Effects of temperature on ecology, behavior, and physiology in desert-dwelling jumping spiders

By

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Committee in charge:

Professor Damian O. Elias, Chair
Professor Robert J. Full
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Abstract

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Temperature affects ectotherms in a variety of ways that are critical to fitness. This work focuses on how temperature impacts the behavior, physiology, species distribution, and ecology of jumping spiders to better understand their evolution and natural history. As my study system, I used spiders in the genus *Habronattus*, mostly *H. clypeatus*. I chose to study these animals because they live in the desert, an extremely thermally-variable habitat. *Habronattus* are also ecologically-important mid-level predators and have striking complex courtship signals.

My first chapter assessed how temperature influences sexual behavior. Temperature has been known to affect behavior in different ectotherm species. The effects on sexual behavior can be especially complex, as different sexes may be affected differently by temperature. I examined this in *Habronattus clypeatus*. In this species, males court females using visual and vibratory signals. I tested whether key intersexual behaviors would change with temperature in similar, predictable ways across males and females. I first measured temperature and apparent activity of individuals in *H. clypeatus* habitat across the day. I found that *H. clypeatus* are active across a wide range of temperatures (11-56 °C) and are most active at times of day when temperature ranges from 13-46 °C. Next, I performed mating experiments across behaviorally relevant temperatures. Females were more likely to allow males to progress to later stages of courtship and had higher mating rates at higher temperatures. Male visual and vibratory courtship behaviors generally became faster, higher-pitched and lower in amplitude at higher temperatures. This relationship between temperature and signal aspects generally attained a roughly curvilinear shape, with an asymptote around 40 °C. Intriguingly, mating rates in the lab were highest at temperatures potentially above those during peak spider activity in the field. My results suggest that temperature's effects on behavior are complex and can affect males and females differently. This work emphasizes that understanding temperature effects on mating is critical to understanding sexual selection patterns particularly in species which use complex signals.

There is evidence of environmental temperature influencing species distributions. However, despite the importance of a broad understanding of an animal's thermal biology, few studies incorporate more than one of these metrics of thermal biology. I explored how temperature influences species distributions in six different species of *Habronattus* distributed along an elevational cline. I measured several different aspects of their thermal biology including thermal limits (CT_{min}, CT_{max}), thermal preference, $\dot{V}CO_2$ as proxy for metabolic rate, locomotor behavior, and warming tolerance. I used these data to test whether thermal biology helped

explain how species were distributed across elevation. *Habronattus* had very high CT_{max} values (~ 52°C), which did not differ among species across the elevational gradient. The highest-elevation species had a lower CT_{min} than any other species. All species had a strong thermal preference around 37°C. With respect to performance, one of the middle elevation species was significantly less temperature-sensitive in metabolic rate. Differences between species with respect to locomotion (jump distance) were likely driven by differences in mass, with no differences in thermal performance across elevation. I suggest that *Habronattus* distributions follow Brett's Rule, a macrophysiological principle that predicts more geographical variation in cold tolerance than heat. Additionally, I suggest that physiological tolerances interact with biotic factors, particularly those related to courtship and mate choice to influence species distributions. *Habronattus* also had very high warming tolerance values (<20°C, on average). Taken together, these data suggest that *Habronattus* are resilient in the face of climate-change related shifts in temperature.

The most common studies of thermal biology are undertaken with lab experiments. Far less work has been done to understand natural thermal environments, particularly on the spatial and temporal scales relevant to the animal in question. In my final chapter, I sought to put into context the various laboratory measurements I had taken in the previous two chapters, specifically for *H. clypeatus.* I conducted a study to assess (1) the variability of thermal environments, (2) the ability of animals to thermoregulate and (3) substrate usage. I used a number of thermal ecology methods, including focal observations and a variety of different habitat temperature measurements (thermal cameras, ambient temperature loggers, and operative temperature models). I first found that males and females differed in their thermal preferences in the lab. Although spiders were able to thermoregulate remarkably well in the field, the differences between males and females disappeared. I suggest that this is primarily because males search for and follow females through the habitat, and females therefore decide where courtship occurs. This was corroborated by data that showed that adult makes move farther than any other age-sex class. Spider thermal habitats are also extremely thermally variable. This is mostly due to variability in substrates, rather than air temperature. Spiders also used these substrates in non-random ways. This could have important implications for thermoregulation and mate choice. Behavioral differences between the sexes hint at additional potential for conflict between the sexes. Females are active earlier in the day than males. These earlier times correspond to habitat temperatures at which females are less receptive. We also found that males spent more time hiding, which possibly relates to tradeoffs associated with greater activity and exposure to predators. Overall, this chapter study suggests that habitat temperature interacts with animals in complex ways, providing the potential for tradeoffs that could be under selection. Understanding thermal biology in the context of natural environments is therefore key to gaining a wholistic view of how animals interact with temperature.

Overall, this work emphasizes the importance of an integrative view of thermal biology. Temperature affects animals on many different levels, and one must reach across disciplines to gain the full picture. These studies also suggest that temperatures' effects can be subtle. Attention to detail is key, and one must have a thorough understanding of the habitats in which animals live in order to understand how selection acts. In this way, we can make predictions about how individuals, populations, and species might respond in response to changes in climate.

Dedication

This work is dedicated to the memory of my beloved maternal grandparents:

Richard E. Olson

(1933-2005)

and

Pauline “Betty” Olson

(1931-2019)

Your continual support and tireless cheerleading made my 30 years of formal education not only possible, but a joy to undertake.

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My Ph.D. advisor, Dr. Damian Elias, provided the perfect combination of support, critique, and intellectual freedom to allow this work to develop and flourish. His guidance was indispensable at every step of the way. My other committee members, Dr. Bob Full and Dr. Bree Rosenblum, provided key feedback on my dissertation work and provided numerous opportunities to develop my research, teaching, mentoring and critical thinking skills throughout my time as a Ph.D. student. Others who played important mentoring roles at Berkeley include Dr. Roy Caldwell, Dr. Rosemary Gillespie, Dr. Eileen Lacey, Dr. Tom Libby, Dr. Caroline Williams, and the entire Biomechanics IGERT group.

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Introduction

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Berkeley, California

Dear Reader,

I am honored to present you with the studies comprising my doctoral dissertation. This work came about by asking a question and allowing the study organism to guide the work beyond simple answers to new and unexpected places. Because of my integrative approach, the different studies illustrated here ask different questions and use different methods. I hope in these next few pages to provide some context illustrating how they are connected.

As a first-year doctoral student, I came upon thermal biology as a potentially fruitful research topic. Temperature is a major part of the abiotic environment for all organisms. As such, temperature affects all aspects of animals' lives. This proceeds through two main mechanisms. First, cellular damage and even death can occur at temperatures outside of an animal's thermal tolerance range. Second, temperature affects the rates of chemical reactions. Since metabolism consists of a complex network of chemical reactions, even moderate, non-lethal changes in temperature can have outsized effects on various aspects of animals' lives.

With few exceptions, habitats are thermally variable, both spatially and temporally. This variability exposes animals not only to potentially lethal extremes, but also the more subtle, but equally important, effects on metabolism. To deal with this variability, some animals have developed the ability to generate metabolic heat (endothermy). This allows such animals to perform all bodily processes within strictly controlled thermal ranges. However, endothermy is metabolically costly.

Most animals are ectotherms, and therefore ambient temperature has direct effects on all their bodily processes. Although the basis of these effects is chemical in nature, the effects of temperature perfuse all levels of biological organization. This has been long recognized among biologists, and many studies have investigated how temperature influences biophysical properties, physiology, behavior, and higher-order ecological processes. This literature is both deep and expansive, but there are several key gaps. These gaps turned out to be ideally-suited to a beginning doctoral student in need of a project.

First, most animals that are investigated in the context of temperature are vertebrates, especially lizards and fish. Comparatively little work has been done on invertebrates and even less on non-insect vertebrates. This is an important gap for many reasons, but primarily from the perspective of thermodynamics. The thermal properties of a 1kg lizard, a 100g fish, and a 10mg insect are very different. As most animals are invertebrates, and almost all invertebrates are ectotherms, these animals need to be studied more closely before being able to claim any "universal" principles of thermal biology. Invertebrates are also interesting in their own right. Recent studies have uncovered a truly disturbing decline in populations of insects and other arthropods. This is in addition to the current extinction crisis that is occurring across all animal taxa. As one of the main causes of decline is climate change, understanding how arthropods respond to temperature in a changing climate is of pressing importance.

Another gap in the thermal biology literature is the siloed nature of research approaches. Studies of thermal biology are generally divided along disciplinary lines. Molecular studies involving heat shock proteins do not often include behavioral data, and investigations of behavioral data do not generally include information about thermal physiology. Contextualizing any of this information with an animal's natural thermal environment is even rarer. Given the complexity of the hierarchical structure of thermal biology, it is vital that these disciplinary divisions are crossed, and methodologies are freely shared between them in order to ask nuanced questions and understand the whole picture. In the following work, I aimed to study animals that are underrepresented in thermal biology, and to study them in the most integrative way possible.

When choosing a study organism for this work, I wanted to select one that experiences thermal variability at a variety of different scales. Desert habitats are an obvious choice. Deserts are often thought of as areas of high temperature, but these habitats are also exceptional in their high level of thermal variability, both spatially and temporally. I chose to study animals in the Santa Rita Mountains in the Sonoran Desert, in SE Arizona, USA. This region is ideal to study the effects of variable temperatures for its level of thermal variability on many levels. First, there is a great deal of spatial variability in temperature. Across the landscape are dotted a number of isolated mountain ranges, known as the Madrean Sky Islands, interspersed with desert scrub habitat. Within a mountain range, there are many different habitat types and temperature regimes. This thermal variability exists on smaller scales, as well. Within each habitat, there is variability in vegetation types, substrates, moisture, and other factors that influence the type and number of thermal microhabitats. Deserts are also thermally variable across time. Seasonal variations can be extreme, with scorching hot summers, periods of intense rain, and occasional snow in the winter. However, variation occurs on daily timespans as well.

Habronattus jumping spiders proved to be the ideal study system for this work. Not only are they small (~20mg) arthropods, they also comprise a large genus (>110 species) with a hotspot of diversity in the Madrean sky islands. *Habronattus* are also well known to have complex courtship behavior, which varies dramatically between species. As an established study system for sexual communication, *Habronattus* lend themselves well to laboratory study. This meant that I could easily study them in both lab and field contexts.

One of the most rewarding aspects of biological research is to allow your study organism and questions to guide you to areas that you would not have been able to predict. To preserve some of the spirit with which this work was undertaken, I have chosen to present the following studies in the order in which they were conducted.

I started my dissertation work wondering how temperature influences sexual behavior in *Habronattus*. A number of studies have looked at sexual behavior in the context of temperature, but never in a system that uses multimodal communication. *Habronattus* sexual displays use the vibratory and visual modalities. Their signals are also multicomponent. In the vibratory realm, *Habronattus* arrange sounds that differ in frequency, tonality, and production mechanism into songs that vary in length by species. *Habronattus* visual displays, which are tightly coordinated with vibratory displays, consist of colored ornaments and a variety of movements that involve different parts of the body. I chose a specific species, *H. clypeatus*, because of its desert habitat, but also because it signals of medium length for *Habronattus,* (~5-minute displays), making them tractable for repeated measures courtship trials. This study yielded interesting insights about how complex signals and mate choice change with temperature. Additionally, it sparked

many questions about how these animals interact with temperature at levels beyond sexual behavior.

In chapter 2, I investigated whether aspects of *Habronattus* thermal physiology explain species distribution along an elevational cline in the Santa Rita Mountains. I used approaches from thermal physiology and behavior to understand thermal biology on several different levels. I found desert *Habronattus* (even high-elevation species) to be remarkably robust in terms of heat tolerance. Surprisingly, I also found that the six species did not differ in any aspect of thermal biology, except for cold tolerance. This study has important implications not only for understanding species distributions, but also for predicting how species ranges may shift as a result of climate change.

At this point, I had amassed a great deal of data concerning how *Habronattus* respond to temperature. I became interested in understanding to what extent animals experience variable temperatures in the field. For this final chapter, I performed a natural history study, borrowing methods from biophysics, thermal physiology, and behavioral ecology to understand how *H. clypeatus* interacts with temperature on relevant spatial and temporal scales. Principally, I discovered that spiders thermoregulate within desired temperature ranges to a surprising extent. I also found evidence of differences in thermal and movement ecology between the sexes, likely related to mate searching and courtship behavior. The most descriptive of my studies, this third chapter has generated surprising insight into the lives of these animals and a bounty of new hypotheses to be tested in the future.

The work involved in these chapters was equal parts exciting, enlightening, frustrating, and intellectually invigorating. Although at a stopping point for the moment, it is also far from over. My sincere hope is that the framework and questions that I have developed here will form the basis of a career that probes the depths of thermal biology in creative and innovative ways. Through these pages, I hope that I can convey some of the thrill of inquiry and scientific discovery that I experienced during these past seven years. It would be the highest compliment to this work for you, too to become excited by the potential that the field holds.

Sincerely,

Erin Brandt, Ph.D.

Chapter 1. Temperature alters multimodal signaling and mating success in an ectotherm

Introduction

Sexual signals have long been known to have temperature-dependent properties (Edmunds, 1963; Enger & Szabo, 1968; Heath & Josephson, 1970; Shimizu & Barth, 1996). This is especially true in ectotherms, animals whose body temperatures depend primarily on the environment (Abram, Boivin, Moiroux, & Brodeur, 2017). For these animals, ambient temperature is directly tied to metabolic rate and thus influences all higher-order functions, such as physiology, immune defense, growth, and behavior, including sexual behavior (reviewed by (Angilletta, 2009a; Hochachka, 2002). Although sexual signals have been studied extensively in this context (Doherty, 1985; Dunlap, Smith, & Yekta, 2000; Pires & Hoy, 1992; Ueda, Shinoda, & Kamaya, 1994), signaling behaviors only represent half of the picture; chooser preference for courtship signals also may change with temperature. During mating interactions, courter and chooser behaviors may change in concert with one another (signal-preference coupling (Dunlap et al., 2000; Greenfield & Medlock, 2007; Pires & Hoy, 1992) or not (Ritchie, Saarikettu, Livingstone, & Hoikkala, 2001), potentially leading to a mismatch across some temperatures.

Studies examining signal-preference coupling have primarily focused on acoustic/vibratory modalities (Doherty, 1985; Gerhardt, 1978; Greenfield & Medlock, 2007; Ritchie et al., 2001; Symes, Rodríguez, & Höbel, 2017), with fewer studies evaluating visual (Allen & Levinton, 2014; Michaelidis, Demary, & Lewis, 2006), and electric (Dunlap et al., 2000) modalities. However, many animals communicate using signals that involve more than one signaling modality (Partan & Marler, 1999) and there has been a dearth of studies that investigate how temperature impacts these multimodal systems (but see Conrad, Stöcker, & Ayasse, 2017). It is likely that abiotic factors such as temperature will impact the way multimodal signals are produced, transmitted, received, and interpreted. Yet the literature on multimodal signal evolution usually does not explicitly consider such effects (Bro-Jørgensen, 2010; Hebets & Papaj, 2005; Higham & Hebets, 2013; Iwasa & Pomiankowski, 1994; Johnstone, 1996; Moller & Pomiankowski, 1993; Partan & Marler, 2005).

Another critical aspect missing from many studies of thermal effects on intersexual communication is ecological data tying activity patterns in the field with temperature. Field activity information is necessary to interpret laboratory data in many instances. For example, studies conducted on temperate species have demonstrated that signals, preferences, and mate choice may shift across temperatures, but animals may be active only within a narrow range of temperatures, e.g., tree frogs (Gerhardt, 1978); fruit flies (Ritchie et al., 2001); and tree crickets (Symes et al., 2017). Information about the temperatures that animals naturally experience, and their activity patterns across temperatures, is therefore critical to understanding the relationship between temperature and behavior.

Jumping spiders in the genus *Habronattus* offer a unique study system to address the effects of temperature on mating behavior. *Habronattus* is a speciose (~106 species) genus of jumping spiders found primarily in North America (Leduc-Robert & Maddison, 2018). They are known for their striking color, patterning dimorphism, and their elaborate multimodal visual (ornaments and movements) and vibratory displays (Elias, Hebets, & Hoy, 2006; Elias, Hebets, Hoy, & Mason, 2005a; Elias, Maddison, Peckmezian, Girard, & Mason, 2012; Elias, Mason, Maddison,

& Hoy, 2003; Taylor, Clark, & McGraw, 2014). In *Habronattus*, males court while females choose. Male *Habronattus* multimodal displays consist of a series of signal "elements" that are organized in functional units that change in a stereotyped order as courtship progresses (Elias et al., 2012). These male displays are important for mating success (Elias, Hebets, et al., 2006; Elias et al., 2005a) and sexual selection has been suggested to drive diversification in the genus (Hedin & Lowder, 2009; Leduc-Robert & Maddison, 2018; Maddison & McMahon, 2000; Masta & Maddison, 2002). I chose *Habronattus clypeatus* as my study species for several reasons. First, *H. clypeatus* males produce complex visual and vibratory signals, but these displays last only for a few minutes in total, making *H. clypeatus* a tractable choice for laboratory study (Rivera et al, 2018, in review). Second, this species is found in low- to mid-elevation desert scrub in the Sonoran Desert, which is known to be a thermally variable habitat. Finally, females in this species only mate once, meaning that any mating decisions are crucially important to their lifetime fitness (Elias, Hebets, et al., 2006).

I sought to understand how temperature influences sexual behavior in *H. clypeatus* by testing the following hypotheses: (1) *H. clypeatus* habitats vary in temperature throughout the day, and spiders have distinct patterns of activity across these ranges. (2) Mating rates, courtship progression, and female aggressive behaviors change with temperature. (3) Male visual and vibratory displays change with temperature. Specifically, I predicted that rate and frequency of male signals will increase with increasing temperature. I also predicted that across temperatures where animals are active, mating rates will be similar due to coordination between courter and chooser behavior. By addressing these hypotheses, I hope to gain an understanding of how an abiotic factor impacts a complex behavioral suite that takes place across a wide range of temperatures.

Methods

Field temperature and activity measurements

The breeding season of *H. clypeatus* occurs from April to June. I conducted surface temperature monitoring in April 2011 for nine days. On each survey day, I measured surface temperature approximately every hour between 0830-1500h for each of the two main substrate types on which spiders were found (rocks and leaf litter). Leaf litter consisted of a mixture of dried oak leaves, sticks, and dirt. I performed these measurements using an IR thermometer (Dual Laser IR Thermometer, Model 42511, Exetech Corp, Nashua, NH). For each substrate type, I measured three exemplars found in sun and shade. Overall, I measured 12 substrate exemplars for each hour of measurement. I recorded surface temperature measurements on a small piece of masking tape placed on each substrate. The emissivity of the IR thermometer was set to 0.95.

I modeled daily patterns of temperature for leaf litter and rock. I performed the modeling with generalized additive mixed models (GAMMs) in R (v 3.5) using the package mgcv (v 1.8.23) (Wood, 2006) with time modeled using a thin plate regression spline and a Gamma error structure (to account for positive skew and improve normality of residuals). I estimated a separate smoothed term for each light microhabitat and specified day as a random effect (to account for uneven sampling and clustering of values among days due to weather).

I conducted surveys in the Santa Rita Mountains east of Green Valley, Arizona in April of 2012 at the same time as the temperature measurements. Surveys consisted of 140 directed walk surveys conducted by four observers. Observers walked haphazardly within each plot while

recording and capturing all spiders detected. Handheld GPS units (Garmin 60CSx or eTrex Legend Cx), automatically recorded observer location and time of day at 30s intervals. From this information, I calculated mean walking speed, survey distance, and survey duration for each survey. I modeled apparent activity of *H. clypeatus* as a function of observer, time of day, and survey distance for each survey path using generalized additive models (Wood, 2006). I specified a Poisson error structure for these count data, while controlling for any differences in the ability for observers to find spiders. I found spiders in all sites. For the field-based portions of this study, it was not possible to use blinded methods.

Collection and lab maintenance of animals

I collected immature female and adult and immature male *H. clypeatus* in the Santa Rita Mountains in the spring. I housed spiders individually in plastic containers (AMAC) and fed them twice per week on a diet of *Drosophila melanogaster* and first instar *Acheta domesticus*. I kept all animals at room temperature on a 12:12 light cycle with UV-enriched full-spectrum lighting. Pieces of fiberglass window screen were added into the containers to provide environmental enrichment (Carducci & Jakob, 2000). Prior studies suggest that females are receptive to mating approximately two weeks after maturity (Elias et al. 2005; Elias et al. 2006), so I waited two weeks before running females in any experiments.

Overall experimental design for courtship experiments

The goal for these experiments was to characterize how mating rates, receptivity, and courtship behavior change with temperature. Like other species of *Habronattus*, *H. clypeatus* courtship signals follow a distinct stereotyped progression (Rivera et al., in review; Elias et al., 2012). Early phase courtship begins with visual-only sidling bouts (Fig 1a). Next, if the female maintains her interest by visually attending to the male and does not attack him, courtship progresses to late phase (visual + vibratory) courtship when the male is about a body length away (Fig 1b). See Elias et al. (2003) for a detailed description in a closely related species. Mating does not occur unless all stages of courtship are completed and the female allows the male to mate (Elias et al., 2003).

I used two different experiments to assess courtship. Because female feedback is important during the early phases, I measured early stage courtship using trials with live males and females in a single-choice paradigm. I also assessed mating rates with these trials. However, a high percentage of females never permitted males to advance to late phase courtship. For this reason, I performed a second set of experiments in which I used live males and euthanized female lures to entice males to produce courtship displays. All males performed courtship in all temperature treatments under these conditions. I performed live courtship trials in 2013 and 2017. I ran courtship trials measuring only late phase courtship in 2012. All lab experiments were conducted between 1000h and 1500h to capture when spiders are most active in a lab setting (judged by whether a spider was outside of its silken retreat). I used blinded methods to collect and analyze data to minimize observer bias. Observations were made from video recordings without any information about the treatment.

Mating rate experiments

I conducted trials on 23 cm diameter terracotta platters. I used a fresh piece of circular paper to line the platter for each trial to eliminate the transfer of chemical cues between trials. For all courtship experiments, I measured temperatures of the surface of the arena at various points

during the trials using IR thermometers. I recorded the temperature at the beginning of the trial, initiation of courtship, and end of the trial. Only virgin females that matured in the laboratory were used. Both spiders were acclimated to the treatment temperature for five minutes. Preliminary observations on behavior suggested that this acclimation period was sufficient. After the acclimation period, I introduced both spiders into the arena and gave them 5 minutes to recognize one another, defined here as directly facing each other from less than 5 body lengths apart. If this did not occur (approximately 20% of trials), one of the investigators used a paintbrush to gently move one of the spiders to face the other. This was done to minimize any errors in recognition that may trigger predatory events.

I assigned males and females to one of three treatments: cool (20-23 °C), room temperature (25-28°C), or warm (34-47 °C). I chose my temperature treatments to reflect the variation of temperatures that spiders experience in the field (Fig. 2a). For the cool treatment, I placed the arena in a refrigerator until it reached approximately 2°C, measured using an IR thermometer (Dual Laser IR Thermometer, Model 42511, Exetech Corp, Nashua, NH). I then removed the arena and allowed it to warm to about 10°C. At this point, I placed both the male and female spiders onto the plate (while still in their cages) to acclimate. Cool trials began when the arena reached approximately 16°C. I began trials by first releasing the female, and then the male directly onto the plate so that they could interact. Only trials in which the arena stayed below 24°C were included in the cool treatment analyses. For room temperature trials, I kept plates at ambient temperature in the lab, and I placed spiders on the arena for 5 minutes prior to the trial. Room temperature trials were conducted at 26°C (range: 25-28°C). For the warm treatment, I placed the arena on an electric griddle (25 cm x 50 cm griddle, Rival Corp, subsidiary of Sunbeam Products Inc., Boca Raton, FL, USA) until the surface temperature attained approximately 60°C. Next, I removed the arena from the griddle and allowed it to cool to about 54°C, at which time the spiders were placed on the plate to acclimate. When the plate attained 49°C, I began the trial. Only trials in which the arena retained a temperature above 32°C were included in the warm treatment. I used a total of 37 pairs in these trials: 8 in the cool treatment, 19 in the room temperature treatment, and 10 in the warm treatment. Most individuals were used only once (n=35) although I reused one male each in the warm and room temperature treatments.

Once the male began courting, the trial was continued until either: (1) the male successfully mated with the female (acceptance), or (2) the female turned away and/or attacked the male 3 times (rejection). I monitored visual signals with a color video camera (CV-3200, JAI Inc., San Jose, CA, USA) with a macro lens.

In addition to mating rate, I also scored female receptivity and aggression in two ways. First, I recorded whether the male reached the late stage of courtship. Second, I counted how many aggressive behaviors females performed toward males. These behaviors were classified as either: (1) *attacks*, in which a female jumped directly at a male, or (2) *grappling bouts*, in which the female attacked the male and remained in contact as she either consumed or attempted to consume him.

To compare mating, courtship progression, and aggression rates between treatments, I used Pearson's chi-squared tests, followed by pairwise nominal independence tests, both implemented in R.

Male courtship

Early stage courtship

A cartoon of the display can be seen in Fig. 1a. The male begins by facing the female, usually at some distance away (~5 body lengths). He waves his first pair of legs in a rhythmic fashion and begins to walk sideways in a shallow arc. As the male sidles back and forth, the arcs shorten, and he moves closer and closer to the female. This movement is not continuous. The male usually completes each arc in a single bout, but often pauses at the end of arcs before beginning to move again. I define the entire visual display as a "sidling bout," and a period of time in which the male was moving as a "movement bout" (Fig. 1a). Since the "movement bout" corresponds to the time that the male is physically moving, I use aspects of the movement bout as a measure of male effort or vigorousness. Similar measures have been used to evaluate courtship in other species of spider (Byers, Hebets, & Podos, 2010). Females do not move in a manner similar to males. If interested in a male, the female pivots in order to maintain visual contact with the male as he sidles about her but does not move more than about one body length from her initial starting point. Females periodically turn away and then back toward males. Once a male is 1-2 body lengths away from the female, he stops moving and extends his first pair of legs toward the female as he begins the late phase of his display (see below).

I identified and quantified the following components in early stage courtship: *sidling bout*, defined as the time from when the male begins moving laterally with respect to the female until he either turns away from the female or begins vibratory courtship, and *movement bouts*, the periods during the sidling bout in which the male is actually moving. Each trial contained 1-12 (mean: 3.6) sidling bouts and multiple movement bouts per sidling bout. For each trial, I calculated (1) the total number of sidling bouts divided by the time of the trial (sidle rate), (2) the percent of the trial spent sidling, and (3) the average length of a movement bout. I measured the temperature of the plate surface immediately adjacent to the interacting spiders at three different time points: (1) at the beginning of the trial, (2) at the beginning of courtship behavior and (3) at the end of the trial. Video analyses were conducted using the Behavioral Observation Research Interactive Software (BORIS) software package (Friard & Gamba, 2016).

To model the relationship between temperatures and early-stage courtship signals, I fit GAMMs to each relationship between temperature and courtship components, implemented in R with mgcv. I chose to use GAMMs because they give detailed models that easily incorporate complexity and nonlinearities. I thus compared my GAMMs qualitatively using the estimated 95% confidence intervals. To build my models, I tested each of the following signal components as a dependent variable separately: sidling rate, percent time sidling, and movement bout length. For temperature, I averaged the three different temperature measurements taken during the trial and used the mean as a continuous independent variable in my models. I used a gamma error structure as data were bounded by zero and used k = 15 for the number of knots. I also used male identity as a random effect, as I reused one male in each of the warm and room temperature treatments.

Late stage courtship

Vibratory portions of *H. clypeatus* late stage courtship consists of three elements: broadband stridulatory *scrapes*, broadband stridulatory/percussive *thumps*, and tonal tremulatory *buzzes*

(Fig 1b). These components are arranged into distinct functional groupings (motifs) (Elias et al., 2012). As the display proceeds, elements are added to motifs creating a distinctive progression.

Male late stage courtship signals are highly dependent on female feedback. In order to ensure my ability to measure all vibratory signal types, I standardized female feedback by using dead female lures. To construct lures, I used wax to glue a euthanized female to a small dowel inserted into a hole in the arena. A pulley system allowed us to rotate the female in a lifelike manner. This system has previously been effective at stimulating male jumping spiders to court (Elias et al., 2012; Girard, Kasumovic, & Elias, 2011). I used 20 males for these trials in a repeated measures design. I ran each male in each of the three temperature treatments for a total n = 60 for these trials. I used courtship arenas constructed from 23 cm diameter terracotta platters with a 1cm hole drilled in the middle of each one where the female lure was placed. A paper liner was used in the arenas as described above. I ran each male in warm, cool, and room temperature treatments in a randomized order.

All trials were terminated once males attempted to mount and mate with the female model. I excluded one trial from the analysis because the male did not perform all aspects of vibratory courtship. I recorded all vibratory signals with a scanning laser-Doppler vibrometer (PSV-400, Polytec, Irvine, CA) Vibratory signals were recorded directly off the male's abdomen.

H. clypeatus late stage courtship displays consist of tightly coordinated unique visual movements coupled with unique vibratory signals. There is a tight, one-to-one coordination between visual and vibratory aspects the late courtship display (Elias, Land, Mason, & Hoy, 2006), and I did not notice any deviation from this coordination across temperature treatments. Therefore, I analyzed only the vibratory elements of these displays for simplicity. I analyzed the vibratory displays using Audacity v. 2.0.5. *Durations* were measured for all thumps and buzzes, and for a subsample of scrapes (n=20 per song). I used custom Python scripts to calculate average durations of thumps, scrapes, and buzzes in the entire song. *Scrape rate* was measured by identifying regions of the display containing multiple repeated scrapes and calculating average scrape rates for each bout. *Frequency* was measured by running an FFT analysis on each signal component and calculating peak (scrape, thump) and fundamental (buzz) frequency using a modified version of the pypeaks Python module. Average root mean square (RMS) amplitude was also calculated for each signal component. All custom software was deposited in a git repository and is available by request.

To statistically model the relationships between vibratory components and temperature, I used GAMMs as described above. I tested each of the following dependent variables separately: scrape, thump and buzz duration, scrape, thump and buzz RMS, scrape rate, scrape and thump peak frequency, and buzz fundamental frequency. For temperature, I used the temperature measured at the time of the beginning of male courtship. Temperature was used as the fixed effect and male identity was a random effect, to account for the repeated measures nature of the design. As with the visual signals, I used a gamma error structure and k = 15 for the number of knots.

Results

Temperature/activity

H. clypeatus habitat varied in temperature on a daily basis. The range of temperatures throughout the day was 11-37 °C (shade), and 20-56 °C (sun) (Fig. 2a). Apparent activity was bimodal, with a peak around 0900h and then after 1600h. Temperatures at these peak activity periods were approximately 13-24 °C in the morning and 32-46 °C in the afternoon (Fig. 2b) (See Table 1-2 for model statistical details).

Female behavior

Mating rates

In mate choice trials, mating rates differed between the temperature treatments (df =2, X^2 = 24.10, p = 5.83 x 10^{-6}). 0% of pairs mated in the cool treatment, 17% of pairs mated in the room temperature treatment, and 61% of pairs mated in the warm treatment (Fig. 3a). The differences in copulation rate were not significant between cool and room temperature treatments (p = 0.180) but were different between cool and warm treatments (p = 1.65 x 10^{-4}), and room and warm treatments (p = 3.42 x 10^{-3}).

Aggression/Receptivity

The percentage of trials that contained female attacks were 37%, 26%, and 30% respectively for cool, room, and warm treatments (Fig. 3b), although these differences were not significant (X^2 = 3.70, p = 0.16). I saw grappling in only 2 trials (both in the warm treatment). The outcome of both trials was cannibalization of the male by the female.

The percentage of trials that proceeded to late phase courtship were 0%, 32%, and 75% respectively for cool, room and warm treatments (Fig. 3c). These differences were significant between all three treatments (X^2 =29.54, p = 3.84 x 10^{-07}, pairwise: cool-room, p = 7.53 x 10^{-03}, cool-warm, p = 9.87 x 10^{-03}, room-warm, p =1.20 x 10^{-02}).

Male signals

Early stage courtship

The rate of sidling bouts that males perform did not change across temperature treatments (Fig. 4a, p = 0.20). However, males spent more time per trial sidling as the temperature increased (Fig. 4b, p = 0.002). Additionally, the length of each individual movement bout increased with temperature (Fig. 4d, p = 0.02), but the total number of movement bouts did not change (Fig. 4c, p = 0.09).

Late stage courtship

Male late stage courtship signals changed with temperature (Figs 5 and 6). All measured signal components were temperature-dependent (See Tables 1-2 and 1-3 for model details and p values). Durations and amplitudes (RMS) of vibratory components decreased with increasing temperature (Fig. 5), although this relationship was not significant for buzz amplitude. Rates and frequencies of signal components all increased significantly with increasing temperature, although a slight decrease was seen at the highest temperatures (Fig. 6).

Discussion

I found that temperature has a profound effect on *H. clypeatus* courtship (Figs 3-6). Females exhibit higher mating rates at higher temperatures (Fig. 3). Various aspects of male signals also changed with temperature (Figs 4-6). Importantly and contrary to my predictions, different aspects of courtship have different relationships to temperature. Further complicating this story, females mate at very low rates at temperatures at which spiders are particularly active in the field (in the early morning, Fig. 2). Overall, my results suggest that changes in temperature have dramatic effects on sexual selection.

Mating rates

Mating rates are lowest at the temperatures at which I recorded the highest levels of activity in the field. The fact that females seem not to be engaging in sexual behaviors at temperatures corresponding to these times of day is surprising and counter-intuitive. It is important to note that I did not measure operative temperature in my field measurements, so it is difficult to know exact spider body temperatures in the field. Regardless, I do see the trend that spiders are more active at lower field temperatures and tend to mate at higher rates at higher lab temperatures.

Lower mating rates at lower temperatures could be the result of several factors. First, temperature may directly affect a female's ability to mate. I consider this unlikely, given that important behaviors such as foraging occur at the lower temperatures included in this study, suggesting that animals are physiologically able to perform important tasks at these temperatures. Also, males were able to perform courtship behavior at these lower temperatures suggesting that females would also likely be able to perform sexual behaviors. Second, females may be unwilling to mate at lower temperatures, potentially because this behavior carries certain environment-dependent risks. For example, females may experience increased predation risk or other hazards while assessing courting males. This risk may be increased at cool temperatures, since females presumably have slower reaction times and a hindered ability to flee danger, as has been found in other ectotherms (Carlson & Rowe, 2009; Cooper, 2000; Weatherhead & Robertson, 1992).

Third, temperature may alter the attractiveness of male signals. If females prefer faster, higher pitched male courtship, cold temperatures will reduce the attractiveness of all males. If this is the case, males that display in a warmer environment may be at an advantage, regardless of their quality and ability to produce multimodal signals. I might therefore expect that males will preferentially court in warm temperatures. This has been found in crickets, which prefer to call from warm locations which allow them to generate a more appealing call (Hedrick, Perez, Lichti, & Yew, 2002). A more extreme version of this principle has been seen in fiddler crabs, which choose to court at temperatures that are dangerously near their thermal limit, but improve their courtship signal (Allen & Levinton, 2014). In systems like *H. clypeatus,* females only mate once, thus there are potentially large fitness consequences for females that choose mates with misleading signals. In most species in which this has been studied, animals have been shown to use signal-preference coupling to coordinate signaling changes in temperature (Doherty, 1985; Gerhardt, 1978; Pires & Hoy, 1992; Symes et al., 2017). Signal-preference coupling predicts that across temperatures, mating rates should be similar, as the same quality males would be selected even as signaling behavior shifts. Signal-preference coupling is not supported in this study, similar to a few studies in flies and frogs (Gerhardt & Mudry, 1980; Ritchie et al., 2001). In these studies, it was suggested that reproductive behavior only occurs in a subset of thermal

conditions. Ectotherms are well known to exhibit temperature shuttling in which they move between different temperature regimes in their environment to maintain a narrow range of preferred body temperature (Casey, 1981; Clissold, Coggan, & Simpson, 2013; Kearney, Shine, & Porter, 2009; Martin & Huey, 2008a). I suggest that females may select particular thermal microhabitats to accurately assess males. This would represent an alternative solution to environment signal-preference mismatch that obviates the need for physiological coupling. Future work will examine whether signal-preference coupling based on microhabitat selection is occurring.

In this study, I observed differences in how far courtship progressed for the different temperature treatments (i.e. early phase, late phase). None of the cool trials progressed beyond the early phase, whereas most of the warm trials did. For many jumping spider species including those in the genus *Habronattus*, female attention is critically important for male success (Elias, Hebets, et al., 2006; Elias, Hebets, Hoy, & Mason, 2005b; Lim, Land, & Li, 2007; Lim, Li, & Li, 2008). Female receptivity in the form of attention is predicted to drive the progression through distinct phases. This study suggests that temperature changes the likelihood that females will allow males to progress through the necessary phases. Overall, these data suggest that although female mate choice behaviors are affected by male courtship behaviors, female behavior is affected by temperature in substantially different ways than male behavior. In other words, female behaviors do not simply get "faster" as a result of increases in temperature like males. Instead, variance in the numbers of males selected changes with temperature suggesting complex shifts in mate choice mechanisms (i.e. preference and choosiness).

Male signals

In addition to mating rate, I also looked at male visual and vibratory signaling and how they responded to temperature. In general, male signal components decreased in duration, increased in speed, and increased in frequency with increasing temperature (Figs 4-5). This occurred in both visual and vibratory modalities. These relationships broadly attained a curvilinear shape, in which rates and frequencies increased to a point, and then flattened out or decreased slightly. This pattern suggests a threshold at which males are no longer able to increase their performance. These peaks occur at about the same temperature (~40°C) across different signal features. In other studies of signals, the relationships with traits and temperature tended to be linear although the temperatures used in these other studies were much narrower than those described here (Gerhardt, 1978; Michaelidis et al., 2006; Pires & Hoy, 1992; Ritchie et al., 2001). The presence of a threshold and the overall shape is consistent with what I might expect from a typical ectotherm thermal performance curve (Huey & Kingsolver, 1989). In thermal performance curves, the peak or plateau is considered the "thermal optimum", or the temperature at which a given behavior is performed at its best. Although I do not yet know what aspects of male signals are most attractive to females, it is interesting that mating success is higher nearer this presumptive thermal optimum point for most signal components.

These thermal curves also have interesting implications for changing climate. If temperatures increase beyond peak threshold, signal aspects will increase more slowly, stop changing, or even decrease. This scenario could lead to further breakdowns in courter-chooser relationships. I suggest the possibility that breakdowns such as these may occur in other animal systems. While past studies have not shown this pattern, those experiments did not examine the ranges of temperatures explored in this study. It will be interesting to examine other species, particularly

ones that have wide thermal ranges to see if and to what extent these patterns may be broadly applicable to other ectotherm groups.

Although males performed visual sidling bouts at a similar rate across temperature treatments (Fig. 4a), they spent more time sidling in warmer temperatures (Fig. 4b). At first glance, this may seem to be a counterintuitive result, as one would expect the lengths of signal components to decrease with temperature (as they do in vibratory displays). However, in ectotherms, it is generally recognized that "performance" increases with increasing temperature. In many spiders, short, high-frequency signal components have been shown to be more attractive (Gibson & Uetz, 2008; Girard, Elias, & Kasumovic, 2015; Kotiaho, Alatalo, Mappes, & Parri, 1996, 1999), but a long sidling bout might also be more attractive than a short one if it represents an increase in stamina or time investment in courtship. Long sidling bouts may also maintain female attention as jumping spiders are particularly sensitive to movement (Menda, Shamble, Nitzany, Golden, & Hoy, 2014; Zurek & Nelson, 2012b, 2012a).

In general, I find that shorter, faster, higher-frequency signals are correlated with higher mating rates across my three temperature treatments. This agrees with other studies that suggest that higher-frequency, faster, and louder signals are often more attractive in spiders. However, I also found that the amplitude (RMS) of vibratory elements decreased with increasing temperature, and that this relationship was more linear than other signal element properties (Fig. 5d-f) although this pattern was not significant for buzzes. This suggests that there might be a tradeoff between rate/frequency and amplitude. In other systems, it has been suggested that such tradeoffs are important for the maintenance of honesty in acoustic signals, so this may be at play here (Manica, Macedo, Graves, & Podos, 2017; Podos, 1997). Alternatively, females may weigh amplitude less than other components when considering whether to mate with a given male. This result is particularly interesting given that amplitude is rarely addressed in studies of thermally-variable courtship. The sole example I found other than my own study suggested that the amplitude of electric signals increases with increasing temperature in a weakly-electric fish (Dunlap et al., 2000). Additional work is needed to explore how amplitude relates to attractiveness of signals and what potential tradeoffs may exist.

The differential patterns between signaling types lend credence to the idea that early and late display phases serve different functions. One hypothesis for the existence of the initial sidling bout is that it allows males to gauge the level of interest and aggression in females (Uhl & Elias, 2011). By showing increased willingness and stamina to perform long sidling bouts, males may be increasing their chances of being allowed to move onto later phases of courtship. If different information can be conveyed by different parts of a display, this may drive the evolution and maintenance of complex signals (Bro-Jørgensen, 2010; Partan & Marler, 2005; van Doorn & Weissing, 2004; Wilson, Dean, & Higham, 2013). Also, if abiotic factors influence these different signal aspects in different ways, there could be complex and unpredictable changes to the information conveyed. If, on the other hand, similar information is conveyed with different signal aspects, those that are less affected by temperature may buffer those that are more temperature-dependent. Although I did not statistically compare the relationships between specific signal aspects and temperature, there seem to be differences in the shape of these relationships. Future work will address what specific aspects of the display are important for females and how the higher-order structure of the displays change across temperature.

Our results emphasize that sexual communication is dependent on temperature, but many questions about their thermal ecology are still unanswered. My lab experiments greatly simplify the variation inherent in thermal environments. First, I only measured environmental temperatures across a subset of time in the breeding season. Therefore, my lab experiments did not encompass the total variation of temperatures, which are likely to be much hotter in the early summer months. Second, my field temperature measurements were limited to surface temperature and did not incorporate the many other ways that spiders can exchange heat with their environment, particularly direct solar radiation which is likely important in desert environments (Clusella Trullas, van Wyk, & Spotila, 2007). Because of this, my field measurements may underestimate the temperatures that spiders may be experiencing. Third, within time points, there may be large differences in adjacent patches of substrates. Animals may shuttle between temperatures, thereby buffering some of these differences. Regardless, my study demonstrates that mate choice and by extension sexual selection may be dramatically different across the day and even within a few steps.

Conclusions

Temperature has large, wide-ranging impacts on intersexual behavior in *Habronattus clypeatus.* These impacts can be dramatic and complex. Changes in temperature likely have large impacts on the ability for females to discriminate among males, and for males to produce signals that will secure mates. The long-term effects of this interaction are complicated and difficult to predict, particularly when I consider that two parties are involved and that both of those parties' behaviors may be temperature-dependent in different ways. These complexities are compounded by the fact that male signals are multimodal, and different aspects of the signals can be affected differently by changing temperatures. my data suggest that profound differences in mating behavior can result from ecological conditions that vary and that differences between the sexes could potentially amplify these effects. This work underscores the importance of obtaining fine-grained ecological knowledge that is relevant to the size and biology of the organism in question. Understanding how changing climatic regimes will affect the selective landscapes of all animals is one of the greatest challenges facing biologists today and one that must be ultimately tackled in different organisms and at different biological scales.

Figures

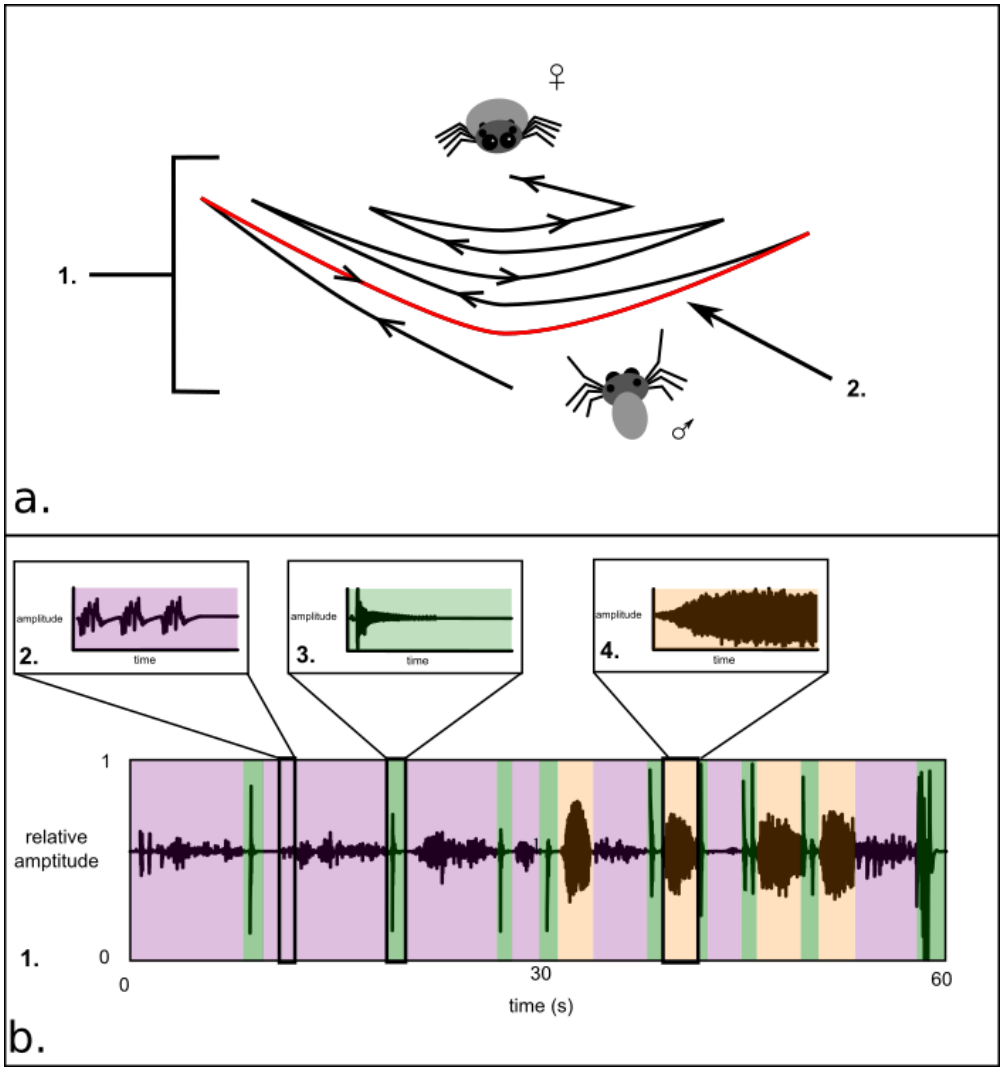

Figure 1-1 Early and late phase courtship of *H. clypeatus*. (a) Depiction of typical early phase courtship display. Female (above) remains in place while male walks in a side-to-side zigzag pattern, moving in arcs of shortening length until he is standing ~1 body length away from the female directly in front of her. Illustrated is (1) the entire sidling bout and (2) a single movement bout. (b) Oscillogram of vibratory aspects of a typical H. clypeatus late phase display. Entire vibratory display is shown in (1). Individual components are indicated in cut-aways: (2) scrapes, (3) thumps, and (4) buzzes.

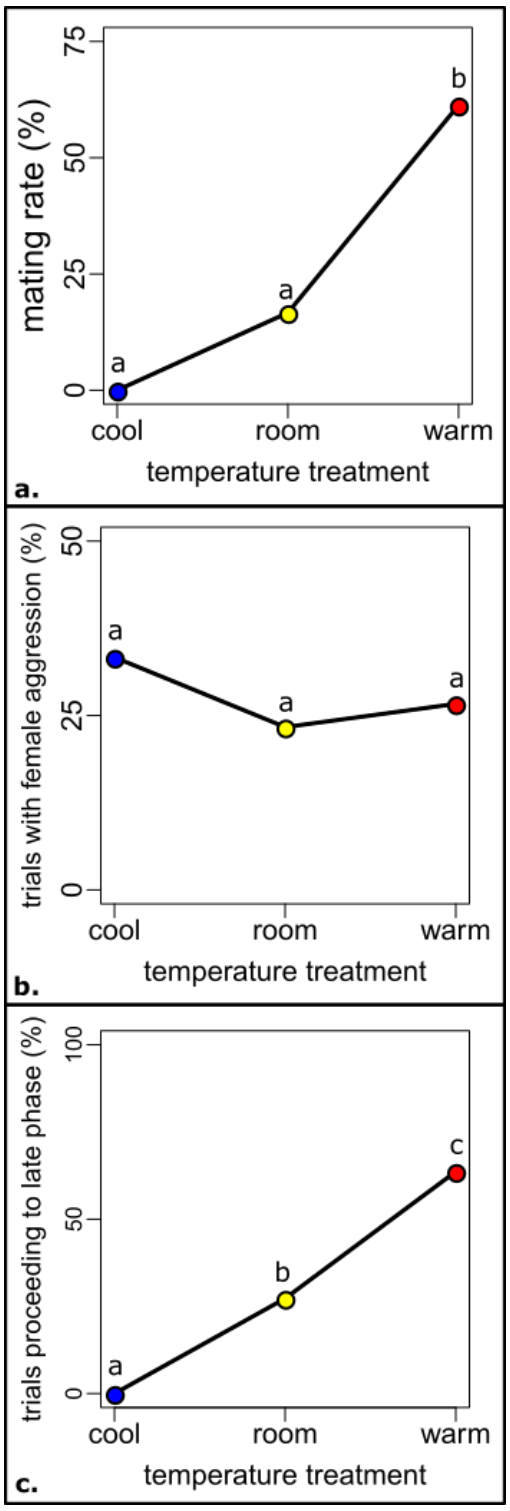

Figure 1-2. *H. clypeatus* female behavior across temperature treatments. Different letters within a panel indicate significant differences between treatments ($p < 0.05$). (a) Percent of females mating in three temperature treatments: cool, room temperature, and warm (n = 37 pairs, 8 in the cool treatment, 19 in the room temperature treatment, and 10 in the warm treatment). (b) Percent of trials that included aggressive female behaviors. (c) Percent of trials in each temperature treatment that proceeded to late phase courtship.

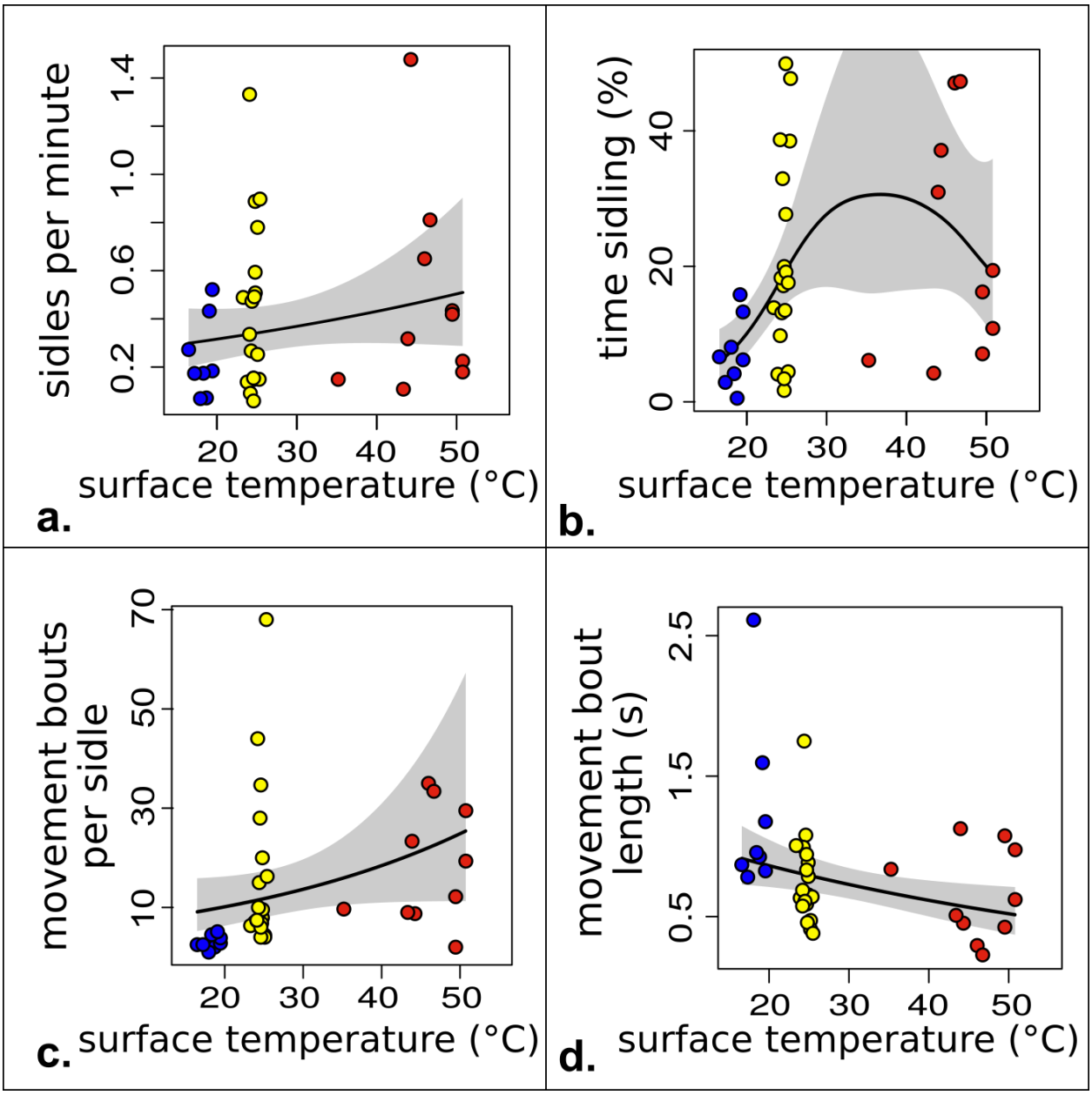

Figure 1-3. Early stage *H. clypeatus* male display. (a) average rate of sidling bouts, (b) average percent of time spent sidling per trial, (c) average number of movement bouts per sidling bout, (d) average length of movement bout per sidling bout (n = 37 pairs, 8 in the cool treatment, 19 in the room temperature treatment, and 10 in the warm treatment). Colored dots indicate whether a sample was collected in cool (blue), room temperature (yellow), or warm (red) treatments. Grey areas indicate 95% confidence interval.

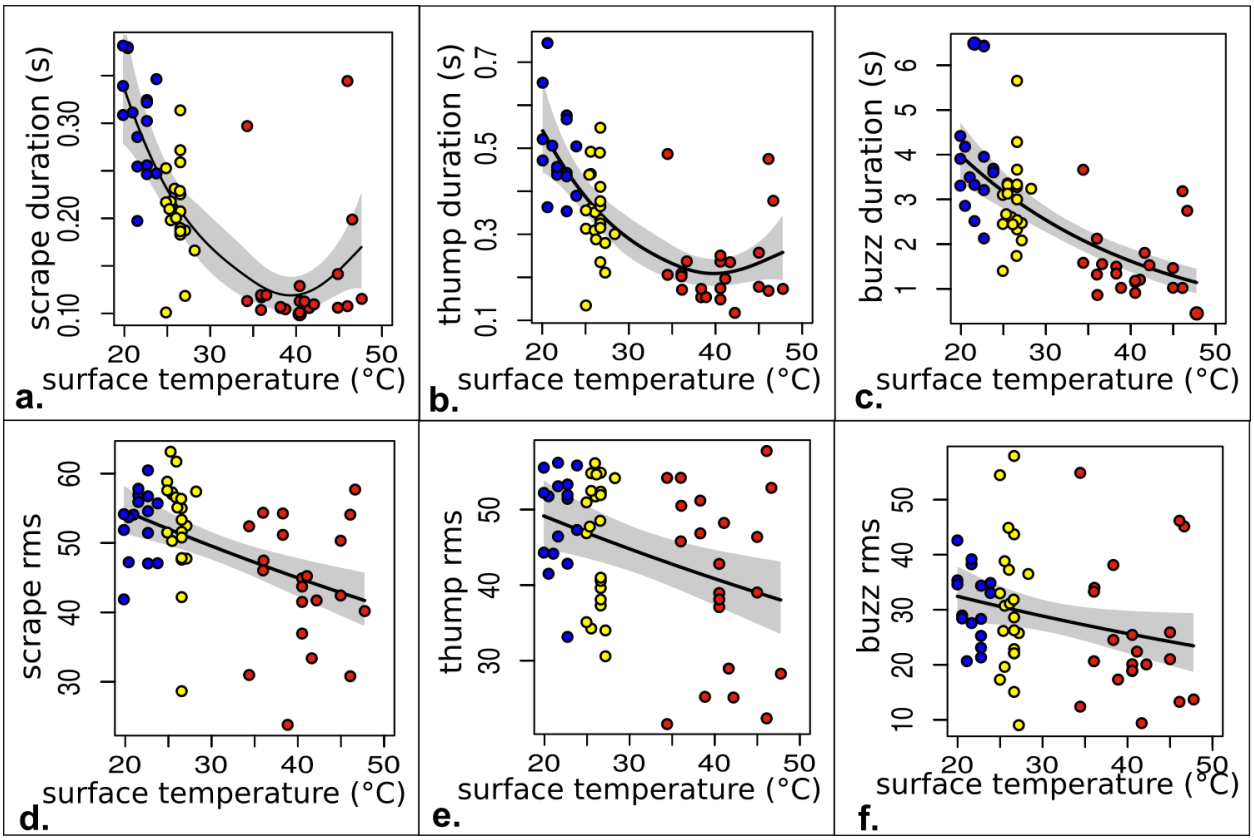

Figure 1-4. Durations and amplitudes of vibratory aspects of *H. clypeatus* male late phase display. (a) average scrape duration, (b) average thump duration, (c) average buzz duration, (d) average scrape RMS, (e) average thump RMS, (f) average buzz RMS. (n = 60 trials, 20 each for cool, room temperature, and warm treatments). Colored dots indicate whether a sample was collected in cool (blue), room temperature (yellow), or warm (red) treatments. Grey areas indicate 95% confidence interval.

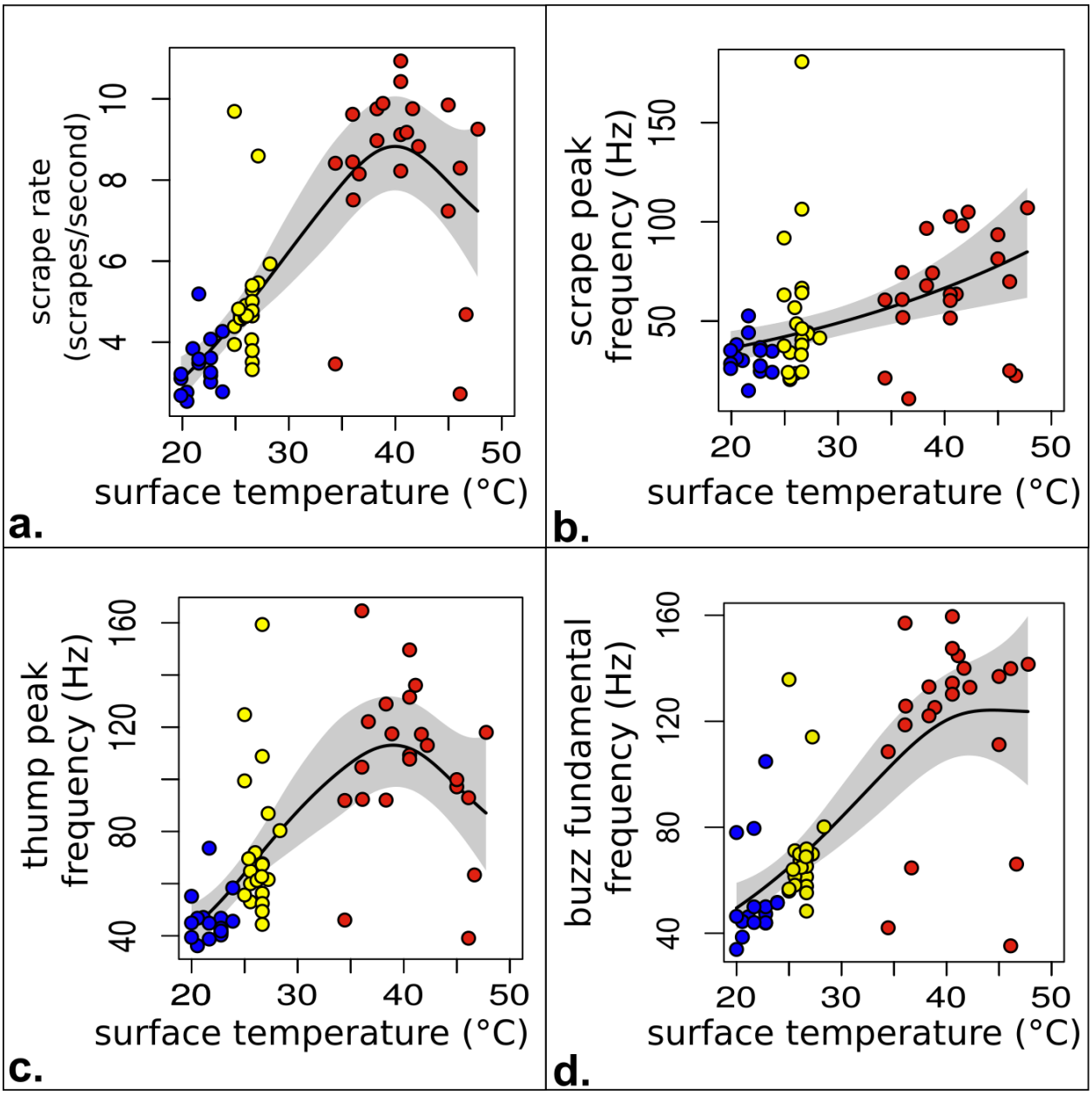

Figure 1-5. Rates and frequencies of vibratory aspects of *H. clypeatus* male late phase display. (a) Rate of scrapes, (b) average peak frequency of scrapes, (c) average peak frequency of thumps, (d) average fundamental frequency of buzzes (n = 60 trials, 20 each for cool, room temperature, and warm treatments). Colored dots indicate whether a sample was collected in cool (blue), room temperature (yellow), or warm (red) treatments. Grey areas indicate 95% confidence interval.

Tables

Table 1-1. Statistical Details for Occupancy Modeling Data. Deviance explained=41.2%. model: number_clypeatus ~ observer + s(time.mid) + s(surv.dist.m) + s(slopedegs.mid); family=Poisson

(a) parametric terms				
model term	β	S.E.	z	p
observer A & April (intercept)	-3.1885	0.9789	-3.257	0.001126
Month (June)	0.4359	0.7375	0.591	0.554528
observer B	0.4001	0.7492	0.534	0.593344
observer C	0.2681	0.7206	0.372	0.709832
observer D	2.1195	0.6332	3.347	0.000816
(b) smoothed terms				
model term	est. df	χ^2	p	
time of day	4.24	21.059	<0.001	
survey distance	1	2.282	0.131	
slope	1	1.177	0.278	

Table 1-2. Statistical details for parametric portion of GAMM models for male visual and vibratory components. k = 15 for all models. P < .05 indicated by *.

visual variable	**estimate**	**S.E.**	**t**	**p**
sidle rate	-1.01	0.13	-7.69	*4.98×10^{-09}
percent time sidling	-1.79	0.11	-15.71	*$<2 \times 10^{-16}$
movement bouts/sidle	2.58	0.19	13.92	*7.86×10^{-16}
movement bout length (s)	-0.30	0.08	-3.97	*0.0003
vibratory variable	**estimate**	**S.E.**	**t**	**p**
scrape duration (s)	-1.63	0.038	-42.63	*$<2 \times 10^{-16}$
thump duration (s)	-1.12	0.039	-28.38	*$<2 \times 10^{-16}$
buzz duration (s)	0.92	0.06	16.22	*$<2 \times 10^{-16}$
scrape rate (scrapes/s)	1.69	0.03	49.84	*$<2 \times 10^{-16}$
scrape peak frequency (Hz)	3.84	0.08	47.89	*$<2 \times 10^{-16}$
thump peak frequency (Hz)	4.31	0.04	97.34	*$<2 \times 10^{-16}$
buzz fundamental frequency (Hz)	4.37	0.04	111.70	*$<2 \times 10^{-16}$
scrape amplitude (RMS)	3.90	0.02	191.90	*$<2 \times 10^{-16}$
thump amplitude (RMS)	3.80	0.03	111.50	*$<2 \times 10^{-16}$
buzz amplitude (RMS)	3.36	0.05	67.00	*$<2 \times 10^{-16}$

Table 1-3. Statistical details for smoothed (non-parametric) portion of GAMM models for male vibratory components. k = 15 for all models. P < .05 indicated by *.

visual variable	**est. df**	**ref. df**	**F**	**p**	**R^2 (adj.)**
sidle rate	1.00	1.00	1.67	0.20	*0.002
percent time sidling	2.38	2.38	6.63	0.002	0.12
movement bouts/sidle	1.00	1.00	3.07	0.09	*0.03
movement bout length (s)	1.00	1.00	6.06	0.02	0.09
vibratory variable	**est. df**	**ref. df**	**F**	**p**	**R^2 (adj.)**
scrape duration (s)	3.11	3.11	25.9	4.68×10^{-12}	0.63
thump duration (s)	2.70	2.70	23.0	5.58×10^{-10}	0.55
buzz duration (s)	1	1	52.7	3.03×10^{-10}	0.45
scrape rate (scrapes/s)	2.907	2.907	37.19	6.02×10^{-16}	0.66
scrape peak frequency (Hz)	1	1	14.36	0.0004	0.15
thump peak frequency (Hz)	2.80	2.80	24.71	8.26×10^{-11}	0.50
buzz fundamental frequency (Hz)	2.13	2.13	31.87	3.04×10^{-11}	0.55
scrape amplitude (RMS)	1	1	16.36	0.0002	0.21
thump amplitude (RMS)	1	1	8.88	0.004	0.11
buzz amplitude (RMS)	1	1	3.90	0.05	0.05

Chapter 2. Brett's Rule predicts species distributions of jumping spiders across a desert elevational cline

Introduction

A fundamental goal in biology is to explain species distributions. Temperature is one aspect that has been repeatedly shown to be of great importance in this respect (Gaston et al., 2009; Ghalambor, Huey, Martin, Tewksbury, & Wang, 2006; Sunday et al., 2019). Environmental temperature is a particularly salient factor for ectothermic animals (those which cannot generate metabolic heat)(Angilletta, 2009b; Hochachka, 2002), especially small animals whose body temperatures rapidly equilibrate with environmental temperature (Dillon, Liu, Wang, & Huey, 2012; Hochachka, 2002). Specifically, thermal tolerance (the ability to withstand extreme temperatures) is frequently measured to assess an animal's thermal biology (Angilletta, 2009b).

Geographic clines in temperature shape ectotherm thermal tolerances at the inter- and intra-specific levels (Gaston et al., 2009; Sinclair, Williams, & Terblanche, 2012). A number of macrophysiological "rules" have emerged from this literature. One of these, Brett's rule, predicts more geographic variation in cold, rather than heat tolerance (Gaston et al., 2009). Brett's rule has some compelling support. Species from cold habitats at high latitudes are generally found to be more cold-tolerant than those found in warm, low-latitude environments. Heat tolerance however tends to be more constrained across latitudes and environments (Addo-Bediako, Chown, & Gaston, 2000; David, Gibert, Moreteau, Gilchrist, & Huey, 2003; Kellermann et al., 2012; Kimura, 2004; Sunday et al., 2019). Among these studies, there has been strong evidence for Brett's rule in tropical populations of insects, lending support to the idea that tropical species have narrower thermal breadths and are thus more vulnerable to climate change (Janzen, 1967; Polato et al., 2018). However, there have been relatively few studies looking at these principles across elevational gradients, particularly outside of tropical forest habitats (Polato et al., 2018; Sunday et al., 2019). In fact, among arthropods found across elevational gradients, support for Brett's rule has only been found in grasshoppers, ants, and aquatic insects, all in tropical systems (Arnan, Cerdá, & Retana, 2014; Nowrouzi, Andersen, Bishop, & Robson, 2018; Polato et al., 2018). It is crucial to increase habitat and taxonomic diversity to better understand the underlying patterns and to test the generality of macrophysiological "rules" (Gaston et al., 2009).

In addition to expanding habitat and taxonomic diversity, methodological diversity in examining thermal biology is needed. The totality of an animal's thermal biology cannot be collapsed down to only its ability to tolerate extreme temperatures. Moderate temperatures can exert selection through their effects on growth, reproduction, and other fitness characters, expressed as thermal performance curves (Angilletta, 2009b; Hochachka, 2002). Shifts in thermal performance curves for these physiological or performance traits can occur in response to divergent climatic regimes and can be used to predict responses to climate change (Sinclair et al., 2016). Animals can also select favorable microhabitats that optimize performance through thermoregulation. The ability to behaviorally thermoregulate within a desired preference range can dramatically limit the subset of temperatures to which animals are exposed, and act as a buffer against thermally-stressful temperatures (Martin & Huey, 2008b). Behavioral thermoregulation can thus reduce selection on both thermal limits and thermal performance curves (Muñoz et al., 2016). Despite the importance of a broad understanding of an animal's thermal biology, few studies of species distributions incorporate more than one of these metrics of thermal performance (Overgaard,

Kearney, & Hoffmann, 2014). In the following study, I measured aspects of thermal tolerance, thermal preference, and thermal performance to examine whether thermal biology explains species distributions across an elevational gradient.

Jumping spiders (Family Salticidae) are one of the most diverse arachnid families (< 6000 species) and are ecologically important predators (Foelix, 2010; Michalko, Pekár, & Entling, 2019). Among jumping spiders, *Habronattus* is a particularly diverse genus (~110 species) found across North America. Communities of *Habronattus* species have been described in many habitats (Griswold, 1983; Richman, 1973, 1977) often spread across elevational gradients. Few studies have explored thermal biology in any arachnid, and none has explicitly measured this in the context of habitat gradients (Bennett et al., 2018). In this study, I examined *Habronattus* species found across an elevational gradient in the Santa Rita mountain range in the Sonoran Desert. Habitats on this mountain range from lowland desert (hot/dry/open) to pine forest (cool/wet/shaded) (DeBano, 1999). I hypothesized that aspects of thermal biology would explain the distribution of *Habronattus* species across the Santa Rita Mountains in Southeastern Arizona (hereafter referred to as SR *Habronattus*).

I first collected *Habronattus* species found at four sites along an elevational gradient in the Santa Rita Mountains (Fig. 1a-b). I then performed experiments to assess different aspects of the animals' thermal biology. I tested thermal tolerance by measuring the critical thermal limits (CT_{min} and CT_{max}) and determined whether this assemblage obeys Brett's Rule. I also measured thermal performance (respiration and locomotion), and temperature preference. Finally, I compared these aspects of thermal biology to current and future environmental temperatures to assess vulnerability to climate change.

Materials and Methods

Description of sites and species

I collected animals from four sites along an elevational gradient in the Santa Rita Mountains in SE Arizona: a low elevation (average 1201 m) desert scrub site, a middle elevation (average 1266 m) desert grassland site, a middle-high elevation (average 1532 m) oak woodland site, and a high elevation (average 1672 m) pine woodland site (Fig. 1a). I collected individuals of six *Habronattus* species: *H. virgulatus* Griswold, 1987, *H. clypeatus* (Banks, 1895), *H. conjunctus* (Banks, 1898), *H. hallani* (Richman, 1973), *H. pugillis* Griswold 1987 and *H. geronimoi* Griswold 1987. In general, each species was most associated with one elevational site although I occasionally found individuals at other sites. I only collected and performed experiments upon individuals that were found in their typical site. Specifically, *H. virgulatus* was found exclusively at the low elevation site. *H. clypeatus* was found at the middle site, but also at the low site and infrequently at the middle-high site. *H. hallani* and *H. conjunctus* were found at the middle elevation site. *H. pugillis* is known to be an oak forest specialist (Maddison & McMahon, 2000) and was only found at the middle-high site. Finally, *H. geronimoi* was mostly found at the high elevation site (one individual was found in 2018 at the middle-high site).

Habitat data

I tested whether the different elevational sites were climatically and thermally distinct. I did this by recording GPS collection data for every spider collected in 2017 (sample sizes for each site: low: 82, middle: 580, middle-high: 74, high: 48). I then imported the GPS points into R v. 3.5.2,

and used the WorldClim2.0 dataset (Fick & Hijmans, 2017) implemented using the raster package to extract Bioclim variables for those points. I used two different Bioclim variables: Maximum temperature of the warmest month, and minimum temperature of the coldest month. The first variable is thought to correspond well to CT_{max}, and the second, to CT_{min} (Kellermann et al., 2012). I also calculated a third variable, annual breadth, calculated as maximum temperature of warmest month minus minimum temperature of coldest month. I compared the climatic variables of my four different sites using an ANOVA followed by post-hoc Tukey tests.

Animal collection and maintenance

I collected spiders during March and April of 2017 and 2018. I collected animals at all life stages, but only performed experiments on individuals that had been sexually mature for at least two weeks. I used approximately equal numbers of males and females (assessed by looking for male or female genitalia) for each experiment. Animals were kept in small plastic cages and fed *Drosophila melanogaster* and pinhead *Gryllodes sigillatus* crickets once per week. I acclimated all animals to lab conditions (~24°C) for at least two weeks before running experiments. Animals were not fed within 48 hours of any experiment. I performed $CT_{min/max}$ trials during fall 2017, thermal performance studies during spring and summer of 2018, and preference studies during summer 2018.

Thermal tolerances

To assess thermal tolerances, I used a ramping assay that used loss of righting ability as the indicator of a limit being reached. Ramping assays have been suggested to be faster and more accurate than static assays (Kovacevic, Latombe, & Chown, 2019; Rezende, Tejedo, & Santos, 2011; Terblanche, Deere, Clusella-Trullas, Janion, & Chown, 2007). To perform these experiments, I used an incubator (MIR-154-PA, Panasonic Healthcare, Tokyo, Japan) and a custom device that allowed us to flip spiders throughout the ramp procedure to see at which point they lost their righting ability. See Appendix for more detailed methods.

For CT_{max} trials, I started the incubator at 40°C, Spiders were acclimated to this temperature for ten minutes. I then began the trial and ramped the incubator to 60°C. For CT_{min} trials, I used the same method, but set the incubator to 15 °C for the initial temperature and ramped to 0°C. The average ramp rate for both types of trials ranged from 0.5 to 1.0°/min. I flipped the spiders every 2 minutes and observed the spiders until they were unable to right themselves within 20 seconds. I measured the mass of each individual after each trial. Due to the experimental setup, I was unable to immediately remove animals from the setup after their CT_{max} had been achieved. Because of this, animals frequently died after the CT_{max} trials, so I ran CT_{min} trials first, and CT_{max} trials after at least 24 hours of recovery time.

Thermal Preference

I designed a temperature gradient device to assess thermal preference, spanning a thermal gradient of about 15-60 °C. See Appendix for details of setup. In short, the thermal gradient was heated on one end with silicone heaters (model SRFG-110/-10P, Omega Engineering, Norwalk, CT, USA) and ceramic heat bulbs (Zoo Med Laboratories Inc, San Luis Obispo, CA, USA). The entire device was placed into a room regulated at 7°C. The setup was divided into six lanes, so that each spider could be isolated from the others. Perpendicularly, the lanes were divided into 6 different “zones”, from the hot to cold end, so that the initial placement of spiders along the gradient could be randomized.

During the thermal preference trials, the thermal gradient was first turned on and allowed to thermally equilibrate for 30 minutes. Next, six different spiders were each randomly assigned to one of the six lanes. I then observed spiders and recorded their body temperature every 10 minutes with a non-contact IR thermometer (Dual Laser IR Thermometer, Model 42511, Exetech Corp, Nashua, NH). At the end of 40 minutes, I recorded the spiders' final temperatures and concluded the trial. These final temperatures were used in analyses. I compared final temperatures between species using ANOVA and post-hoc Tukey tests. I also calculated T_{pref} range as the middle 50% of temperatures (Angilletta, 2009b). Thermal preferences are not static or only associated with one behavior or set of behaviors. Ectotherms can have multiple ranges of preferred body temperatures that they employ to accomplish different physiological tasks and developmental stages (Clissold et al., 2013; Dillon, Wang, Garrity, & Huey, 2009). However, I use this method to achieve baseline information about thermal preference that can be easily compared.

Thermal Performance

Respirometry

I used stop-flow respirometry using a LiCor7000 CO_2 analyzer (Li-Cor Biosciences, Lincoln, NE) and Sable Systems respirometry system (Sable Systems, Las Vegas, NV) to measure rate of CO_2 production ($\dot{V}CO_2$), a proxy for resting metabolic rate. I used a modified repeated measures design, in which I measured each individual at each of seven temperatures, from 10 °C to 40°C, at 5° intervals (total of 1361 samples, individual sample sizes: *H.* virgulatus = 44, *H. clypeatus* = 58, *H. conjunctus* = 35, *H. hallani* = 42, *H. pugillis* = 51, *H. geronimoi* = 33). See Appendix for detailed experimental setup. If an individual died between trials, I substituted an individual of the same species and sex for the remainder of the original individual's trials. I chose a range of temperatures that fell well below the CT_{max} of the spiders to avoid stress. I selected seven temperatures from 10 °C - 40°C, at 5° intervals for 7 total temperature treatments.

I modeled the relationship between $\dot{V}CO_2$ and temperature using nlme in R (Pinheiro, Bates, DebRoy, & Sarkar, 2018). I log-transformed data to approximate a normal distribution. Fixed effects were temperature, species, weight, and the species-by-temperature interaction. Random effects were individual and days since last fed. Due to heteroskedasticity in data with respect to temperature, I set a fixed variance structure for temperature, allowing it to increase with increasing temperature. I also calculated mass-scaling exponents using the respirometry package in R (Birk, 2019). Finally, I calculated Q_{10} coefficients, a dimensionless measure of the thermal sensitivity of biological processes. It is calculated as $Q_{10} = (R2/R1)^{10°C/(T2-T1)}$, where R1 and R2 are the beginning and ending rates of interest (in this case, mL VCO_2/hr), and T1 and T2 are the temperatures associated with each rate. The Q_{10} for most processes is around 2, indicating that for each 10 degree increase, the rate doubles (Hochachka, 2002).

Locomotion

As jumping is a major means of locomotion for jumping spiders (Chen, Liao, Tsai, & Chi, 2013; Foelix, 2010; Richman & Jackson, 1992), I used a jumping assay to evaluate the effects of temperature on locomotion. I used seven temperatures ranging from 15° to 45° C, at 5°C intervals (460 total samples, sample sizes: *H. virgulatus* = 11, *H. clypeatus* = 20, *H. conjunctus* = 15, *H. hallani* = 15, *H. pugillis* = 15, *H. geronimoi* = 4). Each spider was run at every temperature if possible. If an individual died between trials, I replaced it with another of the

same sex and species for the remainder of the original individual's trials. I shifted the set of temperatures by 5°C warmer compared to the respirometry experiment to measure locomotor performance closer to the animals' thermal maxima. For each trial, a spider was placed in the incubator at a given temperature for ten minutes to allow its internal body temperature to equalize with the incubator. I then stimulated spiders to jump by pushing a wooden block toward the spider from behind until it jumped. I attempted to elicit three jumps for each trial. I recorded jumps from above with a GoPro Hero 5 Black set to 240 fps. I used the MTrackJ plugin (Conn, 2012) for ImageJ (Schindelin, Rueden, Hiner, & Eliceiri, 2015) to measure jump distance.

For each trial, I averaged the distances for up to three jumps. I then modeled jump distance in R with lme, with temperature and species as fixed effects and individual as a random effect. I also set a fixed variance structure to allow variance to increase with increasing temperature. Finally, I calculated mass-scaling exponents and Q_{10} values.

Warming tolerance

Temperatures are predicted to increase in the Santa Rita Mountains by 1.7°C-2.8°C in the next fifty years (Coe, Finch, & Friggens, 2012). I therefore calculated the buffer between spiders' CT_{max} measures and environmental temperature to assess their ability to cope with future temperature regimes. This is often done with warming tolerance, a metric calculated by subtracting mean annual environmental temperature from the animals' CT_{max} values (Deutsch et al., 2008). I calculated a modified version of this metric by using maximum annual temperatures in calculating warming tolerance to provide the most conservative measure possible. I compared this measure across species using an ANOVA and post-hoc Tukey tests.

Evolutionary history

I generated a pruned phylogenetic tree of SR *Habronattus* species using data from Leduc-Robert & Maddison, 2018 using Mesquite version 3.51 (The Mesquite Project Team, 2018). I tested for phylogenetic signal in elevation (as a continuous variable) and thermal biology data. I used Blomberg's K, implemented using phytools in R (Revell, 2012). For respiration, I calculated Blomberg's K on slopes of the log-transformed linear relationship between $\dot{V}CO_2$ and temperature. For jumping, I calculated K on slopes of the linear relationship between jump distance and temperature. Due to small sample sizes, I used a K value of > 1 to suggest phylogenetic signal within the data (Hebets, Vink, Sullivan-Beckers, & Rosenthal, 2013).

Results

Thermal habitat differences

All four sites differed in their minimum and maximum temperatures (Figs 1c-d). Both maximum and minimum temperatures differed between all elevational sites (max temperature: $F = 14774$, $p < 0.0001$; min temperature: $F=10554$, $p < 0.0001$). I found the same pattern with annual breadth (Fig. 1e) ($F = 6656$, $p < 0.0001$). More closely-related species were not found at more similar elevations than more distantly-related species. ($K = 0.789$, $p = 0.3000$).

Thermal limits

H. geronimoi (the highest-elevation species) had a lower CT_{min} than all other species. Low, middle, and middle-high elevations all had similar CT_{min} values ($F=12.14$, $p < 0.0001$). *H. clypeatus*, a mid-elevation species, had a higher CT_{max} than the other middle and the middle-high

species (F=5.6635, p = 0.0002). Relatedness of species had no bearing on their thermal limit values (CT_{min}: K= 0.789, p =0.0350; $CT_{max:}$ K = 0.986, p = 0.1390)

Thermal preference

All spider species had a mean preferred temperature: 37°C, and a T_{pref} range of 34.2°C – 41.2°C (Fig. 3).

Thermal Performance

Spiders produced more CO_2 at higher temperatures, with a Q_{10} of 1.94. Temperature (F = 637.047, p < 0.0001), species (F =4.148, p = 0.1001), weight (F = 254.712, p < 0.0001), and a temperature by species interaction (F=6.390, p <0.0001) all affected CO_2 production. *H. conjunctus* (a mid-elevation species) produced less CO_2 than the other species, particularly at high temperatures (Fig. 3a). More closely-related species had more similar metabolic rates (K = 1.34, p = 0.0350). Heavier spiders also produced more CO_2 than lighter spiders, with a mass-scaling exponent of 0.669 averaged across temperatures.

Spiders also jumped farther at higher temperatures, with a Q_{10} of 1.37. Temperature (F = 141.474, p =0.0130), weight (F = 24.75370, p <0.0001), species (F = 3.264062, p = 0.0071), the temperature by weight interaction (F =16.47918, p < 0.0001), and the weight by species interaction (F =2.86450, p = 0.0150) all affected jump distance. *H. hallani* jumped significantly farther than *H. clypeatus* (p = 0.0032). *H. hallani* also jumped significantly farther than *H. geronimoi* (p = 0.0030) (Fig. 3b). There was no phylogenetic signal in these results (K = 0.591, p = 0.5970). Heavier spiders also jumped farther than lighter spiders, with a mass-scaling exponent of 0.23 averaged across temperatures.

Warming Tolerance

All species showed high warming tolerance, broadly recapitulating the species differences in CT_{min} (F = 46.77, p < 0.0001. *H. geronimoi* had the highest warming tolerance, followed by *H. pugillis* (Fig. 5). There was more variation among the middle and low elevation species. *H. clypeatus* had a higher warming tolerance than both *H. conjunctus* and *H. hallani.* Relatedness was not a factor in these results (K = 0.035, p = 0.3860).

Discussion

My data suggest that cold tolerances are more variable across elevation than heat tolerances, a pattern commonly known as Brett's Rule. I also found interactions between thermal performance and thermal preference, suggesting that thermal biology in SR *Habronattus* represents a complex interplay between physiology and behavior. Finally, I find support for high warming tolerance and species resilience in SR *Habronattus* to global climate change.

Evidence for Brett's Rule

Of all the thermal variables measured, CT_{min} was the only one that showed clear differences between elevational sites. I found the lowest CT_{min} in the highest-elevation species, *H. geronimoi* (Fig. 2b), corresponding with the lower minimum temperatures in high elevation habitats. However, this was not recapitulated with a similarly low CT_{max} in this species (Fig. 2a). In fact, I found that all SR *Habronattus* species had extraordinarily high CT_{max} measurements that were conserved across the elevational gradient. CT_{max} values ranged from 52-55°C on average, among

the highest of any spider species recorded using similar methods, including species living in extreme thermal conditions in the Namib (Lubin & Henschel, 1990) and Australian Deserts (van den Berg, Thompson, & Hochuli, 2015). SR *Habronattus* thermal limits approached those of the most thermally-tolerant terrestrial ectotherms known, *Cataglyphis bombycina*, a desert-adapted ant species from the Saharan desert (CT_{max} of 56.7°C) (Christian & Morton, 1992).

This pattern of invariant heat tolerance and variable cold tolerance supports Brett's Rule (Nowrouzi et al., 2018; Polato et al., 2018; Slatyer, Nash, & Hoffmann, 2016; Slatyer & Schoville, 2016). Recent work has suggested that Brett's rule applies most strongly in tropical habitats with low thermal variability (Polato et al., 2018). My study provides support for Brett's Rule in desert habitats that have high thermal variability and suggests that this rule may be more universal than previous considered.

The mechanisms behind Brett's rule fall broadly into three non-exclusive categories (Sunday et al., 2019). First, there may be elevationally invariant selection on CT_{max}. That is, either all elevations are exposed to similarly hot temperatures at some point during the year (Sunday et al., 2014), or animals behaviorally thermoregulate such that they do not experience their thermal maxima (Huey, Hertz, & Sinervo, 2003; Muñoz et al., 2016). Second, in recent range expansions, there may a lag between distribution shifts and concordant changes in CT_{max} (Lancaster, 2016). Finally, there may be genetic or physiological constraints preventing CT_{max} from reaching habitat-appropriate values (Araújo et al., 2013). It is possible that any (or all) of these hypotheses could be in play for SR *Habronattus.* With respect to elevationally invariant selection, we know that SR *Habronattus* have strong thermal preferences that do not vary between species (Fig. 3.). If spiders can thermoregulate within their preferred ranges, this could potentially relax selection on CT_{max} and produce the observed patterns. With respect to biogeographical history, it is hypothesized that the Santa Rita mountain range was part of a glacial refugium, suggesting that SR *Habronattus* diversified and dispersed to their current ranges sometime between 30 kya and 2 mya (Leduc-Robert & Maddison, 2018; Masta, 2000). Given this relatively short time frame, it is possible that there has been insufficient time for CT_{max} to evolve. Finally, it is unclear whether genetic or physiological constraints may be impacting CT_{max} in SR *Habronattus.* Studies examining the genetic basis of thermal tolerance across *Habronattus* could evaluate whether there is support for this hypothesis.

Despite the lack of elevational signal in CT_{max} measurements, some interesting patterns in thermal limits can be seen at intermediate elevations. For example, *H. clypeatus* had a higher CT_{max} than the other middle and middle-high species but was indistinguishable from either the low or high species (Figs 2a-b). Because we find differences in CT_{min} and CT_{max} between species found at the same middle elevation site, this site may be more thermally diverse. The measured thermal limits of some species fit well with what I know about their ecology. For example, *H. hallani* had a low CT_{max} and has been shown to exhibit swimming behavior (rare in salticids), and suggests specialization for a cool microhabitat (Stratton, Suter, & Miller, 2004). This suggests that my measured thermal limits may reflect differences in thermal microhabitats

Integration of Behavior and Physiology

It is striking that despite a large thermal breadth in SR *Habronattus* (~40°C), the thermal preference (T_{pref}) range only encompassed about 7°C and does not vary between species (Fig. 3). The T_{pref} range of SR *Habronattus* was higher than most spiders (particularly fossorial species) but fell within the range of wolf spiders found in the Alps (Frick, Kropf, & Nentwig, 2007).

Having narrow thermal preference is one way that animals may behaviorally buffer against their thermal limits. If spiders consistently seek out microhabitats within a narrow range of temperatures, there is less risk of exposure to temperatures exceeding their CT_{max}. This assumes that microhabitats that are within the preferred temperature range are consistently available and requires future study to assess the thermal heterogeneity of relevant microhabitats. It may also be more difficult to buffer against CT_{min}, given that environmental heterogeneity tends to be decreased at night, when minimum temperatures occur (Ghalambor et al., 2006; Muñoz et al., 2016). SR *Habronattus* seem more limited by cold tolerance rather than by heat, especially given that their habitats attain temperatures well below their CT_{min} (Fig. 1c). This could pose a substantial challenge to their thermoregulatory ability.

Interestingly, the T_{pref} range sits among the warmest temperatures that I measured in performance trials (Fig. 4). Metabolic rate increased steadily throughout this range. Although I did not directly measure T_{opt} (the optimal temperature for a given process/behavior), this suggests that for metabolic rate, T_{opt} is well above T_{pref}. I found a similar pattern with jumping, however jumping is less temperature-sensitive than metabolic rate (Q_{10} of 1.3 vs. 1.9). This suggests that maintenance costs rise faster than performance ability, perhaps indicating that a decline in aerobic scope may constrain performance at high temperatures (Pörtner, 2001, 2010). I have also found previously that *H. clypeatus* courtship signals plateau around 40°C (Chapter 1). Taken together, this suggests that spiders are choosing temperatures that optimize courtship behavior, and not metabolism or locomotion.

I found few differences between species with respect to thermal performance, and none that appear to relate to elevation. Again, this could be related to either invariant selection or constraints. There is some evidence that metabolic rates are phylogenetically constrained. *H. conjunctus* had lower respiration and was less temperature-sensitive with respect to metabolic rate (Fig. 3a). *H. conjunctus* is the most distantly-related of all SR *Habronattus* species (Fig. 1b). (Leduc-Robert & Maddison, 2018). With respect to locomotion, the only differences between species are between the two smallest by mass and the largest, with no effect of relatedness.

Implications for future species distribution patterns under climate change

SR *Habronattus* show large thermal breadths and warming tolerances (Figs 2,5). One goal of this study was to bring these data to bear on the question of resilience in *Habronattus* given changing global climate and the massive worldwide declines in arthropod populations (Hallmann et al., 2017; Lister & Garcia, 2018). SR *Habronattus'* large warming tolerances may represent a substantial buffer against future increases in habitat temperature (Fig. 4). The predicted increase of 1.7-2.8°C should make little difference to SR *Habronattus*, with a warming tolerance of > 20°C for each species (Fig. 5). This suggests that these species are relatively robust to future thermal shifts due to climate change. There are important caveats, however.

First, I used average environmental temperatures that do not necessarily reflect the daily extremes that may be driving selection (Sunday et al., 2019). Second, although the increases in temperatures may appear modest compared to the large warming tolerance, vegetation types are also predicted to shift (Coe et al., 2012), which could influence thermal microhabitat distribution. Third, thermal stress can disrupt animals in ways that cannot be measured with physiological assays. In at least one species of SR *Habronattus*, courtship and mating are affected by temperature, with female receptivity and mating rate increasing with temperature (Chapter 1). Finally, there is evidence of widespread historic introgression across the group (Leduc-Robert &

Maddison, 2018). If changing climate puts allopatric *Habronattus* species into contact, complex dynamics involving temperature-dependent courtship signals, mate preference, and physiological tolerances may come into play.

Conclusions

By studying multiple measures of thermal biology and behavior, I provide support for Brett's Rule along an elevational gradient, suggesting that extreme low temperatures are an important selective agent in determining species distributions. Specifically, I found that only cold tolerance, and no other thermal biology metrics, had explanatory power regarding species distribution patterns in SR *Habronattus*. This lends weight to the hypothesis that Brett's Rule applies broadly across latitudinal (Addo-Bediako et al., 2000; Ghalambor et al., 2006; Kimura, 2004) and elevational clines in both tropical (Ghalambor et al., 2006; Polato et al., 2018; Slatyer et al., 2016) and desert habitats. I also found that SR *Habronattus* have among the highest CT_{max} measurements of any known arthropod with little variation across the species studied. The lack of variation may reflect (1) geographically invariant selection on responses to hot temperatures, (2) genetic constraints on the evolution of these traits and/or (3) the importance of behavioral thermoregulation. Given that temperature preferences are conserved between species, I suggest that behavioral plasticity might be important in allowing these physiologically-similar species to live in very different microhabitats. Finally, SR *Habronattus* have high warming tolerances, suggesting that these species should be robust to future increases in habitat temperature. Ongoing work will build upon these results by examining variation in spider habitats at scales relevant to the animals, and further integrating how behavior, especially sexual behavior, interacts with their thermal biology. Additionally, studies comparing different species assemblages across the landscape can be leveraged to test hypotheses about how thermal tolerance differences have arisen (e.g.: local adaptation, environmental filtering).

Figures

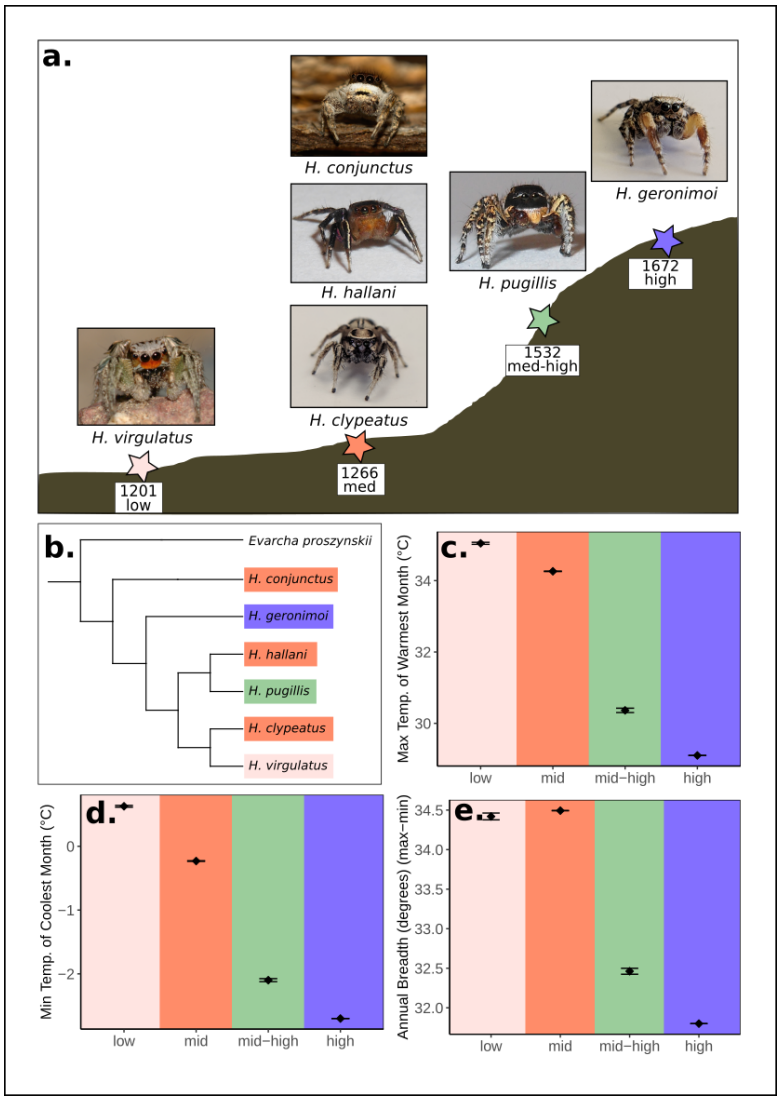

Figure 2-1. Santa Rita *Habronattus* elevational distribution and environmental conditions (a) schematic of elevational distribution of *Habronattus* species found in the Santa Rita mountains (diagram not to scale). Collection sites are indicated with labels used throughout the paper (low, middle, middle-high, high) along with average elevation. Color coding for elevational sites is consistent across figures. All photos are of adult males. (b) Phylogeny showing relationships between the six SR *Habronattus* species. Data were taken from Leduc-Robert & Maddison, 2018. Species are highlighted with the same colors as in panel a to illustrate their elevational site. (c) Maximum temperature of warmest month for each site ± SE. All sites are significantly different from one another ($p < 0.0001$). (d) Minimum temperature of coldest month for each site ± SE. All sites are significantly different from one another ($p < 0.0001$). (e) Annual temperature breadth (minimum temperature of coldest month minus maximum temperature of warmest month) of all sites ± SE. All sites are significantly different from one another ($p < 0.0001$).

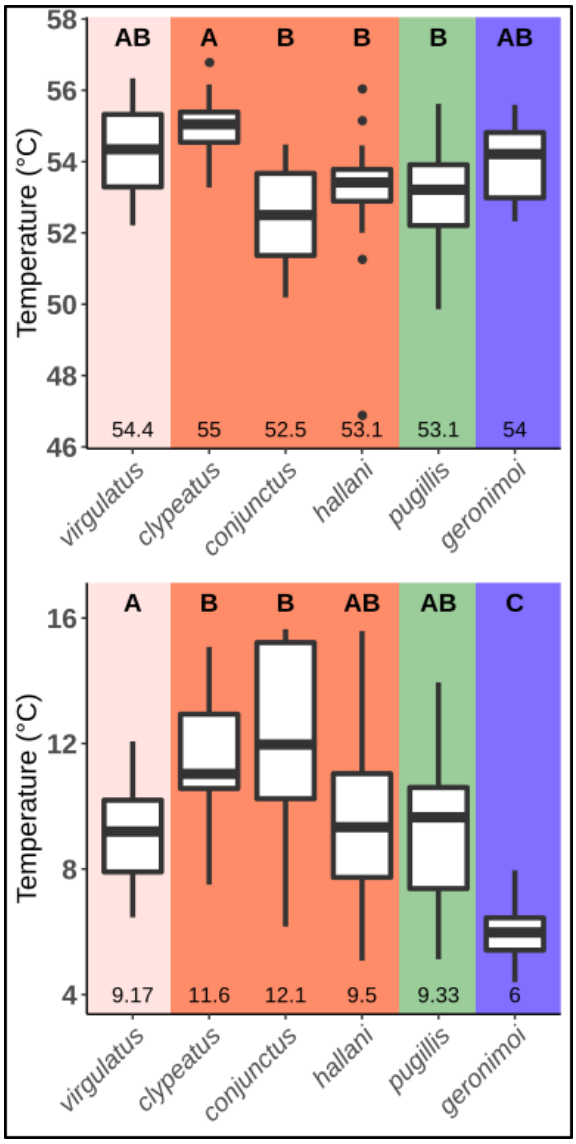

Figure 2-2. Critical thermal limits in Santa Rita *Habronattus***.** (a) Critical thermal maxima. Mean values are shown for each species. *H. clypeatus* is significantly different from *H. hallani*, *H. conjnctus*, and *H. pugillis*. No other differences are seen. (b) Critical thermal minima. Mean values are also shown for each species. Note that *H. geronimoi* is significantly different from all other species except for *H. virgulatus*. Background colors indicate site, from lowest (left) to highest (right) elevation.

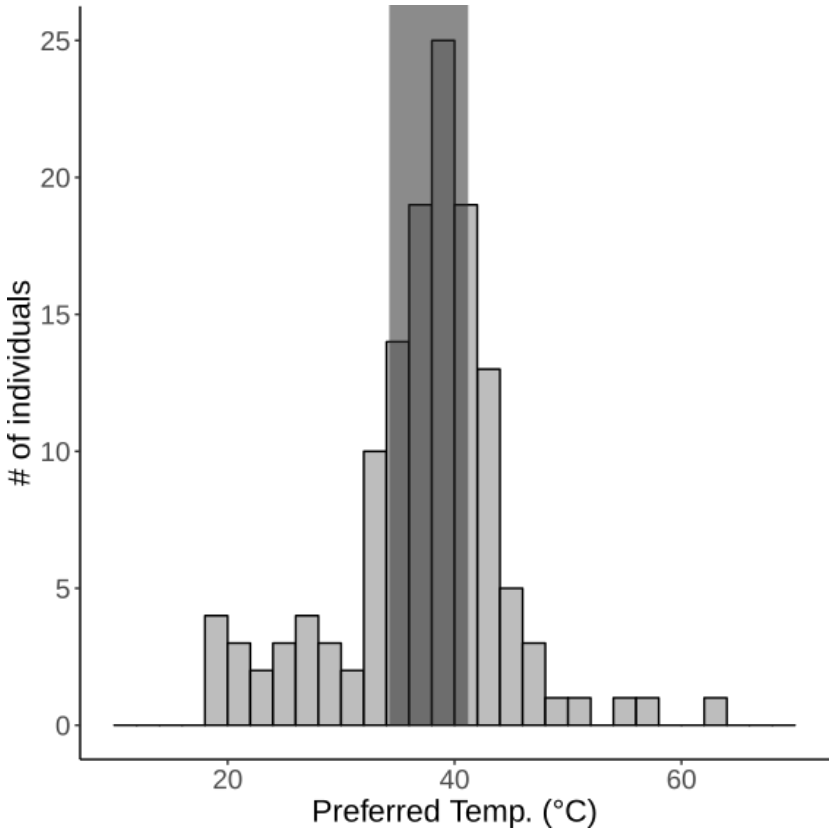

Figure 2-3. Thermal preference in Santa Rita *Habronattus*. (a) overall histogram of T_{pref} for all species. There are no differences between species. Dark bar indicates T_{pref} range (temperatures of middle 50% of individuals).

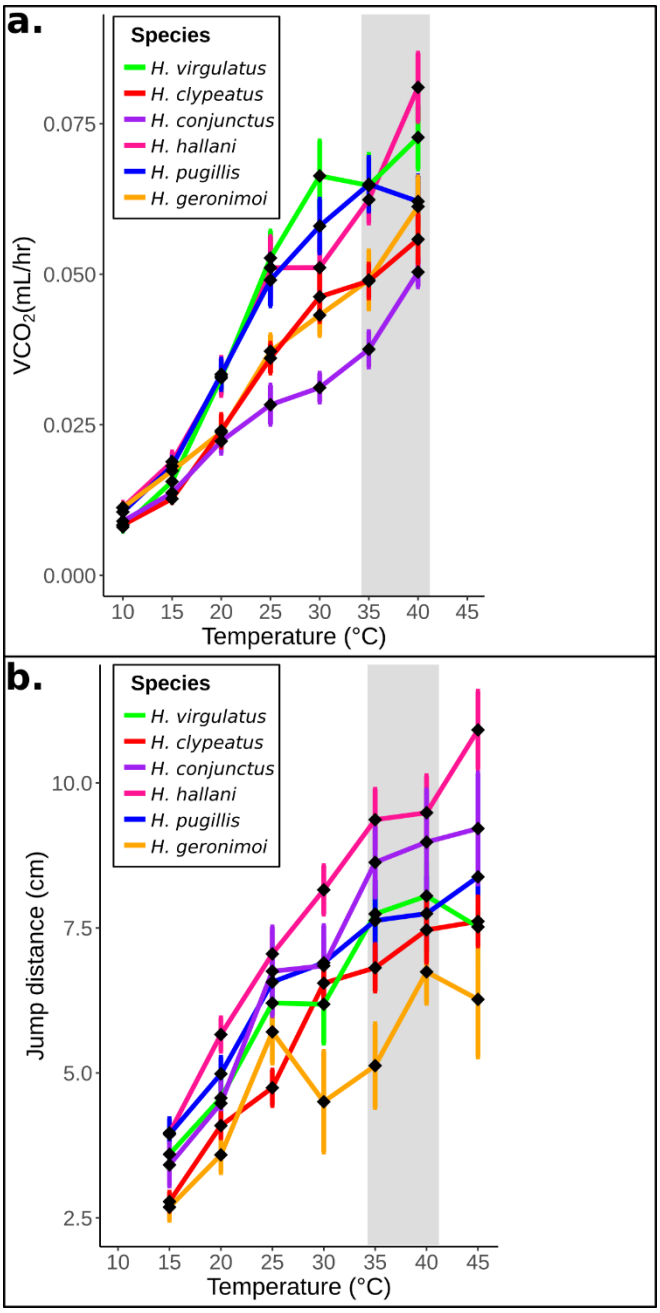

Figure 2-4. Thermal performance in Santa Rita *Habronattus* (a) carbon dioxide emission of SR *Habronattus* across seven different temperatures. *H. conjunctus* has significantly lower carbon dioxide emission than the other six species. Grey bar indicates the T_{pref} range (middle 50% of individuals) (b) jump distance in SR *Habronattus* species across seven different temperatures. No differences are seen between species when body size is taken into account. Grey bar indicates the T_{pref} range (middle 50% of individuals).

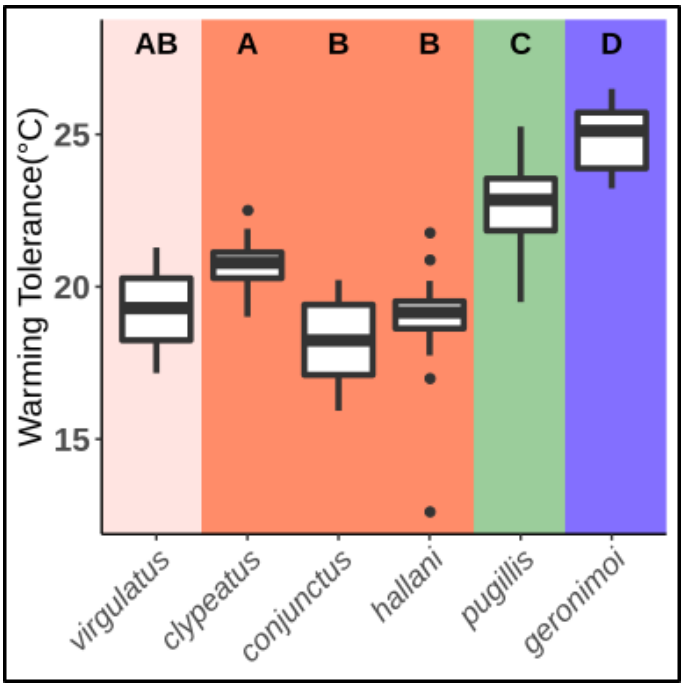

Figure 2-5. Warming tolerance for Santa Rita *Habronattus* species**.** Warming tolerance was calculated by CT_{max} – max temp of warmest month. Background colors indicate site, from lowest (left) to highest (right) elevation.

Chapter 3. Thermal ecology in miniature: thermoregulation and substrate use in desert jumping spiders

Introduction

A large body of work has shown that ambient temperatures influence many aspects of life history for ectotherms, from survival, to growth, to behavior (Angilletta, 2009b; Gibert, Chelini, Rosenthal, & DeLong, 2016; Hochachka, 2002). Although ectotherms cannot metabolically regulate their temperature, they are not typically passive in the face of fluctuating environmental temperatures. Behavioral thermoregulation is a process by which animals take advantage of thermal variability present in the environment, typically by moving between patches of microhabitat to attain desired body temperatures (Bogert, 1949; Hertz, Huey, & Stevenson, 1993). Successful behavioral thermoregulation has two main requirements. First, the animal in question must have a temperature preference (set point). Second, the animal's thermal environment must be sufficiently variable to give options to move within it. Behavioral thermoregulation has been found in many ectothermic taxa, including lizards, fish, and a variety of insects (May, 1979; Neill, Magnuson, & Chipman, 1972; Sears et al., 2016).

Ectothermy, in combination with behavioral thermoregulation, can give animals metabolic and behavioral advantages that endotherms do not have. Since endotherms maintain a relatively constant body temperature (unless hibernating or experiencing fever), they must perform all metabolic and behavioral processes at one temperature. Ectotherms, however, can optimize their body temperature for the task at hand (Dillon, Liu, Wang, & Huey, 2012). In many taxa, gravid females have different temperature preferences than non-gravid females (Gardner-Santana & Beaupre, 2009; Paranjpe, Bastiaans, Patten, Cooper, & Sinervo, 2013). Thermal preferences also vary on shorter timescales. For example, grasshoppers have been shown to prefer different temperatures depending on the macronutrient composition of the food that they are currently digesting (Clissold et al., 2013). This sets up the potential for tradeoffs, in which an animal must choose a temperature that optimizes one physiological state or behavior over another. For example, ectotherms can experience "behavioral fever" in which they temporarily shift their temperature preference in order to fight infection by a pathogen or parasite. This can come at the cost of other physiological processes that must be performed at sub-optimal "fever" temperatures (Thomas & Blanford, 2003; Woodhams, Alford, & Marantelli, 2003). Other such thermoregulatory tradeoffs can occur in the context of courtship and mating. For example, male fiddler crabs temporarily prefer temperatures during the breeding season that are warm enough to cause thermal stress, but are also more attractive in courtship contexts (Allen & Levinton, 2014).

Further complicating matters, other factors (e.g., conspecifics, predators, prey, physical substrates within the environment) can create additional tradeoffs in the context of thermoregulation (Nielsen & McGaw, 2016). Understanding how these complex interactions play out in nature requires careful observation in their natural environment (Sears et al., 2016).

We previously found that high mating success in *Habronattus clypeatus* (a desert-dwelling jumping spider) occurs at relatively high temperatures, which occur at times of day when spiders are less active (Chapter 1). However, information regarding how spiders are using and moving through their thermal microhabitats is lacking. For this project, we assessed behavioral thermoregulation, particularly temperature preference, substrate selection, and thermal

heterogeneity in order to fill these gaps. I set out to follow up these lab experiments with field observations. I specifically set out to measure spider thermal preferences and determine whether spiders can behaviorally thermoregulate in the field. Additionally, I sought to understand whether there existed any tradeoffs or differences between the sexes regarding the choice of temperature, substrate, and various aspects of behavior.

I first measured thermal preferences in the lab. Next, I measured body temperature in their natural habitat (T_b) to test the hypothesis that *H. clypeatus* are behaviorally thermoregulate. Next, I quantified several aspects of the animal's thermal environment to determine the level of thermal variability. Finally, I measured aspects of substrate usage to assess the interaction between sexual behavior, temperature, and substrate choice.

Materials and Methods

Temperature preference (T_{pref})

To determine *H. clypeatus'* temperature preference (T_{pref}), I conducted a laboratory study in which I exposed animals to a thermal gradient and allowed them to choose where they preferred to settle. See Chapter 2 methods for detailed methods. I used 22 adult females and 18 adult males for this study. I measured spider body temperatures every 10 minutes and used body temperatures after 40 minutes in the analysis. I compared average T_{pref} of males and females using an ANOVA and calculated T_{pref} range as the range between the first and third quartiles of temperatures (Angilletta, 2009b).

Field body temperatures (T_b)

I took temperature measurements of animals in the field using an operative temperature model, following (Kingsolver, Ragland, & Shlichta, 2004). These models consisted of copper shaped and painted (Matte paint, Humbrol Limited, Kingston upon Hull, UK) to thermally mimic the spiders, with an embedded thermocouple wire (40-guage K-type, Omega Engineering, Norwalk, CT, USA). As these measures are proxies for body temperature, I denote these field temperatures as T_b and T_b range for the average and middle 50% quartiles, respectively. See below for details on the sampling scheme. I used a total of 78 individuals in the analyses (N = 24 mature females, 54 mature males).

Field observations

Overall design

To measure how spiders interact with temperatures and substrates in the field, I performed focal observations of individuals in their natural habitat. First, to determine my sampling area, I referred to three years of *H. clypeatus* collection records. Next, I used the QGIS software package (QGIS Development Team, 2019) to draw a polygon around areas of the greatest spider densities. I generated 50 random points (minimum 10m point spacing) within this polygon to be used as locations for my focal observation plots. I spent 9 days during the first two weeks of April 2019 performing these focal observations. Observations were conducted by teams of two observers. On each sampling day, each team selected a point at random from those previously determined above. In the morning, each team arrived at their point and marked out a plot approximately $25m^2$ at the selected point. If the point was found within an area of entirely

unsuitable habitat (e.g., point positioned on cliff edge or in stream), the team placed a plot near their given point or chose another generated point at random.

Plots were randomized throughout the habitat patch to sample across different microhabitat types in which spiders are found. The main goals were to (a) find sufficient numbers of individuals, and (b) sample across the known habitat area. Each team remained at the plot from approximately 9:00 am until 5:00 pm, performing 10-minute observations for each spider found and then collecting the spider.

Whenever a spider was located within a sampling plot, one observer described everything that the spider did for the next ten minutes into a digital voice recorder. This included any substrates on which the spider sat, the animal's position within the substrate (top, side, under), the light conditions (sun, shade, partial shade), and any behaviors that the animal performed (See table 1). I analyzed voice recordings using the BORIS behavior coding program (Friard & Gamba, 2016). Simultaneously, the other observer followed the animal with numbered markers, placing markers at the locations that the spider moved throughout the observation period.

After performing an observation, the spider was collected, and age-sex class (adult female, immature female, mature male, and immature male) was verified. I then preserved the animals in 80% ethanol. Observers also took photographs of the numbered markers (hereafter referred to as "path images"). At this point, I also collected spider body temperatures with the operative temperature model along the spider path. These two measurements were later averaged together.

Habitat temperature measurements

I measured habitat temperature using two different methods. First, I deployed iButtons (Thermochron temperature logger, Maxim Integrated, San Jose, CA, USA) throughout the study area to record air temperatures. I suspended iButtons within opaque plastic cups to shield them from the sun and hung them from trees and cacti at approximately breast height. Second, I took substrate temperature measurements using a thermal camera (model TI30, Fluke Corporation, Everett, WA, USA). Each day, I chose a random exemplar plot of spider habitat (~ 60x60 cm) within the boundaries of one of the focal observation plots. I mounted the thermal camera on a tripod and aimed it at this photo-plot and captured an image every thirty minutes. I monitored a total of 8 plots (one per day) between April 3 and April 12, 2017.

Movement

I calculated the distance that spiders traveled during an observation by measuring distances between markers in the path images. I did this by first spatially calibrating the images using the path markers as an object of known size. For longer paths, I stitched multiple images together using the Inkscape image manipulation program. I calculated distances between markers using ImageJ (Schindelin et al., 2015). I recorded a total of 201 movement paths (37 mature female, 49 immature female, 65 mature male, and 50 immature male).

Substrate usage

I classified substrates into seven different categories: (leaf, rock, stick, dirt, cow pie, grass, and other). I then compared habitat substrate availability to the amount of time spiders spent on each substrate type. To determine substrate availability, I generated 3 random coordinates for each path image (n = 201 path images) and determined substrate type for each random point. I

calculated proportions of each substrate type, pooled across all path images.To determine substrate usage by spiders, I calculated the total amount of time spent on a given substrate for each age-sex class (mature female, mature male, immature female, immature male), divided by the total amount of time spent observing that age-sex class. I then used chi-square tests to compare the distribution of spider substrate usage to substrate availability, and substrate usage by different age-sex classes to each other.

Results

Spider temperatures

In the lab, spiders had strong thermal preferences, with a T_{pref} range (middle 50% of temperatures) of 34.5-40.8 °C, with an average T_{pref} for all spiders at 36.7° (Fig 1a). Males had a significantly higher average T_{pref} than females ($F=6.873$, $p=0.0125$) (Fig. 1b). Spider body temperatures in the field were similar to lab-assessed thermal preferences, with a T_b range of 32.3-41.4°C (Fig. 1c). Although males and females had different average T_{pref} values, male and female body temperatures did not differ in the field (Fig. 1d). In the field, male and female body temperatures did not differ (Fig. 1d). Note that for the purposes of comparing field body temperatures data to the lab thermal preference data., I used only adult individuals in the analysis.

Habitat Temperatures

Three measures of field temperature can be seen in Figure 2. Air temperatures were lower than spider T_{pref} ranges overall (Fig. 2a). Substrate temperatures varied a great deal, both throughout the day and between individual plots (Fig. 2c). Figure 2c shows how spider body temperatures compare with measures of field temperature.

Substrate usage

Distributions of spider substrates across habitats are shown in Figure 3a. Spiders spent time on substrates non-randomly. Each age-sex class used substrates differently from one another and from the null expectation based on the availability of substrates in the habitat ($X^2 = 4054$, $df = 21$, $p < 0.001$) (Fig. 3b).

Field behavior

Mature males traveled significantly farther during the ten-minute observations than any age-sex class (Fig. 5) (sex: $F = 31.02$, $p < 0.001$; age: $F = 33.03$, $p < 0.002$; sex by age interaction: $F=13.51$, $p < 0.001$; Tukey HSD, $p < 0.001$). Females were active significantly earlier than males, irrespective of age class (Fig. 4) ($F=6.973$, $p=0.01$). Males spent significantly more time underneath substrate than females ($F=0.233$, $p=0.04$) (Fig.6).

Discussion

Habitat enables behavioral thermoregulation

In the face of extreme thermally-variability in their habitat, spiders were able to thermoregulate in the field within their preferred range to a remarkable degree (Fig. 1a, 1c). In 5/7 of the image-plots monitored with thermal cameras, temperatures within the spiders' T_{pref} range were attained at some point during the day (Fig 2b). However, air temperatures rarely overlapped with spider

body temperatures at any time of the day. (Fig. 2a). This suggests that substrate temperatures are the most important factor in determining spider body temperatures.

Females lead – males follow

In lab, males prefer temperatures at which mating rates are high (Fig. 1b). These preferred temperatures also correspond to temperatures at which rates and frequencies of male vibratory courtship signals are at their highest (Chapter 1). Females, however, prefer slightly lower temperatures (Fig 1b).

These differences in preferred temperatures between the sexes do not translate to differences in field body temperature (Fig. 2d). One likely explanation is that in this system, males must locate females before they can proceed to courtship displays. *Habronattus* courtship occurs at close range (Elias, Mason, & Hoy, 2004; Elias et al., 2003). However, male jumping spiders in some species use chemicals in female dragline silk to locate and gather information about potential mates (Clark & Jackson, 1995; Hoefler, 2007). I suggest that this is also the case in *H. clypeatus*, as males were often seen tapping their palps (sexual and sensory organs) on the substrate when they were following a female at a distance. Adult males also travel longer distances than any other age-sex class (Fig. 4). Together, this suggests that males are following females throughout the environment. Therefore, females choose the substrates and temperatures at which courtship interactions occur. This presents a tradeoff for males: they can either remain in habitats suitable for courtship but encounter females less frequently or seek and encounter females at higher rates but in conditions less favorable for mating success.

It may be in females' best interests to maintain body temperatures below the males' thermal preferences. High mating rates, such as those seen at high temperatures, may indicate a decrease in female choosiness. This could result in females mating with a broader range of males, rather than only selecting the most attractive ones. By choosing slightly lower body temperatures than those preferred by males, females may therefore be able to better exert choice. This could play out on temporal as well as spatial contexts. For example, females are more active at earlier times of day than males (Fig. 4), when temperatures are lower (Chapter 1).

Although the sexes do not differ with respect to their field body temperatures on average, there is still a great deal of variation between individuals (Fig. 2c). This likely translates to variation in the temperatures at which courtship takes place and could select for males that preferentially locate and court females that are at warmer temperatures. This could potentially lead to males using additional cues, such as substrate type, to determine whether a female is at a "favorable" warm temperature.

Substrate choice is complex

If spiders had no substrate preference, we would expect to find that spiders spend the most time on substrates that are more available in their habitat. However, I found the opposite to be true. Spiders spent the most time on rare substrates and less time on more common ones (Fig. 3). There are a multitude of possible reasons for this. First, desert substrates have previously been shown to differ with respect to their thermal properties (Ahnesjö & Forsman, 2006). As spiders seem to be behaviorally thermoregulating (Fig. 1), it is likely that this plays at least a partial role. However, substrates affect spider behavior in other ways as well.

For *Habronattus*, substrate properties play an important role in how vibratory courtship signals are transmitted from courter to chooser. In a closely-related *Habronattus* species, leaves transmit vibrations more efficiently than other available substrates. This could also partially explain the pattern of males preferring leaves over rocks, whereas females prefer rocks over leaves (Elias et al., 2004).

Substrates also vary in their 3-dimensional structure, which can facilitate behaviors such as hiding. This has been shown in other spiders to lead to tradeoffs involving foraging, competition, and predation risk (Rypstra, Schmidt, Reif, DeVito, & Persons, 2007). In *H. clypeatus*, I find that males prefer to remain under substrate (as opposed to on top) at higher rates than females (Fig. 6). Males also prefer leaves, a complex substrate, over rocks, whereas females prefer rocks over leaves. It is therefore possible that males choose more complex habitat to remain hidden from potential predators. The propensity for males to hide may be the result of an additional habitat choice tradeoff. Mature males travel farther than any other age-sex class, suggesting that they spend a great deal of time mate searching (Fig. 4). This could potentially expose them to risk from visual predators at much higher rates than other age-sex classes. However, if they remain hidden while mate searching, mature males can potentially decrease this risk.

Overall, substrate choice cannot be explained by one or even a few factors. One such factor that we could not assess is mating history. *H. clypeatus* females likely only mate once. This means that the population of mature females that we monitored likely contained unmated and mated (gravid) individuals. Although it was impossible to assess mating status of females in the field, this difference in mating history likely has important implications for substrate choice and thermal biology. For example, mated females may intentionally choose substrates where they are unlikely to encounter males. Further substrate choice experiments will help pinpoint the exact causal factors and tradeoffs associated with substrate choice. However, these data highlight the importance of understanding not only substrate choice, but also the multitude of competing reasons for choosing a given substrate in a given situation.

Conclusions

Temperature is clearly an important aspect of the lives of *H. clypeatus*, and they thermoregulate surprisingly well in their natural environment. However, understanding the specific ways that spiders interact with temperature nuanced and involve the interaction between thermal physiology, sexual selection, and habitat selection. It is likely that spiders experience tradeoffs and potentially sexual conflict as it relates to body temperature and substrate choice. In this way, temperature sets the stage for selection and understanding how animals interact with their environment is critically important. By thermoregulating, animals have some modicum of control over this aspect of their selective environment. Animals that are better able to thermoregulate may be therefore be better able to optimize their behavior better than others and thus would have higher fitness. Additionally, differences between the sexes suggest that there may be conflict over the optimal thermal environments.

My results also underscore the importance of quantifying habitat variability on scales relevant to the animal in question. For example, spiders were observed spending time on the underside on single blades of grass, which experienced different light and temperature conditions than the top side. This level of variability would have been impossible to quantify without detailed observational methods.

Finally, I emphasize the importance of placing the results of controlled experiments in the context of an animal's natural history. Thermal biology must be assessed using a diversity of approaches in order to generate hypotheses and insight that would have been otherwise hidden. Natural history therefore has a powerful and often-overlooked role to play in the study of thermal biology.

Figures

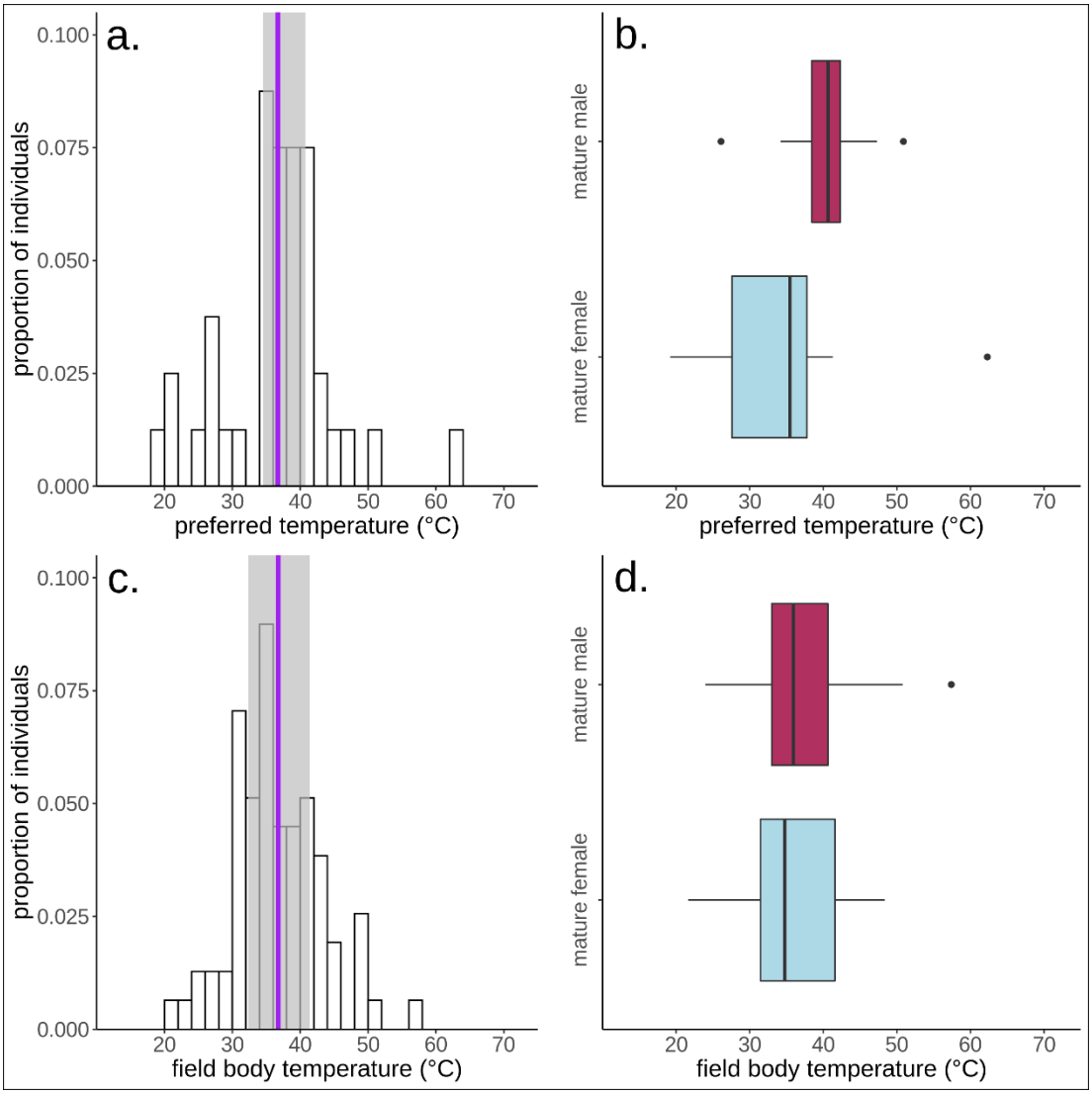

Figure 3-1. Body temperatures of *H. clypeatus* in lab thermal preference gradient and in the field. (a) distribution of spider body temperatures after 40 minutes in thermal gradient. Gray bar indicates 50% preference range (middle two quartiles), and purple bar represents average. (b) Average temperature preferences in males and females. (c) Distribution of spider body temperatures measured in the field. Gray bar indicates 50% preference range (middle two quartiles) and purple bar represents average. (d) Average field body temperatures in males and females.

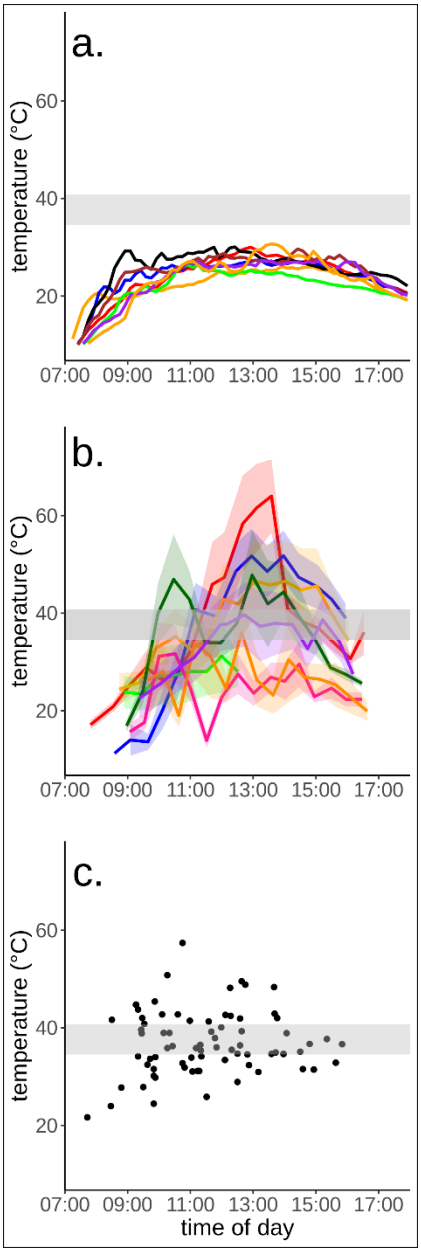

Figure 3-2. Temperatures of spider habitat. Gray bar in all plots represent spider T_{pref} range. (a) Air temperatures throughout the day. Each line represents a different temperature logger in a different location within the broader spider collection area. Temperatures for each logger were averaged over the two-week deployment period. Gray bar represents spider T_{pref} range. (b) Substrate temperatures throughout the day. Each line represents mean and standard deviation of a given thermal imaging plot. Each plot was monitored for one day. Results are compiled here to show only time of day, and not date. (c) Spider body temperatures in the field throughout the day. Different spiders were measured on different days off the 9-day observation period, however they are all presented here to show only time of day, and not date.

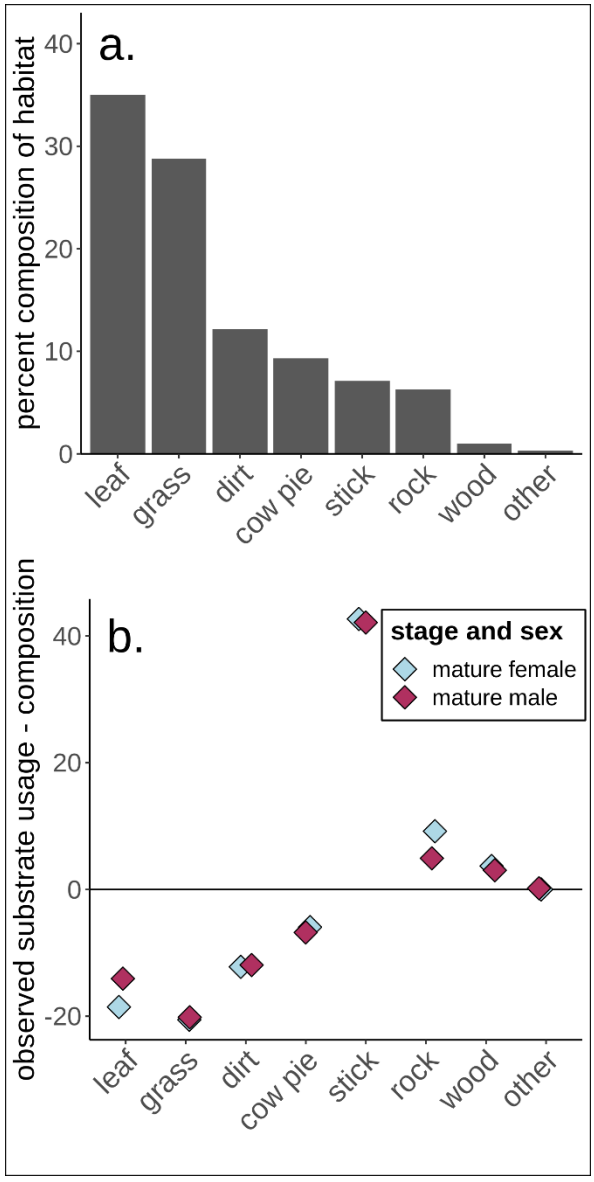

Figure 3-3. Spider substrate usage. (a) Substrate availability, ordered from most to least-common substrates. Results are summed across all locations at which spiders were observed. (b) Spider substrate preference. Substrate usage was calculated by summing the total amount of time spent on each substrate by a given age-sex class by the total time spent observing that age-sex class. The observed habitat distribution in (a) was then subtracted from this observed substrate usage. Points above the line indicate that animals preferred substrates more than expected, whereas points below the line indicate less preference than expected. Each age-sex class differed significantly from all others, as well as the distribution of habitat availability.

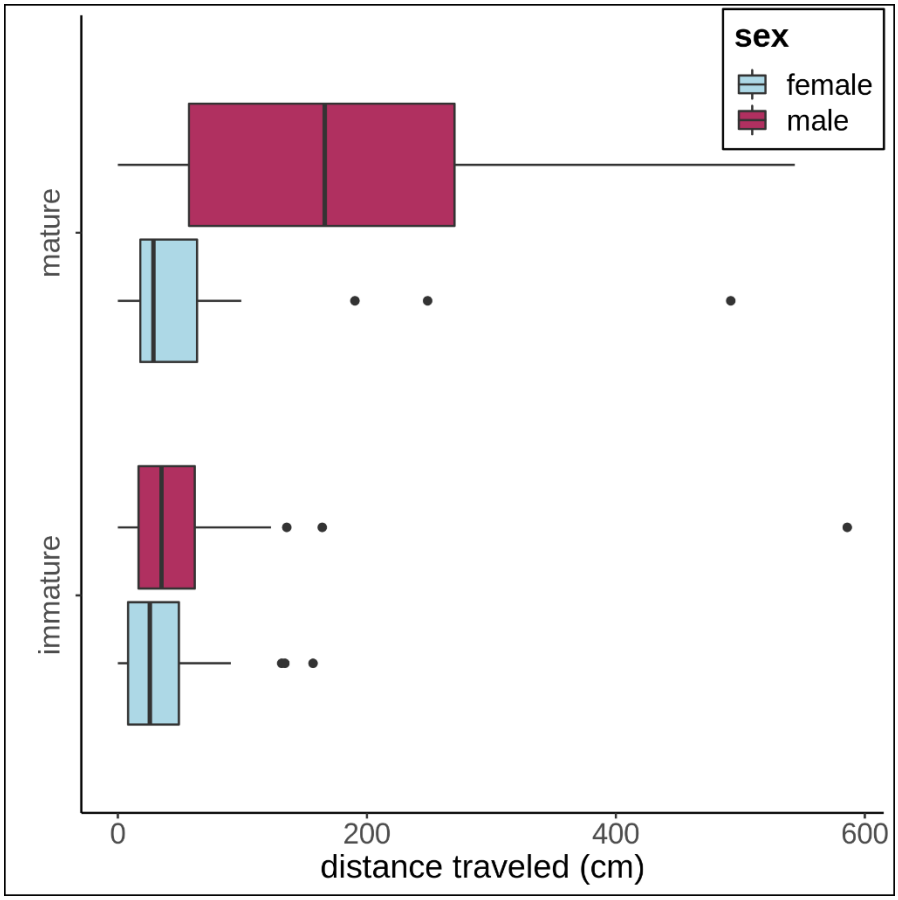

Figure 3-4. Average distance traversed by spiders during the 10-minute observation periods, divided by age-sex class. Adult males traveled significantly farther than immature males and females of any age.

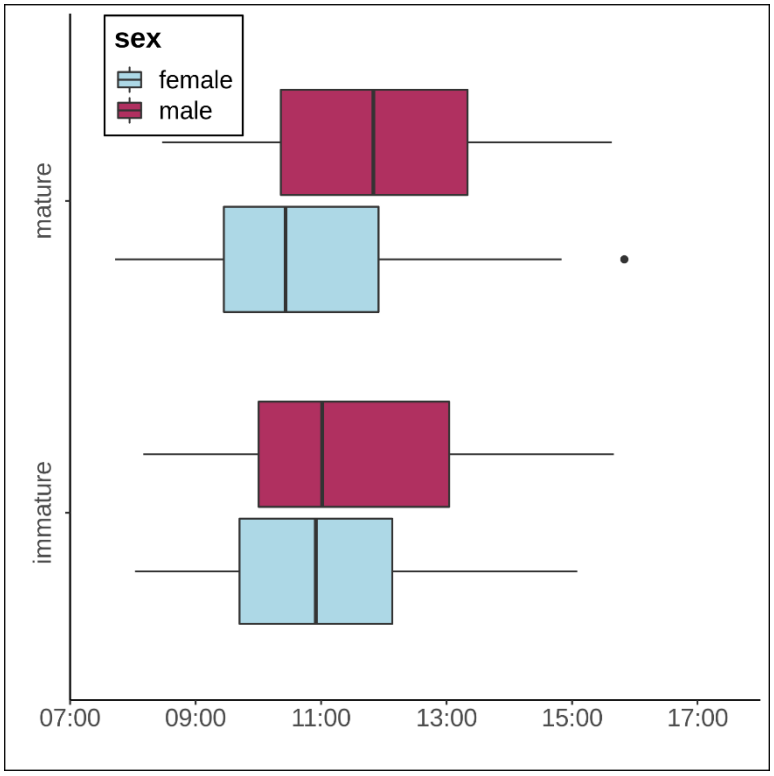

Figure 3-5. Time of day at which spiders were observed, separated by age-sex class. Females were active significantly earlier than males on average, however there were no differences between age class.

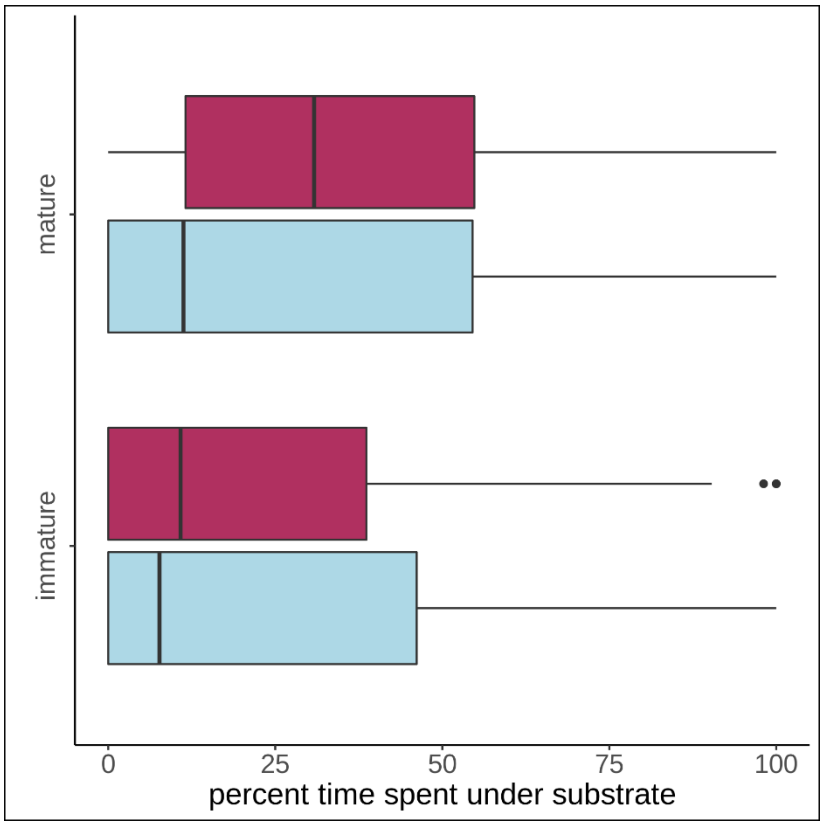

Figure 3-6. Average amount of time spent underneath substrate, separated by age-sex class. Mature males spent more time under substrate than immature males, and females of any age.

Tables

Table 3-1. Ethogram of focal observations

category	options	description
substrate		
	leaf litter	material on ground composed primarily of leaves, either dry or fresh
	dirt	soil composed of organic matter and/or sand
	rock	stone larger than about 2x size of the spider
	cow pie	dry or fresh cow cattle feces
	grass	dry or fresh grass
	other	item not fitting into the other categories, typically manmade, such as observer's clothing or equipment
	wood	stick of diameter 2x or more of the spider's bodylength
light		
	sun	full direct sunlight
	partial shade	light conditions between full sun and full shade
	shade	completely shaded
behavior		
	jump	all of the spider's legs leave the ground and the spider is propelled away from starting position
	walk	spider moves > 1 bodylength away from starting position
	eat	spider consumes prey item
	groom	spider strokes itself using pedipalps or scratches itself with its legs
	turn	spider rotates in place or moves < 1 bodylength from starting position

Concluding Remarks

Thermal biology is not a particularly young field. Biologists have long recognized the importance of temperature in a variety of biological processes and a great deal of work has been done to generate knowledge on this topic. Recently, this has become more pressing with the understanding that temperatures in all habitats will likely undergo substantial changes due to anthropogenic causes. This has resulted in a redoubled flurry of effort seeking to understand in more detail how temperature will change species distributions and the ability for organisms to survive in human-altered climates. This change is occurring rapidly – studies have noted changes in species distributions, thermal tolerances, and other temperature-related affects within human lifetimes. These studies can at times not be completed quickly enough – species are disappearing at an alarming rate, often exceeding the rate that studies can be conducted, published, and disseminated to those who can affect change. As thermal biologists adapt to this new breakneck pace, it will be increasingly important to leverage diversity in a variety of ways. Specifically, we must seek to be more diverse in terms of organisms studied and the biologists who study them. In doing so, we will be able to produce meaningful and timely data that inform how animals will respond to changing climates.

First, we must embrace diversity of study systems in thermal biology. As mentioned in the introduction, size is a key aspect that can completely change how animals respond to temperature. The easiest way to sample across size ranges is to sample across taxa. Also, even within a given size range, animals possess an extreme diversity in terms of their thermal tolerance and thermal sensitivity in many axes. Sampling diverse taxa within a given environment can also give an overall impression of how species compositions will change over time. Diversity in environments cannot be ignored either. Studies comparing the thermal biology of temperature versus tropical animals have yielded surprising revelations about key differences between them. Habitats vary with respect to their thermal variability. Understanding how animals partition thermally-variable habitats can result in important insight into species' latent plasticity and their resilience to change.

Human diversity is another and, I would argue, more important aspect of propelling thermal biology studies into the future. First, temperature has broad, multifaceted effects on animals. In order to understand big questions such as, "Will this species survive a 5° C shift in temperature?", one must look at the animal's thermal sensitivity in physiology, behavior, and ecology, just to name a few aspects. No single biologist, working in isolation, could possibly possess the necessary expertise to examine this in its totality. Multidisciplinary teams of thermal biologists will need to assemble in order to tackle these questions. One subfield of biology that is particularly lacking in many of the studies to date is natural history. We must study animals in their natural environments and on spatial and temporal scales relevant to them. This is hard work, but the insight and hypotheses generated through watching an animal in its natural habitat cannot be gained in any other way.

I argue that the future of the field of thermal biology is bright – and complicated. On the one hand, new study systems, methodologies and collaborations have the potential to produce exciting breakthroughs in the field. On the other hand, we often find ourselves documenting truly disturbing climate trends, and watch populations and species slip between our fingers before we get to know them. The one shining light in the sometimes-depressing reality of thermal biology is that we do not need to wrestle with these issues on our own. It is our responsibility to

recognize the social and cultural nature of science and make room for social scientists, policy makers, and other stakeholders, including those with embedded and indigenous knowledges. Regardless of how "small" our individual subfields might seem, we can (and must) tap into broader scientific and global communities to deal with issues of climate and biodiversity. This is the only way that our work will become meaningful in the context of preserving the animals, habitats, and biomes for which we also inevitably gain a deep abiding affection.

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Appendix. Protocols for thermal biology experiments

Thermal Limits ($CT_{min/max}$)

Rationale

This protocol is used to determine the critical thermal minima (CT_{min}) and maxima (CT_{max}) in a group of desert-dwelling jumping spiders. This method involves continuously increasing or decreasing the temperature of animals until they could no longer right themselves. This is a ramping assay, with ramp rates comparable to similar studies conducted in other terrestrial arthropods (Birkett, Blackburn, & Menendez, 2018; Slatyer et al., 2016). Temperature is recorded continuously, and a voice recorder is used to note when spiders are unable to right themselves. The timing is matched to the temperature data later, using BORIS and a custom Python program.

Supplies

- Panasonic MIR-154-PA incubator
- "Spidey-flip" device consisting of 4 plastic chambers with thermocouple leads taped to a wooden stick extending from the side of the incubator
- Lid and rubber band for each chamber
- Temperature recording device that outputs to computer
- Ethanol + paper towel for cleaning chambers between trials
- Incubator door shim (for cold treatment)
- Data sheet + pen
- Voice recorder
- PLW Recorder software and Windows machine

Method

1. Select 4 spiders of the same sex and species to be used. Make sure:
    a. They have been maintained at lab temperature (other than in trials) for at least 2 weeks
    b. They have not eaten less than 48 hours previously
    c. They have not been run in a temperature trial less than 48 hours previously
    d. If you are running a hot treatment, make sure the spiders have already been run in the cold treatment
2. On data sheet, fill out spider numbers and other pertinent data for the trial
3. Place magnetic door shim over button at the top of the door to keep the button pressed (convinces incubator that door is always closed)
4. Set incubator to start temperature (15 for cold treatment, 40 for hot treatment)
5. Moisten paper towel with ethanol and wipe chambers and chamber lids. Be careful not to dislodge the tape holding the incubators to the chambers.
6. Replace any tape if needed
7. Place one spider in each chamber and secure the lid with a rubber band
8. Start computer and plug in temperature logger
9. Launch PLW Recorder
10. NOTE: If PLW recorder ever disappears, don't panic! It is still running. Go down to taskbar (near the clock) and double-click the PLW icon there to bring it back up.
11. In PLW Recorder, go to "File -> new data"
12. Name the new data file after the page and trial number, eg: page 5, trial 1, would be called "5-1.PLW"

13. Do not yet begin collecting data, but monitor temperature of chambers until they all read within 2 degrees of the start temperature
14. If it takes a long time for the start temperature to be reached, you can change the temperature on the incubator to slightly over or under-shoot the temp. Eg: for cold, change it to 13 degrees, for hot, change it to 43 degrees. Make sure to change the temp back to the original starting temp when you begin the acclimation period.
15. When start temperature +/- 2 deg is attained, simultaneously press the "record" button on PLW recorder and the "record" button on the voice recorder
16. On the voice recorder, say the following information: trial number, date, spider species and sex, temperature treatment.
17. Set a timer for 10 minutes and allow the spiders to sit at this temperature. Leave the voice recorder running
18. When 10 minutes have passed, set the incubator to the "goal" temperature (0 for cold, 60 for hot). If you are doing a cold treatment, use the pushpin box to shim open the door.
19. After you set the temperature, flip the spiders to make sure that no one was knocked out during the acclimation period. To flip:
    a. Say the word "flip".
    b. Remove the sponge from the hole in the side of the incubator
    c. Rotate the wooden handle of the "spidey flip" 180 degrees and shake it, to make sure that the spiders all flip onto the lid of the chamber. Make sure that they are not attached to the chamber with silk.
    d. Rotate the handle back the same way you originally rotated it. This will keep the thermocouple wires from becoming wrapped around the handle.
    e. Carefully check each spider to see if it is on its back or if it is upright, but its legs are curled underneath. If either of these conditions are present, the spider is "down". Otherwise, the spider is "up". Try not to say "out" instead of down. "out" sounds a lot like "up", in the recordings.
    f. Announce how many spiders are down or up, "all up", "1 and 2 are up, 3 and 4 are down", "all down"
    g. If it is difficult to tell if a spider is up or down, you can flip once or twice more to see if they right themselves quickly.
20. Set the timer for 2 minutes
21. At the end of 2 minutes, reset the timer, flip and check the spiders
22. Once a spider is down, watch it carefully to see if it comes back up. Announce when/if it comes up.
23. Once the first spider goes down in the hot treatment, change the timer to 1-minute intervals.
24. Repeat steps 18-20 until all spiders have been down for 2 minutes (cold) or 20 seconds (hot)
25. Announce the end of the trial, repeating all information you gave at the beginning
26. Stop the voice recorder and the temperature data logger
27. Reset the incubator to either the "start" temperature (if you are running more trials) or to 25 degrees (if you are done for the day)
28. Remove spiders from chambers and place them back in their vials
29. Weigh all spiders and fill out their masses on the data sheet

Analysis

Supplies

- Audio recordings from trials
- BORIS software and computer
- Data table from PLW Recorder

Method

1. Prepare temperature log files
    a. In PLW Player, open the .PLW file
    b. Click the "show spreadsheet" button on the right-hand side of the player
    c. Check the time to make sure it's in terms of time elapsed, not samples (seconds). If it is in seconds, click the checkmark and select "time since start", and then "OK"
    d. Click the "Select" button to highlight the entire table
    e. Click the "write to disk" button on the lefthand side
    f. Save the file as a .txt
    g. Open the .txt file in Excel
    h. Delete rows 2 and 3
    i. Save the file as a .csv
2. Code audio recordings
    a. Transfer audio recordings from recorder to folder on computer
    b. Rename audio recordings to match trial number (1-1, 1-2, etc.)
    c. Open BORIS
    d. Open the (xxx) project
    e. Go to observation -> new observation
    f. Name the observation after the audio recording number
    g. Add the appropriate audio recording to the observation
    h. Begin the recording:
    i. There are only two behaviors that need to be coded: "D" for down, and "F" for flip. When the recording announces "flip", make sure the "N" (none) subject is selected, and then type "F". When the numbers of spiders that are down is announced, stop the recording, then go back to the flip. Select the relevant subject (spider 1, 2, 3 or 4) and mark it as down. For example, a recording says: "Flip", and then 45 seconds later, "1, 2, and 4 are down, 3 is up". Code the flip exactly when it happens. After you find out who was down at the flip, go back in time to where the flip happened and set 1, 2, and 4 as "down". Continue listening to the recording and make note of whether spiders come back up. When this happens, type "D" again with the appropriate subject selected. At the end of the recording, select each of the subjects and type "D" again as if they all came up. If you don't do this, BORIS will not display the graph properly.

3. Determine temperatures
    a. In BORIS, go to analyze -> plot events, and select the appropriate observation
    b. You should see a plot with 5 rows: one for each of the individuals, plus a row at the bottom for the "none" individual with red triangles representing the flips. Time is on the x axis.
    c. In Excel or another spreadsheet program, open the appropriate temperature file
    d. Open the "CTminmax" Google sheet and enter all of the information for the individuals in the trial that you're working on.
    e. To figure out the temperature at which the spider went down, we actually need to record two separate temperatures: the temperature at which the spider was visibly down (at the "flip"), and the last known timepoint at which the spider was definitely up (the previous "flip"). Since we assume that the spider actually went down somewhere between these two points, we average these two temperatures.
    f. For individual 1, find the first place that it goes down (beginning of the first blue bar). Move your cursor to the very beginning of this bar and note the time. This is KO1(time)1. This means that it's the first time the spider was knocked out "KO1", the time listing (not

the temperature), and this is timepoint 1. To get timepoint zero “KO1(time)0”, find the timepoint of the previous flip, where the spider was still up.

g. With the timepoints entered, go to the temperature data sheet and find those time points for that individual. Enter those in “KO1(temp)0” and “KO1(temp)1” respectively.
h. Repeat f and g for individuals 2, 3, and 4.
i. For each individual, you will need to find three total timepoints/temperatures: first knockdown (as described above), when the spider goes down for 20 seconds, and when the spider goes down for 2 minutes. They are not necessarily mutually exclusive. Sometimes a spider goes down and stays down for the entire trial. Other times, the spider will get up several times.

Thermal Preference

Rationale

This experiment is conducted to determine whether spiders have thermal preferences. It seeks to answer the question, "Do spiders have a specific temperature that they prefer to be at?" The method involves a thermal gradient, which is a device that is very hot at one end and cool at the other. To achieve heat, we are using two different heat sources: a series of silicone heaters attached to a heat sink, and ceramic heat bulbs. To achieve a cold temperature at the other end, the entire apparatus is placed in a cold room (~12℃). The spiders are placed in the thermal gradient device and their body temperature is measured at 10-minute intervals to give them time to move to their preferred temperature.

Supplies

- Thermal gradient setup
    - Put the following on the cart, which holds the setup:
        - Power strip
        - IR thermometer
        - Bookshelf
        - Paper covering for bookshelf
        - Heat sink with silicone heaters attached
            - Temperature controller on bottom shelf of cart
            - Thermocouple attached to heat sink and temperature controller
            - Aluminum foil to cover the back, sides, and top of heat sink
        - Metal slats to create lanes for spiders
        - Clear acrylic end piece to hold metal slats
        - Ceramic heating bulbs (2-3) attached to cart
- Light stands with fluorescent bulbs and diffusers attached
- 6 spiders per trial
- Styrofoam cooler to hold spiders until use
- Note-taking sheet: "Thermal Preference Data Sheet"

Method

1. Prepare the setup:
    a. Remove old paper from the shelf
    b. Wrap one side of the shelf with craft paper. Wrap it around to the other side and tape it in place. Make the paper as tight as possible, so it doesn't bulge out around the ends.
    c. On the papered side of the bookshelf, draw a line going across it every 10 cm. There should be a total of 5 lines.
    d. Wipe the metal slats and acrylic end piece with ethanol
    e. Place the bookshelf on the cart, close to one end but not touching it.
    f. Place the heat sink on the end of the shelf closest to the edge of the cart. Do not let the heat sink touch the cart.
    g. Attach the end of the thermocouple to the heat sink with tape. Attach it between two of the fins on the front. Make sure the very tip of the wire is touching the heat sink. IMPORTANT: make sure the thermocouple wire never becomes detached from the heat sink.
    h. Plug the other end of the thermocouple into the temperature controller
    i. Plug the three heating element cords into the back of the temperature controller.
    j. Make sure the temperature controller is switched off (switch in the down position)

    k. Slide the slats between the fins of the heat sink. There should be three "spaces" between each slat
    l. Slide the end piece over the slats, pushing it flush against the end of the bookshelf (the opposite side of the heat sink). Place the two wooden dowels so as to prevent the end piece from moving.
    m. Clip the heating bulbs onto the same end of the cart as the heat sink. Space them as evenly across the lanes as possible.
2. Select a random number between 1 and 2 (you can use a coin). This will be the direction that you face the cart in the cold room
3. Set up the light stands. They should be set up next to the cart on either end. Plug the lamps into the power strip on the cart.
4. Select 6 spiders to use in the trial (3 males, 3 females)
    a. Weigh all 6 spiders
    b. Place the spiders into a square container, labeled with their name, this label should be transferred to their lane during the trial.
5. Bring the setup to the cold room and orient it according to the random number you chose. NOTE: do not bring the spiders into the cold room until you are ready to begin the experiment (after the thermal equilibration period).
6. Loosely cover the back, sides, and top of the heat sink with aluminum foil, shiny side facing in.
7. Plug the heat lamp bulbs directly into the power strip on the cart. They will immediately begin to become very hot.
8. Plug the temperature controller into the power strip on the cart.
9. Turn on the temperature controller. Push the button with the circular arrows until you see "SP" in the bottom corner of the display. Once you see this, adjust the temperature with the up and down arrow buttons until it reaches the desired temperature. I have been setting it to **200**, but this may need to be adjusted. Once it is set, press the circular arrow button again repeatedly until you don't see any letters on the display. This is the temperature that the thermocouple is currently reading. Keep in mind that the temperature controller is in Farenheit, but everything else you will do will be in Celsius. After this point, be careful not to touch the heat sink. It will become very hot quickly.
10. Let the entire setup thermally equlibrate for **about a half hour**.
11. After the equilibration period, check with an IR thermometer that the lanes go from at least **~50℃** at the heat sink end to **~17℃** at the far end.
12. Bring the spiders into the cold room, bring them into the room in the cooler, and keep them in the cooler with the lid on until you physically place them into the setup. They should not be exposed to the temperature in the cold room beforehand.
13. To place each spider in a lane:
    a. The lane number on the data sheet is the lane each the spider will use. If you are standing behind the end piece, lanes go 1- 6 from your left to your right.
    b. Use a random number generator to determine which position each spider will be placed at. These numbers correspond to the numbers you drew on the paper before. Position 1 is the area between the heat sink and the first line, etc.
    c. Note the ID and initial position (P_0) in your notes
14. Set a timer for **10 minutes**
15. Carefully monitor the position of the spiders so that they do not escape. Use the paint brush to knock them off of the metal slats if they climb up them.
16. At 10 minutes, measure the temperature with the IR thermometer off of the body of each spider. Mark this as T_{10}
17. Set the timer for 10 minutes again, repeat steps 16 and 17.
18. Set the timer for 10 minutes again, repeat steps 16 and 17.

19. This protocol and the notes sheet allows for **4 time measurements**, at 10, 20, 30, and 40 minutes. You may decide to do more or fewer sampling periods.
20. Use the comments section of the notes to include any additional pertinent notes

Analysis

There is no special analysis for this experiment. The last temperature measurement for each individual is the one that will be used in analyses.

Important safety notes:

1. The heat sink and heating bulbs will be VERY HOT. Avoid touching them if at all possible. If you absolutely must move them, use pot holders or another barrier to protect your hands
2. Always make sure the silicone heaters are plugged into the temperature controller, NOT directly into an outlet.
3. Always make sure the thermocouple wire is attached to the heat sink
4. If the silicone heaters get too hot, they may start to produce smoke. If this happens, immediately turn of the temperature controller and let everything completely cool before investigating and using it again.

Stop-Flow Respirometry

Rationale

This protocol describes an experiment that will measure the amount of carbon dioxide produced by spiders at different temperatures.

Supplies needed

- Live animals to be measured
- 10 mL glass gas-tight syringes to incubate animals (Hamilton 81601)
- Racks for syringes
- Expedata software (Available for Windows only)
- Respirometer with scrubber
- Incubator for each simultaneous temperature treatment
- Sensitive balance
- 1 small piece of mesh per animal

Method

1. Before running trials
2. At least 1 hour before running trials, set the incubator to the desired temperature
3. Determine how many syringes you will need, plan on one per spider plus a blank for each temperature treatment.
4. Remove the plunger
5. Load each syringe (including the blanks) with a piece of mesh
6. Transfer a spider into each syringe except for the blanks
7. Replace the plunger, setting doesn't matter
8. Incubate syringes for ½ hour
9. Prepare Respirometer
10. Make sure rig is turned on and set to a flow rate of ~101 mL/min
11. Make sure the scrubber is bypassed. This means that the valves on the tube coming from the middle of the machine are set to the middle (Figure 1)
12. Purge syringes. To do this:
    a. Remove plunger from syringe
    b. Place tip of syringe into valve on right side of rig labeled "purge" (Figure 2)
    c. Open the valve so that air passes through the syringe. Allow approximately 30 seconds to pass.
    d. With the syringe still inserted, *carefully* begin to replace the plunger into the syringe. As soon as it is almost fully inserted, close the valve and remove the syringe in one smooth motion. It is very important not to apply too much back-pressure to the respirometer, or it will cause problems with the sensor.
    e. Press the plunger until it reads 5 mL of volume
    f. Place cap on end of syringe
13. Turn off scrubber
14. Write down the time (This is T_0 for the trial)
15. Put syringes back into incubators (write down the time that you do this.)
16. Incubate syringes for 2 hours
17. Prepare Expedata for measurement
18. Start about 15 minutes before the 2 hours are up
19. Turn on the scrubber, then let it equilibrate for a few minutes
20. Launch Expedata

21. Go to acquire -> setup data acquisition
22. Select use the UI2 -> connected -> ok
23. Setup -> select file, "beetle stop flow 4-14-16"
24. Change axes to max of 50 (you can't do this after the recording has started)
25. Select "record"
26. Allow recording to go for about 10 minutes (at least 600 samples)
27. Inject samples
    a. Remove syringe cap
    b. Insert syringe into port labeled "inject" also on right side of setup (Figure 3)
    c. Open the valve, and quickly and smoothly press the plunger until 2 mL of volume is left, injecting a total of 3 mL of gas. Be careful not to crush the spider in the syringe.
    d. Write down the time that you injected the sample. This is your T_1
    e. Wait 2 minutes for Expedata to re-stabilize
28. Inject all other syringes in turn, 2 minutes apart with the blank at the end
29. Let the recording run for at least 10 minutes at the end.
30. Stop recording
31. Save file in appropriate folder. Make sure to be consistent with naming files
32. Using a high-sensitivity balance, weigh each spider and note down the weight of each in mg.

Important Notes

- The syringes are *very* fragile and expensive. Some tips to keep them intact:
    - Be very mindful when handling syringes.
    - Do not set them down on hard surfaces.
    - Do not handle them over a hard surface unless absolutely necessary.
    - Put down a padded mat on the desk if you are worried about them slipping out of your hand.
    - The most common place for the syringes to break is the lip at the end (where you hold it when injecting). Avoid putting the syringes in a rack where the lip rests on the rack.
    - When injecting the syringe, be careful to avoid putting any torque on the syringe. The tip can easily break off.
    - If you do not wash the syringes between each trial, designate "male" and "female" syringes. If males are placed into a syringe where a female was previously present, he will perform courtship behavior and substantially increase his metabolic rate.
- Syringes do not need to be washed frequently, but any waste left by animals does need to be cleaned out periodically. To wash syringes:
    1. Wear gloves that give you additional grip -- syringes get very slippery while cleaning
    2. Remove the plungers and set aside
    3. Remove any stickers from syringes
    4. Fill one container (bowl, or shoebox-sized plastic container) with hot water and a small amount of dish soap. Fill another with hot water only.
    5. Add syringes to container, and let sit for a few minutes
    6. Scrub syringes inside and out with a bottle brush
    7. Transfer syringes to rinse container
    8. Dip and briefly swish plungers into wash water, then transfer to rinse water. Scrub if needed.
    9. Transfer syringes to drying oven set to ~ 100°C for a few hours
    10. Turn off oven, and let syringes cool slowly before handling
- Make sure that each animal has not been fed more than 48 hours prior to trial being run.

- If an animal has been subjected to a temperature trial within 24 hours, do not subject it to a temperature greater than 5 degrees different from yesterday's trial. 48 hours between trials is best.