The Biophysical Ecology of a Desert Bee

by

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ABSTRACT

Deserts are natural laboratories in which to study the acute effects of extreme heat and aridity on animal physiology, as well as the physiological adaptations that these animals develop to survive. For small, endothermic fliers in the desert, heat balance and water balance are challenging due to high surface area to volume ratios and the additional heat load imposed as a result of endothermy. Much of the previous fifty years of thermo- and hydroregulation research has focused on larger, charismatic megafauna; extremophiles; or only part of the heat or water balance story. Here, I calculate the first heat budget for an important desert bee, *Centris caesalpiniae*. As is common in many mammals, avians, and other flying insects, I find that *C. caesalpiniae* males use an appendage – in this case the abdomen – as a convective radiator to dispel excess heat produced by the thoracic flight muscles at high air temperatures. The few heat budgets measured for flying endotherms are conducted in the shade so as to eliminate the effects of solar radiation. To further refine the accuracy of my heat budget model, I consider how heat gain from solar radiation affects the thermal balance of flying desert endotherms. To this effect, I find that solar radiation contributes 43 to 54% of the total heat gain of a desert *Centris pallida* bee. I additionally show that large morph male, small morph male, and female *C. pallida*, use different thermoregulatory tactics while flying in shaded versus sunny conditions; large males and females rely on the abdominal convector in the sun while small morph males increase convective conductance in the sun, but do not use an abdominal convector. Given that evaporative cooling was not a significant part of the heat budget for neither *C. caesalpiniae* nor *C. pallida*, I investigated the effects of water loss rates and critical thermal maxima during flight on duration of activity period. I found that male *C. caesalpiniae* limited their activity period due to high water loss rates rather than overheating, and that *Centris* critical water contents ranged from 48 to 54%, limiting flight activity to about 3 hours.

DEDICATION

Thank you to everyone who has supported me, and even to those who have not, for the fodder that lit my deep passion for biological research, dedication to protecting the natural world, and commitment to making everyone feel comfortable and valued in science.

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PREFACE

What is a desert?

Deserts minimally make up, 25-33% of terrestrial land (Polis, 2023; Ward, 2016). However, their ecologies are often simplified and misunderstood – labeled as lifeless, hot, and dry. In fact, deserts are full of life. Deserts can be cold. One desert in particular receives torrential rainfall during two seasons. This (sometimes) rainy desert, the Sonoran Desert, receives between 76 to 380 mm of rain per year, with water arriving via a few massive and unpredictable rain events in the late summer and mid-winter (Dimmitt et al., 2015). Most other times of the year can indeed be classified as a "dry heat", as the Sonoran Desert lies in the rain shadow of large mountain ranges – the Rocky Mountains, Cascades, and Sierra Nevada. As a result, the Sonoran's residents, both human and non-human, have adapted to take advantage of varied biogeography and cope with water's unpredictable arrival, high solar radiation, and fluctuating environmental temperatures.

How will the Sonoran Desert change due to climate change?

These important and interactive abiotic patterns are changing due to anthropogenic climate change (Weiss & Overpeck, 2005; Williams et al., 2020) and damaging ecological decisions generally associated with the overuse of water – i.e., groundwater depletion, overallocation of river water, and damming (Castle et al., 2014; Tecle, 2017). The United States Southwest has been in its driest twenty year period since 800 CE (Williams et al., 2022). A key source of water in the Sonoran Desert, the Colorado River, is predicted to decrease in flow rate by more than 30% by the end of the century (Udall & Overpeck, 2017) due to high temperatures and drought (Overpeck & Udall, 2020). Decreasing water availability will almost certainly affect plant growth and phenology, which in turn changes the constitution and quantity of landcover and further increases land surface temperatures (Duman et al., 2021). Native desert flora and fauna will likely respond by restricting their ranges to high elevations or moving northward, adapting to the changing abiotic environment, or dying out (Archer & Predick, 2008; Kimball et al., 2010; Zachmann et al., 2021).

Why should humans care about deserts?

The Sonoran Desert is home to well over 10,000 insect species (including ~700 bee species), over 100 bird, ~130 mammal, 20 amphibian, ~100 reptile, ~30 native fish, 2,500 plant, and even important fungal and bacterial species that live in the soil crust (Dimmitt et al., 2015). This massive abundance is spread across seven types of biomes and associated elevations that the Sonoran encompasses. Near Flagstaff, Arizona, Doko'oosliid (Humphreys Peak) stretches close to 3,900 meters and supports alpine tundra (Dimmitt et al., 2015). As one travels south to Phoenix, Arizona and beyond, the landscape shifts from coniferous forest to deciduous forest to grasslands and to desert (Dimmitt et al., 2015). One may even encounter thornscrub areas and riparian communities along rivers.

Beyond appreciating the sheer abundance of biodiversity in the Sonoran Desert, it is a moral imperative to interact with nature responsibly and protect its many inhabitants. Otherwise, the short-term economic and environmental effects of climate change will be catastrophic to most living organisms (Carleton & Hsiang, 2016). In the Arizona portion of the Sonoran Desert, many hundreds of important pollinator species are important to crop pollination (e.g., citrus trees), as well as to the floral biodiversity that draws thousands of tourists to the Sonoran Desert

(Simpson & Neff, 1987). Monoculture, ranching, agrochemicals, habitat fragmentation, urbanization, and other land cover changes, are major threats to these pollinators, either killing them outright, or wiping out important, native floral resources and nesting locations (Potts et al., 2010). As native insect and pollinator numbers decline (Potts et al., 2010; Woodard et al., 2020), animals in higher tropic levels, such as the birds and bats that rely on insects for energy, might also decline (Goulson, 2019). As a result, disease-carrying mosquitos and flies may increase in number, increasing the prevalence of vector-borne diseases in previously unaffected areas (Kolivras & Comrie, 2004).

The desert and its inhabitants may be a lifeline in combating the challenges posed by anthropogenic climate change. Certain desert species may be just fine in the face of radical abiotic changes. Despite, or perhaps because of, their apparent hardiness, humans should endeavor to understand and catalogue the physiological mechanisms and structures of inhabitants, arguably some of the most successful living things on Earth. What wisdom can humans gain from the desert? How can humans make better desert-inspired materials to wear? To build? To improve technology? For example, many desert insects possess structural adaptations that reflect heat gain from solar radiation (Barrett & O'Donnell, 2023; Krishna et al., 2020; Shi et al., 2015) and even mid-infrared radiation from the ground and other hot objects in the environment (Krishna et al., 2021; Shi et al., 2015). These structures are not only found on insects; many desert plants also have pubescence, or "hairs", that reflect near-infrared radiation from sun (Barthlott et al., 2017; Ehleringer & Björkman, 1978). Anthropogenic applications of this knowledge could be geared toward improving solar energy harvesting or coating the surfaces of outdoor structures with a different material so as to alter heat gain or heat loss.

The desert also holds economically valuable minerals (like gypsum and copper), but humans face a critical tradeoff between mining for important elements and destroying biodiversity and eradicating cultural landscapes (i.e., in Oak Flat, Arizona). The destruction of deserts (via mining, for example) will hugely affect the natural carbon and nitrogen cycling and sequestration in the soil. Deserts serve as large carbon sinks, trapping greenhouse gasses (Biederman et al., 2018; Li et al., 2015), and certain desert plants even act as important nitrate filters (Schade et al., 2001). In 2021, 40.9 million people visited Arizona, collectively spending $23.6 billion, with $703 million of this spent at campgrounds (Dean Runyan Associates, 2022). Clearly, a large amount of tourism is driven by the climate and beauty of the Sonoran Desert in Arizona. It is hopefully evident that the Sonoran Desert is a precious resource, biodiversity hotspot, and cultural landscape that merits protection and study.

How do animals survive in deserts?

Scientists have been fascinated by the physiological mechanisms and behavior of desert organisms for centuries (Merriam, 1890; Morong, 1891). Their ability to withstand drastic temperature swings, desiccation, and solar radiation loads is indeed unique among living things (Rocha et al., 2021). In hot desert conditions, animals can reduce metabolic heat production, reduce heart rate, reduce blood pressure, reduce water losses, store and metabolize fats, and more, all through a number of mechanisms depending on species and environment (Rocha et al., 2021). Though there are many desert-adapted animals and plants, in this dissertation, I will focus on desert bees. I have extensively reviewed the effects of high temperature on the physiology and behavior of flying pollinators, including the bees (Johnson et al., 2023), but will briefly highlight the traits of flying bees relevant to my dissertation.

Large desert bees maintain elevated thorax temperatures and limit water losses through a number of mechanisms during flight. Certain bees can increase and decrease metabolic heat production, likely though the modulation of wingbeat frequency (Glass & Harrison, 2022; Roberts et al., 1998; Roberts & Harrison, 1999). Others may regurgitate crop contents to evaporatively cool the head (Heinrich, 1980; Roberts & Harrison, 1999). It is imaginable that others may defecate not only to maintain water balance (Nicolson & Louw, 1982), but also to cool the abdomen (Lahondère & Lazzari, 2012). Changes in flight speed could increase convective cooling but would also theoretically result in increased water loss rates from the cuticle. Many large endothermic bees dump heat to the abdomen via a countercurrent heat exchanger (Heinrich, 1976; Johnson et al., 2022), resulting in increased convective cooling (Johnson et al., 2022).

In addition to these physiological mechanisms, bees are small (relative to other flying desert animals like birds and bats) and mobile which allows them to select thermally and/or hydrically beneficial microclimates. Like butterflies, bees may very well adapt a posture or location that minimizes or maximizes heat gain from solar radiation. If temperatures and humidities become dangerous, bees can limit their activity periods to cooler hours during the day (Willmer, 1986). Even more broadly, bees can limit activity to certain times of the year, spending up to eleven months diapausing in sealed chambers underground or in aboveground cavities (Danforth et al., 2019). In particularly hot and dry years, some bees may not emerge and instead wait until the next year, effectively “betting” that the next year will be better (Danforth et al., 2019).

Aside from the diverse behavioral and physiological mechanisms that desert bees rely on to survive, the morphology of a bee is quite important. Surface area and volume are two important measures that influence the rate of heat gain and loss from an animal, as well as the rates of water loss from exposed surface areas. To limit heat gain, the surface structure of a bee’s cuticle and hair like structures called setae can reflect/absorb near- and mid-infrared radiation from the sun and surrounding environment (Barrett & O’Donnell, 2023; Johnson et al., 2023a; Shi et al., 2015). The cuticle’s thickness and chemical constitution may also prevent desiccation. Other body parts could be specialized, for example, long legs to lift the bee away from the ground.

Very few biophysically-focused, peer-reviewed research papers have been published concerning native, desert bees. Unsurprisingly, there are yet many unknowns concerning their physiology, behavior, and morphology (Johnson et al., 2023a). This is likely because the majority of species are exceedingly difficult to locate and track from year to year, and because sample sizes are often very small for solitary bees (except in the case of aggregating species such as *Centris pallida* and *Diadasia diminuta*, for example).

The Centris genera

The genus *Centris* is comprised of 230 species found in the New World, from the deserts of the United States to the neotropics (Martins et al., 2014). Many of these bees are important crop pollinators in the neotropics (Freitas, 1997; Oliveira & Schlindwein, 2009). Twenty five species have been found and collected in Arizona (Ascher & Pickering, 2020); some species form mating aggregations (i.e., *C. cockerelli*) and others are completely solitary (i.e., *C. rhodopus*) (Alcock et al., 1976;

Rozen & Buchmann, 1990). In the Sonoran Desert, *Centris* play an important role in pollinating iconic plants such as *Parkinsonia microphylla*, *Olneya tesota, Prosopis velutina, Psorothamnus spinosus*, *Krameria bicolor,* and *Larrea tridentata* (Alcock et al., 1977; Rozen & Buchmann, 1990). The best studied desert *Centris* species, in terms of their physiology and behavior, are *C. pallida* and *C. caesalpiniae* (Alcock et al., 1977; Barrett & Johnson, 2022; Chappell, 1984a; Johnson et al., 2022; Roberts et al., 1998; Spangler & Buchmann, 1991). Both species form enormous mating aggregations, with individuals numbering in the many thousands. Aggregating desert *Centris pallida* and *Centris caesalpiniae* thus provide abundant sample sizes, males and females are easy to catch, and nesting sites are obvious and plentiful. *Centris pallida* is a specialist of *P. microphylla* and emerges in late April, whereas *C. caesalpiniae* females specialize on *K. bicolor* and emerge in May-June. After three to four weeks of mating activity, both species diapause underground as prepupae until the next year (pers. obs.). *Centris pallida* and *C. caesalpiniae* males are dimorphic with size-associated alternative reproductive tactics (Alcock et al., 1977). In both species, large males are active on the ground, searching and digging for females or making patrolling fights. *Centris pallida* large males form mating “bee balls” (Alcock et al., 1977), but *C. caesalpiniae* do not to the same extent (pers. obs.). Small morph *C. pallida* males choose hovering points high in the palo verde trees and appear to defend this position against other small males. Small *C. caesalpiniae* males make sweeping flights higher above the bushes, but do not hover like *C. pallida* small males.

Few researchers have focused on desert *Centris* physiology (Barrett et al., 2022; Chappell, 1984a; Johnson et al., 2022; Roberts et al., 1998; Spangler & Buchmann, 1991). I conducted an exploratory study of the thermal biology and heat balance of *C. caesalpiniae* for the first time in Chapter 2. Prior to this study, the wingbeat frequency of large *C. caesalpiniae* males was shown to decline with increasing air temperatures (Spangler & Buchmann, 1991). In Chapter 3, I create a full heat budget for *C. pallida* bees, measuring heat balance in the sun and shade. The thermal biology of *C. pallida* small morph males has been previously studied (Chappell, 1984a; Roberts et al., 1998); small males were purportedly the best-known thermoregulators (m=0.15 thorax temperature on air temperatures; Roberts et al., 1998), but in Chapter 3, I present new information concerning the heat balance of small and large *C. pallida* males as well as females, showing that solar radiation significantly contributes to the heat balance of flying *Centris* bees for the first time. In Chapter 4, I present water loss rates in flight and at rest for *C. caesalpiniae* large morph males, as well as critical water contents of both species and sexes, showing that water loss rates limit the activity period.

A thorough exploration of the common themes of adaptation and limitation in desert organisms is critical as global temperatures rise, precipitation patterns change, and other anthropogenic impacts take a toll on the Sonoran and its inhabitants. In this dissertation, it is my aim to provide a foundation for the accurate biophysical modeling of native bees to predict how an important group of desert pollinators will fare in the wake of the climate crisis.

CHAPTER 1

A DESERT BEE THERMOREGULATES WITH AN ABDOMINAL CONVECTOR DURING FLIGHT

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 of increase in air temperature may be fatal (McKechnie & Wolf, 2019). Other animals that currently 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 & Huey, 2016; Buckley & Kingsolver, 2021). Here, I investigate the mechanisms used for thermoregulation during the flight of a 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 & 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 & Harrison, 1998a). The mechanisms of thermoregulation by endotherms, and how and why these vary with phylogeny and environment remain poorly understood (Gilmour & Ellington, 1993; Roberts & Harrison, 1998a; P. Willmer & Stone, 1997). For example, in birds, there are strong phylogenetic effects on the mechanisms used for evaporative cooling, with the Columbidae utilizing cuticular transpiration and passerines panting (McKechnie et al., 2021). In a marsupial species, the desert dwellers are less likely to utilize evaporative cooling than their temperate counterparts (Hulbert & Dawson, 1974).

Among the flying endothermic insects, thermoregulatory mechanisms are highly variable, for reasons that are unclear (Table 1.1). Honey bees have been reported to decrease metabolic heat production and decrease wingbeat frequency as air temperature increases (Roberts et al., 1998; Roberts & Harrison, 1999), though some dispute this (Heinrich & Esch, 1994; W. A. Woods et al., 2005). Similarly, the desert digger bee, *Centris pallida*, hovering orchid bees, and dragonflies, reduce flight metabolic rates and wing beat frequencies as air temperature rises (Borrell & Medeiros, 2004; May, 1976, 1995; Roberts et al., 1998). In contrast, bumble bees and carpenter bees do not vary flight metabolic rates with air temperature (Heinrich, 1976; Nicolson & Louw, 1982). In the bees that have been examined, evaporative water loss rates increase 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 & Louw, 1982; Roberts et al., 1998; Roberts & Harrison, 1999).

Table 1.1 Thermoregulatory mechanisms of flying endothermic insects. While all of the listed species are endothermic and partially homeothermic, how metabolic rates (MR), wing beat frequencies (WBF), evaporative water loss rates (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 with air temperature, ↓ = decreased with air temperature, ↑ = increased with air temperature.

Species	**Mass (mg)**	**MR**	**WBF**	**EWL**	**Rab**	**Rh**	**Reference**
ANTHOPHORIDAE							
Anthophora plumipes	185	.	.	.	↑	.	Stone, 1993
Centris pallida	131.4	↓	↓	–	–	↓	Roberts et al., 1998; Chappell, 1984
C. caesalpiniae	335	.	↓	.	.	.	Spangler and Buchmann, 1991
Xylocopa capitata	1,245	–	.	↑	.	.	Nicolson and Louw, 1982
X. californica	587	–	.	.	.	.	Chappell, 1982
X. varipuncta	673	.	.	.	↑	↑	Heinrich and Buchmann, 1986
X. virginica	370	.	.	.	↓	↓	Baird, 1986
APIDAE							
Apis mellifera	75.3	↓	↓	↑	–	–	Roberts and Harrison, 1999; Cooper et al., 1985
A. mellifera	.	–	.	.	–	.	Heinrich, 1980; Stevenson and Woods, 1997
Bombus bimaculatus	.	.	–	.	.	.	Joos et al., 1991
B. fervidus	.	.	–	.	.	.	
B. vagans	.	.	–	.	.	.	
B. pratorum	122	.	↓	.	.	.	Unwin and Corbet, 1984
B. prascuorum	131	.	↑	.	.	.	
B. vagans	120	.	.	.	–	.	Heinrich, 1972

B. vosnesenskii	645	–	.	.	↑	.	Heinrich, 1976
Euglossa imperialis	160.8	↓	↓	↓	↓	↓	Borrell and Medeiros, 2004
Melipona subnitida	47.4	.	.	.	↑	↑	Souza-Junior et al., 2020
VESPIDAE							
Sphecius grandis	.	.	.	.	–	↓	Coelho et al., 2007
Vespula germanica	.	.	.	.	–	↓	Coelho and Ross, 1996
V. maculifrons	.	.	.	.	–	↓	Coelho and Ross, 1996
DIPTERA							
Chrysomya megacephala	.	.	.	.	↓	↓	Gomes et al., 2018
Sarcophoga subvicina	68	.	.	.	↑	.	Willmer, 1982
S. carnaria	73	.	.	.	↑	.	
LEPIDOPTERA							
Hyles lineata	650	–	.	.	↑	.	Casey, 1976
Manduca sexta	2070	–	–	.	↑	↓	Hegel and Casey, 1982; Heinrich and Bartholomew, 1971
ODONATA							
Anax junius	1200	↓	↓	.	–	.	May, 1995b
Zenithoptera lanei		.	.	.	–	–	Guillermo-Ferreira and Gorb, 2021
COLEOPTERA							
Scarabaeus sacer	.	.	.	.	–	.	Verdú et al., 2012
S. cicatricosus	.	.	.	.	↑	.	

One of the most important mechanisms of thermoregulation in endotherms is variable perfusion of the skin and appendages (Morrison & Nakamura, 2019). Increasing blood flow to the skin and appendages raises their surface temperatures, 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 (Dzialowski & O'Connor, 1999), and a dragonfly can lose heat through the wings (Guillermo-Ferreira & Gorb, 2021).

As for other mechanisms of thermoregulation, the reported use of variable blood flow to surfaces is inconsistent (Table 1.1). 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 & Bartholomew, 1971). *Bombus vosnesenskii* queens use a countercurrent heat exchanger in the petiole to prevent heat transfer between 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 & Harrison, 1998a). *Centris vittata* females and *C. lanosa* males have aortic cardiovascular structures similar to *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 thorax and the abdomen or head, Baird (1986) suggested using the temperature excess ratio (R_{tagma}):

$$R_{\text{tagma}} = \frac{(T_{\text{tagma}} - T_{\text{air}})}{(T_{\text{thorax}} - T_{\text{air}})} \quad (1.1)$$

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 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 1.1). The abdominal temperature excess ratio 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 & Casey, 1982; Heinrich, 1972, 1976; Heinrich & Buchmann, 1986; P. Willmer & Stone, 1997), but not for *Centris pallida* small morph males, or honey bee workers or drones (Roberts et al., 1998; Coelho, 1991).

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 on overall heat exchange. A true quantitative determination requires a heat budget model. Each parameter in a heat budget is converted to the unit, Watts, or Watts·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 & Casey, 1982; Roberts & Harrison, 1999; Stupski & 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 use of variable heat transfer to body surfaces can be performed by calculating the convective conductance (κ), 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, I expect to observe an increase in the convective conductance due to the increase in the fraction of body surface area that is warm, and the reduction in average insulation. Calculation of κ 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 US 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 one to two months most years. Male and female *C. caesalpiniae* emerge from brood cells located 8 to 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°C to nearly 40°C (Rozen & Buchmann, 1990; Spangler & 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 & 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*, *Centris pallida* males thermoregulate in flight, with slopes of thorax temperature plotted against air temperature of 0.15 to 0.3 (Chappell, 1984b; 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 & Buchmann, 1991). In this study, I asked two questions: 1) Do *C. caesalpiniae* males thermoregulate during flight? 2) If so, then how? I measured the tagma temperatures, metabolic rate, water loss rate, and wingbeat frequency of large morph *C. caesalpiniae* males through the morning activity period at an aggregation site. I calculated a heat budget and convective conductances to quantitatively assess the relative importance of heat loss/gain pathways.

Methods

Animals: I located an active mating aggregation of *C. caesalpiniae* at a rural property in Scottsdale, Arizona (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 hind legs. I distinguished small morph males by their dark black, hairless abdomen and behaviorally by flight above the creosote bushes (Fig. 1.1). Large morphs, however, are typically found crawling, digging, or flying near female nest entrances. I caught large morph males in flight during late June and early July 2020, using sweep nets.

This study focuses on the thermoregulatory mechanisms of the large morph males due to abundance and ease of capture.

Figure 1.1. 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.

Carbon dioxide and water vapor emission measurements: To determine the metabolic and water loss rates of *C. caesalpiniae* large morph males during flight, I used flow-through respirometry. I 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 that of their natural, ambient conditions. Shaded air temperatures ranged from 19 to 38 °C across and within four days of measurements.

A SS-4 Sub-Sampler Pump (Sable Systems, Las Vegas, NV, USA) pumped ambient air at 1000 $mL \cdot min^{-1}$ 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_2-free air. Output of the chamber was directed to the sample cell of a LI-7000 CO_2/H_2O Gas Analyzer with the reference cell maintained at zero CO_2 by circulation through a scrubbing column (LI-COR, Lincoln, NE, USA). I recorded a baseline measurement, without an animal, for at least one minute. After introducing the bee to the chamber, I covered it with a dark cloth and allowed the system to flush for another three minutes to eliminate all vestiges of outside air. After removing the cloth, I stimulated the bees to fly by gently tilting the chamber, and recorded CO_2 and H_2O production during two to three minutes of flight. Bees hovered well in the chambers with little need for external stimulation. I calibrated the CO_2 analyzer, which is accurate to 0.1 $\mu mol \cdot mol^{-1}$ from 0-3000 $\mu mol \cdot mol^{-1}$, with dry, CO_2-free air and a compressed air tank containing certified (resolution: 0.01 $\mu mol \cdot mol^{-1}$) 252 ppm CO_2 span gas. The water analyzer is accurate to 1% of full scale from 0-60 $mmol \cdot mol^{-1}$. I digitized the analog data using a Sable Systems UI2 and recorded at 1 Hz using ExpeData (Sable Systems, v. 1.7.2) for Windows. I calculated average CO_2 and H_2O levels for 2-3 minute periods when bees were observed to be steadily hovering. I recorded flight behaviors for each bee, but all bees flew well and consistently, and I found no relationship between the flight behavior scores and flight metabolic rates, so these behavioral data are not reported.

To measure shaded air temperature, I used a BAT-12 thermometer and thermocouple. To assess that the metabolic chamber was perfectly air-tight, I measured CO_2 and H_2O levels over three to four minutes without an animal in the chamber; under these conditions there were no significant changes in CO_2 concentration. The 95% washout of CO_2 from the metabolic chamber occurred in approximately 90 seconds.

I calculated CO_2 production rate ($ml\cdot h^{-1}$) using Equation 1.2, with FR equal to flow rate ($m\cdot h^{-1}$), and F_{CO2} equal to the fractional CO_2 level ($\mu mol\cdot mol^{-1}$) in the excurrent air from the respirometry chamber during flight.

$$VCO_2 = FCO_2 * \mathrm{FR} \quad (1.2)$$

I calculated water loss rate, reported in mg $H_2O\cdot h^{-1}$, using Equation 1.3, where F_{H2O} equals to the average fractional level of H_2O ($mmol\cdot mol^{-1}$) in the excurrent air from the respirometry chamber during flight.

$$VH_2O = \frac{(FH_2O*\mathrm{FR}*18\mathrm{g/mol})}{(22400\ \mathrm{mL/mol})} \ (1.3)$$

Bee tagma temperatures: Immediately following the respirometry measures, I transferred the bee into a plastic bag, which I flattened onto a Styrofoam board to reduce conduction and restrict the bee's movement. I then measured head, thorax, and abdomen temperatures (T_h, T_{th}, T_{ab}, respectively) in random order within five seconds after cessation of flight by inserting a hypodermic thermocouple (Physitemp, MT-29/5HT Needle Microprobe, time constant = 0.025s) into the center of each tagma. I recorded the tagma temperature data with a Pico Technology USB TC-08 Thermocouple Data Logger (Tyler, TX, USA). I recorded the shaded air temperature values following the temperature measurements for each individual. I calculated temperature excess ratio (R_{tagma}) using Equation 1.1 (Baird, 1986).

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

Wingbeat frequency and flight score: I recorded the sound of wing movements during hovering flight in the flight chamber prior to each respirometry measurement for 20 to 30 seconds using the iPhone 7+ microphone. After wingbeat frequency measurement, I closed the chamber to flush CO_2 and H_2O before the respirometry measurement. Using a sound editing program, Audacity version 2.4.2 for Windows, I visualized the wingbeats. I calculated average wingbeat frequency by dividing the number of wing beats by the time duration for three separate measures of 10 wingbeats.

Total body surface area calculations: I used a digital caliper (accurate to 0.01 mm) to approximate body surface area using geometrical calculations. I assumed that the head of the bee is a cylinder, measuring head width as the diameter and head thickness as the height. I assumed that the thorax is a sphere measuring thorax width as the diameter. I assumed that the abdomen is a cylinder and a cone, with the 1st through 3rd terga of the abdomen being the cylinder and the 4th and 5th tergi being a cone (Roberts & Harrison, 1999). I 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 air. I calculated average T_{bee} (°C) using Equation (1.4) which weights each tagma according to its relative surface area (SA):

$$T_{\text{bee}} = \frac{(SA_{\mathrm{h}} * T_{\mathrm{h}}) + (\mathrm{SA}_{t} * \mathrm{T}_{t}) + (\mathrm{SA}_{ab} * \mathrm{T}_{ab})}{(SA_{\mathrm{h}} + SA_{\mathrm{t}} + SA_{\mathrm{ab}})} \quad (1.4)$$

Dorsal vessel dissection: I collected four, large morph *C. pallida* bees on April 29th, 2022, and stored them in Prefer™ (Anatech Ltd., Battle Creek, MI) fixative for four weeks before dissection occurred. To visualize the dorsal vessel, I removed the legs and wings, and made a coronal cut about 1 mm on either side of the petiole. I 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: I assumed that bees were flying at thermal equilibrium between 19 °C and 38 °C in steady-state conditions. This assumption is supported by observations for honey bees that body temperatures are stable during 1-5 min of flight (Roberts and Harrison 1999), the prolonged steady hovering exhibited by most of the bees, and the steady CO_2 emission traces I observed. Using Equation 1.5, I calculated a heat budget for flying bees at every degree between 19 °C and 38 °C where $Q_{\mathrm{metabolic}}$ indicates metabolic heat production, $Q_{\mathrm{radiation}}$ indications net radiative heat, $Q_{\mathrm{evaporation}}$ indicates evaporative heat loss, and $Q_{\mathrm{convection}}$ indicates net convective heat flux.

$$0 = Q_{\mathrm{metabolic}}+Q_{\mathrm{radiation}}+Q_{\mathrm{evaporation}}+Q_{\mathrm{convection}} \quad (1.5)$$

$Q_{\mathrm{metabolic}}$ and $Q_{\mathrm{evaporation}}$ were calculated from VCO_2 and VH_2O. Bees have mostly been reported to utilize carbohydrates as fuel for flight (Bertsch, 1984; Gäde & Auerswald, 1999; Suarez et al., 2005). Therefore, I assumed a respiratory quotient of 1 and 21.4 $J{\cdot}mL^{-1}$ CO_2 to calculate metabolic heat production in Watts. I then multiplied by 0.96 (the fraction of power input liberated as heat during flight) (Ellington, 1984; J. F. Harrison et al., 1996; Roberts & Harrison, 1999). To calculate evaporative heat loss in Watts, I multiplied VH_2O by the latent heat of evaporation of water, 2.45 $J{\cdot}mg^{-1}$ H_2O.

As I performed respirometry measurements in shade, I assumed shortwave radiation to be negligible. I summed the longwave (infrared) net radiation (R_{loss} – R_{gain}) for the head, thorax, and abdomen of each bee using the Stefan-Boltzmann equation:

$$Q_{\mathrm{radiation}} = \sigma(\varepsilon_s SA_h T_h^4 + \varepsilon_s SA_t T_t^4 + \varepsilon_s SA_{ab} T_{ab}^4 - a\varepsilon_c T_i^4) \quad (1.6)$$

I assumed that the bee's emissivity, ε_s, is 0.97, and that bee surface temperature equals bee internal temperature (Stupski & Schilder, 2021). I assumed that the bee's body surface absorptivity, *a,* is 0.97, that the emissivity, ε_c, of the glass metabolic chamber is 0.90, and that air temperature equals the wall temperature, T_i, of the glass chamber (Bolz & Tuve, 1973; Campbell, 1977; Stupski & Schilder, 2021). To estimate whole-bee radiative exchange, I summed $Q_{radiation}$ for the head, thorax and abdomen and multiplied by tagma surface area. I calculated convective heat exchange using Equation 1.7.

$$Q_{convection} = (-Q_{metabolic} - Q_{radiation} - Q_{evaporation}) \quad (1.7)$$

To calculate mass-specific convective conductances (κ is a measure of the capacity of the bee to transfer heat) in Watts·mm^{-2}·°K^{-1}, I divided convective heat transfer ($Q_{convection}$) in Watts by the total surface area of the bee and the gradient between air temperature and average bee surface temperature (in °K) by combining Equations 1.4 and 1.7:

$$\kappa = \frac{Q_{Convection}}{(T_{bee} - T_{air})} \quad (1.8)$$

Finally, to calculate Q_{10}, the factor by which metabolic rate increases over a 10 °C increase in air temperature, I used Equation 1.9 where R indicates metabolic rate and T indicates temperature in °C:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)} \quad (1.9)$$

Data Analysis: I tested data for normality, log base ten transformed the data if necessary, and ran all statistical analyses in RStudio (Version 1.3.1093 for Windows; RStudio: Integrated Development Environment for R. PBC, Boston, MA, http://www.rstudio.com/). I created figures in GraphPad Prism (Version 8.0.0 for Windows; GraphPad Software, San Diego, CA, www.graphpad.com). I included only large morph males in all analyses and excluded data if more than two standard deviations from the mean. I determined two-tailed significance at $\alpha = 0.05$. I used linear models to test for the effect of air temperature on wet and dry mass, tagma temperature, excess temperature 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 I felt confident using this data in my linear models due to my large sample size (*n*=68) and the fact that Gaussian models are robust against normality variations (Knief & Forstmeier, 2021). I report significant results below and all data are archived on Dryad.

Results

Body size trends and morphology: As air temperature increased through the morning, total wet body mass decreased (Fig. 1.2). Dry body mass showed the same trend (Fig. 1.2). Total body surface area (mean mm^2 = 481.69±8.76) declined with increasing air temperature (Fig. 1.2).

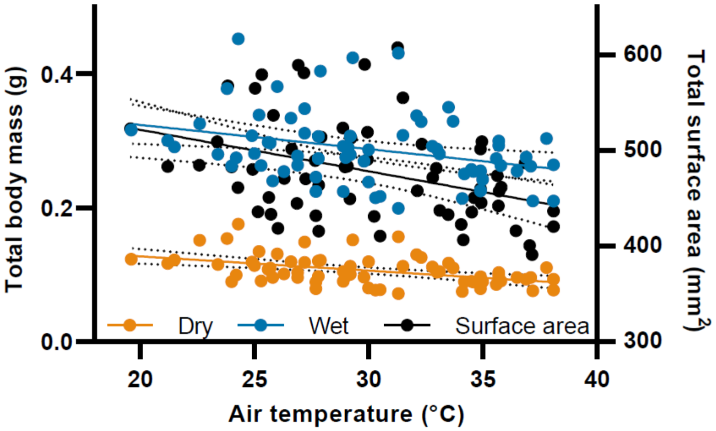

Figure 1.2. Total body mass and surface area versus air temperature for *C. caesalpiniae* large morph males. As air temperature increased through the day, wet mass, dry mass and total body surface area decreased. Dotted lines indicate 95% confidence intervals (CI). Linear regression: wet mass=−0.0036Tair+0.40, n=69, r2=0.10, P=0.0068, slope 95% CI (−0.0062, −0.0010); dry mass=−0.0021Tair+0.17, n=69, r2=0.21, P<0.0001, slope 95% CI

(−0.0031,−0.0011); total body surface area=−4.34Tair+608.1, n=62, r2=0.15, P<0.005, slope 95% CI (−6.99, −1.69).

Heads and thoraxes scaled hypometrically with body mass, while the abdomens scaled hyperallometrically (Fig. 1.3), indicating that heavier bees had relatively smaller heads and thoraxes, but larger abdomens compared to smaller bees. I found that *Centris pallida* large morph males had a dorsal vessel without petiolar loops that ran from the thorax to the abdomen.

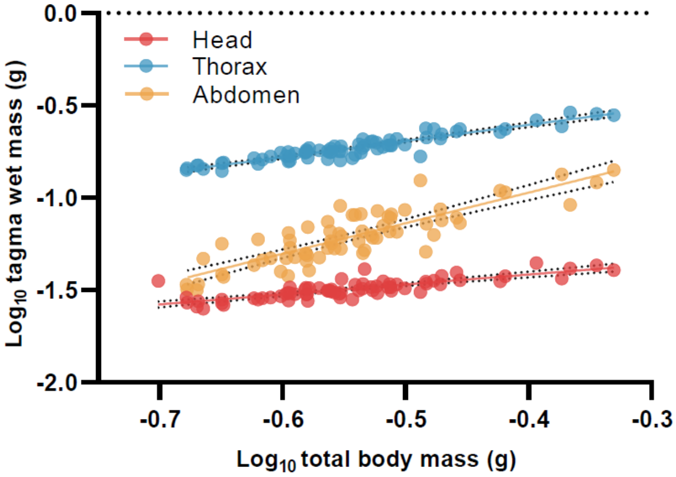

Figure 1.3. Allometric scaling of C. caesalpiniae large morph male body tagma. Head (M_h; g) and thorax mass (M_{thorax}; g) scaled hypometrically with body mass (M_b; g), while abdomen mass (M_{ab}; g) scaled hyperallometrically. Linear regression: $[M_h]=0.54*[M_b]-1.20$, $r^2=0.66$, $P<0.0001$, slope 95% CI (0.45, 0.63); $[M_{thorax}]=0.88*[M_b]-0.25$, $r^2=0.88$, $P<0.0001$, slope 95% CI (0.80, 0.95); $[M_{ab}]=1.65*[M_b]-0.32$, $r^2=0.73$, $P<0.0001$, slope 95% CI (1.41, 1.91). n=69 for all parameters.

Tagma temperatures following hovering flight in a shaded, metabolic chamber: Order of temperature measurement did not affect tagma temperatures, suggesting that stress or time effects associated with the measurements on body temperatures were not significant. Thorax temperatures increased by 0.37°C for every 1 °C increase in air temperature (Fig. 1.4). 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. 1.4).

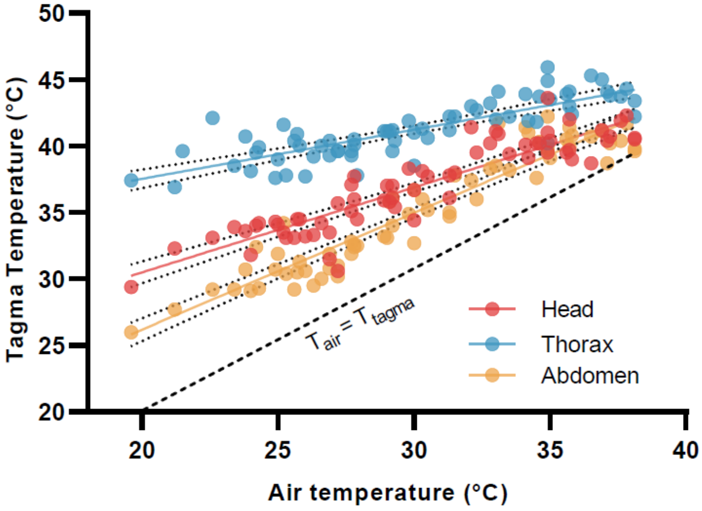

Figure 1.4. Tagma temperature versus air temperature for *C. caesalpiniae* males flying in a shaded glass chamber. Tagma (T_{tagma}), head (T_h), thorax (T_{thorax}), and abdomen (T_{ab}) temperature increased with air temperature (T_{air}). Linear regressions: $[T_h]=0.64*[T_{air}]+17.70$, n=67, $r_2=0.83$, $P<0.0001$, slope 95% CI(0.57,0.71); $[T_{thorax}]=0.37*[T_{air}]+30.13$, n=68, $r_2=0.68$, $P<0.0001$, slope 95% CI(0.31,0.43); $[T_{ab}]=0.88*[T_{air}]+8.62$, n=66, $r^2=0.89$, $P<0.0001$, slope 95% CI (0.80,0.96). Dashed line indicates where T_{air} equals T_{tagma}.

The abdominal excess temperature ratio increased with air temperature (Fig. 1.5), indicating that heat is actively transferred from the thorax to abdomen at higher air temperatures. In contrast, heat transfer from the thorax to the head appeared to be passive (Fig. 1.5).

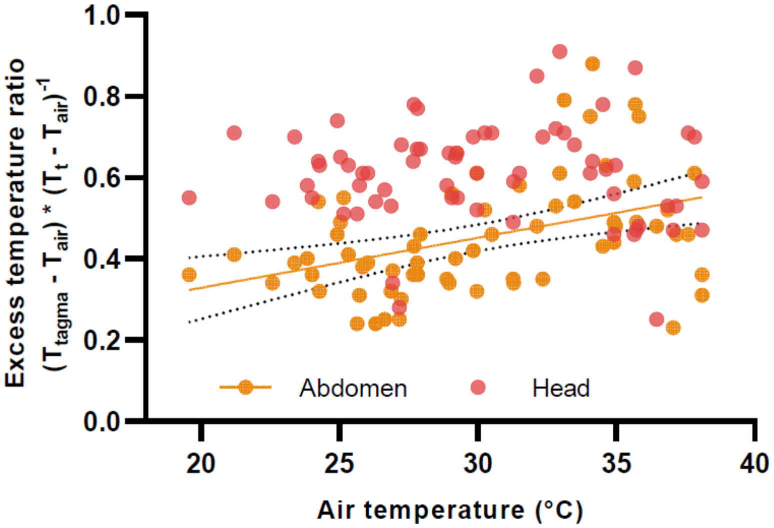

Figure 1.5. Temperature excess ratio versus air temperature for *C. caesalpiniae* males flying in a shaded glass chamber. Abdominal temperature excess ratio $[R_{ab}=(T_{ab}-T_{air})\cdot(T_{thorax}-T_{air})^{-1}]$ increased with increasing air temperature (T_{air}). Linear regression: $[R_{ab}]=0.012*[T_{air}]+0.082$, 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±0.015, n=66.

Metabolic rate, water loss rate, and wingbeat frequency: The mean body mass of the *C. caesalpiniae* males used for respirometry was 290±0.053 mg (range 199–467 mg). Metabolic rate (measured in watts) significantly increased with body mass measured in grams (Fig. S1.1). Mass-specific metabolic rate (mL $CO_2\cdot g^{-1}\cdot h^{-1}$) increased linearly with air temperature (Fig. 1.6A), while metabolic rate not corrected for body mass (mL $CO_2\cdot h^{-1}$) did not vary with air temperature (Fig. 1.S2A).

The Q_{10} for mass-specific metabolic rate (mL CO_2·g^{-1}·h^{-1}) was 1.13. Mass-specific water loss rates (mL H_2O·g^{-1}·h^{-1}) were highly variable but increased with air temperature when log-corrected (Fig. 1.6B), and water loss rate not corrected for body mass showed no variation with air temperature (Fig. 1.S2B). Wingbeat frequency did not vary with air temperature or body mass (mean Hz = 166±2, Fig. 1.6C).

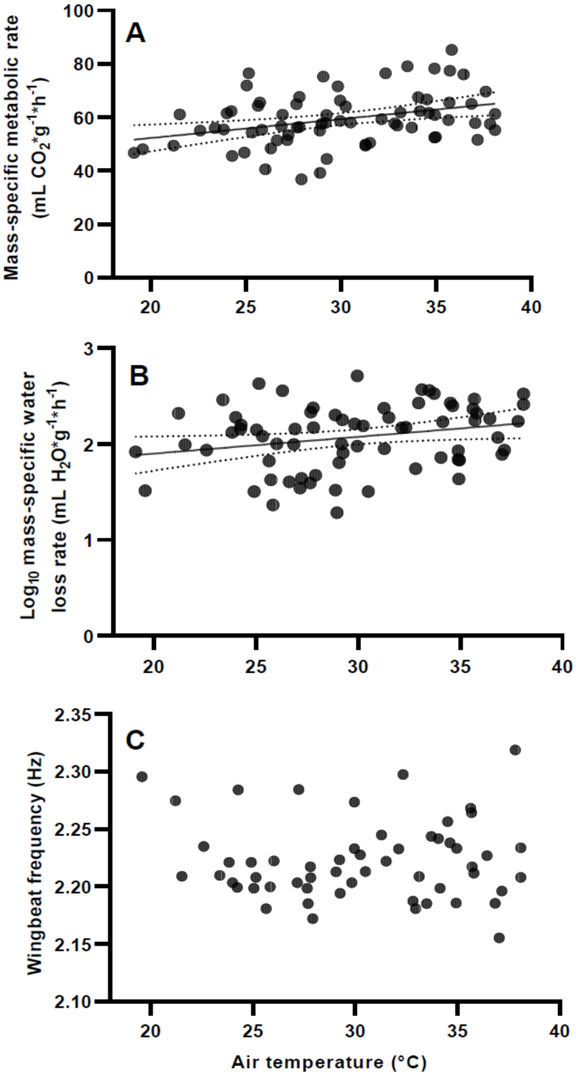

Figure 1.6. Mass-specific metabolic and water loss rate and wingbeat frequency versus air temperature for *C. caesalpiniae* males flying in a shaded glass chamber. (A) Mass-specific metabolic rate (VCO_2) increased with air temperature. Linear

regression: $[VCO_2]=0.71*[T_{air}]+38.02$, $r^2=0.12$, $P=0.0027$, n=71, slope 95% CI (0.26,1.17). (B) Log-transformed mass-specific water loss rate (VH_2O) increased with air temperature. Linear regression: $[Log_{10}(VH_2O)]=0.018*[T_{air}]+1.55$, $r^2=0.063$, $P=0.0364$, n=70, slope 95% CI (0.0011,0.034). (C) Wingbeat frequency (n=56) did not vary significantly with air temperature.

Heat budget model: Mass-specific metabolic heat production increased with air temperature (Fig. 1.7). Evaporative heat loss was a minor part of the heat budget, and slightly but significantly increased with air temperature (Fig. 1.7). Mass-specific convective heat loss significantly increased, and mass-specific radiative flux decreased with air temperature.

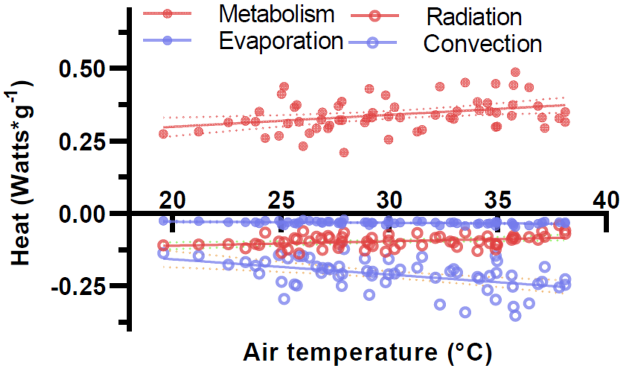

Figure 1.7. Effect of air temperature on mass-specific routes of heat production and loss for *C. caesalpiniae* males flying in a shaded metabolic chamber. Metabolic ($Q_{metabolic}$), evaporative ($Q_{evaporation}$), convective ($Q_{convection}$) and radiative ($Q_{radiation}$) heat flux are shown against air temperature (T_{air}). Linear regression: $[Q_{metabolic}]=0.0041*[T_{air}]+0.22$, $r^2=0.12$, $P=0.0054$, slope 95% CI (0.0013, 0.0070); $[Q_{evaporation}]=-0.00040*[T_{air}]-0.020$, $r^2=0.12$, $P=0.0047$, slope 95% CI (−0.00068, −0.00013); $[Q_{convection}]=-0.0053*[T_{air}]-0.053$, $r^2=0.23$, $P<0.0001$, slope 95% CI (−0.0079, −0.0028); $[Q_{radiation}]=0.0013*[T_{air}]-0.18$, n.s. n=63 for all parameters.

Convective conductance increased with air temperature (Fig. 1.8). 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 watts with temperature (Fig. 1.S3).

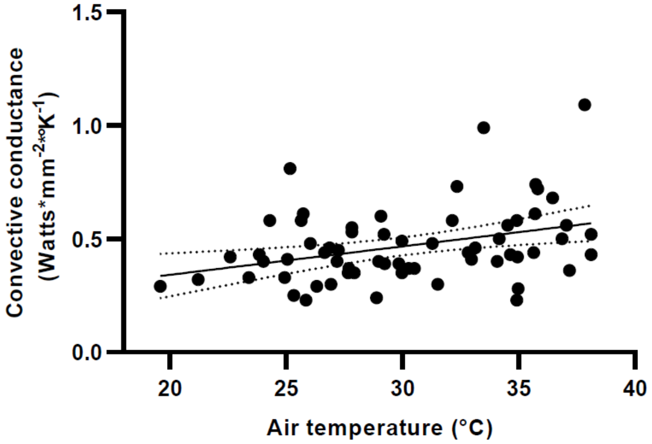

Figure 1.8. Mass-specific convective conductance versus air temperature for *C. caesalpiniae* males flying in a shaded glass chamber. Mass-specific convective conductance (κ) increased as air temperature (T_{air}) rose. Linear regression: [κ]=0.013*[T_{air}]+0.091, r^2=0.13, P<0.005. n=63; absolute values are plotted for clarity.

Discussion

Centris caesalpiniae use an abdominal convector during flight

To partially regulate thorax temperatures during flight, *Centris caesalpiniae* males actively varied heat transfer from the thorax to the abdomen, as evidenced by the convergence of thoracic and abdominal temperatures as air temperature rose (Fig.

4) and the increase in the abdominal excess temperature 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. 4,5). As the air warmed, the rise in the abdominal temperature excess ratio indicated active, increasing heat transfer from the thorax to the abdomen, likely by circulating hemolymph.

I found that *C. pallida* large morph males had a dorsal vessel that runs directly from the abdomen through the petiole without petiolar loops like those that occur in *A. mellifera*, possibly to act as counter-current heat exchangers (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 as documented for the sphinx moth, *Manduca sexta*, and the bumblebee, *Bombus vosnesenskii*. In these animals, the rate of warm hemolymph flow from the thorax to the abdomen increases due to 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. 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. This active transfer of warm blood to the uninsulated abdomen 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

I found that the large morph *C. caesalpiniae* males used an abdominal convector heat loss mechanism, whereas, small morph *C. pallida* males did not (Roberts et al., 1998). At present, it is not clear whether this represents a species or a 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-to-volume ratio and likely experience high conductive and radiative heat gain from the ground (ground temperatures can reach 58°C), and likely experience 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 principle means to thermoregulate during flight (Roberts et al., 1998). In contrast, I 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 *C. caesalpiniae* males flown in the shade in my studies. One plausible explanation for this difference is that metabolic rates only decrease under conditions in which flight muscle temperatures rise well above optimal (J. F. Harrison & Fewell, 2002) . In my study, thorax temperatures for large morph *C. caesalpiniae* averaged 41°C and peaked at about 44°C (Fig. 2), whereas thorax temperatures 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 & Buchmann, 1991), suggesting that with solar heat load and possibly higher thorax temperatures, metabolic heat production might decline in *C. caesalpiniae*

Another possibility is that large morph *C. caesalpiniae* males cannot reduce their metabolic rates 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, on the other hand, constituted only 15% of body mass (Chappell, 1984b). 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 sizes 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 rates. 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 38°C (Roberts & Harrison, 1999).

Bigger bees are active earlier in the activity period

A striking and somewhat surprising finding was that the wet mass and dry mass decreased as the day progressed by 22% and 32%, 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 due to desiccation stress. Plausibly, larger bees were more active in the cool early morning because they are more capable of endothermic elevation of thorax temperatures due to their lower surface-to-volume ratios. Additionally, because most *C. caesalpiniae* females emerged early in the day (Johnson and Glass, pers. obs.), 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 for larger males is reduced.

Surface area and mass affects 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 temperatures rose strongly affected my heat budget and data interpretation. I 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 I had ignored the decline in body mass and simply used watts, I 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 temperatures 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). I found that convective conductance ($\text{Watts}\cdot\text{mm}^{-2}$) 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 large, relatively bare abdomen. This provides the first quantitative estimate of the thermoregulatory value of the abdominal convector. In contrast, net radiative heat loss declined significantly with rising air temperature (Fig. 7), as the temperature gradient between the bee and the air significantly decreased through the morning.

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. My heat budget model utilized literature values for absorbances and emissivities; however, these can vary substantially among the insects (Shi et al., 2015; Stupski & Schilder, 2021; Tsai et al., 2020; Wang et al., 2021), and have the potential to affect the 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 temperatures and performance will be affected by climatic warming. Additional critical research needs will be to determine how flight performance (e.g., foraging load carriage, mating success) is affected by air and body temperature to predict how environmental conditions will influence fitness.

For *Centris* species in particular, and for other large flying endothermic insects, I should endeavor to untangle the limitations on activity period, i.e., whether high temperature, low water availability and desiccation (or neither) causes 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 investigate 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.

CHAPTER 2

THE EFFECTS OF SOLAR RADIATION ON A FLYING DESERT BEE, *CENTRIS PALLIDA*

Introduction

Anthropogenic climate change is predicted to result in increased air temperatures by at least 1.5 °C on average in the next twenty years (Kikstra et al., 2022). However, certain areas, such as the Sonoran Desert, are expected to be differentially affected, becoming even hotter and drier (Weiss & Overpeck, 2005) with a predicted increase in extreme heat events (Perkins & Alexander, 2013). As a result, desert vegetative landcover may shift in range, change phenology, and/or decline in abundance (Bowers, 2007; Herrmann et al., 2016), altering the microclimatic landscape for animals. These trends make it very important to understand the thermal biology of important insects such as pollinating desert bees, as the impact of climate change on pollinators may add yet another challenge to plant species persistence as a result of climate change (Janeba, 2009; Simpson & Neff, 1987). Here, I assess the impact of solar radiation on the thermal balance of a flying endothermic bee, *Centris pallida*, a solitary bee native to the Sonoran Desert. *Centris pallida* is a major pollinator of important local plants including *Parkinsonia microphylla*, *Olneya tesota, Prosopis velutina, Psorothamnus spinosus*, *Krameria bicolor,* and *Larrea tridentata* (Alcock et al., 1977; Rozen & Buchmann, 1990).

Solar radiation can be a critical determinant of body temperature for terrestrial ectotherms, both vertebrate and invertebrate, many of whom shuttle between sun and shade to thermoregulate (Kearney et al., 2009; Ma et al., 2018; Seebacher & Franklin, 2005). Surprisingly, there has been relatively little study of how solar radiation affects the thermal biology of terrestrial endotherms, and to my knowledge, no studies of the effect of solar radiation on heat budgets of flying endotherms. Direct solar radiation can impose a heat load twenty times the surface area-specific metabolic rate of mammals; however, the pelage of many mammals and birds acts as a heat shield, preventing much of the theoretical heat-loading (Maloney & Dawson, 1995; Tattersall et al., 2012; Walsberg & Wolf, 1995; Wolf & Walsberg, 1996). In nonflying mammals and birds, solar radiation can strongly depress metabolic rate, increase evaporative water loss rates (Cain et al., 2006; Wolf & Walsberg, 1996), and induce movement to cooler microclimates (Fuller et al., 2016). Given the well-known tendency of heat-stressed mammals and birds to dump heat by increasing thermal conductance by mechanisms such as shunting blood flow to poorly-insulated surfaces (Tattersall et al. 2012), it seems likely that this may occur in response to solar radiation in insects. I am unaware of studies that document such an effect, though there is evidence that butterflies modulate conductance between wings and body to vary heat transfer during solar basking (Kingsolver & Moffat, 1982). Hyperthermia is also a well-known response of vertebrate endotherms to high temperatures (Tattersall et al. 2012), and so seems to be a plausible response to solar radiation for flying invertebrates.

Solar radiation effects on flying endothermic insects have mainly focused on the benefits of solar radiation as it facilitates the warming of flight muscles in order to fly (Heinrich, 1986; Kovac et al., 2009, 2010; Stabentheiner & Kovac, 2023). Solar radiation allows small butterflies to attain the thorax temperatures necessary for flight, with minimal contribution from metabolic heat production (Tsuji et al., 1986). Like ectothermic nonflying insects, many endothermic flying insects change body positions and location, basking to raise body temperatures (Clench, 1966; Heinrich, 2013; Kevan & Shorthouse, 1970; Stabentheiner & Kovac, 2023; Whitman, 1987). In the context of climatic warming, it is also important to examine the potential role of solar radiation in influencing the thermal balance of endothermic flying insects. Plausibly, for endothermic fliers at high temperatures, the addition of heat gain due to solar radiation could be deadly.

As for vertebrate endotherms, the potential responses to solar radiation combined with high air temperatures for flying insect endotherms include hyperthermia, reduction in metabolic heat production, increased thermal conductance, and increased evaporative heat loss. Most flying insect endotherms imperfectly regulate their thorax and to a lesser extent, head temperatures, as air temperature rises (Heinrich, 2013; Johnson et al., 2022, 2023a). It is therefore reasonable to predict that solar radiation will increase the body temperatures of flying bees. Flight metabolic rates decrease with air temperature in some flying insect endotherms, including honey bees (Borrell & Medeiros, 2004; Glass & Harrison, 2022; Roberts & Harrison, 1998b, 1999) and *Centris pallida* small morph males (Roberts et al., 1998), but *C. caesalpiniae* maintained constant flight metabolic rates (mL $CO_2 \cdot h^{-1}$) across air temperatures of 19 to 38 °C (Johnson et al., 2022). Many, but not all, flying endotherms increase thermal conductance at higher air temperatures, so this is a plausible response to solar radiation. *Centris caesalpiniae* large morph males strongly increase convective conductance at higher air temperatures, likely by shifting hot hemolymph from the well-insulated thorax to the relatively bare abdomen (Johnson et al., 2022). Similar responses have been seen in *Bombus vosnesenskii* queens, *Hyles lineata, Manduca sexta*, *Sarcophoga,* and *Melipona subnitida*, but not *Apis mellifera*, *B. vagans*, *Vespula*, or *Anax junius* (Johnson et al., 2022). Increasing evaporative water loss is a major mechanism of thermoregulation in honey bees, but plays a minor role in thermoregulation at higher air temperatures for *C. pallida* small morph males and *C. caesalpiniae* large morph , which range in mean mass from approximately 150 to 293 mg, respectively (Johnson et al., 2022; Roberts et al., 1998). Small body sizes and high surface-to-volume ratios may cause natural selection to favor flying insect pollinators that minimize evaporative water losses to prevent dangerous desiccation.

As noted above, *C. pallida* males are dimorphic. The *C. pallida* male morphs differ in mating strategies (Alcock et al., 1977), body size, and coloration, with hair-like structures (setae) conferring possible thermal benefits (Barrett & O'Donnell, 2023). Large morph males are highly reflective in the near-infrared (NIR) on the dorsal surface compared to small morph males due to the presence and abundance of setae, reducing absorbed solar energy (Barrett & O'Donnell, 2023). Based on size, I expect surface-to-volume ratios to be greatest in small morph males, intermediate in females, and smallest in large morph males. Based on the surface area and volume relationships, I predicted that all responses (body warming, as well as any thermoregulatory response) would be greatest in small morph males, intermediate in females, and least in large morph males. Alternatively, differential microclimate use by the morphs and sexes may cause differences; large male morphs are mostly found on the hotter bare ground, while smaller morphs and females are more often in cooler air well above ground or within shaded vegetation. Plausibly, large morph males may have evolved greater capacities to cope with radiant heating because they spend more time in hot microclimates. I tested the various possible mechanisms by which these desert-adapted bees would respond to solar radiation; do they reduce flight metabolic rate, increase evaporative cooling, store incident heat, increase convective or radiative heat loss, or some combination of these?

Methods

Animals: In April 2022, I studied an active mating and nesting aggregation of *C. pallida* males and females (Fig. 2.1) in the flood plains near the Salt River (GPS coordinates: 33.55, –111.56). Males are dimorphic and exhibit sized-based mating strategies (Alcock et al., 1977; Barrett et al., 2021). Large morph males are often found on sunny ground, digging and fighting for unmated females, but also make sweeping patrol flights between shaded and sunny microclimates. Small morph males hover in partially shaded microclimates, also flying between shade and sun. I divided males into large and small categories based on body mass, morphological features, and behavior at the time of capture. *Centris pallida* males are behaviorally rigid, with I selected fresh-looking bees with very little to no wing wear or “balding” on the thorax for all measures conducted.

Figure 2.1 *Centris pallida* bees. (A) A large morph male *C. pallida* (top) mating with a female (bottom) (B) Large morph males fighting in a “bee ball” for an unmated female. Photos by Bruce Taubert.

Flight score: I assigned a score of from zero to five during the respirometry measures to indicate the quality of hovering flight in the metabolic chamber. 0 = no flight, 1 = buzzing and crashing with little flight control, 2 = unstable flight (<25% of the time) with frequent crashing (>10) , 3 = some hovering flight (~50% of the time) with frequent crashes (3-6), 4 = good hovering flight (~75% of the time) with infrequent crashes (<3), 5 = great hovering flight with almost no crashes (<1).

Surface area measures: I used a digital caliper (accurate to 0.01 mm) to measure tagma surface areas assuming that the head is a cylinder, the thorax is a sphere, and the 1-3rd terga are a cylinder, and the 4-5th terga are a cone (Johnson et al., 2022; Roberts & Harrison, 1999). I did not include antennae, wing, or leg surface areas in the total body surface area calculation.

Field respirometry and heat budget calculations: I used flow through respirometry to measure the CO_2 and H_2O emissions of *C. pallida* bees during flight in the shade versus in full sun. I set up the respirometry station on a table beneath a grove of shady palo verde trees (*Parkinsonia microphylla*) in the middle of the aggregation site to achieve full shade. For the "sunny measurements", I held a 500 mL borosilicate chamber in which the bee flew in full sun, keeping equipment in the shade. Borosilicate transmits 87% all of the wavelengths of solar radiation (300-2500 nm; Zhu et al., 2017). I set up the respirometry equipment in the same configuration as Johnson et al., 2022. Following flight in the chamber, I recorded head, thorax, and abdomen temperatures in addition to air temperature using a BAT-12 thermometer and hypodermic thermocouple (Physitemp, MT-29/5HT Needle Microprobe, time constant = 0.025 s). I stored the bees in individual vials, placed in an insulated cooler. I measured wet masses within three hours of leaving the field site. I calculated heat budgets and convective conductances (κ) as in Johnson et al., 2022 with the addition of heat gain from solar radiation for bees flying in the sun (Equation 2.1).

Excess temperature ratio (R_{tagma}) calculations: Excess temperature ratio is the amount that tagma (head or abdomen) temperature is elevated over air temperature relative to thorax temperature elevation over air temperature. To determine if there was active transfer of heat (i.e., movement of heat facilitated by an activity such as abdominal pumping) between the thorax and the abdomen or head, I calculated excess temperature ratios of the head (R_h) and abdomen (R_{ab}) as in Johnson et al, 2022. R_{tagma} will be constant and independent of air temperature (T_{air}) if heat moved from the thorax to the head or abdomen by mainly passive conduction. If heat was actively transferred from the thorax to the head or abdomen at high T_{air}, then R_{tagma} will increase with rising air temperature.

Solar radiation measurements and calculations: To calculate the heat gain from solar radiation (Q_S) for bees flying in the sun, I summed the solar heat gained directly from the beam (Q_b), reflected from the surroundings (Q_{ref}), and from diffuse radiation (Q_d).

$$Q_s = [\alpha_b \cdot A_b \cdot I_b] + [\alpha_r \cdot A_r \cdot I_r] + [\alpha_d \cdot A_d \cdot I_d] \quad (2.1)$$

α_b, α_r, and α_d represent the bee absorption coefficient, A_b, A_r, and A_d represent the bee surface area (m^2) exposed to each route of solar radiation exposure, and I_b, I_r, and I_d represent the irradiance ($\text{Watts} \cdot m^{-2}$) of the beam, reflected, and diffuse source, respectively. I assumed that $A_b = 0.25 \cdot A_s$, where A_s=total surface area, and both A_d and $A_r = 0.5 \cdot A_s$ (Stupski & Schilder, 2021). I assumed α_d to be equal to α_b (Stupski & Schilder, 2021). I obtained and calculated α_r and α_b values from Barrett and O’Donnell, 2023. The coefficient of absorption differs between the thorax and abdomen for *C. pallida* females, but not for males (Barrett & O’Donnell, 2023). Because the coefficient of absorption has not been measured for heads, I assumed that head coefficients of absorption were identical to thoraxes as heads are similarly colored and covered in setae, and calculated the heat gain from solar radiation separately for the head plus thorax and the abdomen:

$$Q_s = (Q_{b\text{-head+thorax}} + Q_{b\text{-abdomen}}) + (Q_{ref\text{-head+thorax}} + Q_{ref\text{-abdomen}}) + (Q_{d\text{-head+thorax}} + Q_{d\text{-abdomen}}) \quad (2.2)$$

I measured $I_b + I_d$ with a pyranometer and assumed that $I_r = 0.245\cdot I_b$ for Sonoran Desert soils (Marion, 2020). I set up a LI-COR LI-200 pyranometer, calibrated such that 1000 W*m^{-2} yielded 90 μA) output, connected to a type SZ sensor on a level surface in full sun to obtain measures of direct beam plus diffuse solar radiation ($I_b + I_d$; Watts*m^{-2}) during each respirometry trial for bees flying in the sun. I checked the older LI-200 pyranometer against an Eppley Model 8-48 Black and White pyranometer on a sunny, clear day in Philadelphia, PA, comparing simultaneous readings from both pyranometers over the course of the morning. The calibration against the newer Eppley pyranometer suggested that my LI-200 readings underestimated the amount of incident solar radiation. Multiplying the LI-200 reading by 1.1958 corrected this underestimation.

I calculated I_d, assuming it was constant through the day, using a formula derived from Becker, 2001 (Equation 2.3):

$$I_d = C_j\cdot(D_R + D_B) \quad (2.3)$$

C_j is a seasonal correction = $1+0.11\cdot\cos[(J-15)\cdot(2\pi\cdot 365^{-1})]$; J = the number of days in the year. D_R indicates diffuse radiation by Raleigh dispersion in watts·m^{-2} = $39.78\cdot\sin(h)^{0.35}$; h = solar angle. D_B indicates diffuse radiation by vapor dispersion in watts·m^{-2} = $2.6\cdot\sin(h)^{0.66}\cdot(10^3\cdot B-12)^{0.81}$; B = turbidity coefficient from Randerson 1973. I assumed that cloud interference at low, medium, and high altitudes was zero, and therefore omitted this portion of the equation. I subtracted I_d from the pyranometer reading to get I_b.

Data Analysis: I tested data as to whether the assumptions of parametric statistics were met, log base ten transformed the data if necessary, and ran statistical analyses in GraphPad Prism (Version 8.0.0 for Windows; GraphPad Software, San Diego, CA, www.graphpad.com). I included and compared both male morphs and female *C. pallida* bees in all analyses. I excluded data if it was more than two standard deviations from the mean, and, if the data in question corresponded with field notes indicating a problem with the quality of measurement. I included only bees that were flying in all respirometric analyses, as determined by a non-zero flight score. If means and slopes are presented, I included the 95% confidence limits. I determined two-tailed significance at $\alpha = 0.05$ for all analyses and chose the best general linear models (GLMs) based on AIC.

I tested for effects of air temperature on tagma temperatures of bees flying in the sun and shade. I used a GLM to test for two- and three-way interactions between location (sun or shade), morph (large morph male, small morph male, or female), and air temperature, with tagma temperature as the dependent variable.

I used a one-way ANOVA to compare R_h, R_{ab} VCO_2 , VH_2O, Q_M, Q_C, Q_R, Q_E, and κ as dependent variables, with location (shade or sun) as the independent variable. I used the Šídák multiple comparisons test, as each comparison (sun versus shade for each morph) was independent of each other (Abdi, 2007). I compared the scaling of $\log_{10}$ transformed total body wet mass and VCO_2 using an ANCOVA. I tested for interactive effects of location, morph, and air temperature for all dependent variables using a GLM.

Results

Sunny versus shaded tagma temperatures in the respirometry chamber

Bees measured in the sun versus shade did not differ in mass or surface area regardless of morph (Table 2.1, ANOVA, $F_{(1,139)}=3.49$, $P=0.64$). Large morph males had the smallest surface area to volume ratios, followed by females, and small morph males.

Air temperatures were higher for bees measured in the sun (Table 2.1; unpaired t-test: $t_{(151)}=5.68$, $P<0.0001$). Higher air temperatures increased all tagma temperatures (Table 2.1). I analyzed each body tagma's response to solar radiation and temperature separately.

Sex affected how head temperature elevation was affected by air temperature (significant 2-way morph*air temperature interaction, Table 2.2). The elevation of head temperature relative to air temperature (T_h – T_{air}) did not vary between shaded and sunny conditions (Table 2) but declined from about 15 °C to 5 °C as air temperature rose from 18 to 37 °C. Females had shallower slopes of T_h – T_{air} on air temperature than males (Fig. 2.2A).

Thorax temperature elevations over air temperature were significantly increased by solar radiation and decreased as air temperature rose (Fig. 2.2B, Table 2.3). Thorax temperature elevation did not differ between male morphs and females (Table 2.3). Across all bee types (large males, small males, and females), solar radiation increased thorax temperatures by 1.7 °C at 30 °C air temperature.

I found that solar radiation elevated abdomen temperatures in the sun (Table 2.4). Given the diverse trends, I analyzed the bee types separately. For females (Fig. 2.2C), solar radiation caused an abdominal temperature elevation difference of 5 °C at 30 °C air temperature. For large male morphs, 3.4 °C (Fig. 2.2D). For small morph males, 2.3 °C (Fig. 2.2E). On average, the abdominal temperature elevation was 3 °C hotter for bees flying in the sun (Table 2.4).

Location	**Variable**	**Female**	**Large morph male**	**Small morph male**
Sun and shade	Mean wet mass (mg) mean ± 95% CI and range	207.9, 95% CI (198.8, 217.1), range (108.5, 278.7)	270.4, 95% CI (261.6, 217.1), range (158.1, 279.2)	160.1, 95% CI (156.2, 232.7), range (93.42, 163.9)
Sun and shade	Mean total body surface area (mm^2)	370.6, 95% CI (355.7, 413.3), range (312.5, 493.3)	400.9, 95% CI (388.5, 413.3), range (312.5, 493.3)	317.7, 95% CI (303.1, 332.3), range (229.4, 440.1)
Shade	Air temperature (˚C)	26.1, 95% CI (25.2, 27.0), range (16.4, 33.4)		
Sun	Air temperature (˚C)	29.2, 95% CI (28.6, 29.8), range (23.4, 37.3)		
Shade	$T_{th} \sim T_{air}$	$T_{th} = 0.24 * T_{air} + 35.06$, n=21, **n.s.**	$T_{th} = 0.23 * T_{air} + 35.7$, $r^2=0.23$, 95% CI slope (0.054 to 0.41), n=26	$T_{th} = 0.40 * T_{air} + 31.12$, $r^2=0.28$, 95% CI slope (0.099 to 0.70), n=22
Shade	$T_{ab} \sim T_{air}$	$T_{ab} = 0.87 * T_{air} + 9.57$, $r^2=0.91$, 95% CI slope (0.74 to 1.00), n=21	$T_{ab} = 0.82 * T_{air} + 10.8$, $r^2=0.86$, 95% CI slope (0.68 to 0.96), n=26	$T_{ab} = 0.81 * T_{air} + 10.91$, $r^2=0.72$, 95% CI (0.58 to 1.05), n=22
Shade	$T_{h} \sim T_{air}$	$T_{h} = 0.61 * T_{air} + 20.53$, $r^2=0.66$, 95% CI slope (0.40 to 0.82), n=21	$T_{h} = 0.28 * T_{air} + 29.5$, $r^2=0.35$, 95% CI slope (0.12 to 0.44), n=26	$T_{h} = 0.59 * T_{air} + 20.75$, $r^2=0.53$, 95% CI (0.33 to 0.85), n=22
Sun	$T_{th} \sim T_{air}$	$T_{th} = 0.98 * T_{air} + 15.08$, $r^2=0.69$, 95% CI slope (0.64, 1.31), n=19	$T_{th} = 0.21 * T_{air} + 38.4$, $r^2=0.08$, 95% CI slope (-0.057 to 0.48), n=31	$T_{th} = 0.38 * T_{air} + 33.03$, $r^2=0.38$, 95% CI slope (0.12 to 0.64), n=18
Sun	$T_{ab} \sim T_{air}$	$T_{ab} = 1.71 * T_{air} - 11.81$, $r^2=0.80$, 95% CI slope (1.27, 2.16), n=19	$T_{ab} = 0.78 * T_{air} + 15.4$, $r^2=0.52$, 95% CI slope (0.50 to 1.07), n=31	$T_{ab} = 0.60 * T_{air} + 20.03$, $r^2=0.66$, 95% CI slope (0.37 to 0.83), n=18
Sun	$T_{h} \sim T_{air}$	$T_{h} = 1.08 * T_{air} + 8.00$, $r^2=0.64$, 95% CI slope (0.66, 1.51), n=19	$T_{h} = 0.58 * T_{air} + 23.2$, $r^2=0.49$, 95% CI slope (0.35 to 0.81), n=31	$T_{h} = 0.48 * T_{air} + 25.78$, $r^2=0.55$, 95% CI slope (0.25 to 0.71), n=18

Table 2.1. Effects of location (sun versus shade) and morph/sex on wet masses, surface areas, air temperatures, and the slopes of tagma temperatures on air temperatures for *C. pallida* bees flying in a respirometry chamber. Equation of the linear regression, r^2, 95% confidence interval of the slope, and sample size (n) are reported in each box. All linear regressions are significant unless otherwise indicated by n.s.

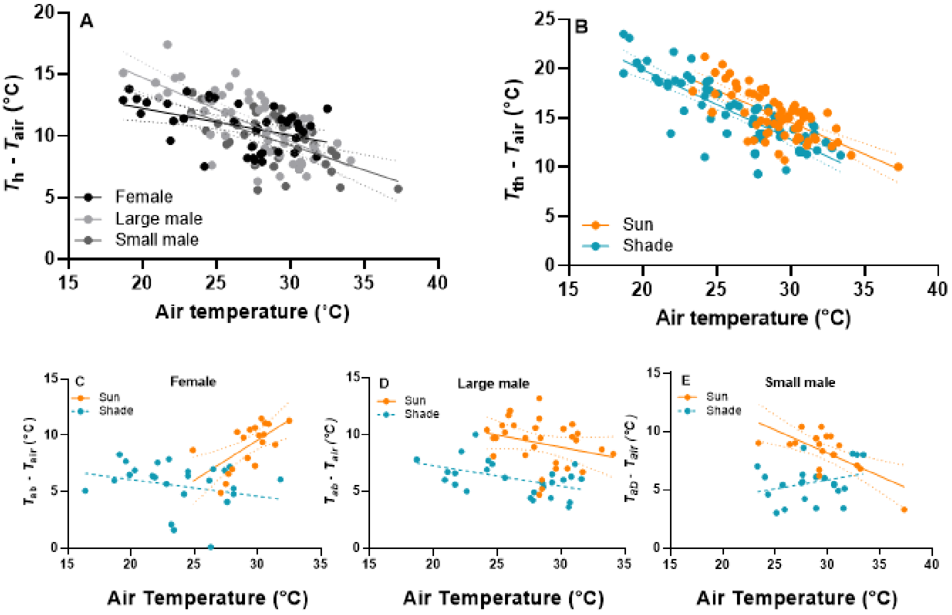

Figure 2.2 Tagma temperature elevation of *Centris pallida* males and females flying in a shaded or sunny 500 mL respirometer. (A) Head temperature elevation decreased with air temperature for all bees. Large males: $[T_h - T_{air}]=-0.52*[T_{air}]+25.05$, n=57, $r^2=0.46$, $P<0.0001$, slope 95% CI (-0.66,-0.37). Small males: $[T_h - T_{air}]=-0.40*[T_{air}]+21.26$, n=40, $r^2=0.33$, $P<0.0001$, slope 95% CI (-0.59,-0.21). Females: $[T_h - T_{air}]=-0.22*[T_{air}]+16.62$, n=40, $r^2=0.22$, $P<0.0001$, slope 95% CI (-0.36,-0.082). (B) Thorax temperature elevation decreased with air temperature for all bees with an effect of location, but not morph. Sun: $[T_{th} - T_{air}]=-0.65*[T_{air}]+34.03$, n=68, $r^2=0.48$, $P<0.0001$, slope 95% CI (-0.82,-0.48). Shade: $[T_{th} - T_{air}]=-0.70*[T_{air}]+33.97$, n=69, $r^2=0.66$, $P<0.0001$, slope 95% CI (-0.83,-0.58). (C) Female abdomen temperature elevation. Sun: $[T_{ab} - T_{air}]=0.71*[T_{air}]-11.81$, n=19, $r^2=0.40$, $P=0.0035$, slope 95% CI (0.27,1.16); Shade: $[T_{ab} - T_{air}]=-0.15*[T_{air}]+9.05$, n=26, n.s. (D) Large male abdomen temperature elevation. Sun: $[T_{ab} - T_{air}]=-0.22*[T_{air}]+15.35$, n=30, n.s.; Shade: $[T_{ab} - T_{air}]=-0.18*[T_{air}]+10.84$, n=26, $r^2=0.22$, $P=0.016$, slope 95% CI (-0.32,-0.037). (E) Small male abdomen temperature elevation. Sun: $[T_{ab} - T_{air}]=-0.40*[T_{air}]+20.032$, n=18, $r^2=0.45$,

P=0.0022, slope 95% CI (-0.63,-0.17); Shade: [T_{ab} - T_{air}]=0.15 *[T_{air}]+1.22, n=22, n.s.

Table 2.2. GLM results for the independent and interactive effects of morph, air temperature, and location on the elevation of head temperature over air temperature. T_h - T_{air} ~ Intercept + Morph + Air temperature + Location + Morph*Air temperature + Morph*Location + Air temperature*Location.

Model					
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value
Regression	406.0	9	45.11	F (9, 132) = 8.325	P<0.0001
Residual	715.3	132	5.419		
Total	1121	141			
Variable	**Estimate**	**Standard error**	**|t|**	**P value**	**P value summary**
Intercept	25.38	3.811	6.659	<0.0001	****
Morph[small]	-4.541	4.726	0.9609	0.3383	ns
Morph[female]	-13.12	4.579	2.864	0.0049	**
Air temperature	-0.4968	0.1330	3.736	0.0003	***
Location[shade]	2.984	4.014	0.7434	0.4586	ns
Morph[small] : Air temperature	0.1407	0.1599	0.8803	0.3803	ns
Morph[female] : Air temperature	0.4358	0.1555	2.802	0.0058	**
Morph[small] : Location[shade]	0.6573	1.022	0.6434	0.5211	ns
Morph[female] : Location[shade]	-0.1732	1.162	0.1491	0.8817	ns
Air temperature : Location[shade]	-0.1834	0.1429	1.283	0.2018	ns

Table 2.3. GLM results for the independent effects of morph, air temperature, and location on the . Heat gain and heat loss across increasing air temperatures over air temperature: T_{th} - T_{air} ~ Intercept + Morph + Air temperature + Location.

Model					
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value
Regression	706.9	4	176.7	F (4, 132) = 51.35	P<0.0001
Morph	8.248	2	4.124	F (2, 132) = 1.198	P=0.3050

Air temperature	649.0	1	649.0	F (1, 132) = 188.6	P<0.0001
Location	75.38	1	75.38	F (1, 132) = 21.90	P<0.0001
Residual	454.3	132	3.442		
Total	1161	136			
Variable	**Estimate**	**Standard error**	**|t|**	**P value**	**P value summary**
Intercept	35.68	1.491	23.93	<0.0001	****
Morph[small]	-0.1527	0.3951	0.3864	0.6999	ns
Morph[female]	-0.5905	0.3850	1.534	0.1275	ns
Air temperature	-0.6986	0.05087	13.73	<0.0001	****
Location[shade]	-1.630	0.3484	4.680	<0.0001	****

Table 2.4. GLM results for the independent effects of morph, air temperature, and location on the elevation of abdomen temperature over air temperature: $T_{ab} - T_{air} \sim$ Intercept + Morph + Air temperature + Location.

Model					
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value
Regression	265.2	4	66.31	F (4, 138) = 2.559	P=0.0413
Morph	9.522	2	4.761	F (2, 138) = 0.1838	P=0.8323
Air temperature	83.11	1	83.11	F (1, 138) = 3.208	P=0.0755
Location	211.6	1	211.6	F (1, 138) = 8.168	P=0.0049
Residual	3575	138	25.91		
Total	3841	142			
Variable	**Estimate**	**Standard error**	**|t|**	**P value**	**P value summary**
Intercept	12.24	3.478	3.519	0.0006	***
Morph[large]	0.05427	1.028	0.05278	0.9580	ns
Morph[small]	-0.5662	1.164	0.4863	0.6275	ns
Air temperature	-0.2423	0.1353	1.791	0.0755	ns
Location[sun]	2.699	0.9443	2.858	0.0049	**

Abdominal excess temperature ratio (R_{ab}) varied complexly, with a significant three-way interaction between bee type, air temperature and presence/absence of solar radiation (Table 2.5). All potential two-way interactions were also significant

(Table 2.5). Abdominal excess temperature ratio (R_{ab}) increased in the sun for all bee types, indicating that bees exposed to the sun likely shifted heat from the thorax to the abdomen (Fig. 2.3A, Table 2.5). Females flying in the respirometry chamber increased R_{ab} with air temperature in both the shade and sun (Fig. 2.3B-C), and solar radiation intensified the rate of R_{ab} increase with air temperature, suggesting that females shift more hot blood from the thorax to the abdomen in response to the elevation of thorax temperatures resulting from solar radiation (ANOVA: $F_{(1, 35)}=15.05$, P=0.0004). In contrast, in the sun, large and small morph males did not change $\mathrm{R_{ab}}$ with air temperature (Fig. 2.3C).

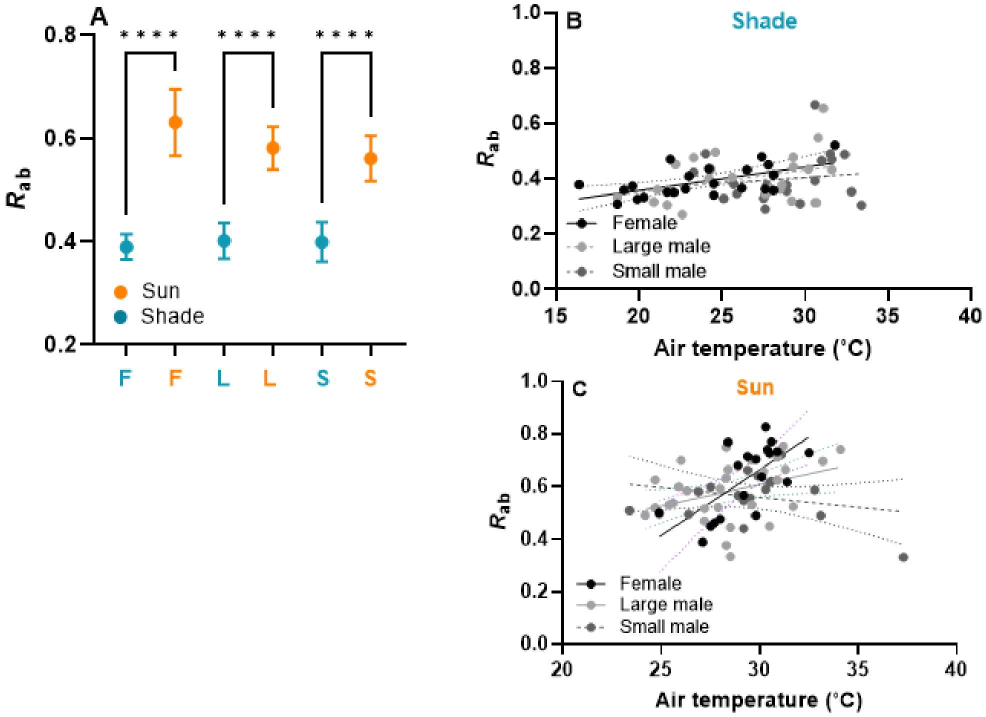

Figure 2.3 (A) Mean abdominal excess temperature ratio (R_{ab}) increased in the sun for females, large morph males, and small morph males. (B) In the shade, excess temperature ratio of the abdomen increased with air temperature ($[R_{ab}] = 0.0073*[T_{air}]+0.21$; add stats), but the slopes of these relationships did not differ significantly different among bee types ($F_{(2,64)}=0.23$, P=0.79). (C) In the sun, the slopes of abdominal temperature elevation on air temperature differed among bee

type. Significant 3-way morph*location*air temperature interaction: Morph[large]*Location[sun]*Air temperature |t|=2.56, P=0.01; Morph[small] : Location[sun] : Air temperature |t|=3.10, P=0.0024. Linear regression: Females: [R_{ab}]=0.056*[T_{air}]-0.99, n=17, r^2=0.50, P=0.0016, slope 95% CI (0.025,0.086); large morph males: [R_{ab}]=0.016*[T_{air}]+0.12, n=30, r^2=0.15, P=0.036, slope 95% CI (0.0012,0.032); small morph males: [R_{ab}] = -0.0075*[T_{air}] + 0.78, n=18, n.s. F=female, L=large morph male, and S=small morph male.

Table 2.5. GLM results for the independent and interactive effects of morph, air temperature, and location on the abdominal excess temperature ratio: R_{ab} ~ Intercept + Morph + Location + Air temperature + Morph*Location + Morph*Air temperature + Location*Air temperature + Morph*Location*Air temperature

Model					
Analysis of Variance	**SS**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Regression	1.658	11	0.1507	F (11, 129) = 6.563	P<0.0001
Residual	2.962	129	0.02296		
Total	4.619	140			
Variable	**Estimate**	**Standard error**	**|t|**	**P value**	**P value summary**
Intercept	0.9826	0.2012	4.883	<0.0001	****
Morph[large]	-0.7920	0.2894	2.737	0.0071	**
Morph[small]	-0.6986	0.3756	1.860	0.0651	ns
location[sun]	-1.817	0.6264	2.900	0.0044	**
Air temperature	-0.02372	0.008330	2.848	0.0051	**
Morph[large] : Location[sun]	1.743	0.7274	2.396	0.0180	*
Morph[small] : Location[sun]	2.318	0.7949	2.916	0.0042	**
Morph[large] : Air temperature	0.03173	0.01143	2.777	0.0063	**

Morph[small] : Air temperature	0.02775	0.01384	2.005	0.0471	*
Location[sun] : Air temperature	0.07365	0.02184	3.373	0.0010	***
Morph[large] : Location[sun] : Air temperature	-0.06532	0.02556	2.556	0.0118	*
Morph[small] : Location[sun] : Air temperature	-0.08521	0.02747	3.102	0.0024	**

CO$_2$ emission rates for bees flying in the sun versus shade across a range of air temperatures

Both location (sun versus shade) and bee type affected mass-specific $V\mathrm{CO}_2$, as did their interaction (Table 2.6). Mass-specific $V\mathrm{CO}_2$, however, was higher for bees flying in the sun relative to the shade (Table 2.6, sun: 1.44, 95%CI [1.33,1.54]; shade: 1.21, 95% CI [1.07, 1.35]). In the shade, small morph males significantly decreased $V\mathrm{CO}_2$ as air temperature rose (|t|=2.8, P=0.0059; Fig. 2.4A), but air temperature did not affect $V\mathrm{CO}_2$ in large morph males and females. In the sun, large morph males decreased $V\mathrm{CO}_2$ as air temperature rose (Fig. 2.4B) but small morph males and females did not significantly vary $V\mathrm{CO}_2$ with air temperature (Fig. 2.4B).

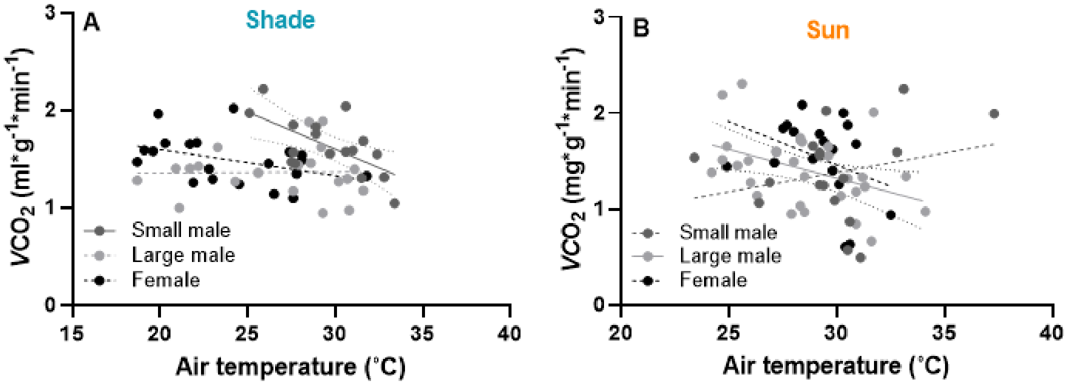

Figure 2.4 (A) Mass-specific $V\mathrm{CO}_2$ decreased with air temperature for small males flying in the shade in a respirometry chamber: $[V\mathrm{CO}_2]=-0.078*[T_{\mathrm{air}}]+3.89$, n=17, $r^2=0.41$, P=0.0059, slope 95% CI (-0.13,-0.026). Large male and female $V\mathrm{CO}_2$ did

not vary with air temperature: [large morph $V\mathrm{CO}_2$]=0.00098*[T_{air}]+1.34, n=20, n.s.; [female $V\mathrm{CO}_2$]=-0.027*[T_{air}]+2.13, n=20. (B) Mass-specific $V\mathrm{CO}_2$ decreased with air temperature for large males flying in the sun in a respirometry chamber: [$V\mathrm{CO}_2$]=-0.060*[T_{air}]+1.11, n=31, r^2=0.18, P=0.018 slope 95% CI (-0.11,-0.011) but did not vary for small males or females: [small morph $V\mathrm{CO}_2$]=0.040 *[T_{air}]+0.17, n=17, n.s.; [female $V\mathrm{CO}_2$]=-0.090 *[T_{air}]+4.17, n=18, n.s.

Table 2.6. GLM: $V\mathrm{CO}_2$ ~ Intercept + Air temperature + Location + Morph + Air temperature*Location + Air temperature*Morph + Location*Morph.

Model					
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value
Regression	5.366	9	0.5963	F (9, 129) = 2.385	P=0.0157
Residual	32.26	129	0.2500		
Total	37.62	138			
Variable	**Estimate**	**Standard error**	**|t|**	**P value**	**P value summary**
Intercept	1.315	0.6266	2.098	0.0379	*
Air temperature (°C)	-0.006159	0.02590	0.2378	0.8124	ns
Location[sun]	2.551	0.9112	2.800	0.0059	**
Morph[large]	-0.8126	0.8573	0.9478	0.3450	ns
Morph[small]	-2.691	1.044	2.577	0.0111	*
Air temperature (°C) : Location[sun]	-0.07374	0.03115	2.367	0.0194	*
Air temperature (°C) : Morph[large]	0.03059	0.03390	0.9026	0.3684	ns
Air temperature (°C) : Morph[small]	0.1013	0.03867	2.619	0.0099	**
Location[sun] : Morph[large]	-0.2362	0.2516	0.9387	0.3497	ns
Location[sun] : Morph[small]	-0.4366	0.2647	1.649	0.1016	ns

I found an effect of the interaction between air temperature and location on $V\mathrm{H}_2\mathrm{O}$ (Table 2.7). Pooled mean mass-specific $V\mathrm{H}_2\mathrm{O}$ differed between the sun and shade (sun: 0.53, 95%CI [0.22,0.36]; shade: 0.29, 95% CI [0.22,0.36]). Females and males had statistically similar slopes and intercepts of mass-specific $V\mathrm{H}_2\mathrm{O}$ on air temperature in the shade (Fig. 2.5A; slope: $F_{(2,65)}$=0.42, P=0.66; intercept:

$F_{(2,67)}$=1.57, P=0.22), but did not vary significantly as air temperatures rose (Table 7). Only females and small morph males flying in the sun showed significantly increased mass-specific VH_2O with air temperature (Fig. 2.5B).

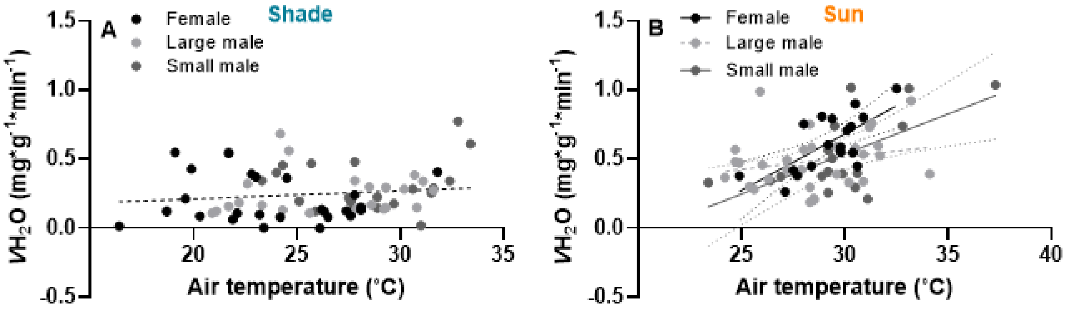

Figure 2.5 (A) Males and females did not vary mass-specific VH_2O in the shade. Pooled data: [VH_2O]=0.011*[T_{air}]-0.00059, n=73, n.s. (B) Females flying in the sun in a respirometer increased water loss rates with air temperature: [VH_2O]=0.081 *[T_{air}]-1.76, n=17, r^2=0.47, P<0.005 slope 95% CI (0.034,0.13) as did small males: [VH_2O]=0.058 *[T_{air}]-1.20, n=17, r^2=0.39, P<0.05 slope 95% CI (0.018,0.098).

Table 2.7. GLM results for the independent and interactive effects of morph, air temperature, and location on the rate of water loss during flight: VH_2O~ Intercept + Morph + Location + Air temperature + Morph*Location + Morph*Air temperature + Location*Air temperature

Model					
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value
Regression	3.429	9	0.3810	F (9, 129) = 5.812	P<0.0001
Residual	8.458	129	0.06556		
Total	11.89	138			
Variable	**Estimate**	**Standard error**	**|t|**	**P value**	**P value summary**
Intercept	0.1194	0.3209	0.3720	0.7105	ns
Morph[large]	0.2460	0.4390	0.5604	0.5762	ns

Morph[small]	-0.5226	0.5347	0.9774	0.3302	ns
Location[sun]	-0.5910	0.4666	1.267	0.2075	ns
Air temperature	0.005297	0.01326	0.3993	0.6903	ns
Morph[large] : Location[sun]	-0.07481	0.1289	0.5806	0.5625	ns
Morph[small] : Location[sun]	-0.2490	0.1356	1.837	0.0685	ns
Morph[large] : Air temperature	-0.009654	0.01736	0.5562	0.5790	ns
Morph [small] : Air temperature	0.02205	0.01980	1.114	0.2675	ns
Location[sun] : Air temperature	0.03198	0.01595	2.005	0.0471	*

Comparison of sunny versus shaded heat budgets

Mean metabolic heat production did not significantly differ between sunny and shaded locations (Fig. 2.6A). Overall, convection strongly and significantly increased for all bees flying in shaded conditions compared to sunny ones (Fig. 2.6B) as did evaporative heat loss (Fig. 2.6D), though it was a minor route of cooling in the heat budget. Females additionally increased longwave radiative flux in the sun (Fig. 2.6C).

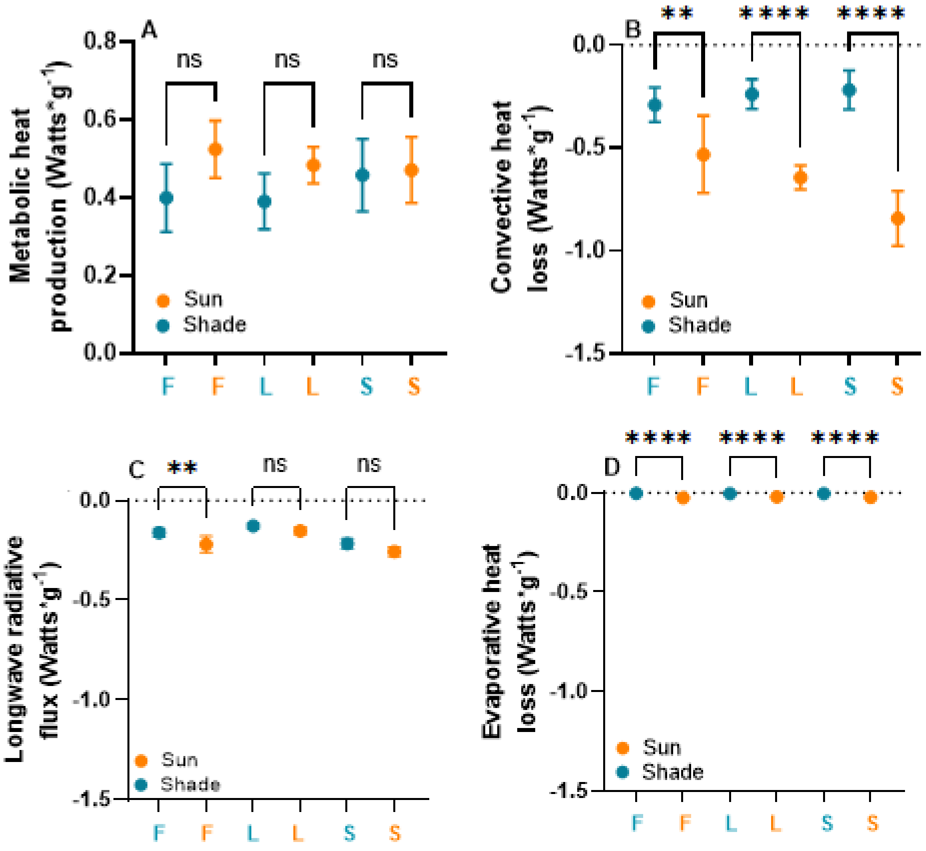

Figure 2.6. Comparison of (A) metabolic heat production, (B) convective heat loss, (C) longwave radiative heat flux, and (D) evaporative heat loss for both male morphs and females flying in a 500 mL respirometry chamber either in the shade or the sun. F=female, L=large morph male, and S=small morph male

Table 2.8. GLM results for the independent and interactive effects of morph, air temperature, and location on heat loss due to radiative cooling: Q_R ~ Intercept + Location + Morph + Air temperature.

Model					
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value
Regression	0.2483	4	0.06208	F (4, 126) = 23.08	P<0.0001
location	0.03882	1	0.03882	F (1, 126) = 14.43	P=0.0002
Morph	0.2064	2	0.1032	F (2, 126) = 38.37	P<0.0001
Air temperature	1.447e-005	1	1.447e-005	F (1, 126) = 0.005381	P=0.9416
Residual	0.3389	126	0.002690		
Total	0.5873	130			
Variable	**Estimate**	**Standard error**	**|t|**	**P value**	**P value summary**
Intercept	-0.1679	0.03645	4.607	<0.0001	****
Location[sun]	-0.03788	0.009972	3.799	0.0002	***
Morph[large]	0.05072	0.01117	4.543	<0.0001	****
Morph[small]	-0.04594	0.01280	3.590	0.0005	***
Air temperature	-0.0001038	0.001415	0.07335	0.9416	ns

Table 2.9. GLM results for the independent and interactive effects of morph, air temperature, and location on heat loss due to convective cooling: Q_C ~ Intercept + Location + Morph + Air temperature + Location*Morph + Location*Air temperature + Morph*Air temperature.

Model					
Analysis of Variance	SS	DF	MS	F (DFn, DFd)	P value
Regression	7.610	10	0.7610	F (10, 120) = 17.43	P<0.0001
Residual	5.238	120	0.04365		
Total	12.85	130			

Variable	Estimate	Standard error	|t|	P value	P value summary
Intercept	0.04296	0.2818	0.1524	0.8791	ns
Location[sun]	0.09497	0.3787	0.2508	0.8024	ns
Morph[large]	-0.2643	0.3671	0.7200	0.4729	ns
Morph[small]	1.197	0.4563	2.622	0.0099	**
Air temperature	-0.007878	0.01180	0.6679	0.5055	ns
Location[sun] : Morph[large]	-0.2538	0.1127	2.252	0.0262	*
Location[sun] : Morph[small]	-0.3827	0.1193	3.208	0.0017	**
Location[sun] : Air temperature	-0.008134	0.01295	0.6282	0.5311	ns
Morph[large] : Air temperature	0.01169	0.01468	0.7959	0.4277	ns
Morph[small] : Air temperature	-0.03617	0.01693	2.137	0.0347	*

Convective conductance was significantly higher in the sun for females (Fig. 2.7A; $F_{(3, 79)}$=155.1, P<0.0001), large males (Fig. 2.7A; $F_{(3, 96)}$=180.5, P<0.0001), and small males (Fig. 2.7A; $F_{(3, 62)}$=56.40, P<0.0001). Only small morph males showed increasing convective conductance with air temperature in the sun and shade (Fig. 2.7B). There was no effect of location, nor was there any effect of air temperature on radiative conductance (Table 2.10).

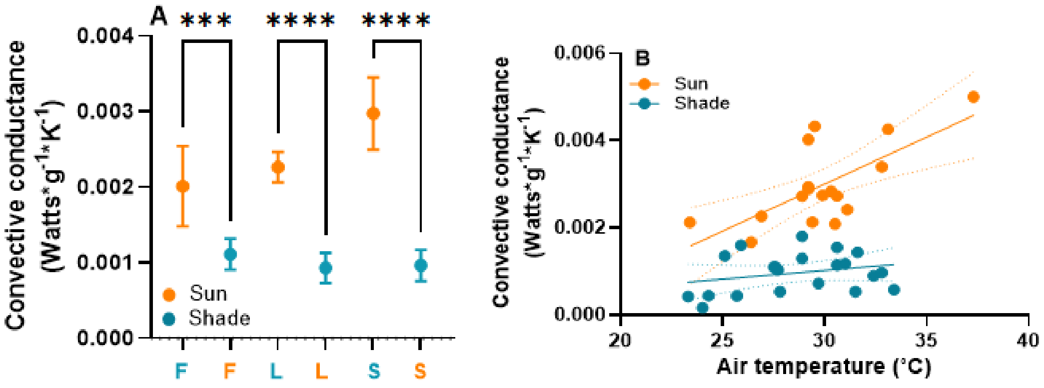

Figure 2.7. Convective conductance (κ; Watts*g^{-1}*K^{-1}) for bees flying in either a shaded or sunny 500 mL respirometry chamber. Absolute values shown for clarity. (A) All bees showed higher mean convective conductance in the sun than in the shade (B) Small morph males increased convective conductance with rising air temperature in the sun and shade. F=female, L=large morph male, and S=small morph male.

Table 2.10. GLM results for the independent and interactive effects of morph, air temperature, and location on convective conductance during flight: κ ~ Intercept + Morph + Air temperature + Location + Morph*Air temperature + Morph*Location + Air temperature*Location.

Model					
Analysis of Variance	**SS**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Regression	9.144e-005	9	1.016e-005	F (9, 121) = 17.88	P<0.0001
Residual	6.877e-005	121	5.684e-007		
Total	0.0001602	130			
Variable	Estimate	Standard error	|t|	P value	P value summary
Intercept	-0.0001169	0.001011	0.1157	0.9081	ns
Morph[large]	-0.001046	0.001323	0.7905	0.4308	ns
Morph[small]	0.003386	0.001607	2.107	0.0372	*
Air temperature	-3.846e-005	4.234e-005	0.9084	0.3655	ns
Location[sun]	0.0003302	0.001366	0.2418	0.8093	ns
Morph[large] : Air temperature	5.064e-005	5.280e-005	0.9592	0.3394	ns
Morph[small] : Air temperature	-0.0001030	6.013e-005	1.713	0.0893	ns
Morph[large] : Location[sun]	-0.0008430	0.0004063	2.075	0.0401	*
Morph[small] : Location[sun]	-0.001368	0.0004304	3.178	0.0019	**
Air temperature : Location[sun]	-3.283e-005	4.672e-005	0.7026	0.4836	ns

Morph and sex differences in the heat budget for bees flying in the sun

Heat gain from solar radiation increased with air temperature for both male morphs and for female bees (Figs. 2.8A-C, Table 2.8). Large morph males flying in the sun gained heat from solar radiation (in Watts*g^{-1}) more slowly than small morph males (One-way ANOVA: $F_{(2, 58)} = 9.589$, $P<0.0005$) with no difference between the females and either male morph. Metabolic heat production decreased with air temperature for only the large morph males (Fig. 2.8A) with no significant difference between the mean metabolic heat production for males of both morph and females. Evaporative cooling increased with air temperature for small males and females (Figs. 2.8B-C) with no significant difference in the rates of evaporative heat loss between small males and females, or between the means of all bees. Longwave radiative heat loss increased for females (Fig. 2.8C) with large males having the lowest radiative heat loss compared to small males and females (one-way ANOVA: $F_{(2,\ 57)}$ = 11.75, P<0.0001). Convective cooling increased for small males with air temperature (Fig. 2.8B) and small males had higher mean convective cooling than large males ($F_{(2,\ 58)}$= 3.363, P<0.05).

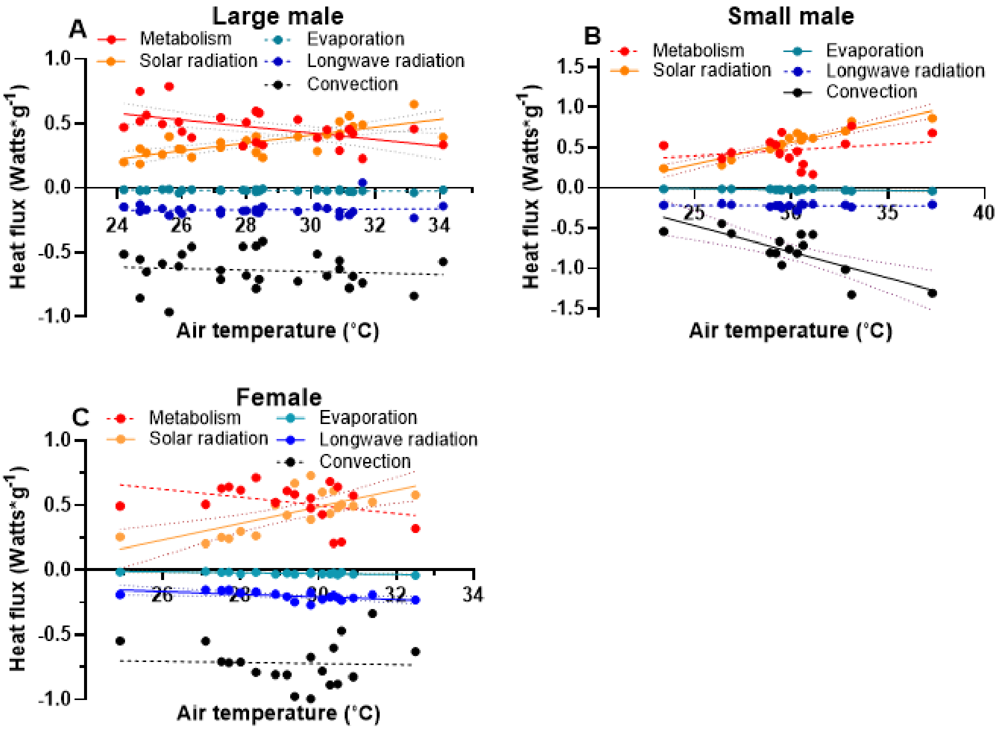

Figure 2.8. Heat gain and heat loss across increasing air temperatures (T_{air}) for *Centris pallida* large males, small males, and females flying in a sunny 500 mL glass borosilicate chamber. (A) Large morph males decreased metabolic heat production (Q_{M}) and increased heat gain from solar radiation (Q_{S}). [Q_{M}]=-0.026[T_{air}]+1.20, n=27, r^2=0.30, P=0.0028, slope 95% CI (-0.042,-0.0097); [Q_{S}]=0.031*[T_{air}]-0.52,

n=27, r^2=0.58, P<0.0001, slope 95% CI (0.020,0.042). Evaporative heat loss (Q_E), longwave radiative flux (Q_L), and convective heat loss (Q_C) did not vary with air temperature: [Q_E]=-0.00077*[T_{air}]+0.0010, n=27, n.s.; [Q_L]=0.0013*[T_{air}]-0.21, n=27, n.s.; [Q_C]=-0.0058*[T_{air}]-0.48, n=27, n.s. (B) Small morph males increased heat gain from solar radiation (Q_S), increased evaporative heat loss (Q_E), and increased convective heat loss (Q_C): [Q_S]=0.054*[T_{air}]-1.04, n=15, r^2=0.89, P<0.0001, slope 95% CI (0.042,0.065); [Q_E]=-0.0023*[T_{air}]+0.046, n=15, r^2=0.40, P<0.05, slope 95% CI (-0.0040,-0.00061);); [Q_C]=-0.065*[T_{air}]+1.16, n=15, r^2=0.62, P<0.005, slope 95% CI (-0.096,-0.034). Metabolic heat production (Q_M), and longwave radiative flux (Q_L) did not vary with air temperature: [Q_M]=0.014*[T_{air}]+0.046, n=15, n.s.; [Q_L]=-0.00039*[T_{air}]+0.046, n=15. (C) Females increased heat gain from solar radiation (Q_S), increased evaporative heat loss (Q_E), and increased longwave radiative heat loss (Q_L): [Q_S]=0.064*[T_{air}]-1.43, n=19, r^2=0.51, P<0.005, slope 95% CI (0.032,0.096); [Q_E]=-0.0033*[T_{air}]+0.072, n=18, r^2=0.48, P<0.05, slope 95% CI (-0.0052,-0.0015); [Q_L]=-0.010*[T_{air}]+0.093, n=19, r^2=0.30, P<0.05, slope 95% CI (-0.018,-0.0023). Metabolic heat production (Q_M), and convective heat loss (Q_C) did not vary with air temperature: [Q_M]=-0.031 *[T_{air}]+1.43, n=18, n.s.; [Q_C]=-0.0042*[T_{air}]-0.60, n=19.

Discussion

Main findings

I found that, on average, solar radiation was responsible for 43% of total heat gain during flight in the sun of large morph males, 46% to females, and 54% to small morph males (Fig. 2.8A-C), representing a significant additional heat load to flying

Centris pallida bees. By subtracting air temperature from weighted body temperature, multiplied by body mass (in g), and multiplying by 3.9 J*g-1*°C^{-1} (the specific heat of tissue), I found that, unlike vertebrate non-flying endotherms, bees flying in the sun do not store additional heat compared to bees flying in the shade despite significantly increased temperatures in sunny conditions. To counteract the additional heat gain from the sun, both male morphs and females showed increased mean convective cooling in the sun compared to the shade (Fig. 2.7A). The mechanism by which large morph males and females increase convective cooling is likely through the use of their abdominal convector, which is supported by the increase in abdominal excess temperature ratio with air temperature (Fig. 2.3A). I found that small morph males do not use an abdominal convector (Fig. 2.3B-C), they may instead be increasing flight speed at high air temperatures as a mechanism to increase convective cooling.

My hypothesis that desert bees would regulate their body temperature like that of nonflying desert vertebrates is rejected. *Centris pallida* do not store heat in body tissues (Schmidt-Nielsen, 1997), nor do they rely on evaporative cooling (Fig. 2.7D) or suppression of metabolic heat production (Fig. 2.7A). I found that large morph males had the smallest surface area to volume ratios and small morph males had the largest surface area to volume ratios, supporting my hypothesis that this ratio differs between morphs.

Small morph males were poor thermoregulators overall, with slopes of thorax temperature to air temperature in the shade and sun of 0.40 and 0.38 (Table 2.1), respectively, supporting my hypothesis that body warming in shaded conditions was greatest in small morph males, intermediate in females, and lowest in large morph males. However, females were poor thermoregulators in the sun, with a slope of thorax temperature on air temperature of 0.98.

I found support for my hypothesis that microclimatic conditions (sun versus shade) shifted the physiological responses of *C. pallida* male morphs and females (Fig. 2.6A-D). Females used the abdominal convector in the shade (Fig. 2.3B), and both large males and females exhibited use of the abdominal convector in sunny conditions (Fig. 2.3C). Females flying in the sun additionally increased longwave radiative cooling and evaporative cooling (Fig. 2.6C) and large males flying in the sun decreased metabolic heat production (Fig. 2.6A). Small morph males strongly increased convective cooling in the sun (Fig. 2.6B) and increased mean evaporative heat loss (Fig. 2.6D) but did not exhibit other strong physiological mechanisms of thermoregulation.

Of the bees measured in the sun, females and large morph males showed strong mechanisms of physiological thermoregulation, with large males decreasing metabolic heat production and females evaporatively cooling. Whereas small morph males did not show any evaporative cooling or decrease in metabolic heat production. This evidence partially rejects my hypothesis that a large surface area to volume ratios drives the magnitude of physiological response. In the case of convective cooling, however, small morph males showed the strongest response followed by large morph males and females (Fig. 2.7A-B).

Water loss rates and evaporative heat loss patterns

Despite the abundance of palo verde blooms, meaning bountiful water availability for *C. pallida*, evaporative heat loss was not a major mechanism of cooling for either male morph or females (Fig. 2.8A-C), suggesting water loss is minimally adjustable based on water availability. But, if water or nectar is in short supply, *Centris pallida* may run out of water and desiccate fairly quickly given that critical water content ranged from 47.6% to 53.7% (Johnson et al., 2023 unpublished). Low reliance on evaporative cooling is common for other desert-adapted bees (Chappell, 1984; Johnson et al., 2022, 2023; Willmer, 1986; Willmer & Stone, 1997). However, evaporative heat loss increased for males and females flying in the sun (Fig. 2.6D) indicating that the additional heat load imposed by solar radiation induced increased water loss rates. I did not observe defecation or extruded liquid on the proboscis by flying bees, so future work might clarify the routes of water loss for *C. pallida* bees in flight. Small morph male cuticular permeabilities were quite low (Roberts et al., 1998), so water loss must occur via the spiracles, mouth, or anus.

Comparative role of solar heat gain for desert animals

Mammals and birds mainly resort to panting and cuticular evaporative heat loss to keep cool, though panting is quite dependent on body size (Fuller et al., 2016; Pessato et al., 2020; Schmidt-Nielsen, 1997). Desert reptiles also may use evaporative cooling to lower body temperatures at high air temperatures, though these body temperatures are rarely attained in the field (Dawson & Templeton, 1963; DeNardo et al., 2004). Diet and availability of water would play a role in the use of this mechanism, for example, desert passerine birds that drink water have higher evaporative cooling capacities than non-drinking species (Czenze et al., 2020). Desert bees, such as *Centris,* also drink water via nectar consumption, but do not seem to heavily rely on evaporative heat loss (Chappell, 1984a). Desert grasshoppers show minimal evaporative heat loss, and then exponentially increased evaporative cooling above air temperatures of 45 °C, similar to desert birds and the Gila monster (DeNardo et al., 2004; McKechnie & Wolf, 2019; Roxburgh et al., 1996) as do other insects at high air temperature (Prange, 1996). It would seem that insects, as well as man other desert inhabitants, “turn on” evaporative cooling as a last resort at high air temperatures, but rely mainly on other mechanisms, such as cooling via convection (for flying desert animals) or behavioral avoidance/toleration of hyperthermia, to lower body temperatures.

Solar radiation and emissivity

Heat gain from solar radiation increased linearly with air temperature for all morphs and sexes, surpassing metabolic heat production at different points in the morning (Fig. 2.8A-C). Solar heat gain surpassed metabolic heat production at air temperatures of 27.2 °C for small morph males, whereas large morph males were heated more by solar radiation above 30.2 °C, and females above 30.1 °C. Animals with high surface area to volume ratios (in this case, the small morph males) would gain and lose heat more quickly than larger counterparts, assuming similar insulation capacity.

The dorsal thoracic and abdominal coefficients of absorption differed greatly for large morph males and small morph males (Barrett and O’Donnell, 2023). Small morph males had a high thoracic and abdominal coefficient of absorption (an emissivity 0.83 and 0.82, respectively), meaning that they absorbed more heat from solar radiation than large males (Barrett and O’Donnell, 2023). Large morph males, on the other hand, had relatively lower coefficients of absorption, 0.76 for both the dorsal thorax and abdomen (Barrett and O’Donnell, 2023). Large males spend the majority of their activity period on the ground, fighting and digging for emerging, unmated females (Alcock et al., 1977), and may certainly benefit from reflecting as much heat gain from solar radiation as possible in a hotter microclimate in combination with strong physiological responses such as decreasing metabolic heat production, heat shunting to the abdomen, and a small amount of evaporative cooling.

Hair-like structures called setae were responsible for the variation in coefficients of absorption for the male morphs and females (Barrett and O'Donnell, 2023). Dense hair on the thoraxes of flying facultative endotherms was thought to help insulate the thorax and maintain high thorax temperatures in cool mornings (Heinrich, 1976; Johnson et al., 2022), and a relatively bare abdomen was thought to allow for increased heat transfer from the abdomen to the environment. However, female *C. pallida* had more densely hairy abdomens than large or small morph males (Barrett and O'Donnell, 2023), yet females relied greatly on the abdominal convector for heat dumping while flying in both shaded and sunny conditions (Fig. 3B-C). Clearly, a densely hairy abdomen does not necessarily preclude a large flying endotherm from dumping heat from the thorax to the abdomen at high air temperatures. One possible explanation is that reflection could be determined by hair length, rather than density. Females appeared to have much shorter abdominal hairs than male bees, as well as significantly differing coefficients of absorption for the thorax and abdomen (0.80 and 0.75, respectively; Barrett and O'Donnell, 2023).

The mid-infrared spectrum (2.5- 25 μm; MIR) also plays an important role in heating (Johnson et al., 2023a; Krishna et al., 2021; Shi et al., 2015). I do not currently have values for the coefficient of absorption in this spectrum for *C. pallida*, so I used an assumed value of 0.96 (Stupski & Schilder, 2021). This would alter the longwave radiative flux value, i.e., the heat gain from hot objects in the environment like the desert floor, and heat loss from the body of the bee to the environment, further refining the heat budget.

Conclusions

For the first time, I show that solar radiation is a key heat source for endothermic desert bees flying in sunny conditions. Even at the highest air temperatures, convective cooling was most important for heat balance. *Centris pallida* males and females regulated body temperatures via different mechanisms, with large males and females dumping heat to the abdomen. Likely, this was due to their varied use of microclimates in the field with large males in the hottest microclimates, and small males in the coolest.

CHAPTER 3

WATER LOSS, NOT OVERHEATING, LIMITS THE ACTIVITY PERIOD OF AN ENDOTHERMIC SONORAN DESERT BEE

Introduction

The Sonoran Desert, like many regions in the world, is experiencing rising temperatures and more frequent and severe droughts (Weiss and Overpeck, 2005; Williams et al., 2020). Desert birds and mammals use many strategies to survive the extraordinary heat load and high water potential gradient (Rozen-Rechels et al., 2019; Terrien, 2011; Walsberg, 2000). For diurnal flying insects, however, desert conditions may pose an exceptional challenge. Their relatively high surface area to volume ratio results in high radiant and/or convective heat loading and water losses (Merckx et al., 2008; Stevenson, 1985). Many of the biggest flying insects also substantially elevate thorax temperatures, which aids flight in cool conditions, but the added heat load provides a further thermoregulatory challenge during hot conditions (Heinrich, 1974; Johnson et al., 2022; Johnson et al., 2023). Understanding how abiotic conditions limit flight is critical for developing accurate predictive models of activity, geographical distribution, and organismal fitness.

Behavioral thermoregulation is an important strategy for desert animals. For small, mobile desert insects, there is considerable thermal heterogeneity available. While behavioral thermoregulation strategies are well-known to be critical to the survival of flying ectothermic insects (e.g. Gols et al., 2021; Ørskov et al., 2019), the role of behavioral thermoregulation via microclimate selection has received substantially less attention for flying endothermic insects. In addition to thermoregulation by changing location, changes in daily activity times are another important strategy (Casey, 1976; Contreras et al., 2013; Willmer and Stone, 1997).

Desert animals also use physiological mechanisms to offload heat, such as the controlled vasodilation of blood vessels near the surface of the skin to increase heat transfer (Hill and Veghte, 1976; Klir and Heath, 1992). Certain flying desert invertebrates use a similar mechanism, with the abdomen acting as a convective radiator at high air temperatures, wherein heat is transferred from an insulated thorax to a relatively bare abdomen at high air temperatures to offload heat (Casey, 1976; Heinrich, 1976; Johnson et al., 2022; May, 1976). Other physiological tactics of flying invertebrates include the suppression of metabolic heat production at high air temperatures via decreased wingbeat frequency (Roberts et al., 1998) or alternative use of gliding and powered flight (May, 1976), as well as increased evaporative cooling at high air temperatures via regurgitation (Gomes et al., 2018).

It is unknown whether endothermic insects have adapted to hot desert conditions by evolving higher capacities to tolerate heat. The evolution of upper thermal limits ($CT_{\max}$) appears to be tightly constrained, with limited potential to change in response to climate warming (Bennett et al., 2021; Boyles et al., 2011; Hamblin et al., 2017; Hoffmann et al., 2013). For example, $CT_{\max}$ does not vary significantly with latitude for terrestrial ectotherms (Johnson et al., 2023; Sunday et al., 2019). Possibly, this is due to constraints that make it near-impossible for animals to offset the negative effects of temperature on membrane lipids and proteins (Bennett et al., 2021). Alternatively, across a wide variety of environments, ectotherms may have the potential to be exposed to similar high temperatures due to solar heating (Kaspari et al., 2015). Nonetheless, there is some data suggesting that certain desert species have notably high upper thermal tolerances (Freeman et al., 2020) and that there is some potential for plasticity in thermal tolerances for non-flying invertebrates (Diamond et al., 2018).

Desert animals must also conserve water. Desiccation is an especially high risk for flying insects due to their high surface area to volume ratios (Gibbs, 2002) and high air speeds that increase water loss rates relative to metabolic water production (McCluney, 2017). Desert insects generally have low cuticular water loss rates relative to insects from wet, humid regions (Ajayi et al., 2020; Hadley, 1994; Zachariassen, 1996). Long-term desiccation can result in lethal water loss (Chown et al., 2011; Lighton and Feener, 1989). The critical water content (the percent of total body water content at death) of arthropods ranges from 17 to 89% (Burdine and McCluney, 2019; Hadley, 1994). As yet, there is no evidence that critical water content of insects varies across habitat types (Hadley, 1994), or with increasing urbanization (Burdine and McCluney, 2019).

Desert *Centris* bees are responsible for pollinating iconic plants in the Sonoran Desert (Alcock et al., 1977; Rozen and Buchmann, 1990). Males of *C. caesalpiniae* are dimorphic (Fig. 1A-B), and the morphs differ in both behavior and appearance (Snelling, 1984). Large morph males are active from sunrise to midday; they are typically found on, or near the ground digging or flying near female emergence sites (Johnson et al., 2022; Spangler and Buchmann, 1991). Small morph males are active later in the morning and fly above creosote bushes (Johnson et al., 2022; Spangler and Buchmann, 1991). These dimorphic *C. caesalpiniae* males seem to have similar mating strategies as documented for *C. pallida* (Alcock et al., 1977). Large morph male *C. caesalpiniae* thermoregulate during flight, and the major mechanism of thermoregulation is variable use of the abdominal convector (Johnson et al., 2022)..

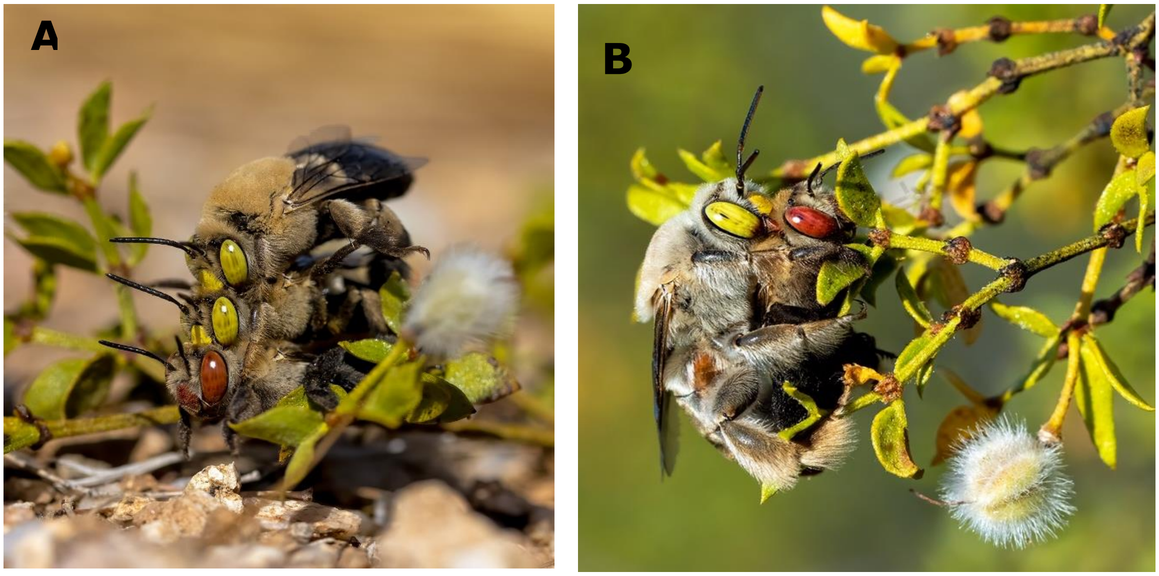

Figure 3.1. (A) Two small morph *Centris caesalpiniae* males (green eyes) attempting to mate with a female (red eyes) on *L. tridentata*. (B) A large morph male mating with a female on *L. tridentata*. Photos by Bruce Taubert.

Here, I ask whether high temperature or desiccation is a greater constraint on the activity period of *C. caesalpiniae*. Determining whether water loss or overheating more greatly limits activity in the field is often extremely challenging because it is rare for researchers to know the timing and duration of activity in the field. I know that *Centris caesalpiniae* males fly from sunrise to near mid-day in mating aggregations, allowing us to directly compare threats to survival posed by overheating versus desiccation. If males cease flying mid-day to avoid high air temperatures, I predict that the thorax temperatures of flying bees will approach the critical maximum temperature (CT_{max}) near the end of the activity period. If males ceased flying mid-day to avoid desiccation, I predicted that the water loss rates of flying bees would cause bees to approach lethal desiccation during a single morning of flight. Plausibly, neither high temperature nor desiccation constrain activity period*;* flight activity may cease at mid-day due to a biotic factor, such as lack of female emergence or depletion of energy stores.

Materials and Methods

Animals: In June 2020, I studied a mating aggregation of *C. caesalpiniae* males in rural Scottsdale, Arizona (GPS coordinates: 33.727, -111.799), focusing on their heat balance (Johnson et al., 2022) and critical maximum temperatures. In May 2021, *C. caesalpiniae* males and females emerged at the same site. I measured crop volume and sugar content, water loss rate, and microclimate selection. In 2022, I measured critical water content for *C. pallida* males and females collected near the flood plains of the Salt River (GPS coordinates: 33.552, –111.566), and for *C. caesalpiniae* females collected in Cottonwood, Arizona (GPS coordinates: 34.726, -112.017). I took all mass measures using a Mettler Toledo XPE56 XPE micro-analytical balance (accurate to 0.000001 g) unless otherwise specified.

*Crop volume and sugar content***:** I measured the crop volume and sugar content of large *C. caesalpiniae* males flying across 24 to 45 °C air temperatures in 2021. After netting a bee, I squeezed the abdomen caudally to cranially until the proboscis extended to extrude crop contents. I collected crop contents with a 10 µL glass microcapillary tube, using multiple tubes if necessary. I measured the length of the microcapillary tube using digital calipers (accurate to 0.01 mm) to estimate volume. I transferred the contents of the microcapillary tube(s) to a BRIX refractometer (V·RESOURCING, model VLT032) to measure sugar content.

*Mark-recapture***:** To provide measures of how long males persisted at an aggregation site within a day and across days, I conducted a mark-recapture study. On May 11th, 2021, from 0630 to 0730, I marked ~200 large morph *C. caesalpiniae* males on the thorax with blue acrylic paint. On May 12th, 13th, 17th, 18th, and 20th, I netted bees through the morning (0630 – 1130), counting the total number of marked and unmarked males. I released all captured males.

*Microclimate selection***:** To record changes in microclimate selection through the activity period in 2021, I marked four, one by one-meter patches with two in shade and two in direct sunlight. Every 30 minutes, from sunrise to midday, I recorded 20 second videos of each patch, and measured ground and air temperature with a BAT-12 thermometer and copper-constantan thermocouple (Type T, AWG 36). I analyzed videos by manually counting the number of large males on the ground or in flight. I did not count small morph males or females; both were easily identifiable by their dark black abdomens (Fig. 3.1A). I coded behavior based on the initial observed location of the individual. I summed the number of bees in each location for each patch to determine the relative number of bees in sun or shade and flying versus on the ground.

*Water loss rates and metabolic water production for flying C. caesalpiniae males***:** In 2020, Johnson et al., 2022 measured the water loss and CO_2 emission rates of flying *C. caesalpiniae* large morph males from 19 to 38 °C using flow through respirometry. I calculated metabolic water production from the previously published data on carbon dioxide emission rates (Johnson et al., 2022) and also calculated net water balance from the previously measured water loss rate data (from Johnson et al., 2022) and as a function of metabolic water production. I estimated metabolic water production (*MWP*) assuming carbohydrate metabolism. Although the respiratory quotient of flying *C. caesalpiniae* has not been measured, bees have been reported to utilize carbohydrates (Bertsch, 1984; Gäde and Auerswald, 1999; Suarez et al., 2005). I estimated metabolic water production in mg $H_2O \cdot h^{-1}$ in Equation 3.1:

$$MWP = [VCO_2 \cdot (22.4\ L \cdot mol^{-1})^{-1}] \cdot 18000\ mg \cdot mol^{-1} \quad (3.1)$$

Critical water content: In 2022, I recognized the need for *critical water content* measures, but were unable to capture *C. caesalpiniae* males. Therefore, I measured *critical water content* for *C. pallida* males and females and *C. caesalpiniae* nesting females. For both species, I netted the first bees to begin flying in the early morning and those with little to no wing wear or hair loss on the thorax. Bees were transported from the field sites (45 – 70 minutes) to the laboratory in pre-weighed 10 mL tubes with ventilation holes. Bees remained still in the tubes and did not fly, defecate, or regurgitate. Because these *Centris* bees would not consume any sugar water I offered, I expressed crop contents to standardize nutrient and water availability. I weighed the bees and placed them in a sealed plastic container containing silica gel, at a relative humidity < 1% at 30 °C. I recorded masses every hour, and immediately after death of the bee. I dried all individuals in an oven at 50 °C until masses were constant (dry mass, m_d). Critical water content was calculated as:

$$critical\ water\ content = [(m_f - m_d) \cdot m_i^{-1}] \cdot 100\% \quad (3.2)$$

where m_f is the final wet mass (at death) and m_i is the initial wet mass.

Field validation of water loss rates: Because wind and solar radiation conditions differ for bees in the field versus in the respirometer, and because water loss rates during flight were only measured for a few minutes in the respirometer, I performed a field-validation of water loss rates for large *C. caesalpiniae* males in 2021. Before sunrise, I caught ten large morph males, and individually marked them with acrylic paint on the thorax or abdomen. I put five in a cage in the sun and five in a shaded location. The 30 cm^3 cages were constructed with aluminum 6.4 mm mesh, allowing bees to fly or walk. Bees in the sun were usually flying, while bees in the shade tended to remain still. Every 30 minutes, I measured air temperature, ground temperature, and total body mass of each bee using a portable field balance sufficiently accurate for tracking fluctuations in body mass of these large bees (Fisher SLF103, accurate to 0.001 g). Water loss rates were calculated as the change in mass per unit time.

*Critical thermal maxima (CT_{max})***:** To ensure the ecological relevance of my CT_{max} measures, I defined CT_{max} as the temperature at which bees lost control of flight. I caught large and small morph *C. caesalpiniae* males in the field in 2020 and transported them to my kitchen in individual tubes within a dark, insulated bag. Before the experiment, I allowed bees to adjust to room temp (26-30 °C) for one hour. I placed one individual in a 500 mL glass chamber coated with fluon to prevent landing. I turned on a 200-Watt lightbulb in an insulated box (Fig. 3.2), and gently stimulated the bee to fly if they were not already flying. The chamber heated at a rate of about 0.3°C per min starting at an initial temperature of 30 °C. This heating rate was based on preliminary field measures of heating rates of dead *C. caesalpiniae* on sunny ground in the field. I measured chamber temperature using a copper-constantan thermocouple thermometer (Type T, AWG 36) connected to a Pico Technology USB TC-08 Thermocouple Data Logger (Tyler, TX, USA). I defined flight failure as occurring when the bees could not sustain flight; usually they continued to buzz around the bottom of the chamber. Within five seconds of flight failure, I transferred the bee to a Styrofoam board and measured head, thorax, and abdomen temperature as described in Johnson et al., 2022.

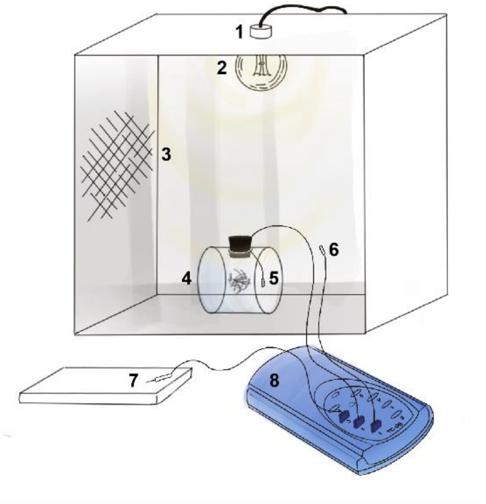

Figure 3.2. Critical thermal maximum setup. A 200 Watt light bulb (1) heated the chamber at a rate of ~0.3 Cmin-1 (2). Walls were insulated (3) to maintain a more constant ramping rate. Bees were placed in a 500 mL glass chamber (4) and stimulated to fly until failure. I recorded chamber temperature (5), box temperature (6), and took bee tagma temperatures (7) with a hypodermic thermocouple immediately after flight failure. I recorded temperature data with a Pico Logger (8).

Data Analysis : I tested data as to whether the assumptions of parametric statistics were met, log base ten transformed the data if necessary, and ran statistical analyses in GraphPad Prism (Version 8.0.0 for Windows; GraphPad Software, San Diego, CA, www.graphpad.com). I included only large morph *C. caesalpiniae* males in the analyses of mass and body water content, microclimate selection, respirometry, and validation of water loss rates. Laboratory *critical water content* studies included *C. pallida* females and males, and *C. caesalpiniae* females. I used both large and small morph *C. caesalpiniae* males in my measures of $CT_{\max}$. I excluded data if it was more than two standard deviations from the mean, and, if the data in question corresponded with field notes indicating a problem with the quality of measurement (for example, if I measured the tagma temperature of a bee more than five seconds after catching the bee). If means and slopes are presented, I included 95% confidence limits. I determined two-tailed significance at $\alpha = 0.05$ for all analyses.

I tested for associations between air temperature and wet and dry masses, and surface area, with a linear regression. I compared the regression lines of bees in different conditions (forward flight in the sun and flight in the shaded respirometer) using an ANCOVA. I used a general linear model to determine whether changes in body water content of bees in forward flight in the sun or shaded respirometer changed with increasing air temperature and dry mass. I used a linear regression to test for a scaling relationship between body water content or tagma water content with wet mass.

In the mark-recapture study, large males were not individually marked, resulting in possible pseudo replication across time points within days and between days. Therefore, I did not run any statistical analyses as the pattern was clear.

In the microclimate selection study, I assessed the same patches every thirty minutes, with the possibility of recounting bees as I was unable to individually identify bees. I pooled individual counts in the sun, and individual counts in the shade, then calculated the proportion of bees flying in each location: flying sun, flying shade, ground sun, and ground shade. I did not include any statistical analyses as the pattern was clear.

I used linear models to test for an association between total body mass and water loss rate during flight, as well as at rest in the *critical water content* experiment, and the effect of mass-specific water loss rate on mass-specific metabolic water production. To determine the difference in water loss rates for bees in positive versus negative water balance I ran a t-test. I ran a one-way ANOVA to test for differences in *critical water content* between *Centris* species, differences in water loss rates across conditions, and for $CT_{\max}$ comparisons between morphs. I corrected for multiple comparisons using Tukey's HSD test. I chose the multivariable models based on the lowest AIC. All data are available on Dryad (https://doi.org/10.5061/dryad.7pvmcvdzb).

Results

Body size, body mass, and crop content

The wet and dry mass of *C. caesalpiniae* males declined through the day, correlating with increased air temperature (Fig. 3.3A, F=1.53, DF= (3, 347), *P*=0.206, pooled m= -0.0091).). Air temperature increased at a rate of 2.8 $^{\circ}\mathrm{C}\cdot\mathrm{h}^{-1}$ (pooled sun and shade air temperature) and sunny ground temperature increased faster, at a rate of 9.6 $^{\circ}\mathrm{C}\cdot\mathrm{h}^{-1}$ (Fig. 3.3B). Ground temperature reached 50-60 °C depending on the day, at least 20 °C higher than peak air temperatures (Fig. 3.3B). Total body surface area of bees in the respirometer also declined with increasing air temperature. The mean wet mass of the large morph males was 234 mg, 95%CI [284.0, 303.0], (*n*=226) and the mean dry mass was 113 mg, 95% CI [108.6, 116.6], (*n*=224). Smaller crop volumes had higher sugar content (mean crop volume was 11.0 ±1.90 μL and mean sugar content was 71.4 ± 2.23 %), but I did not find any relationships between air temperature and sugar content or crop volume. On average, crop contents constituted 7.3 ± 1.3% of the total body water content.

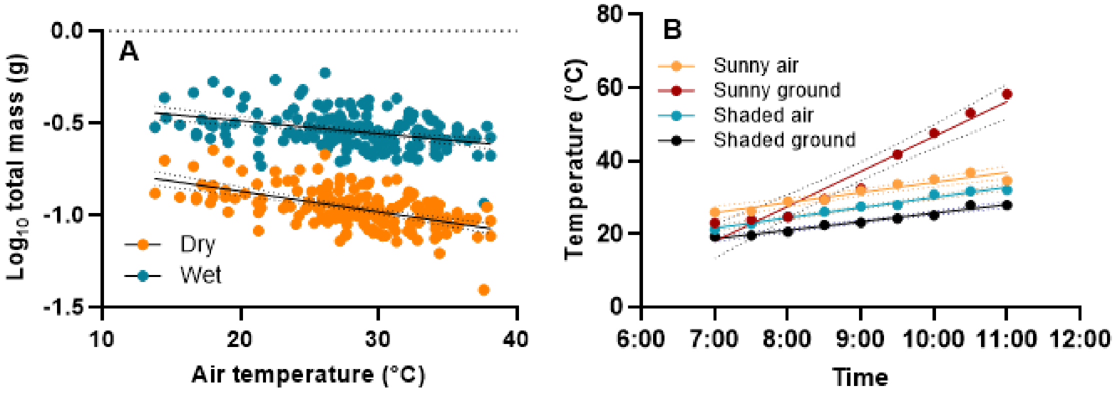

Figure 3.3 (A) As air temperature (T_{air}) increased through the day, the wet mass (g) of large morph *Centris caesalpiniae* males collected flying in the sun and flying in the shaded respirometry chamber decreased. ($\mathrm{Log}_{10}[\mathrm{mass}_{wet}]=-0.0069*[T_{air}]-0.35$, $n=179$, $r^2=0.15$, $P<0.0001$, slope 95% CI (-0.0094,-0.0044); as did dry mass (g): ($\mathrm{Log}_{10}[\mathrm{mass}_{dry}]=-0.011*[T_{air}]-0.65$, $n=176$, $r^2=0.31$, $P<0.0001$, slope 95% CI (-0.014,-0.0087). (B) Air (T_{air}) and ground temperatures (T_{ground}) increased through the activity period of *C. caesalpiniae* males. Ground temperature increased at the highest rate: $[T_{ground}]=9.6*[Time]-49.0$, $n=9$, $r^2=0.95$, $P<0.0001$, slope 95% CI (7.6,11.5). The slopes of sunny air, shaded air, and shaded ground were not significantly different ($F_{(2,21)}=1.39$, $P=0.2704$, pooled m= 2.6).

Body water content and scaling relationship to body mass

Total body water content declined with air temperature for bees free flying over the aggregation in the sunny field and in the shaded respirometer, with no significant difference between these groups (ANCOVA: $F_{(2,318)}=0.30$, $P=0.74$; Fig. 3.4A). Total body water content scaled isometrically (Fig. 3.4B). This resulted from differential scaling of water content in the various body tagma, as water content scaled hypometrically in *C. caesalpiniae* male heads, isometrically in thoraxes, and hypermetrically in abdomens. Thoraxes had the highest water content, followed by abdomens, then heads.

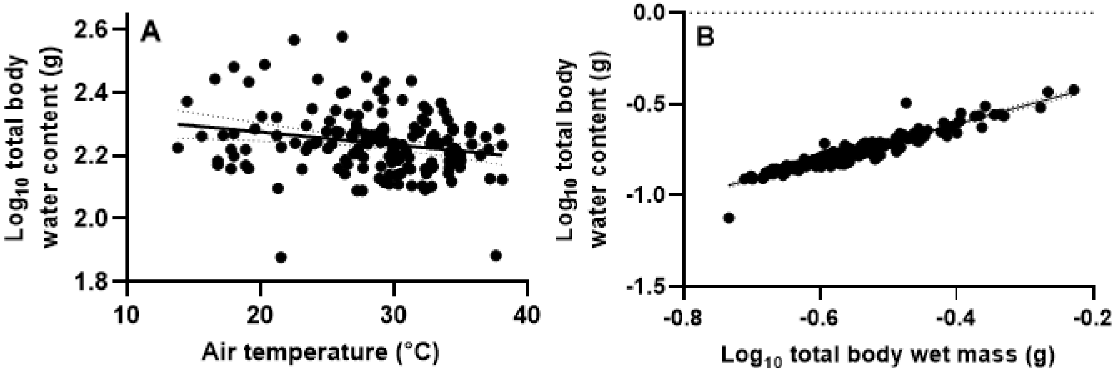

Figure 3.4 (A) The total body water content (g) of bees declined with air temperature: $\text{Log}_{10}[W_b]=-0.0040*[T_{air}]+2.36$, $n=162$, $r^2=0.048$, $P<0.01$, slope 95% CI (-0.0068,-0.0012). (B) Total body water content of flying male *C. caesalpiniae* scaled isometrically with wet mass: $\text{Log}_{10}[W_b] = 1.01*[\text{Log}_{10}(\text{mass}_{wet})]-0.021$, $n=162$, $r^2=0.90$, $P<0.0001$, slope 95% CI (0.96, 1.06).

Mark-recapture and microclimate selection

The proportion of marked bees declined with time of day (Fig. 3.5A) and declined further over subsequent days (GLM: day effect, $F_{(4,27)}=17.5$, $P<0.0001$). Over 20% of captured bees were marked on the second day after marking, but by the ninth day after marking, less than 4% of captured bees were marked (Fig. 3.5B).

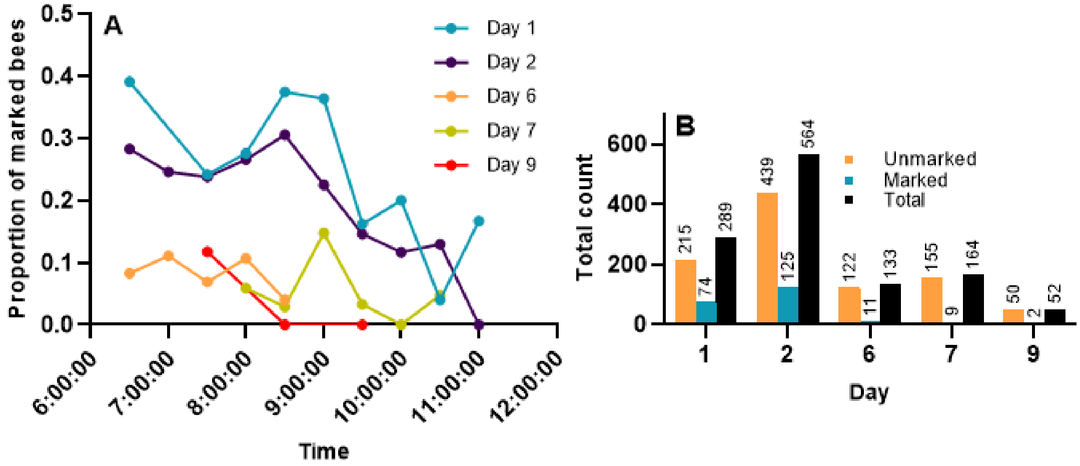

Figure 3.5 (A) The proportion of marked *C. caesalpiniae* males recaptured over the activity period declines over the activity period). (B) The total number of marked and unmarked netted bees each day is also shown.

Bees tended to fly in sunny locations early in the day and shady locations later in the day (Fig. 3.6A). Similarly, bees were often found on sunny ground early in the day, and shady ground later in the day (Fig. 3.6B).

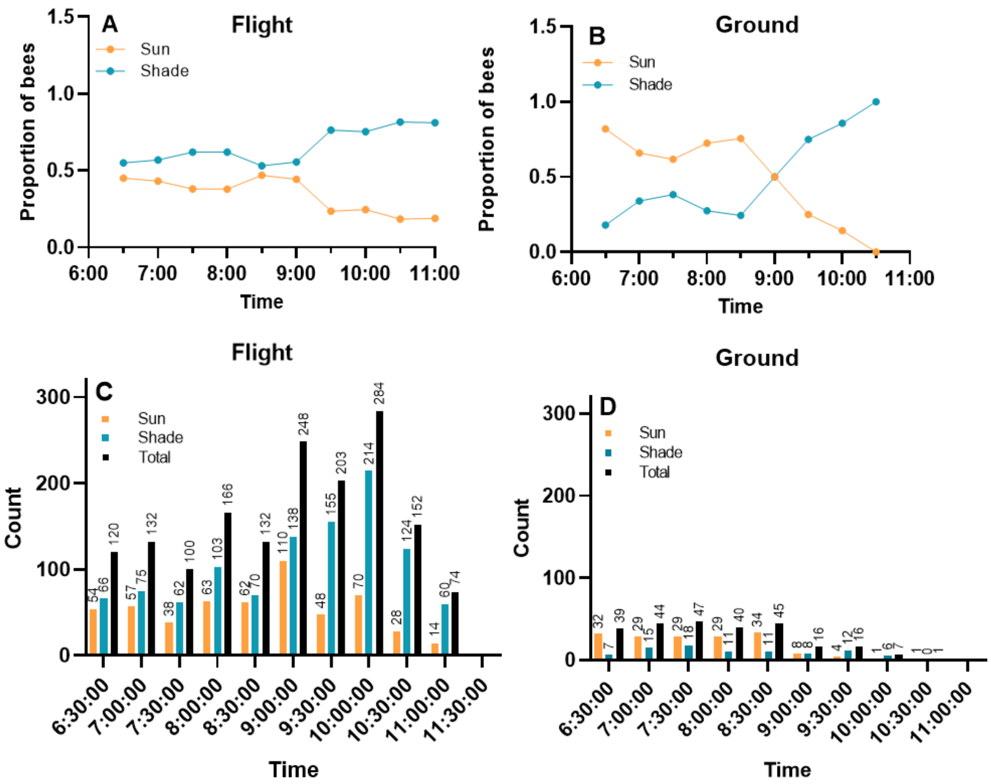

Figure 3.6 (A) *Centris caesalpiniae* males were more likely to be flying in shade as the morning progressed. (B) The proportion of *C. caesalpiniae* males on shady ground increased as the day progressed. (C) Counts of flying *C. caesalpiniae* in the sun or shady plots as a function of time of day. (D) Counts of *C. caesalpiniae* on sunny or shady ground as the day progressed.

Metabolic water production and water loss rate

During flight, mass-specific water loss rates (*WLR*) were positively correlated with mass-specific rates of metabolic water production (*MWP*) for large-morph *C. caesalpiniae* males, but on average, water loss exceeded metabolic water production by 33% (Fig. 3.7A). Johnson et al., 2022 showed that metabolic rate scales with body mass$^{0.67}$; using data from Johnson et al., 2022, I found that water loss rate was independent of body mass (Fig 3.7B). I found no effect of water content, head or thorax temperature, air temperature, or wet or dry mass on mass-specific water loss rates. Accounting for metabolic water gain, the net loss of water was 12.9% of total body water per hour.

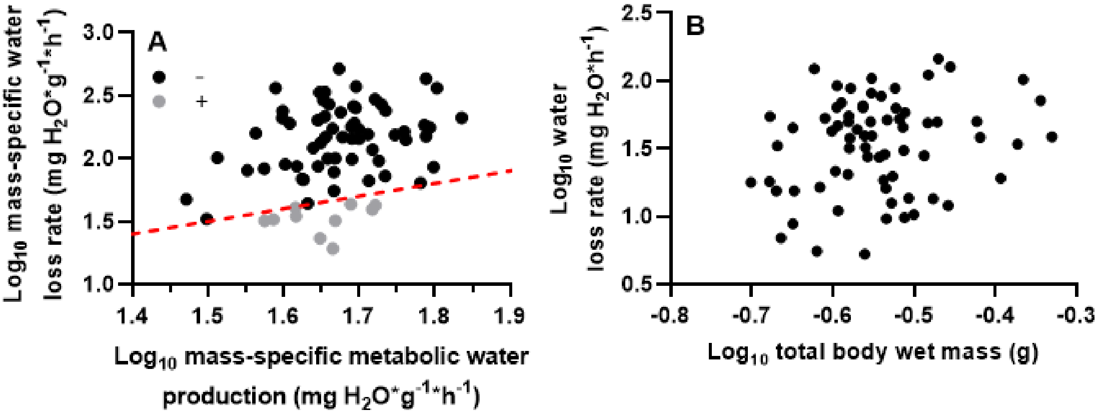

Figure 3.7 (A) Mass-specific water loss rates (calculated from Johnson et al. 2022) exceeded mass-specific metabolic water production for the majority of flying *Centris caesalpiniae* large morph males in a shaded metabolic chamber ($\text{Log}_{10}[WLR]=0.067*\text{Log}_{10}[MWP] + 1.53$, $n=71$, $r^2=0.10$, $P<0.05$, slope 95% CI (0.0194,0.1186). The red dashed line indicates equality of water loss and metabolic water production. Black data points indicate positive water balance and gray data points indicate negative water balance. (B) Water loss rate was not significantly linearly related to wet mass, but the estimated slope did not differ from isometric scaling.

Critical water content of Centris bees

C. caesalpiniae females had the lowest critical water content (47.6 ±0.82%) and *C. pallida* females had the highest (Fig. 3.8A, 53.7 ±0.55%). As bee dry mass increased, the rate of water loss increased isometrically with dry mass while at rest in the 30 °C, 0% humidity chamber (Fig. 3.8B).

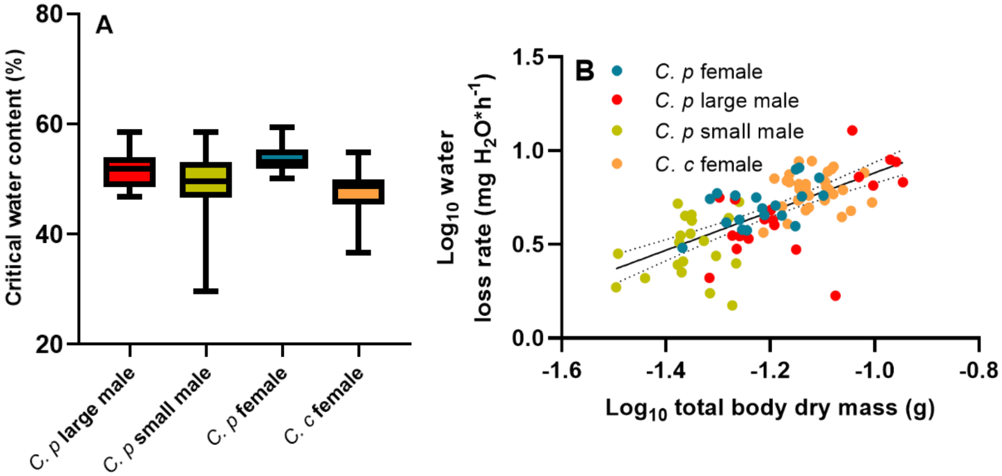

Figure 3.8 (A) Critical water content of *Centris* bees varied between species. One-way ANOVA: $F_{(3,\ 89)}$ = 7.87, *P* = 0.0001. (B) Larger bees (dry mass, g) had higher water loss rates (mL H_2O*h^{-1}). [Log_{10}(*WLR*)] = 1.03*[Log_{10}(m_d)] + 1.91, r^2=0.45, *P*<.0001, slope 95% CI (0.79,1.27). M_d = dry mass, *WLR* = water loss rate, *C. p* = *Centris pallida*, *C. c* = *Centris caesalpiniae.*

Comparison of water loss rates for flying bees in the respirometer versus field conditions

The water loss rates of large male *C. caesalpiniae* flying in sunny cages (measured gravimetrically) were similar to water loss rates measured with a respirometer (Fig. 3.9). Bees flying in sunny cages lost 43.1% of total body water over four hours (10.8% of total body water content per hour) then died, whereas those mostly standing in the shaded cages lost 11.3% of total body water (2.83% of total body water per hour) and lived. Water loss rates for resting bees in the lab (nonflying, 30 °C, during the critical water content experiments) were similar to those measured for *C. caesalpiniae* males in shaded field-cage conditions (Fig. 3.9). “Resting water loss rates” averaged five times lower than bees in flight in the shaded respirometer or sunny cages.

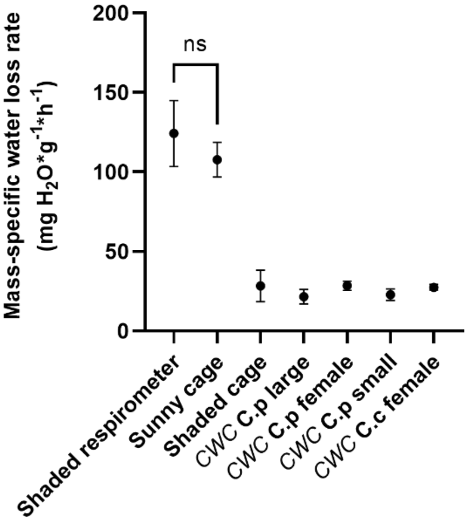

Figure 3.9. The mean mass-specific water loss rate of flying *C. caesalpiniae* males in the respirometer or in sunny field cages was higher than that of non-flying Centris bees in shaded field conditions or measured in the lab at 30 °C. One-way ANOVA: $F_{(6, 166)} = 21.2$, $p < 0.0001$. *C. p = Centris pallida*, *C. c = Centris caesalpiniae.*

Critical maximum temperature

Mean thoracic CT_{max} of *C. caesalpiniae* large males was 51.2 °C ± 0.4 (Fig. 3.10), or 6 °C higher than the hottest thorax temperature measured in sunny field conditions (45.2 °C; Johnson et al., 2022). Mean thoracic CT_{max} of small morph males was 49.4 °C ± 0.8 (Fig. 3.10). CT_{max} did not significantly differ between large and small morph males ($|t|=1.95$, $P=0.053$); however, the thorax was hotter than the head ($|t|=6.56$, $P<0.0001$) and the abdomen at CT_{max} ($|t|=8.34$, $P<0.0001$). Total body wet mass did not affect tagma CT_{max} (pooled thoracic temperatures: [body mass]=12.49*[T_{thorax}]+47.82, P=0.060, n=21).

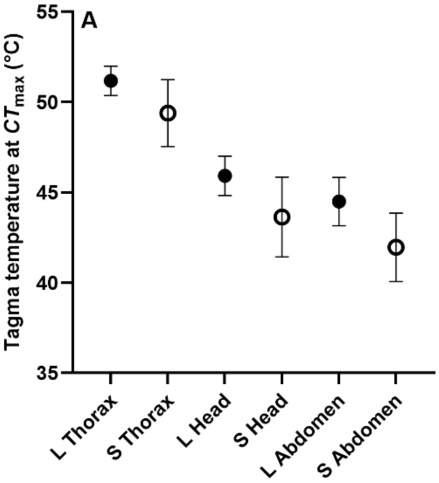

Figure 3.10. Critical maximum temperature of large and small morph *C. caesalpiniae* males. Thorax, head, and abdomen temperatures of large and small males were not significantly different at CT_{max}, but the thorax was significantly hotter than the other two tagma at $CT_{max.}$ For large morphs (n=13) and for small morphs (n=8). *L* = large morph; *S* = small morph.

Discussion

Desiccation is a more critical limit than overheating

Several lines of evidence suggest that desiccation, not overheating, is a more important limit on the activity of large *C. caesalpiniae* males. Mean flight water loss rates (13% of body water lost per hour) would result in lethal water loss in 3.9 hours based on the mean *critical water content* (50%) for *Centris* bees, similar to the duration of activity for *C.* caesalpiniae large males during a single day. Both the mark-recapture data, and the declines in body mass over time suggest significant turnover of males within a morning, with larger males active in the early morning and smaller males active in the late morning. I hypothesized that males leave the aggregation site in two to three hours, before their body water content approaches dangerous levels.

In contrast, my data do not support the hypothesis that overheating forces *Centris* males to cease activity. The flight CT_{max} of *C. caesalpiniae* males was at least 6 °C higher than the hottest thorax temperatures in the field, so these bees would appear to have a relatively larger thermal safety margin (Fig. 3.10). Of course, conceivably, thermal damage to sensitive tissues such as sperm may occur at temperatures below CT_{max}. Additionally, the CT_{max} of *C. caesalpiniae* was not exceptional among bees (Table 3.1), suggesting that *C. caesalpiniae* have not been selected to survive high body temperatures. I used a different method for measuring CT_{max} compared to most investigators, as I measured CT_{max} during flight rather than using quiescent insects. Plausibly, the CT_{max} of C. caesalpiniae would be higher if measured on nonflying bees. I also used a faster ramping rate than most previous studies; my ramping rate was based on measurements of how fast the bees can heat in the field. In general, a faster ramping rate tends to raise CT_{max} (Gonzalez et al., 2022; Johnson et al., 2023) and the fact that failure to sustain flight may be an earlier metric of CT_{max}. Because methods and measures of CT_{max} vary widely across and within species (Table 3.1), I did not conduct formal statistical tests on these data.

Size patterns

As found in a prior study (Johnson et al., 2022), I found that larger males were active in the early morning, and smaller males were active in the later morning as evidenced by the decline in dry mass, wet mass, and surface area with time of day

(Fig. 3.3A). Plausibly, this occurred because larger males had a greater capacity to endothermically raise thorax temperatures during cool mornings when females were most abundant (Johnson et al., 2022), and then left the site in two to three hours to prevent excessive water loss. I found no evidence that body size affected relative water balance, as water content scaled isometrically (Fig. 3.4B), and water loss rates and metabolic water production rates patterns versus body mass were too noisy to accurately determine mass-scaling patterns (Fig. 3.7A-B).

Using mark-recapture to estimate within and across-day male persistence

The number of recaptured, marked males, declined by 50% through the morning (Fig. 3.5A), suggesting that marked males left the aggregation site as the morning progressed. Male *C. caesalpiniae* returned in similar numbers on Days 1 and 2 (Fig. 3.5A), indicating that males live longer than one day, and therefore likely replenish water content outside of the activity period. Marked males were captured up to Day 9, though the numbers of returning males dwindled each day after Day 2 (Fig. 3.5B).

Behavioral thermoregulation

Males shifted from sunny to shady locations, and from ground to air, as the morning progressed, and temperatures warmed (Fig. 3.6A-B). Males may dig and fly near sunny ground in the early morning to warm up flight muscles, and then move to flying above the aggregation in shade to avoid overheating and minimize water losses, as water loss increases with temperature (Johnson et al. 2022).

An important remaining question: What are the major routes of water loss during flight?

To address the question of whether desert bees such as *C. caesalpiniae* have evolved adaptations to reduce the risks of desiccation during flight, it will be necessary to determine the major avenues of water loss during flight. Water loss may occur across the cuticle, through the spiracles associated with breathing, via oral regurgitation (as in honey bees, Heinrich, 1980), or by defecation. I did not observe regurgitation or defecation in the respirometry or the critical water content experiments, and since evaporative heat loss is a minimal portion of the heat budget (Johnson et al. 2022), I do not think that these pathways are likely important routes of water loss for flying *Centris* males. My experiments did not allow me to separate cuticular versus respiratory water loss. Water loss for quiescent bees at a body temperature of 30 °C was approximately 20% of those measured during flight, at an average weighted body temperature of 36.6 °C. Assuming a Q_{10} of ~2.3 (Chown et al.-+, 2011), water loss for quiescent bees may increase to approximately 40% of those observed in flight. Since the majority of water loss is cuticular for most quiescent insects (Hadley, 1994), this may represent a lower estimate for cuticular water loss rates for flying bees. However, cuticular water loss rates are expected to increase strongly with wind speed, due to the reduction in boundary layer (Chappell, 1982). Water loss can be estimated from the molar rate of oxygen uptake times the relative gradient for water relative to oxygen (Woods and Smith, 2010). At 36.6 °C, water vapor pressure is 6.1 kPa. The gradient for oxygen in *Centris* bees is unknown, but in bumblebees, muscle P_{O2} during flight is near ~7 kPa (Komai, 2001); suggesting a 14 kPa P_{O2} gradient. If this oxygen gradient applies to flying *Centris*, the molar rate of water loss should be approximately 40% of the molar rate of oxygen uptake, and respiratory water loss can be estimated as 19 $\text{mg}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$, or approximately 15% of total water loss rate during flight. However, conceivably, the gradient for oxygen across the spiracles could be much lower, and the majority of total water loss could be respiratory. Direct measures of cuticular and respiratory water loss are needed to determine the primary routes of water loss during flight of *C. caesalpiniae.*

Limitations of my study

Large *C. caesalpiniae* males did not show evidence of active water loss (Johnson et al., 2022) nor did small morph *C. pallida* males (Roberts et al., 1998), supporting the idea that desert *Centris* minimize water losses used for cooling during flight. On the other hand, *Apis mellifera* (Roberts and Harrison, 1999), *Xylocopa capitata* (Nicolson and Louw, 1982) and the wasp, *Lissopimpla excelsa* (Tomlinson and Phillips, 2012) use evaporative cooling at high air temperatures, at least in some cases, using regurgitation to cool the head. I did not fly *C. caesalpiniae* in air temperatures greater than 38 °C. If forced to fly above this air temperature, desert Centris may possibly activate evaporative heat loss mechanisms.

My measurements of critical water content in the lab and field lacked controls in which *Centris* bees were kept without food but with water, to demonstrate that death occurred due to desiccation. However, the fact that the critical water contents I measured are quite similar to those measured for a sweat bee, and lower than measured for honey bees and a bumble bee (Burdine and McCluney 2019), suggests that death did occur due to desiccation. However, the fact that the critical water contents I measured are quite similar to those measured for a sweat bee, and lower than measured for honey bees and a bumble bee (Burdine and McCluney 2019),

suggests that death did occur due to desiccation. Another issue with the critical water content trials was that I was unable to test *C. caesalpiniae* large morph males due to lack of availability, and so I was forced to estimate flight durations for these males using critical water contents of the other *Centris* species and morphs.

I do not know the fuels used by *C. caesalpiniae* large males during flight. It is plausible that these bees could catabolize fats rather than carbohydrates as I assumed. Catabolized oleic acid yields 17 moles of water for every 18 moles of carbon dioxide produced, whereas glucose yields a 1:1 ratio of H_2O to CO_2. If *C. caesalpiniae* do utilize fats as metabolic fuels, this would be a small correction that would not appreciably alter the conclusions of this study.

However, oxidation of glycogen could provide an additional source of water not included in my calculations, as could loss (due to catabolism) of tissues. Glycogen is stored in the fat body and flight muscle of bees (Harrison, 1986). Glycogen binds 1.6 to 3.8 grams of water per gram of glycogen (Shiose et al., 2022). If all of the 60 $ml \cdot g^{-1} \cdot h^{-1}$ of oxygen consumption was supported by glycogen catabolism, water released from glycogen catabolism could replace all water lost. Another source of water may stem from the breakdown of muscles during flight as in birds (Lindstrom et al., 2000). However, while such alternative sources of "metabolic water" may influence the source of water, this will not alter the relationship between water loss and critical water content. My finding that bees in sunny cages in the field lost 43% of body water and died within four hours suggests that my estimates of how long bees can fly without desiccating to death are reasonable.

Conclusions

The high water loss rates of large morph *Centris caesalpiniae* males plays the major role in limiting mating activity period. However, temperature remains an important limiting factor in certain contexts. For example, flying males often land on hot ground to search for emerging females and mate on the ground, possibly heating up near to critical thermal maxima. Understanding the interactive effects of heating and water loss in different microclimates and during different behaviors will be important to generate predictive models. Native bees are critical components of many desert ecosystems and merit special attention in order to understand the threat of climate change to community function.

Table 3.1. Critical thermal maxima values for bees range from 38.2 to 58.5 °C.

Species	**CT_{max} (°C)**	**Reference**
Agapostemon sericeus	50.3	Burdine and McCluney, 2019
Agapostemon virescens	44.6	Hamblin et al., 2017
Apis mellifera	49.2	Kovac et al., 2014
Apis mellifera	50	Kovac et al., 2014
Apis mellifera	50.7	Sánchez-Echeverría et al., 2019
Apis mellifera	50.9	Sánchez-Echeverría et al., 2019
Apis mellifera	49.1	Burdine & McCluney, 2019
Apis mellifera	42	Gonzalez et al., 2022
Apis mellifera	47.6	Gonzalez et al., 2022
Apis mellifera	47.3	Gonzalez et al., 2022
Apis mellifera	48.9	Gonzalez et al., 2022
Apis mellifera	47.6	Gonzalez et al., 2022
Apis mellifera	50.7	Atmowidjojo et al., 1997
Apis mellifera	42.8	Atmowidjojo et al., 1997
Bombus bifarius	39.6	Oyen et al., 2016
Bombus bifarius	43.8	Oyen et al., 2016
Bombus bimaculatus	44.7	Hamblin et al., 2017
Bombus griseocollis	45.3	Hamblin et al., 2017
Bombus huntii	44.8	Oyen et al., 2016

Bombus huntii	45	Oyen et al., 2016
Bombus impatiens	53.1	Burdine & McCluney, 2019
Bombus impatiens	53.1	Oyen and Dillon, 2018
Bombus impatiens	48.7	Oyen & Dillon, 2018
Bombus impatiens	58.5	Oyen & Dillon, 2018
Bombus impatiens	46.1	Hamblin et al., 2017
Bombus sylvicola	38.2	Oyen et al., 2016
Bombus sylvicola	39.8	Oyen et al., 2016
Bombus terrestris audax	50	Maebe et al., 2021
Bombus terrestris audax	49.8	Maebe et al., 2021
Bombus terrestris canariensis	50.2	Maebe et al., 2021
Bombus terrestris canariensis	49.9	Maebe et al., 2021
Bombus terrestris terrestris	49.6	Maebe et al., 2021
Bombus terrestris terrestris	50.1	Maebe et al., 2021
Bombus vosnesenskii	51.6	Pimsler et al., 2020
Braunsapis puangensis	47.7	da Silva et al., 2021
Centris caesalpiniae small male	49.4	present study
Centris caesalpiniae large male	51.2	present study
Centris pallida small male	45.41	Barrett et al., 2022
Centris pallida large male	44.7	Barrett et al., 2022
Centris pallida female	45.99	Barrett et al., 2022
Ceratina calcarata	48.1	Hamblin et al., 2017
Ceratina dentipes	49.7	da Silva et al., 2021
Ceratina strenua	51.3	Hamblin et al., 2017
Halictus ligatus/poeyi	47.3	Hamblin et al., 2017
Homalictus fijiensis	43.9	da Silva et al., 2021
Lasioglossum bruneri	44.6	Hamblin et al., 2017
Lasioglossum imitatum	45.9	Hamblin et al., 2017
Megachile campanulae	45.3	Hamblin et al., 2017
Megachile exilis	46.2	Hamblin et al., 2017
Megachile mendica	47.7	Hamblin et al., 2017
Megachile rotundata	48.2	Hamblin et al., 2017
Ptilothrix bombiformis	47.8	Hamblin et al., 2017
Xylocopa iris	46.6	Gonzalez et al., 2020
Xylocopa olivieri	45.2	Gonzalez et al., 2020
Xylocopa violacea	45.7	Gonzalez et al., 2020
Xylocopa virginica	49.8	Hamblin et al., 2017

CONCLUSIONS

Major findings

I demonstrate that that the abdominal convector plays a major role in the heat balance of *Centris* bees, and quite possibly, this mechanism extends to other large-bodied desert bees (Johnson et al., 2023). I created the most precise heat budget to date for a flying desert bee: incorporating the heat gain from solar radiation, direct VCO_2 and VH_2O measures during flight, tagma temperatures, and precise values of the thoracic and abdominal coefficients of absorption (Chapter 2). Given that evaporative heat loss is a small part of the heat budget for *Centris* (Fig. 1.7; Fig. 2.7D), yet water loss rates are quite high and variable (Fig. 3.7; Fig. 3.8), I consider whether heat balance or desiccation is a greater limit on the mating activity of *Centris* males*.* Using a combination of respirometric measures, critical water content assays, critical thermal maximum assays, and video footage of the aggregation activity in different microclimates, I show that water loss, not overheating, limits the activity period of males (Chapter 3).

Centris are important pollinators in the Sonoran Desert, and a thorough understanding of their thermal biology will certainly improve the understanding of the effects of climate change on native bee pollinators. I conducted the majority of physiological measures in field conditions rather than laboratory conditions, providing a direct insight to the effect of real-time abiotic conditions on an animal's physiology. I used native desert, non-Apis bees as a representative of the responses of a desert-adapted organism rather than something invasive or introduced. More broadly, however, this dissertation represents a significant contribution to the biophysical ecology and heat budget modeling literature using *Centris* as a model organism for large-bodied flying desert invertebrates.

Centris and climate change

What will happen to desert *Centris* as arid environments further heat up and dry out? The answer to this question depends on the confluence of a number of abiotic and biotic factors. Availability of floral resources and nesting habitats, microclimatic diversity, and changes in temperature and precipitation patterns will almost certainly interact with each other. Since the 1970s, *C. pallida* foraging male head width (a proxy for body size) has declined by 5.0–5.2 mm in 1974 to 4.6–4.8 mm in 2022 at a longstanding aggregation site near the floodplains of the salt river in Arizona (Barrett & Johnson, 2022). Evidence from this population also suggests that *C. pallida* have advanced their emergence phenology in the spring by approximately two weeks (Barrett & Johnson, 2022). However, this phenological advancement is unlikely the sole reason for smaller male body sizes, as their host trees, the foothills palo verde, does not present as a clear phenological mismatch with the timing of *C. pallida* emergence (Barrett & Johnson, 2022; Zachmann et al., 2021). An Alternative explanations for decreased body sizes are that increased ambient temperatures may cause rapid development and thus smaller body sizes (Kingsolver et al., 2021).

What if air temperature increases 1 °C, 2 °C, or 3 °C? With greater increases in air temperature, the abdominal convector becomes obsolete, so the activity period of *Centris* bees would likely be restricted. In addition to this, any increases in air temperature would also cause increased water loss rates, which I showed to limit activity of *C. caesalpiniae* (Chapter 3).

Broad patterns of adaptation in deserts: From microbes to mammals to man

As many regions in the world become hotter and drier, comparing the mechanistic responses of taxonomically diverse desert organisms will help humans understand how and why certain species were able to adapt and evolve to thrive in arid environments. Predicting how changing abiotic factors affect physiology, behavior, and morphology of keystone species, such as the native insect pollinators, will yield a better understanding of how broader ecological connections will shift in the future. For example, if large-bodied desert insect pollinators were to limit their activity period as a result of increased air temperatures and water loss rates, their pollination services to crops, home gardens, and wild plant communities would conceivably decline. At higher trophic levels, birds, bats, and other animals (including humans) that consume or rely on the pollinated plant would likely be affected (nutritionally and/or habitat-wise) by the decreased seed set, small plants, or reduced fruit size. Through an abiotic lens, reduced size or absence of plants would decrease groundcover, resulting in hotter and drier conditions than ever, perpetuating the cycle of decreased activity period.

The commonly cited main “problem” in deserts is that they are both hot and dry. High temperatures cause organisms to heat up, and most must cool in some way in order to avoid heat-associated damage at best and dying at worst. For many organisms, evaporation is the quickest way to cool. Yet, water is a scarce resource in deserts during most times of year, so evaporation is not sustainable for long term cooling. *Centris* bees are not alone in their adaptation to desert environments. Numerous reviews of desert mammals (Rocha et al., 2021; Schmidt-Nielsen, 1997; Walsberg, 2000), birds (Maclean, 2013; J. B. Williams & Tieleman, 2005), spiders (Cloudsley-Thompson, 1983), scorpions (Hadley, 1974), insects (Hadley, 1994; P.

Willmer & Stone, 1997), bacteria (Powell et al., 2015), fungi (Friedmann & Galun, 1974; Santiago et al., 2018), reptiles (Cloudsley-Thompson, 2012), plants (Mulroy & Rundel, 1977; Smith et al., 1997; Wood, 2005), and even humans (Jochim, 2013) show that desert dwellers use a number of mechanisms (behavioral, physiological, and morphological) to maintain low body temperatures, and limit water losses.

Many similar patterns to combat overheating and water losses have emerged among the native desert taxa, but a comprehensive review of their similarities and differences is needed to fully understand any patterns of adaptation in arid environments. From microbes to mammals, activity period, quiescence, and diapause appear to be critical to surviving through the hottest and driest times. Native ground-nesting bees commonly spend up to eleven months underground, emerging when their host plant blooms (Danforth et al., 2019). Plants allocate energy to reproduction usually after the rains, or the rainy season, and certain plants drop their leaves to minimize surface area exposed to solar heating. Small rodents are nocturnal to avoid daytime heat (Walsberg, 2000), and time of day affects the activity patterns of other smaller mammals and birds as well (Goldstein, 1984; Levy et al., 2016; Robbins, 1981). Even cyanobacteria grow only during the wet periods in the desert, remaining just below the surface to avoid UV exposure during hotter times (Powell et al., 2015). It would appear that a key strategy for survival is waiting, timing, and rapid responses to changes in the environment.

Physiological mechanisms of regulating heat and water balance are also critical, and desert organisms hugely vary in which mechanism(s) they use. Changes in metabolism, ventilatory patterns, convective cooling, increased urine concentration, photosynthesis patterns and location of photosynthesis, changes in lipid coatings on the organism surface, and more - are commonly found throughout the many species of native desert taxa. Even morphologically, reduced surface area (i.e., basking position, small plant leaves) is a common trait to minimize heat gain, and a massive array of surface structure modifications to alter heat gain/loss can be found on desert organisms from ants (Shi et al., 2015), to plants (Barthlott et al., 2017; Ehleringer & Björkman, 1978), bees (Barrett & O'Donnell, 2023), and mammals (Walsberg, 1983, 1988; Walsberg & Schmidt, 1989).

Outstanding questions

I hope this dissertation has sparked interest in the exciting fields of biophysical ecology, desert biology, and native pollinator thermal biology. There is much yet to understand and learn about the desert environment. To facilitate future work, I have outlined some interesting gaps in the literature identified by working on this dissertation (Table 4.1).

Topic	**Questions**
Water balance	By what route do flying *Centris* bees lose water?
	Does cuticular water loss differ between related xeric and more mesic bees?
	What is *Centris* cuticle comprised of, and how does it limit water loss?
	What fuel does *Centris* burn while flying, and how does that affect metabolic water production?
Flight kinematics	What is convective heat loss as measured in a wind tunnel for a model *Centris* ?
	Do bees change flight speed to manipulate convective heat loss?
	What role does forced convection by the wings play in cooling?
Optics	What is the mid-infrared emissivity of *Centris*? Is it body region-specific?
	Does mid-infrared emissivity differ between xeric, mesic, and tropical *Centris*?

	How does setal (hair) structure cause reflection or absorption in the near-infrared?
Comparative biology	Do thermoregulatory responses to high temperature differ between desert and tropical endothermic insect fliers?
	How does heat balance and water balance compare across native desert taxa?

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APPENDIX A

A DESERT BEE THERMOREGULATES WITH AN ABDOMINAL CONVECTOR DURING FLIGHT, *JOURNAL OF EXPERIMENTAL BIOLOGY*

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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 (Dzialowski and O'Connor, 1999), and dragonflies can lose heat through their wings (Guillermo-Ferreira and Gorb, 2021).

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Table 1. Thermoregulatory mechanisms of flying endothermic insects

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

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}):

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

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 (κ), 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 κ 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 tagma 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 setup 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^{-1} 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_2-free air. Output of the chamber was directed to the sample cell of a LI-7000 CO_2/H_2O Gas Analyzer with the reference cell maintained at zero CO_2 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

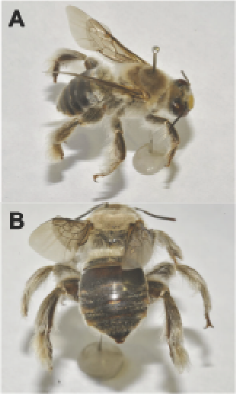

Fig. 1. *Centris caesalpiniae* large male morph. (A) The large morph male has light, densely packed setae on the thorax and a relatively hairless, and darker colored, dorsal abdomen, with long silver hairs on the ventral abdomen and legs. (B) Posterior view of the large morph male abdomen.

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_2 and H_2O production during 2–3 min of flight. Bees hovered well in the chambers with little need for external stimulation. We calibrated the CO_2 analyzer, which is accurate to 0.1 μmol mol^{-1} from 0 to 3000 μmol mol^{-1}, with dry, CO_2-free air and a compressed air tank containing certified (resolution: 0.01 μmol mol^{-1}) 252 ppm CO_2 span gas. The water analyzer is accurate to 1% of full scale from 0 to 60 mmol mol^{-1}. 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_2 and H_2O 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_2 and H_2O levels over 3–4 min without an animal in the chamber; under these conditions. there were no significant changes in CO_2 concentration. During flow-through respirometry, 95% washout of CO_2 from the metabolic chamber occurred in approximately 90 s.

We calculated CO_2 production rate ($\dot{V}_{CO_2}$, ml h^{-1}) using Eqn 2, where FR is flow rate (m h^{-1}) and F_{CO_2} is the fractional CO_2 level (μmol mol^{-1}) in the excurrent air from the respirometry chamber during flight:

$$\dot{V}_{CO_2} = F_{CO_2} \times \mathrm{FR}. \tag{2}$$

We calculated water loss rate ($\dot{V}_{H_2O}$, mg H_2O h^{-1}) using Eqn 3, where F_{H_2O} is the average fractional level of H_2O (mmol mol^{-1}) in the excurrent air from the respirometry chamber during flight:

$$\dot{V}_{H_2O} = \frac{(F_{H_2O} \times \mathrm{FR} \times M}{V_m}, \tag{3}$$

where M is the molar mass of water (18 g mol^{-1}) and V_m is the molar volume of water (22,400 ml mol^{-1}).

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_{thorax}, T_{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_{tagma} data with a Pico Technology USB TC-08 Thermocouple Data Logger (Tyler, TX, USA). We recorded the shaded T_{air} following the temperature measurements for each individual. We calculated R_{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_2 and H_2O 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_{air}. We calculated average bee surface temperature (T_{bee}, °C) using Eqn 4, which weights each tagma according to its relative surface area (SA):

$$T_{bee} = \frac{(\mathrm{SA}_h \times T_h) + (\mathrm{SA}_{thorax} \times T_{thorax}) + (\mathrm{SA}_{ab} \times T_{ab})}{(\mathrm{SA}_h + \mathrm{SA}_{thorax} + \mathrm{SA}_{ab})}. \tag{4}$$

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_2 emission traces we observed. Using Eqn 5, we calculated a heat budget for flying bees at every degree between 19 and 38°C where $Q_{metabolic}$ indicates metabolic heat production, $Q_{radiation}$ indicates net radiative heat loss, $Q_{evaporation}$ indicates evaporative heat loss and $Q_{convection}$ indicates net convective heat flux:

$$0 = Q_{metabolic} + Q_{radiation} + Q_{evaporation} + Q_{convection}. \tag{5}$$

$Q_{metabolic}$ and $Q_{evaporation}$ were calculated from $\dot{V}_{CO_2}$ and $\dot{V}_{H_2O}$. 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^{-1} CO_2 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 $\dot{V}_{H_2O}$ by the latent heat of evaporation of water, 2.45 J mg^{-1} H_2O.

As we performed respirometry measurements in the shade, we assumed shortwave radiation to be negligible. We summed the longwave (infrared) net radiation ($r_{loss} - r_{gain}$) for the head, thorax, and abdomen of each bee using the Stefan–Boltzmann equation:

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

We assumed that the bee's emissivity, ε_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, ε_c, is 0.90, and that T_{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 $Q_{\mathrm{radiation}}$ for the head, thorax and abdomen. We calculated convective heat exchange using Eqn 7:

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

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 (κ is a measure of the capacity of the bee to transfer heat) in W mm^{-2} K^{-1}, we divided convective heat transfer ($Q_{\mathrm{convection}}$) in W by the total surface area of the bee and the gradient between T_{air} and average T_{bee} (in K) by combining Eqns 4 and 7:

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

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

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

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). 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 α=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 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.

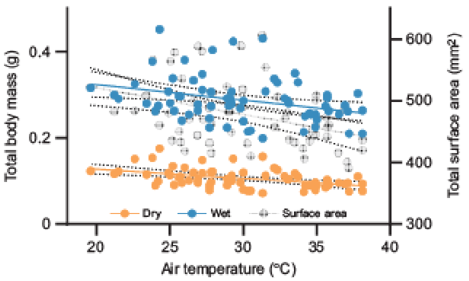

Fig. 2. Total body mass and surface area versus air temperature for *C. caesalpiniae* large morph males. As air temperature increased through the day, wet mass, dry mass and total body surface area decreased. Dotted lines indicate 95% confidence intervals (CI). Linear regression: wet mass=$-0.0036T_{\mathrm{air}}+0.40$,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

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°C for every 1°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_{ab}=$0.012T_{\mathrm{air}}$+0.082, n=65, r^2=0.17,

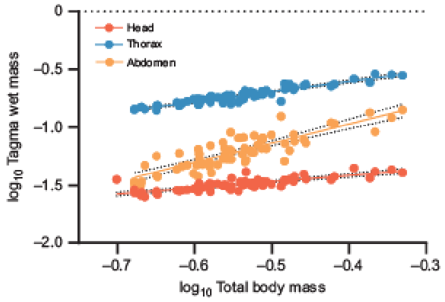

Fig. 3. Allometric scaling of *C. caesalpiniae* large morph male body tagma. Head (M_{h}; g) and thorax mass (M_{thorax}; g) scaled hypometrically with body mass (M_{b}; g), while abdomen mass (M_{ab}; g) scaled hyperallometrically. Linear regression: M_{h}=$0.54M_{\mathrm{b}}-1.20$, r^2=0.66, P<0.0001,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

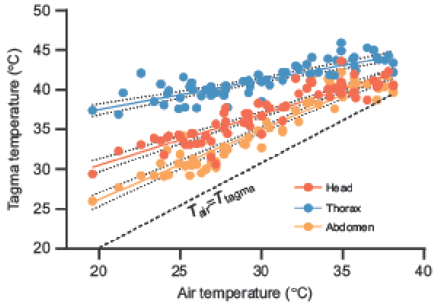

Fig. 4. Tagma temperature versus air temperature for *C. caesalpiniae* males flying in a shaded glass chamber. Tagma (T_{tagma}), head (T_h), thorax (T_{thorax}) and abdomen (T_{ab}) temperature increased with air temperature (T_{air}). Linear regressions: T_h=0.64T_{air}+17.70, n=67, r^2=0.83, P<0.0001, slope 95% CI (0.57, 0.71); T_{thorax}=0.37T_{air}+30.13, n=68, r^2=0.68, P<0.0001, slope 95% CI (0.31, 0.43); T_{ab}=0.88T$_{air}$+8.62, n=66, r^2=0.89, P<0.0001, slope 95% CI (0.80, 0.96). Dashed line indicates where T_{air} equals T_{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_{ab} was a fourth-order polynomial (y=6.5×10$^{-5}x^4$+0.007x^3−0.29x^2+5.17x; Fig. 5). In contrast to R_{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±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 (ml CO$_2$ g^{-1} h^{-1}) increased linearly with air temperature (Fig. 6A), while metabolic rate not corrected for body mass (ml CO$_2$ h^{-1}) did not vary with air temperature (Fig. S2A). The Q_{10} for mass-specific metabolic rate (ml CO$_2$ g^{-1} h^{-1}) was 1.13. Mass-specific water loss rates (ml H$_2$O g^{-1} 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±2 Hz, Fig. 6C).

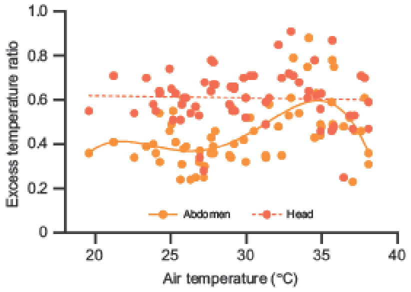

Fig. 5. Temperature excess ratio versus air temperature for *C. caesalpiniae* males flying in a shaded glass chamber. Abdominal temperature excess ratio [R_{ab}=(T_{ab}−T_{air})·(T_{thorax}−T_{air})$^{-1}$] increased with increasing air temperature (T_{air}). Linear regression: R_{ab}=0.012T_{air}+0.082, 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±0.015, n=66.

Heat budget model

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

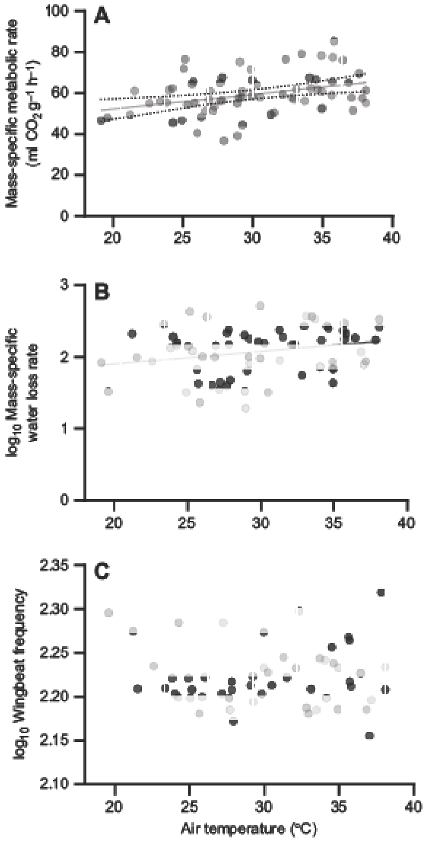

Fig. 6. Mass-specific metabolic and water loss rate and wingbeat frequency versus air temperature for *C. caesalpiniae* males flying in a shaded glass chamber. (A) Mass-specific metabolic rate ($\dot{V}_{CO_2}$) increased with air temperature. Linear regression: mass-specific $\dot{V}_{CO_2}$=0.71T_{air}+38.02, r^2=0.12, P=0.0027, n=71, slope 95% CI (0.26, 1.17). (B) Log-transformed mass-specific water loss rate ($\dot{V}_{H_2O}$; ml H$_2$O g^{-1} h^{-1}) increased with air temperature. Linear regression: log$_{10}$(mass-specific $\dot{V}_{H_2O}$)=0.018T_{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.

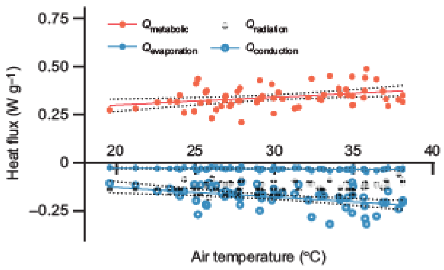

Fig. 7. Effect of air temperature on mass-specific routes of heat production and loss for *C. caesalpiniae* males flying in a shaded metabolic chamber. Metabolic ($Q_{metabolic}$), evaporative ($Q_{evaporation}$), convective ($Q_{convection}$) and radiative ($Q_{radiation}$) heat flux are shown against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

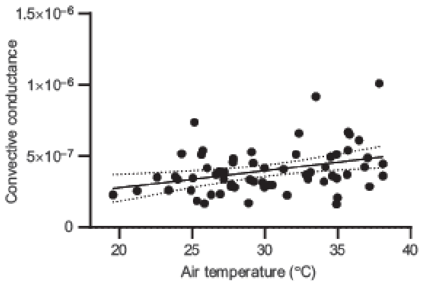

Fig. 8. Mass-specific convective conductance versus air temperature for *C. caesalpiniae* males flying in a shaded glass chamber. Mass-specific convective conductance (κ) increased as air temperature (T_{air}) rose. Linear regression: κ=0.013T_{air}+0.091, r^2=0.13, P<0.005. n=63; absolute values are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

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

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APPENDIX B

REPRODUCTION PERMISSIONS

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