisms and capacities will influence how climate change affects the ecological success of animals. If desert animals are operating near their critical maxima, even a few degrees increase in air temperature may be fatal (McKechnie and Wolf, 2019). Other animals that mostly operate at air temperatures well below their thermal maxima may show increased performance, range expansion and/or higher population numbers when climatic warming occurs (Deutsch et al., 2008). Therefore, understanding how and why thermoregulatory mechanisms vary among species, and how close animals are to critically warm temperatures that cause harm, is of increasing importance in order to

predict which animals will survive as global warming progresses (Buckley and Huey, 2016; Buckley and Kingsolver, 2021). Here, we investigated the mechanisms used for thermoregulation during the flight of the Sonoran Desert bee, *Centris caesalpiniae*.

Maintaining a narrow range of body temperatures across highly variable environmental conditions is thought to enhance the physiological performance of many organisms (Angilletta et al., 2002; Huey and Kingsolver, 1989). Mechanisms of thermoregulation for endotherms include variation in heat production, evaporative heat loss, and radiative and convective heat exchange (Angilletta et al., 2002; Roberts and Harrison, 1998); these mechanisms, and how and why these vary with phylogeny and environment, remain poorly understood (Gilmour and Ellington, 1993; Roberts and Harrison, 1998; Willmer and Stone, 1997). For example, in birds, there are strong phylogenetic effects on the mechanisms used for evaporative cooling, with Columbidae utilizing cuticular transpiration and passerines panting (McKechnie et al., 2021). In a marsupial species, desert animals are less likely to utilize evaporative cooling than their temperate counterparts (Hulbert and Dawson, 1974).

Among the flying endothermic insects, thermoregulatory mechanisms are highly variable, for reasons that are unclear (Table 1). Honey bees have been reported to decrease metabolic heat production and decrease wingbeat frequency as air temperature increases (Glass and Harrison, 2022; Roberts and Harrison, 1999; Roberts et al., 1998), though some dispute this (Heinrich and Esch, 1994; Woods et al., 2005). Similarly, the desert digger bee, *Centris pallida*, hovering orchid bees and dragonflies reduce flight metabolic rate and wing beat frequency as air temperature rises (Borrell and Medeiros, 2004; May, 1976, 1995a; Roberts et al., 1998). In contrast, bumble bees and carpenter bees do not vary flight metabolic rate with air temperature (Heinrich, 1976; Nicolson and Louw, 1982). In the bees that have been examined, evaporative water loss rate increases with air temperature, but it is not always clear when this is active and the extent to which increasing evaporative heat loss contributes to thermoregulation (Kovac et al., 2010; Nicolson and Louw, 1982; Roberts and Harrison, 1999; Roberts et al., 1998).

One of the most important mechanisms of thermoregulation in both vertebrate and invertebrate endotherms is variable perfusion of the skin and appendages (Morrison and Nakamura, 2019). Increasing blood flow to the skin and appendages raises their surface temperature, causing radiative and convective heat loss to increase. This mechanism of thermoregulation is widespread throughout the animal kingdom, and often involves transfer of warm blood from the body core to appendages with large surface area/volume ratios and/or minimal insulation. For example, bird bills are highly vascularized and poorly insulated, allowing for efficient heat dissipation (Tattersall et al., 2017), humans use their hands and feet to regulate body temperature (Taylor et al., 2014), lizards utilize their legs and tail to lose excess heat (Działowski and O'Connor, 1999), and dragonflies can lose heat through their wings (Guillermo-Ferreira and Gorb, 2021).

School of Life Sciences, Arizona State University, 427 East Tyler Mall, Tempe, AZ 85281, USA.

\*Author for correspondence (mgjohn12@asu.edu)

© M.G.J., 0000-0001-6727-088X; J.F.H., 0000-0001-5223-216X

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**Table 1. Thermoregulatory mechanisms of flying endothermic insects**

Species	Mass (mg)	MR	WBF	EWL	$R_{ab}$	$R_h$	Reference
<b>Anthophoridae</b>							
<i>Anthophora plumipes</i>	185	.	.	.	↑	.	Stone, 1993
<i>Centris pallida</i>	131.4	↓	↓	—	—	↓	Roberts et al., 1998; Chappell, 1984
<i>Centris caesalpiniae</i>	335	.	↓	.	.	.	Spangler and Buchmann, 1991
<i>Xylocopa capitata</i>	1245	—	.	↑	.	.	Nicolson and Louw, 1982
<i>Xylocopa californica</i>	587	—	.	.	.	.	Chappell, 1982
<i>Xylocopa varipuncta</i>	673	.	.	.	↑	↑	Heinrich and Buchmann, 1986
<i>Xylocopa virginica</i>	370	.	.	.	↓	↓	Baird, 1986
<b>Apidae</b>							
<i>Apis mellifera</i>	75.3	↓	↓	↑	—	—	Roberts and Harrison, 1999; Cooper et al., 1985; Glass and Harrison, 2022
<i>Apis mellifera</i>	.	—	.	.	—	.	Heinrich, 1980; Stevenson and Woods, 1997
<i>Bombus bimaculatus</i>	.	.	—	.	.	.	Joos et al., 1991
<i>Bombus fervidus</i>	.	.	—	.	.	.	
<i>Bombus vagans</i>	.	.	—	.	.	.	
<i>Bombus pratorum</i>	122	.	↓	.	.	.	Unwin and Corbet, 1984
<i>Bombus prascuorum</i>	131	.	↑	.	.	.	
<i>Bombus vagans</i>	120	.	.	.	—	.	Heinrich, 1972
<i>Bombus vosnesenskii</i>	645	—	.	.	↑	.	Heinrich, 1976
<i>Euglossa imperialis</i>	160.8	↓	↓	↓	↓	↓	Borrell and Medeiros, 2004
<i>Melipona subnitida</i>	47.4	.	.	.	↑	↑	Souza-Junior et al., 2020
<b>Vespidae</b>							
<i>Sphecius grandis</i>	.	.	.	.	—	↓	Coelho et al., 2007
<i>Vespula germanica</i>	.	.	.	.	—	↓	Coelho and Ross, 1996
<i>Vespula maculifrons</i>	.	.	.	.	—	↓	Coelho and Ross, 1996
<b>Diptera</b>							
<i>Chrysomya megacephala</i>	.	.	.	.	↓	↓	Gomes et al., 2018
<i>Sarcophaga subvicina</i>	68	.	.	.	↑	.	Willmer, 1982
<i>Sarcophaga carnaria</i>	73	.	.	.	↑	.	
<b>Lepidoptera</b>							
<i>Hyles lineata</i>	650	—	.	.	↑	.	Casey, 1976
<i>Manduca sexta</i>	2070	—	—	.	↑	↓	Hegel and Casey, 1982; Heinrich and Bartholomew, 1971
<b>Odonata</b>							
<i>Anax junius</i>	1200	↓	↓	.	—	.	May, 1995b
<i>Zenithoptera lanei</i>	.	.	.	.	—	—	Guillermo-Ferreira and Gorb, 2021
<b>Coleoptera</b>							
<i>Scarabaeus sacer</i>	.	.	.	.	—	.	Verdú et al., 2012
<i>Scarabaeus cicatricosus</i>	.	.	.	.	↑	.	

While all of the listed species are endothermic and partially homeothermic, how metabolic rate (MR), wing beat frequency (WBF), evaporative water loss rate (EWL), and abdominal ( $R_{ab}$ ) and head ( $R_h$ ) temperature excess ratios change with air temperature is quite variable. Increasing  $R_{ab}$  or  $R_h$  as air temperature rises is usually considered evidence of use of that tagma as a radiator to increase heat loss. Decreasing  $R_{ab}$  or  $R_h$  as air temperature rises is often considered evidence of evaporation from that tagma. All parameters were evaluated against increasing air temperature: ., no data available; —, no change; ↓, decrease; ↑, increase.

As for other mechanisms of thermoregulation, the reported use of variable blood flow to surfaces for large flying insects is inconsistent (Table 1). In endothermic flying insects, heat is produced primarily in the thorax by the flight muscles. The thorax is often insulated with setae that help maintain elevated flight muscle temperature in cool conditions (Church, 1960). During exposure to exogenous heat while at rest, some insects transfer heat from the thorax to the abdomen, likely using the circulatory system. This mechanism was first documented in bumblebees and hawkmoths (Heinrich, 1970, 1976; Heinrich and Bartholomew, 1971). *Bombus vosnesenskii* queens use a countercurrent heat exchanger in the petiole to prevent heat transfer between the thorax and abdomen under cool conditions, while under warm conditions, they bypass the exchanger to cool the thorax by transferring hot hemolymph to the abdomen (Heinrich, 1976). However, not all endothermic insects appear capable of variable transfer of blood and heat between the thorax and abdomen; for example, there is no evidence for such a mechanism in honey bees (Heinrich, 1980; Roberts and Harrison, 1999). *Centris vittata* females and *Centris lanosa* males have aortic cardiovascular structures similar to those of *Bombus* (Wille, 1958), suggesting that they may be

similar to bumblebees and be able to use their abdomen as a variable radiator.

To determine whether there is active transfer of heat between the thorax and the abdomen or head, Baird (1986) suggested using the temperature excess ratio ( $R_{tagma}$ ):

$$R_{tagma} = \frac{(T_{tagma} - T_{air})}{(T_{thorax} - T_{air})}. \quad (1)$$

$R_{tagma}$  will be constant and independent of air temperature ( $T_{air}$ ) if heat moves from the thorax to the other appendages by passive conduction. If heat is actively transferred from the thorax to the head or abdomen at high  $T_{air}$ , then  $R_{tagma}$  will increase. However,  $R_{tagma}$  is not a perfect indicator of variable transfer of warm blood from the thorax as, if evaporation occurs from a tagma, this will tend to decrease  $R_{tagma}$  (Roberts and Harrison, 1999). Based on changes in abdominal temperature excess ratio ( $R_{ab}$ ) with temperature, use of an abdominal radiator to thermoregulate as air temperature rises is a common but not universal mechanism in endothermic flying insects (Table 1).  $R_{ab}$  increased with air temperature in *Xylocopa californica*, *Xylocopa varipuncta*, *Manduca sexta*, *Bombus*

*vosnesenskii* queens, *Bombus vagans*, *Anthophora plumipes* and some orchid bees (Chappell, 1982; Hegel and Casey, 1982; Heinrich, 1972, 1976; Heinrich and Buchmann, 1986; Willmer and Stone, 1997), but not for *C. pallida* small morph males, or honey bee workers or drones (Coelho, 1991; Roberts et al., 1998).

While use of the temperature excess ratio allows one to determine whether variable heat transfer from the thorax to the abdomen or head is likely occurring, it does not allow for a quantitative analysis of the importance of variable heat transfer to a surface in terms of overall heat exchange. A true quantitative determination requires a heat budget model. Each parameter in a heat budget is converted to  $W$ , or  $W\ g^{-1}$ , which quantifies a real rate of energy transfer between an organism and its environment. To date, heat budget models have only been estimated for honey bees and moths among the flying endothermic insects (Cooper et al., 1985; Hegel and Casey, 1982; Roberts and Harrison, 1999; Stupski and Schilder, 2021), and none of these have attempted to quantitatively address the role of variable heat transfer from the thorax to abdomen. A further quantitative analysis of the importance of the use of variable heat transfer to body surfaces can be performed by calculating the convective conductance ( $\kappa$ ), a measure of the convective heat transfer divided by the body surface area and the temperature gradient that drives convection. If endothermic insects transfer warm blood from an insulated thorax to a relatively less insulated abdomen, we would expect to observe an increase in the convective conductance as a result of the reduction in average insulation. Calculation of  $\kappa$  allows a quantitative estimate of the change in capacity to lose heat by convection as air temperature warms, independent of the thermal gradient.

*Centris caesalpiniae* are bees of the southwestern USA deserts (Ascher and Pickering, 2020). Like many other desert solitary bees, they spend most of the year in underground burrows, emerging to mate, forage and reproduce over 1–2 months most years. Male and female *C. caesalpiniae* emerge from brood cells located 8–25 cm underground around *Larrea tridentata* (desert creosote bush). Males then search for unmated females, flying across a broad range of air temperatures, from 18 to nearly 40°C (Rozen and Buchmann, 1990; Spangler and Buchmann, 1991). There are at least two male morphs of *C. caesalpiniae*, a large morph that typically flies near the ground and a smaller morph that mostly flies near the tops of bushes (Spangler and Buchmann, 1991). The relative mating success of the two morphs has not been studied in *C. caesalpiniae*. In *C. pallida*, large morph males usually are more successful at capturing emerging females, while small morph males are more likely to mate with females that evade the large males (Alcock et al., 1977).

Although there are no prior studies of thermoregulation in *C. caesalpiniae*, we know that *C. pallida* males thermoregulate in flight, with slopes of thorax temperature plotted against air temperature of 0.15–0.3 (Chappell, 1984; Roberts et al., 1998). *Centris pallida* small morph males thermoregulate primarily by reducing metabolic heat production and wingbeat frequency as air temperatures increase (Roberts et al., 1998). There have been no prior studies of thermoregulation in large morphs of *C. caesalpiniae*, but large morph males showed a negative correlation of wingbeat frequency and air temperature in the field, suggesting that they may also vary metabolic heat production with air temperature (Spangler and Buchmann, 1991). In this study, we asked two questions. (1) Do *C. caesalpiniae* males thermoregulate during flight? (2) If so, how? We measured tagna temperature, metabolic rate, water loss rate and wingbeat frequency of large morph *C. caesalpiniae* males through the morning activity period at

an aggregation site. We calculated a heat budget and convective conductance to quantitatively assess the relative importance of heat loss/gain pathways.

## MATERIALS AND METHODS

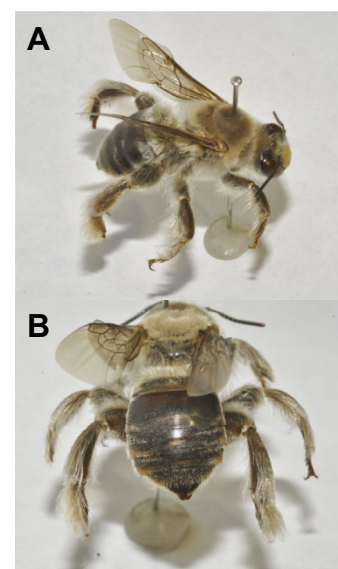
### Animals

We located an active mating aggregation of *Centris caesalpiniae* Cockerell at a rural property in Scottsdale, AZ, USA (GPS coordinates: 33.727, −111.799). Large morph bees were active early in the morning, from sunrise to around midday. These bees have long, tan fur on the thorax, a relatively hairless dorsal abdomen and large hindlegs (Fig. 1). We distinguished small morph males by their dark, hairless abdomen and behaviorally by flight above the creosote bushes. Large morphs, in contrast, are typically found crawling, digging or flying near female nest entrances. We caught large morph males in flight during late June and early July 2020, using sweep nets. This study focused on the thermoregulatory mechanisms of the large morph males because of their abundance and ease of capture.

### Carbon dioxide and water vapor emission measurements

To determine the metabolic and water loss rates of *C. caesalpiniae* large morph males during flight, we used flow-through respirometry. We stationed the set up underneath an outdoor, shaded porch less than 20 m from the locations where bees were captured to measure bees in conditions as close as possible to their natural, ambient conditions. Shaded air temperatures ranged from 19 to 38°C across and within 4 days of measurements.

A SS-4 Sub-Sampler Pump (Sable Systems, Las Vegas, NV, USA) pumped ambient air at 1000 ml min<sup>−1</sup> through a 1000 ml column of silica gel, then a 1000 ml combined column of Drierite and Ascarite II to flush the 500 ml glass metabolic chamber with dry, CO<sub>2</sub>-free air. Output of the chamber was directed to the sample cell of a LI-7000 CO<sub>2</sub>/H<sub>2</sub>O Gas Analyzer with the reference cell maintained at zero CO<sub>2</sub> by circulation through a scrubbing column (LI-COR, Lincoln, NE, USA). We recorded a baseline measurement, without an animal, for at least 1 min. After



**Fig. 1. *Centris caesalpiniae* large male morph.** (A) The large morph male has light, densely packed setae on the thorax and a relatively hairless, and darker colored, dorsal abdomen, with long silver hairs on the ventral abdomen and legs. (B) Posterior view of the large morph male abdomen.



introducing the bee into the chamber, we covered the chamber with a dark cloth and allowed the system to flush for another 3 min to eliminate all vestiges of outside air. After removing the cloth, we stimulated the bees to fly by gently tilting the chamber, and recorded CO<sub>2</sub> and H<sub>2</sub>O production during 2–3 min of flight. Bees hovered well in the chambers with little need for external stimulation. We calibrated the CO<sub>2</sub> analyzer, which is accurate to 0.1 μmol mol<sup>-1</sup> from 0 to 3000 μmol mol<sup>-1</sup>, with dry, CO<sub>2</sub>-free air and a compressed air tank containing certified (resolution: 0.01 μmol mol<sup>-1</sup>) 252 ppm CO<sub>2</sub> span gas. The water analyzer is accurate to 1% of full scale from 0 to 60 mmol mol<sup>-1</sup>. We digitized the analog data using a Sable Systems UI2 and recorded at 1 Hz using ExpeData (Sable Systems, v.1.7.2) for Windows. We calculated average CO<sub>2</sub> and H<sub>2</sub>O levels for 2–3 min periods when bees were observed to be steadily hovering. We recorded flight behaviors for each bee, but all bees flew well and consistently, and we found no relationship between our flight behavior scores and flight metabolic rate, so these behavioral data are not reported.

To measure shaded air temperature, we used a BAT-12 thermometer and thermocouple. To confirm that the metabolic chamber was air-tight, we measured CO<sub>2</sub> and H<sub>2</sub>O levels over 3–4 min without an animal in the chamber; under these conditions, there were no significant changes in CO<sub>2</sub> concentration. During flow-through respirometry, 95% washout of CO<sub>2</sub> from the metabolic chamber occurred in approximately 90 s.

We calculated CO<sub>2</sub> production rate ( $\dot{V}_{\text{CO}_2}$ , ml h<sup>-1</sup>) using Eqn 2, where FR is flow rate (m h<sup>-1</sup>) and  $F_{\text{CO}_2}$  is the fractional CO<sub>2</sub> level (μmol mol<sup>-1</sup>) in the excurrent air from the respirometry chamber during flight:

$$\dot{V}_{\text{CO}_2} = F_{\text{CO}_2} \times \text{FR}. \quad (2)$$

We calculated water loss rate ( $\dot{V}_{\text{H}_2\text{O}}$ , mg H<sub>2</sub>O h<sup>-1</sup>) using Eqn 3, where  $F_{\text{H}_2\text{O}}$  is the average fractional level of H<sub>2</sub>O (mmol mol<sup>-1</sup>) in the excurrent air from the respirometry chamber during flight:

$$\dot{V}_{\text{H}_2\text{O}} = \frac{(F_{\text{H}_2\text{O}} \times \text{FR} \times M)}{V_m}, \quad (3)$$

where  $M$  is the molar mass of water (18 g mol<sup>-1</sup>) and  $V_m$  is the molar volume of water (22,400 ml mol<sup>-1</sup>).

### Bee tagma temperature

Immediately following the respirometry measures, we transferred the bee into a plastic bag, which we flattened onto a Styrofoam board to reduce conduction and restrict the bee's movement. We then measured head, thorax and abdomen temperatures ( $T_h$ ,  $T_{\text{thorax}}$ ,  $T_{\text{ab}}$ , respectively) in random order within 5 s of cessation of flight by inserting a hypodermic thermocouple (Physitemp, MT-29/5HT Needle Microprobe, time constant=0.025 s) into the center of each tagma. We recorded the  $T_{\text{tagma}}$  data with a Pico Technology USB TC-08 Thermocouple Data Logger (Tyler, TX, USA). We recorded the shaded  $T_{\text{air}}$  following the temperature measurements for each individual. We calculated  $R_{\text{tagma}}$  using Eqn 1 (Baird, 1986).

We stored each individual bee in a vial, which was placed in an insulated cooler. Within 3 h of leaving the site, we measured the total wet mass, and that of each tagma, on a Mettler Toledo XPE56 XPE micro-analytical balance (accurate to 0.000001 g). To measure the dry mass of bees, we dried specimens in an oven at 50°C for 3 days.

### Wingbeat frequency and flight score

We recorded the sound of wing movements during hovering flight in the flight chamber prior to each respirometry measurement for 20–30 s using the iPhone 7+ microphone. After wingbeat frequency

measurement, we closed the chamber to flush CO<sub>2</sub> and H<sub>2</sub>O before the respirometry measurement. Using a sound editing program, Audacity v.2.4.2 for Windows, we visualized the wingbeats. We calculated average wingbeat frequency by dividing the number of wingbeats by the time duration for three separate measures of 10 wingbeats.

### Total body surface area calculations

We used a digital caliper (accurate to 0.01 mm) to approximate body surface area using geometrical calculations. We assumed that the head of the bee is a cylinder, measuring head width as the diameter and head thickness as the height. We assumed that the thorax is a sphere and measured thorax width as the diameter. We assumed that the abdomen is a cylinder and a cone, with the first to third terga of the abdomen being the cylinder and the fourth and fifth tergi being a cone (Roberts and Harrison, 1999). We did not include leg and wing surface area in the total body surface area calculation as these are large surface areas, and there is no evidence as yet that these are elevated in temperature relative to  $T_{\text{air}}$ . We calculated average bee surface temperature ( $T_{\text{bee}}$ , °C) using Eqn 4, which weights each tagma according to its relative surface area (SA):

$$T_{\text{bee}} = \frac{(SA_h \times T_h) + (SA_{\text{thorax}} \times T_{\text{thorax}}) + (SA_{\text{ab}} \times T_{\text{ab}})}{(SA_h + SA_{\text{thorax}} + SA_{\text{ab}})}. \quad (4)$$

### Dorsal vessel dissection

Given the rarity of *C. caesalpiniae* male aggregations, we were unable to collect fresh samples. Instead, we collected four large morph males of another desert *Centris* bee, *C. pallida*, on 29 April 2022, and stored them in Prefer™ (Anatech Ltd, Battle Creek, MI, USA) fixative for 4 weeks before dissection occurred. To visualize the dorsal vessel, we removed the legs and wings, and made a coronal cut about 1 mm on either side of the petiole. We located the dorsal vessel on the abdominal side, and followed the tube through the petiole, dissecting away fat, flight muscle and digestive tissue for clear visualization.

### Heat budget model calculations

We assumed that bees were flying at thermal equilibrium between 19 and 38°C in steady-state conditions. This assumption is supported by observations for honey bees that body temperature is stable during 1–5 min of flight (Roberts and Harrison, 1999), the prolonged steady hovering exhibited by most of our bees, and the steady CO<sub>2</sub> emission traces we observed. Using Eqn 5, we calculated a heat budget for flying bees at every degree between 19 and 38°C where  $Q_{\text{metabolic}}$  indicates metabolic heat production,  $Q_{\text{radiation}}$  indicates net radiative heat loss,  $Q_{\text{evaporation}}$  indicates evaporative heat loss and  $Q_{\text{convection}}$  indicates net convective heat flux:

$$0 = Q_{\text{metabolic}} + Q_{\text{radiation}} + Q_{\text{evaporation}} + Q_{\text{convection}}. \quad (5)$$

$Q_{\text{metabolic}}$  and  $Q_{\text{evaporation}}$  were calculated from  $\dot{V}_{\text{CO}_2}$  and  $\dot{V}_{\text{H}_2\text{O}}$ . Bees have mostly been reported to utilize carbohydrates as fuel for flight (Bertsch, 1984; Gäde and Auerswald, 1999; Suarez et al., 2005). Therefore, we assumed a respiratory quotient of 1, and 21.4 J ml<sup>-1</sup> CO<sub>2</sub> to calculate metabolic heat production in W. We then multiplied by 0.96 (the fraction of power input liberated as heat during flight) (Ellington, 1984; Harrison et al., 1996; Roberts and Harrison, 1999). To calculate evaporative heat loss in W, we multiplied  $\dot{V}_{\text{H}_2\text{O}}$  by the latent heat of evaporation of water, 2.45 J mg<sup>-1</sup> H<sub>2</sub>O.

As we performed respirometry measurements in the shade, we assumed shortwave radiation to be negligible. We summed the longwave (infrared) net radiation ( $r_{\text{loss}} - r_{\text{gain}}$ ) for the head, thorax,

and abdomen of each bee using the Stefan–Boltzmann equation:

$$Q_{\text{radiation}} = \sigma(\varepsilon_s SA_h T_h^4 + \varepsilon_s SA_{\text{thorax}} T_{\text{thorax}}^4 + \varepsilon_s SA_{\text{ab}} T_{\text{ab}}^4 - SA_{\text{bee}} \varepsilon_c T_i^4). \quad (6)$$

We assumed that the bee's emissivity,  $\varepsilon_s$ , is 0.97, and that bee surface temperature equals bee internal temperature (Stupski and Schilder, 2021). We assumed that the emissivity of the glass metabolic chamber,  $\varepsilon_c$ , is 0.90, and that  $T_{\text{air}}$  temperature equals the wall temperature,  $T_i$ , of the glass chamber (Bolz and Tuve, 1973; Campbell, 1977; Stupski and Schilder, 2021). Air and tagma temperatures are calculated in K. To estimate whole-bee radiative exchange, we summed  $Q_{\text{radiation}}$  for the head, thorax and abdomen. We calculated convective heat exchange using Eqn 7:

$$Q_{\text{convection}} = (-Q_{\text{metabolic}} - Q_{\text{radiation}} - Q_{\text{evaporation}}). \quad (7)$$

Because body mass and other indices of size declined through the day (see below), we obtained mass-specific heat flux by dividing heat flux for each bee by wet mass. To calculate surface area-specific convective conductance ( $\kappa$  is a measure of the capacity of the bee to transfer heat) in  $\text{W mm}^{-2} \text{K}^{-1}$ , we divided convective heat transfer ( $Q_{\text{convection}}$ ) in W by the total surface area of the bee and the gradient between  $T_{\text{air}}$  and average  $T_{\text{bee}}$  (in K) by combining Eqns 4 and 7:

$$\kappa = \frac{Q_{\text{convection}}}{(T_{\text{bee}} - T_{\text{air}}) \cdot SA_{\text{bee}}}. \quad (8)$$

Finally, to calculate  $Q_{10}$ , the factor by which metabolic rate increases over a  $10^\circ\text{C}$  increase in air temperature, we used Eqn 9 where MR indicates metabolic rate and  $T$  indicates temperature in  $^\circ\text{C}$ :

$$Q_{10} = \left( \frac{MR_2}{MR_1} \right)^{\left( \frac{10}{T_2 - T_1} \right)}. \quad (9)$$

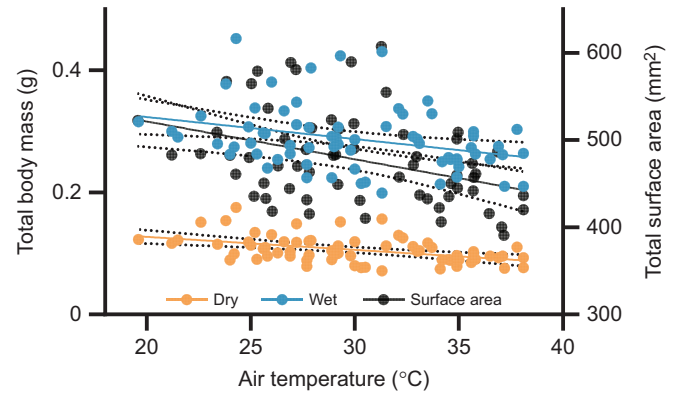
### Data analysis

We tested data for normality,  $\log_{10}$  transformed the data if necessary, and ran all statistical analyses in RStudio (v.1.3.1093 for Windows; RStudio: Integrated Development Environment for R. PBC, Boston, MA, USA; <http://www.rstudio.com/>). We created figures in GraphPad Prism (v.8.0.0 for Windows; GraphPad Software, San Diego, CA, USA; [www.graphpad.com](http://www.graphpad.com)). We included only large morph males in all analyses and excluded data if they were more than 2 s.d. from the mean ( $n=2$  for allometry, and  $n=1$  for the heat budget and conductance calculation). We determined two-tailed significance at  $\alpha=0.05$ . We used linear models to test for the effect of air temperature on wet and dry mass, tagma temperature, temperature excess ratio, flight metabolic rate, water loss rate, heat flux and convective exchange. The  $Q-Q$  plots showed slight deviations from normality for the allometric data, but we felt confident using these data in our linear models because of our large sample size ( $n=68$ ) and the fact that Gaussian models are robust against normality variations (Knief and Forstmeier, 2021). We ran additional non-linear models to test for the effect of air temperature on temperature excess ratio. We used AIC to determine model fit compared with the linear model. We report significant results below and all data are archived in Dryad (doi:10.5061/dryad.3xsj3txjw).

## RESULTS

### Body size trends and morphology

As air temperature increased through the morning, total wet body mass decreased (Fig. 2). Dry body mass and total body surface area also declined with increasing air temperature (Fig. 2). Head and thorax mass scaled hypometrically with body mass, while abdomen

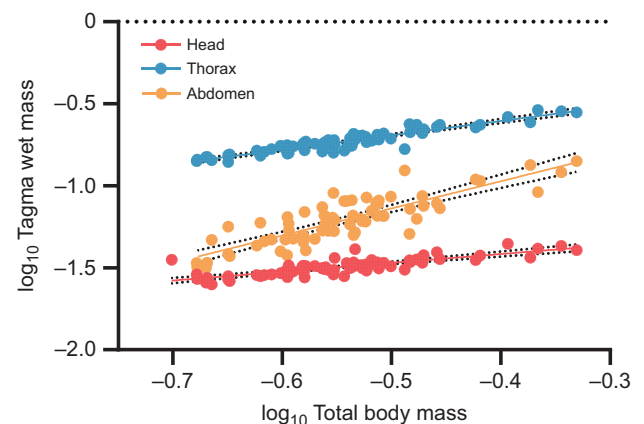


**Fig. 2. Total body mass and surface area versus air temperature for *C. caesalpiniae* large morph males.** As air temperature increased through the day, wet mass, dry mass and total body surface area decreased. Dotted lines indicate 95% confidence intervals (CI). Linear regression: wet mass =  $-0.0036T_{\text{air}} + 0.40$ ,  $n=69$ ,  $r^2=0.10$ ,  $P=0.0068$ , slope 95% CI  $(-0.0062, -0.0010)$ ; dry mass =  $-0.0021T_{\text{air}} + 0.17$ ,  $n=69$ ,  $r^2=0.21$ ,  $P<0.0001$ , slope 95% CI  $(-0.0031, -0.0011)$ ; total body surface area =  $-4.34T_{\text{air}} + 608.1$ ,  $n=62$ ,  $r^2=0.15$ ,  $P<0.005$ , slope 95% CI  $(-6.99, -1.69)$ .

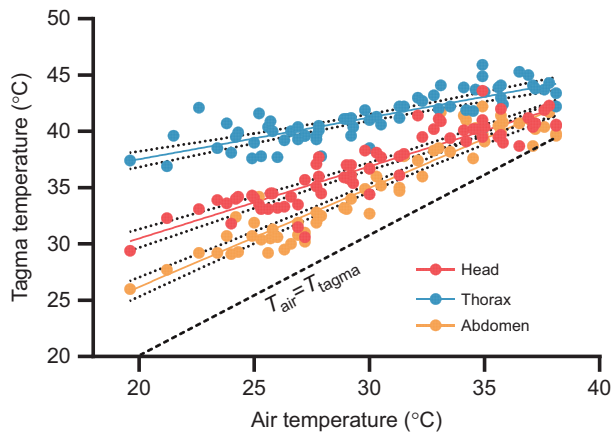
mass scaled hyperallometrically (Fig. 3), indicating that heavier bees had relatively smaller heads and thoraxes, but larger abdomens compared with smaller bees. We found that the large morphs of another desert bee species, *C. pallida*, had a dorsal vessel without petiolar loops in the thorax and abdomen.

### Tagma temperature following hovering flight in a shaded metabolic chamber

Order of temperature measurement did not affect tagma temperature, suggesting that stress or time effects associated with body temperature measurements were not significant. Thorax temperature increased by  $0.37^\circ\text{C}$  for every  $1^\circ\text{C}$  increase in air temperature (Fig. 4). Temperatures of the head and abdomen were regulated less precisely, with the slopes of tagma temperature on air temperature equal to 0.64 and 0.89, respectively (Fig. 4). The abdominal temperature excess ratio increased with air temperature using a linear model [ $R_{\text{ab}}=0.012T_{\text{air}}+0.082$ ,  $n=65$ ,  $r^2=0.17$ ,



**Fig. 3. Allometric scaling of *C. caesalpiniae* large morph male body tagma.** Head ( $M_h$ ; g) and thorax mass ( $M_{\text{thorax}}$ ; g) scaled hypometrically with body mass ( $M_b$ ; g), while abdomen mass ( $M_{\text{ab}}$ ; g) scaled hyperallometrically. Linear regression:  $M_h=0.54M_b-1.20$ ,  $r^2=0.66$ ,  $P<0.0001$ , slope 95% CI  $(0.45, 0.63)$ ;  $M_{\text{thorax}}=0.88M_b-0.25$ ,  $r^2=0.88$ ,  $P<0.0001$ , slope 95% CI  $(0.80, 0.95)$ ;  $M_{\text{ab}}=1.65M_b-0.32$ ,  $r^2=0.73$ ,  $P<0.0001$ , slope 95% CI  $(1.41, 1.91)$ .  $n=69$  for all parameters.

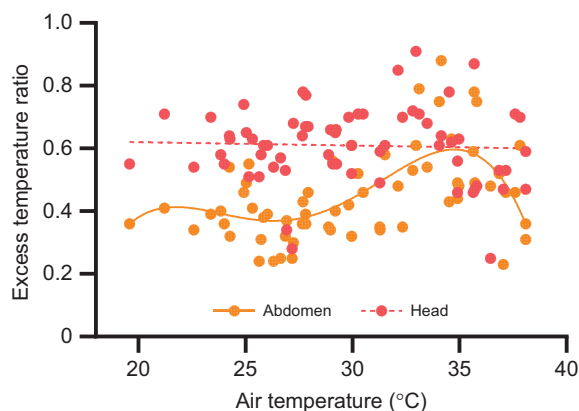


**Fig. 4. Tagma temperature versus air temperature for *C. caesalpiniae* males flying in a shaded glass chamber.** Tagma ( $T_{\text{tagma}}$ ), head ( $T_h$ ), thorax ( $T_{\text{thorax}}$ ) and abdomen ( $T_{\text{ab}}$ ) temperature increased with air temperature ( $T_{\text{air}}$ ). Linear regressions:  $T_h = 0.64T_{\text{air}} + 17.70$ ,  $n=67$ ,  $r^2=0.83$ ,  $P<0.0001$ , slope 95% CI (0.57, 0.71);  $T_{\text{thorax}} = 0.37T_{\text{air}} + 30.13$ ,  $n=68$ ,  $r^2=0.68$ ,  $P<0.0001$ , slope 95% CI (0.31, 0.43);  $T_{\text{ab}} = 0.88T_{\text{air}} + 8.62$ ,  $n=66$ ,  $r^2=0.89$ ,  $P<0.0001$ , slope 95% CI (0.80, 0.96). Dashed line indicates where  $T_{\text{air}}$  equals  $T_{\text{tagma}}$ .

$P=0.0007$ , slope 95% CI (0.0054, 0.019)], supporting the hypothesis that heat is actively transferred from the thorax to the abdomen at higher air temperatures. However, a polynomial fit to the abdominal temperature excess data provided better fits, as judged by lower AIC values. The best fitting model (lowest AIC) to describe  $R_{\text{ab}}$  was a fourth-order polynomial ( $y = 6.5 \times 10^{-5}x^4 + 0.007x^3 - 0.29x^2 + 5.17x$ ; Fig. 5). In contrast to  $R_{\text{ab}}$ , the head temperature excess ratio  $R_h$  did not change with air temperature, supporting the hypothesis that heat transfer between the thorax and the head is unregulated (Fig. 5).

#### Metabolic rate, water loss rate and wingbeat frequency

The mean body mass of the *C. caesalpiniae* males used for respirometry was  $290 \pm 0.053$  mg (range 199–467 mg). Metabolic rate (measured in W) significantly increased with body mass (measured in grams; Fig. S1). Mass-specific metabolic rate ( $\text{ml CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ ) increased linearly with air temperature (Fig. 6A), while metabolic rate

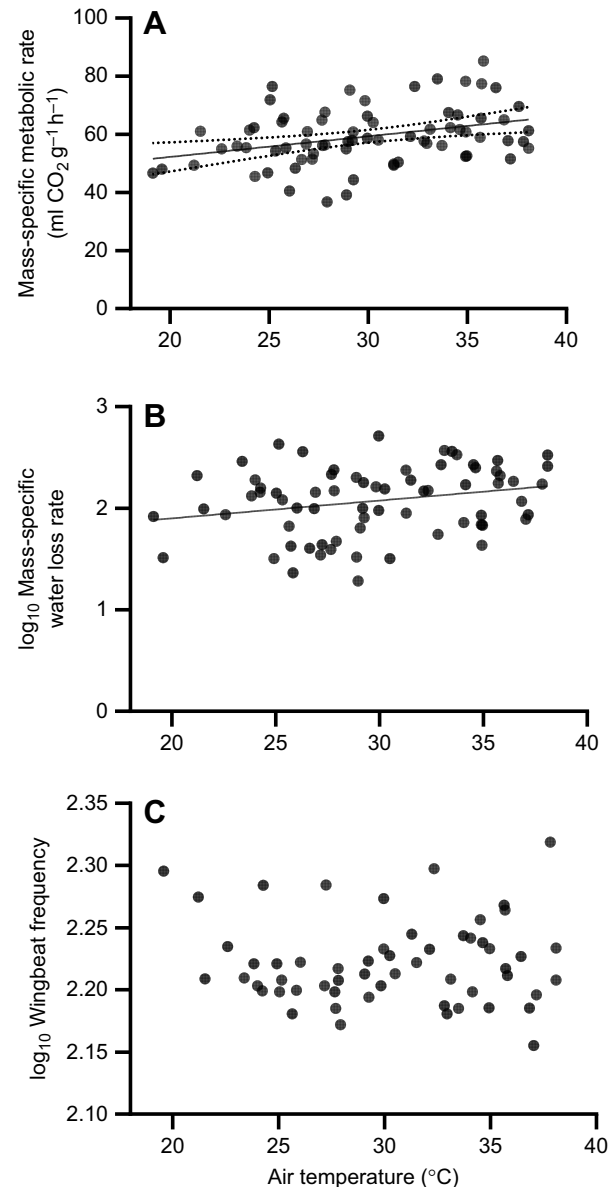


**Fig. 5. Temperature excess ratio versus air temperature for *C. caesalpiniae* males flying in a shaded glass chamber.** Abdominal temperature excess ratio [ $R_{\text{ab}} = (T_{\text{ab}} - T_{\text{air}}) / (T_{\text{thorax}} - T_{\text{air}})^{-1}$ ] increased with increasing air temperature ( $T_{\text{air}}$ ). Linear regression:  $R_{\text{ab}} = 0.012T_{\text{air}} + 0.082$ ,  $n=65$ ,  $r^2=0.17$ ,  $P=0.0007$ , slope 95% CI (0.0054, 0.019). Head temperature excess ratio ( $R_h$ ) did not vary with air temperature, averaging  $0.61 \pm 0.015$ ,  $n=66$ .

not corrected for body mass ( $\text{ml CO}_2 \text{ h}^{-1}$ ) did not vary with air temperature (Fig. S2A). The  $Q_{10}$  for mass-specific metabolic rate ( $\text{ml CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ ) was 1.13. Mass-specific water loss rates ( $\text{ml H}_2\text{O g}^{-1} \text{ h}^{-1}$ ) were highly variable but increased with air temperature when log corrected (Fig. 6B), and water loss rate not corrected for body mass showed no variation with air temperature (Fig. S2B). Wingbeat frequency did not vary with air temperature or body mass ( $166 \pm 2$  Hz, Fig. 6C).

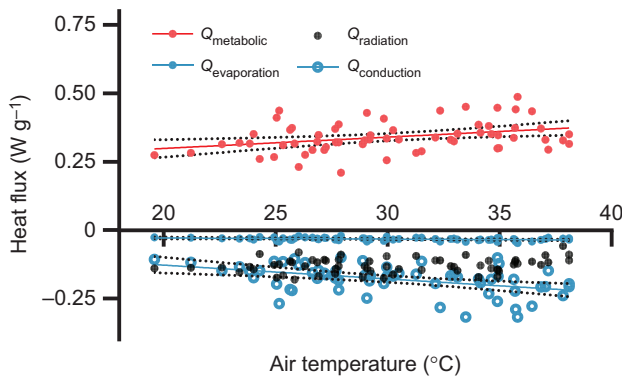
#### Heat budget model

Mass-specific metabolic heat production increased with air temperature (Fig. 7). Evaporative heat loss was a minor part of the



**Fig. 6. Mass-specific metabolic and water loss rate and wingbeat frequency versus air temperature for *C. caesalpiniae* males flying in a shaded glass chamber.** (A) Mass-specific metabolic rate ( $\dot{V}_{\text{CO}_2}$ ) increased with air temperature. Linear regression: mass-specific  $\dot{V}_{\text{CO}_2} = 0.71T_{\text{air}} + 38.02$ ,  $r^2=0.12$ ,  $P=0.0027$ ,  $n=71$ , slope 95% CI (0.26, 1.17). (B) Log-transformed mass-specific water loss rate ( $\dot{V}_{\text{H}_2\text{O}}$ :  $\text{ml H}_2\text{O g}^{-1} \text{ h}^{-1}$ ) increased with air temperature. Linear regression:  $\log_{10}(\text{mass-specific } \dot{V}_{\text{H}_2\text{O}}) = 0.018T_{\text{air}} + 1.55$ ,  $r^2=0.063$ ,  $P=0.0364$ ,  $n=70$ , slope 95% CI (0.0011, 0.034). (C) Wingbeat frequency (Hz;  $n=56$ ) did not vary significantly with air temperature.





**Fig. 7. Effect of air temperature on mass-specific routes of heat production and loss for *C. caesalpiniae* males flying in a shaded metabolic chamber.** Metabolic ( $Q_{\text{metabolic}}$ ), evaporative ( $Q_{\text{evaporation}}$ ), convective ( $Q_{\text{convection}}$ ) and radiative ( $Q_{\text{radiation}}$ ) heat flux are shown against air temperature ( $T_{\text{air}}$ ). Linear regression:  $Q_{\text{metabolic}} = 0.0041T_{\text{air}} + 0.22$ ,  $r^2 = 0.12$ ,  $P = 0.0054$ , slope 95% CI (0.0013, 0.0070);  $Q_{\text{evaporation}} = -0.00040T_{\text{air}} - 0.020$ ,  $r^2 = 0.12$ ,  $P = 0.0047$ , slope 95% CI (−0.00068, −0.00013);  $Q_{\text{convection}} = -0.0053T_{\text{air}} - 0.053$ ,  $r^2 = 0.23$ ,  $P < 0.0001$ , slope 95% CI (−0.0079, −0.0028);  $Q_{\text{radiation}} = 0.0013T_{\text{air}} - 0.18$ , n.s.  $n = 63$  for all parameters.

heat budget, and slightly but significantly increased with air temperature (Fig. 7). Mass-specific convective heat loss significantly increased, and mass-specific radiative flux did not change with air temperature. Convective conductance increased with air temperature (Fig. 8). Heat budgets using non-mass-corrected data also indicate that convective heat loss was the predominant mechanism of heat loss, but there was no significant change in convective heat loss (measured in W) with temperature (Fig. S3).

## DISCUSSION

### *Centris caesalpiniae* use an abdominal convector during flight

To partially regulate thorax temperature during flight, *C. caesalpiniae* males actively varied heat transfer from the thorax to the abdomen, as evidenced by the significant increase of the abdominal temperature excess ratio (Fig. 5). During the cool mornings, the thorax was much warmer than the air and abdominal temperatures, indicating that heat generated by the flight muscles

was conserved in the thorax (Figs 4 and 5). As the air warmed, the non-linear rise in the abdominal temperature excess ratio indicated active, increasing heat transfer from the thorax to the abdomen, likely by circulating hemolymph.

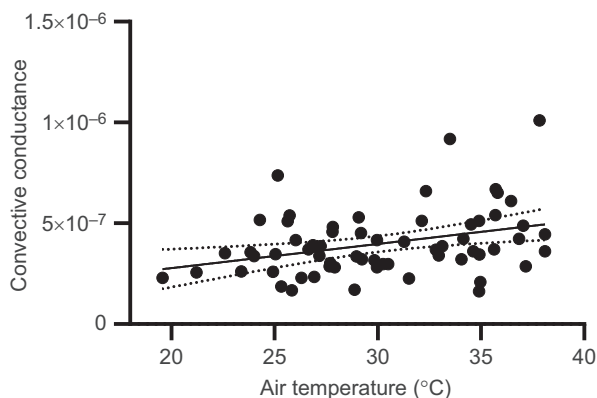
We found that *C. pallida* large morph males had a dorsal vessel that runs directly from the abdomen through the petiole without petiolar loops, which may allow the heart to act as a variable counter-current heat exchanger (Heinrich, 1980). The *C. pallida* heart morphology is similar to that of *Bombus* (Wille, 1958), which also use the abdomen as a variable convector to thermoregulate (Heinrich, 1976). Plausibly, *Centris* large morph males control heat flow from the thorax to the abdomen by a similar mechanism to that documented for the sphinx moth, *Manduca sexta*, and the bumble bee, *Bombus vosnesenskii*. In these animals, the rate of warm hemolymph flow from the thorax to the abdomen increases at higher air temperatures as a result of stronger contractions of the heart and ventral diaphragm, which pulse to allow alternating forward (cool) and reverse (warm) flow through the petiole (Heinrich, 1976).

This warming of the *C. caesalpiniae* abdomen with its large surface area facilitated a rise in mass-specific convective heat loss. Warming of the abdomen raised average bee surface temperature, which will tend to increase both radiative and convective heat loss. Additionally, as air temperature rose, convective conductance more than doubled (Fig. 8), likely because more heat loss occurred from the relatively uninsulated abdomen rather than the highly pubescent thorax. Alternative explanations for the rise in convective conductance at higher air temperatures include the possibility that surface area of the bee increased (perhaps as a result of expansion of the abdomen) or possibly that wind flow increased over the bee, perhaps because of increased flight speeds or increased wing-driven flow over the body. In any case, the active transfer of warm blood to the abdomen combined with an increased convective conductance allowed these bees to effectively lose sufficient heat to balance the increase in metabolic heat production as air and body temperature rose, preventing thoracic overheating.

### Interspecies and morph differences in thermoregulatory mechanisms

We found that the large morph *C. caesalpiniae* males used an abdominal convector heat loss mechanism, whereas small morph *C. pallida* males do not (Roberts et al., 1998). At present, it is not clear whether this represents a species or morph difference. In both species, small morph males were typically found hovering or in forward flight, at a meter or more above the ground (Alcock et al., 1977), while large morph males were usually found on or flying near the ground as they searched for emerging females. Large morph bees, regardless of species, may utilize an abdominal convector mechanism of heat loss because they are more likely to experience overheating. Large morph bees have a lower surface area-to-volume ratio and likely experience high conductive and radiative heat gain from the ground (ground temperatures can reach 58°C), and lower wind speeds than the small morphs, which fly high above the ground. Tests of these hypotheses will require direct comparison of the thermoregulatory strategies of the two morphs.

Another thermoregulatory difference between the large morph *C. caesalpiniae* and the small morph *C. pallida* males was how metabolic heat production responded to temperature. *Centris pallida* small morph males decreased metabolic rate and wingbeat frequency as the principal means to thermoregulate during flight (Roberts et al., 1998). In contrast, we found an increase in mass-specific metabolic rate as air temperature rose (Fig. 6A), and no decrease in wingbeat frequency (Fig. 6C) for the large morph



**Fig. 8. Mass-specific convective conductance versus air temperature for *C. caesalpiniae* males flying in a shaded glass chamber.** Mass-specific convective conductance ( $\kappa$ ) increased as air temperature ( $T_{\text{air}}$ ) rose. Linear regression:  $\kappa = 0.013T_{\text{air}} + 0.091$ ,  $r^2 = 0.13$ ,  $P < 0.005$ .  $n = 63$ ; absolute values are plotted for clarity.

*C. caesalpiniae* males flown in the shade in our studies. One plausible explanation for this difference is that metabolic rate only decreases under conditions in which flight muscle temperature rises well above optimal (Glass and Harrison, 2022; Harrison and Fewell, 2002). In our study, thorax temperature for large morph *C. caesalpiniae* averaged 41°C and peaked at about 44°C (Fig. 2), whereas thorax temperature for the small morph *C. pallida* males averaged 45°C and peaked at about 47°C (Roberts et al., 1998). In support of this hypothesis, *C. caesalpiniae* large morph males flying in the field decreased wingbeat frequency as air temperature increased from 18 to 32°C (Spangler and Buchmann, 1991), suggesting that with solar heat load and possibly higher thorax temperature, metabolic heat production might decline in *C. caesalpiniae*.

Another possibility is that large morph *C. caesalpiniae* males cannot reduce their metabolic rate at high air temperature because they would not generate sufficient lift to fly. Large morph *C. caesalpiniae* males had heavy abdominal loads that constituted 25% of total body mass (Fig. 3). *Centris pallida* male abdomens, in contrast, constituted only 15% of body mass (Chappell, 1984). In *Xylocopa californica* females (Roberts et al., 2004), abdomens also scaled hypermetrically, presumably because larger females had more reproductive tissue (Roberts et al., 2004). Similarly, the relatively large abdominal size and hypermetric scaling of *C. caesalpiniae* abdomens may indicate larger testes or energy stores.

#### ***Centris caesalpiniae* males do not use evaporative water loss as a major thermoregulatory strategy**

As for *C. pallida* small morph males, though evaporative heat loss increased with air temperature, it was a minor part of the heat budget for *C. caesalpiniae* males (Fig. 7). Water loss rates were highly variable among individuals for unknown reasons (Fig. 6B) and were not correlated with flight metabolic rate. However, it is possible that increases in evaporative water loss might be observed at higher body temperatures than measured. For example, *Apis mellifera* strongly increased evaporative water loss at air temperatures above 39°C (Glass and Harrison, 2022).

#### **Bigger bees are active earlier in the activity period**

A striking and somewhat surprising finding was that wet and dry mass decreased as the day progressed, by 22% and 32%, respectively, and surface area of these bees decreased by about 16% (Fig. 2). These results strongly suggest that this pattern resulted from different bees being present at different times of the day, though a mark–recapture study will be required to confirm this. These data suggest that individual bees may only be able to persist at the aggregation site for a portion of the morning, perhaps as a result of desiccation stress. Plausibly, larger bees were more active in the cool early morning because they are more capable of endothermic elevation of thorax temperature because of their lower surface-to-volume ratios. Additionally, because most *C. caesalpiniae* females emerged early in the day (M.G.J. and J.R.G., personal observation), if individual males can only persist at the aggregation site for a few hours, larger males may dominate the early mornings, while smaller males may arrive for the later times when environmental conditions are less favorable, but competition with larger males is reduced.

#### **Surface area and mass affect metabolic rate, water loss rate, and convective and radiative heat exchange**

The decline in male bee size and surface area as the day progressed and air temperature rose strongly affected our heat budget and data

interpretation. We found that metabolic rate, water loss rate and convective heat loss all increased with air temperature if corrected for wet mass, but not when left uncorrected. If we had ignored the decline in body mass and simply used the uncorrected values (in W), we would have concluded that all of these parameters were insensitive to air and body temperature in *C. caesalpiniae*.

Calculation of convective conductance provides an excellent measure of how the capacity of the bee to lose heat varies with air temperature because this corrects for heat loss associated with the changing thermal gradient between a bee and the environment, as well as the decline in surface area as the bees became smaller later in the day (Eqn 8). Radiation and convection characterize a large portion of heat loss to the environment and are affected by several factors such as insect size, surface area, hair and cuticle coloration, hair length and hair density (Church, 1959). We found that convective conductance ( $\text{W mm}^{-2} \text{K}^{-1}$ ) approximately doubled as air temperature rose from 18 to 38°C (Fig. 8). The most likely explanation for the increased convective conductance is the increased transfer of heat to the large, relatively bare abdomen. This provides the first quantitative estimate of the thermoregulatory value of the abdominal convector.

#### **The importance of heat budgets**

The future of biophysical modeling is exciting; with rapidly advancing infrared and spectrophotometric technologies comes increased resolution for small insect measurements, more precise biophysical models, and a deeper understanding of thermoregulatory nuances dependent on insect size, shape and coloration. Our heat budget model utilized literature values for absorbance and emissivity; however, these can vary substantially among insects (Shi et al., 2015; Stupski and Schilder, 2021; Tsai et al., 2020; Wang et al., 2021), and have the potential to affect our understanding of their mechanisms of thermoregulation. In particular, many insects fly in the sun, and the incorporation of models that assess the contribution of solar radiation to thermal balance will be necessary to develop a more comprehensive understanding of how insect body temperature and performance will be affected by climatic warming. Additional critical research is required to determine how flight performance (i.e. foraging load carriage, mating success) is affected by air and body temperature so we can predict how environmental conditions will influence fitness.

For *Centris* species in particular, and for other large flying endothermic insects, we should endeavor to untangle the limitations on activity period (i.e. whether high temperature, low water availability and desiccation – or none of these – cause flight cessation). It is possible that climate-associated heat waves may impose limits on endothermic flight and reproduction, with some thermoregulatory strategies (Table 1) prevailing over others. Future studies should investigate how and why some insects use an abdominal convector and others do not, and similarly why some bees vary metabolic heat production and other species do not. Comparative tests using similar methods to this study, but in a phylogenetic framework, are required to answer these critical questions.

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#### **Competing interests**

The authors declare no competing or financial interests.



## Author contributions

Conceptualization: M.G.J., J.F.H.; Methodology: M.G.J., J.F.H.; Software: M.G.J.; Validation: M.G.J., J.R.G.; Formal analysis: M.G.J.; Investigation: M.G.J., J.R.G.; Resources: M.G.J., J.F.H.; Data curation: M.G.J.; Writing - original draft: M.G.J.; Writing - review & editing: M.G.J., J.R.G., J.F.H.; Visualization: M.G.J., J.F.H.; Supervision: M.G.J.; Project administration: M.G.J.; Funding acquisition: M.G.J., J.F.H.

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## Data availability

All data are available from Dryad (Johnson et al., 2022): doi:10.5061/dryad.3xsj3txjw

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