

# Temperature-related breakdowns in the coordination of mating in *Enchenopa binotata* treehoppers (Hemiptera: Membracidae)

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## Abstract

Temperature influences the expression of a wide range of behavioral traits in ectotherms, including many involved in the initiation of pair formation and mating. Although opportunities to mate are thought to be greatest when male and female activity overlap, sex-specific behaviors and physiology could result in mismatched thermal optima for male and female courtship. Here, we investigate how conflicts in the thermal sensitivity of male and female courtship activity affect patterns of mating across temperatures in *Enchenopa binotata* treehoppers (Hemiptera: Membracidae). These plant-feeding insects coordinate mating with plant-borne vibrational signals exchanged in male–female duets prior to pair formation. We manipulated temperature across an ecologically relevant range (18–36°C) and tested the likelihood of individual male and female *E. binotata* to engage in courtship activity using vibrational playbacks. We then staged male–female mating interactions across the same temperature range and quantified the thermal sensitivity of mating-related behaviors across stages of mating. Specifically, we measured the timing of duetting, the likelihood for key pre-copulatory behaviors to occur, whether the pair mated, and copulation duration. We found sex-specific thermal sensitivity in courtship activity: Males showed a clear peak of activity at intermediate temperatures (27–30°C), while females showed highest activity at the hotter thermal extreme. Mating rates, courtship duets, and copulatory attempts were less likely to occur at thermal extremes. Also, duetting occurred earlier and copulation was shortest at higher temperatures. Overall, our data suggest that sexes differ in how temperature affects mating-related activity and some processes involved in mate coordination may be more sensitive than others across variable thermal environments.

## KEYWORDS

mating behavior, plasticity, sex-specific responses, thermal sensitivity, vibrational communication

## 1 | INTRODUCTION

Temperature plays an essential role in biological functions across multiple levels of organization, from molecular interactions to whole-organism performance (Angilletta et al., 2006). As a result, organismal functions typically peak at intermediate temperatures

and are inhibited at thermal extremes (Angilletta, Niewiarowski, & Navas, 2002; Clusella-Trullas, Blackburn, & Chown, 2011; Huey & Stevenson, 1979; Pörtner, 2002a). While some animals can mitigate these thermal constraints through physiological adaptation and behavioral adjustment (Tattersall et al., 2012; Terrien, Perret, & Aujard, 2011), many ectotherms have limited ability to regulate

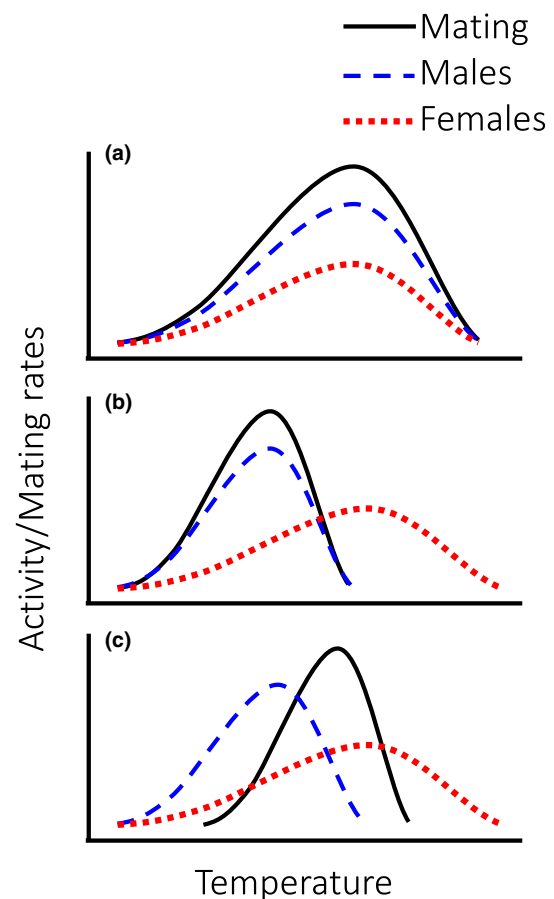
body temperature independently from environmental conditions (e.g., Caillon, Suppo, Casas, Arthur Woods, & Pincebourde, 2014). As a result, relative fitness in ectotherms often varies with temperature (Birch, 1953; Huey & Berrigan, 2001; Kingsolver, 2009; Kingsolver & Huey, 2008; Magnuson, Crowder, & Medvick, 1979). Studies of the effects of temperature on fitness-related traits have primarily focused on physiological traits like body size and metabolic rate (Angilletta et al., 2002; Angilletta, Steury, & Sears, 2004; Bowler & Terblanche, 2008; Gillooly, Brown, West, Savage, & Charnov, 2001; Irlich, Terblanche, Blackburn, & Chown, 2009; Kingsolver & Huey, 2008), or life history and ontogeny (Angilletta et al., 2004; Both et al., 2004; Gotoh, Yamaguchi, & Mori, 2004; Green & Fisher, 2004; Parmesan, 2007; Régnière, Powell, Bentz, & Nealis, 2012). However, a variety of behavioral traits are also affected by temperature with important consequences for fitness (Abram, Boivin, Moiroux, & Brodeur, 2017; Gunderson & Leal, 2015; Sinclair, Williams, & Terblanche, 2012).

Many studies that describe behavioral responses to temperature focus on performance traits like sprint speed and locomotion (Bennett, 1980, 1990; Cullum, 1998; Green & Fisher, 2004; Hertz, Huey, & Nevo, 1983; Lailvaux, Alexander, & Whiting, 2003); in contrast, the thermal sensitivity of mating-related traits has received less attention (Andrew et al., 2013; but see Brandt, Kelley, & Elias, 2018; Rosenthal & Elias, 2019; Macchiano, Sasson, Leith, & Fowler-Finn, 2019). Because reproductive behaviors are closely tied to Darwinian fitness, understanding how temperature affects mating-related behaviors is central to understanding the fitness effects of thermal variation (Sinclair et al., 2012; Visser, 2008). Many ectotherms mate within specific thermal windows because physiological constraints can limit mating-related behavior to intermediate temperatures (Huey & Stevenson, 1979). For example, in sexually reproducing species, temperature-related constraints on activity levels, mate localization, and copulatory behaviors could all influence the coordination of mating (Brandt et al., 2018; Candolin, 2019; Flanagan & Bevier, 2014; Macchiano et al. 2019) with potentially important consequences for reproduction in a thermally variable environment.

Mating necessarily involves the coordination of male and female pre-copulatory activity. Thus, the likelihood to mate may be greatest when patterns of male and female courtship activity overlap. When the thermal sensitivity of courtship activity in males and females are similar, patterns of mating across temperatures are likely to align with the activity levels of both sexes. However, sex-specific thermal responses (e.g., Kvarnemo, 1994; Cullum, 1998; Lailvaux et al., 2003; Darnell, Fowler, & Munguia, 2013; Condon et al., 2015; Brandt et al., 2018; Macchiano et al., 2019) stemming from differences in behavior and physiology—for example, differences in body size, metabolic costs, and extra heat produced by mating-related activity (Beaupre & Duvall, 1998; Block, 1994; Brown & Weatherhead, 2000; Cullum, 1998; Kingsolver & Huey, 2008)—may generate mismatches in the timing of when males and females are most actively seeking or receptive to potential mates. When mismatches between male and female responses to temperature occur, the activity patterns of one sex could disproportionately constrain mating rates or the activity

patterns of both sexes may interact to determine patterns of mating across temperatures.

Here, we test three alternative hypotheses about how thermal sensitivity in pre-copulatory courtship behavior of males and females generates patterns of mating activity across a range of temperatures. The first hypothesis is that patterns of mating activity across temperatures arise from similar thermal constraints on males and females; this hypothesis predicts that activity levels across temperatures are similar for male courtship, female courtship, and mating (Figure 1a). The second hypothesis is that patterns of mating activity across temperatures are primarily determined from constraints on one of the sexes; this hypothesis predicts that patterns of activity across temperatures differ between male courtship and female courtship, and patterns of mating more closely match courtship activity for only one of the sexes (Figure 1b). The third hypothesis is that patterns of mating activity across temperatures emerge from a combination of constraints on male and female activity; it predicts that patterns of activity across temperatures differ between male courtship and female



**FIGURE 1** Graphical predictions of three hypotheses regarding how mating rates emerge from thermal constraints on the courtship activity of males and females: (a) Mating rates result from similar thermal constraints on both sexes; (b) mating rates are primarily constrained by the activity of one sex when temperature affects male and female activity differently; and (c) mating rates arise from a combination of different thermal constraints in each sex

courtship and that patterns of mating do not match courtship activity in either sex but are highest at temperatures where male and female activity overlaps the most (Figure 1c).

In addition to testing the three above hypotheses, we also investigate whether thermal sensitivity varies across pre-copulatory and copulatory behaviors. In particular, we aim to determine if greater thermal constraints during some stages of mating may indicate that different behavioral processes contribute to realized rates of mating across temperatures. We focus on the success and timing of each stage of a mating interaction, from pre-copulatory courtship and copulation attempts to successful mating and duration of copulation. Thermal sensitivity in the latency to initiate courtship may indicate that temperature affects processes that occur before either sex initiates a mating interaction. Thermal sensitivity in the latency to copulate after a male or female initiates a mating interaction may indicate that temperature affects processes facilitating pair formation. These processes can encompass time spent searching for mates or search efficiency (Groot, Čokl, & Virant-Doberlet, 2011; Legendre, Marting, & Coccoft, 2012) including temperature-related attenuations in muscle function during or precluding copulatory attempts by males (Bennett, 1984; Pörtner, 2002b). Finally, copulation duration is known to be thermally sensitive in a variety of arthropods (Costa & Sotelo, 1984; Katsuki & Miyatake, 2009; Michiels, 1992; Yenissetti, Hegde, Venkateswarlu, & Krishna, 2006). Changes in copulation length could further impact the outcome of mating in terms of sperm transfer or fertilization success (Andrés & Cordero Rivera, 2000; Dickinson, 1986; Katsuki & Miyatake, 2009; Laird, Gwynne, & Andrade, 2004; Michiels, 1992; Sato, Yoshida, & Kasugai, 2017; Snow & Andrade, 2004; Yamagishi & Tsubaki, 1990).

Here, we study the thermal sensitivity of mating behaviors in *Enchenopa binotata* treehoppers (Hemiptera: Membracidae). The *E. binotata* species complex is a group of host-plant-specific treehopper species (Coccoft, Rodríguez, & Hunt, 2008, 2010; Rodríguez, Sullivan, & Coccoft, 2004; Wood, 1993) that communicate with plant-borne vibrational signals (Coccoft, Rodríguez, & Hunt, 2008; Hunt, 1994; Rodríguez et al., 2004; Sattman & Coccoft, 2003). Mating in *E. binotata* culminates from many behavioral components. Reproductively active males fly from stem to stem and produce advertisement signals to nearby females when they land (Coccoft et al., 2008; Hunt, 1994). Females generally stay in a fixed position on a plant (Hunt, 1994) and emit a reciprocal vibrational response signal if they find the male signal attractive (Rodríguez & Coccoft, 2006; Rodríguez et al., 2004). Males and females produce one or more of these duets composed of a series of male signals and female responses (Coccoft et al., 2008; Hunt, 1994) during which males assess the direction of female responses to aide in locating the female (Gibson & Coccoft, 2018). After mate localization, the male mounts the lateral side of the female while facing the same direction as the female; during mounting, males and females exchange vibrational signals for a few seconds to hours before the male attempts copulation (Hunt, 1994; Wood & Guttman, 1982). Copulation involves pairing of the male and female genitalia after the male turns to face the opposite direction of the female.

We manipulated ambient temperature across a range of ecologically relevant temperatures (18–36°C; Jocson, Smeester, Leith, Macchiano, & Fowler-Finn, 2019) in a controlled laboratory setting and tested male courtship activity and female courtship activity in isolation using vibrational playbacks to simulate a reproductive context. We then paired males and females in live interactions across the same temperature range and determined mating success as well as the success and timing of each stage of the mating interaction. We found that the influence of temperature on male and female courtship activity was sex-specific. Mating rates more closely matched male courtship activity in the first year of the experiment, and female courtship activity in the second year of the experiment. Furthermore, temperature affected the expression of mating-related behaviors at different stages of mating interactions to varying degrees, with duetting, copulatory attempts and copulation duration being the only thermally sensitive behaviors. Therefore, temperature-related breakdowns in reproduction may occur multiple times throughout the progression of mating.

## 2 | METHODS

### 2.1 | Study system

*Enchenopa binotata* comprises a species complex for which many species are not yet formally described (Hamilton & Coccoft 2009). However, they can be distinguished based on the host plant species they use as well as male signal characteristics. We used the species of *E. binotata* living on *Ptelea trifoliata* (Rutaceae), which produces signals at a frequency of ~350 Hz at 25°C (Rodríguez & Coccoft, 2006). All experiments and animal care complied with the regulations of Saint Louis University and with the current laws of the United States. Insects were reared under non-stressful conditions on healthy host plant exemplars in a controlled greenhouse setting. Nets used to hold insects and plants were adequately cleaned between uses. Insects were returned to net cages after testing in the laboratory. We kept voucher specimens in 70% ethanol in the Fowler-Finn Laboratory collection.

We collected insects as late-instar nymphs from a population in Columbia, Missouri, USA (latitude, longitude: 38.927133, -92.320419) and reared them to adulthood on potted host plant exemplars in the Saint Louis University greenhouse. To control for sexual experience, we placed males and females onto separate netted host plants after they reached adulthood and before they were sexually mature. We tested courtship activity of males and females using vibrational playbacks when individuals reached sexual maturity. This occurs two weeks after the final molt for males, and six weeks after the final molt for females, and is indicated by individuals responding to an artificial signal stimulus (Coccoft et al., 2008; Rodríguez & Coccoft, 2006; Rodríguez, Haen, Coccoft, & Fowler-Finn, 2012; Rodríguez et al., 2004). We ran mating trials approximately seven weeks after the final molt to adulthood. Courtship and mating trials were conducted from late morning to early afternoon (9:00 a.m. to

4:00 p.m.), after which courtship activity was low. All experiments were conducted between June and August 2017 and 2018.

## 2.2 | Vibrational playback and recording protocol

Vibrational playbacks were played from WAV files using the sound editing program Audacity (v. 2.1.1; <http://audacity.sourceforge.net/>) and MATLAB (v. 8.3 2014). The stimuli were recordings of live duets (for males) and male vibrational signals (for females) recorded at the testing temperature. We delivered the stimuli using Roland Duo-Capture USB audio interfaces (Model No. UA-11-MK2) and linear resonant actuators (LRA coin type Z-axis Model G0832012) attached to the plant stem with beeswax. We monitored and recorded male and female signals using an accelerometer (Vibra Metrics Model No. 9002A with signal conditioner and power supply Model P5000), Roland Duo-Capture Ex (Model No. UA-22) and PreSonus AudioBox USB interfaces. We saved and analyzed all sound files as WAV files using the program Audacity (v. 2.1.1) at a sampling rate of 44.1 kHz. We isolated the recording setup from ambient vibrational noise by placing the testing incubator on top of either a heavy iron plank or concrete landscaping tile (each approximately 100 kg) supported by partially inflated bicycle inner tubes on top of a heavy table with rubber casters.

## 2.3 | Courtship activity trials

To determine patterns of activity in male courtship, we tested the likelihood for males to produce courtship signals at seven ecologically relevant testing temperatures that span the range the insects naturally experience in the field (Jocson et al., 2019; 18, 21, 24, 27, 30, 33, and 36°C). We first acclimated each male to a randomly assigned testing temperature within a temperature-controlled incubator for a minimum of 20 min (e.g., Greenfield & Medlock, 2007; Jocson et al., 2019). We then placed each male singly onto a host plant exemplar (which had also been acclimated for more than 20 min at the testing temperature) within a testing incubator set at the testing temperature. After 2 minutes, we played back a vibratory duet primer comprised of two male signals alternating with two female response signals. We played primers every two minutes until ten minutes had passed (four primers per trial) and recorded whether the male responded at any point with his own vibratory advertisement courtship signal. Recording responses to these primers is a common way to test if *E. binotata* males and females are sexually responsive (Cocroft et al., 2008; Rodríguez & Cocroft, 2006; Rodríguez et al., 2004, 2012). We tested 15–17 males at each target temperature in 2017 ( $n = 112$ ) and 10 males at each target temperature in 2018 ( $n = 70$ ).

We determined patterns of courtship activity for females in a similar manner, except that the playbacks involved three different vibratory primers. Both male signal frequency (Jocson et al., 2019; Sattman & Cocroft, 2003) and the frequency that females prefer

(Jocson et al., 2019) increase with increased temperature in *E. binotata*. Furthermore, in some populations, females prefer male signal frequencies that differ from the average male signal across temperatures (Jocson et al., 2019). Thus, to reduce the likelihood of females not responding because of a lack of preference for the male signal frequency, we played back six repetitions of three primers to each female. One primer was a recording of a live male signaling at the female's testing temperature to account for variation in female preference due to temperature coupling of signals and preferences (Gerhardt, 1978; Greenfield & Medlock, 2007; Jocson et al., 2019). For the same reason, we also played either a male calling at a high temperature (33°C) if the female was being tested at 27°C or hotter, or a male calling at a low temperature (21°C) if the female was tested below 27°C. Additionally, we played a male calling at the average signal frequency of the species recorded at 24°C (350 Hz; Rodríguez & Cocroft, 2006) in case females prefer male signals that are less sensitive to temperature (e.g., Conrad, Stöcker, & Ayasse, 2017). We played back primers at a standard amplitude in a random order using a custom script (available upon request) in MATLAB (v. 8.3 2014). We recorded whether the female responded with her own vibratory signal in response to any of these primers. We tested 18–20 females at each target temperature in 2017 ( $n = 133$ ) and 4–6 females at each target temperature in 2018 ( $n = 36$ ). Although female activity remained high at the hottest temperature tested (36°C; see Results), we did not test females at hotter temperatures due to high mortality rates above 36°C.

## 2.4 | Mating trials

We tested patterns of mating activity across the same seven temperatures used for the courtship activity trials (18–36°C). Male–female pairs were randomly assigned and each individual acclimated to the testing temperature for 20 min prior to a trial. We introduced males and females in a standard way across all trials. We placed the female approximately 15 cm above the soil on the main stem of the plant inside the testing incubator, allowed her 5 min to settle on the plant. We then placed the male approximately 10 cm below her current position on the main stem. To reduce any potential effects of plant architecture on the ability for males to locate the female, we used standardized host plant exemplars: All test plants had three clusters of leaves and eight leaves total, with a distance of 32–35 cm between the base of the stem and the tip of the highest leaf. We allowed each pair to freely interact until copulation occurred or until 4 hr had passed, whichever occurred first. Copulating pairs were allowed to remain in copula until they naturally ended copulation.

For both 2017 and 2018 trials, we recorded whether mating occurred. For the 2017 trials, we additionally recorded the success and timing of each stage of mating interactions. To do so, we monitored and recorded interactions in the 2017 trials using digital HD video cameras (Sony HDR-PJ540) and accelerometers coupled to the plant stem approximately 10 cm above the soil. For the success of each stage of mating interactions, we recorded the likelihood to duet

(comprised of a male courtship signal followed by female response); the likelihood for males to attempt copulation if duetting occurred (indicated by stereotypical extension of the wings and positioning of genitalia); the likelihood for copulation to occur if the male attempted copulation (indicated by stereotypical body position and coupling of the genitalia); and the likelihood for copulation to occur on the first attempt if the male attempted copulation. For the timing of behaviors, we measured the latency of first duet (measured from the time the male was introduced to the plant); the latency of the first copulatory attempt (measured from the time of the first duet); the latency to copulation (measured from the time of the first duet and indicated by stereotypical body position and coupling of the genitalia); and the duration of copulation.

All females used in the courtship and mating trials were known to be unmated due to splitting males and females on separate rearing plants upon the final molt to adulthood. Males were used up to three times in the 2017 mating trials (never at the same temperature) and once in 2018. All males used in the courtship activity trials were also known to be unmated. To identify individuals, we painted the pronota using non-toxic paints (Apple Barrel satin acrylic multi-surface paint) in a unique two-color combination. At the end of each mating trial, males were returned to their rearing plant and females were preserved in 70% ethanol as vouchers and stored in the Fowler-Finn Laboratory. For the 2017 mating trials, we used only males and females that were actively signaling during the courtship activity trials. Our intent was to select only individuals we knew to be receptive such that we could test the effects of temperature on copulation likelihood independently from effects of temperature on courtship likelihood. However, in 2018, we initially selected both responsive and non-responsive males and females at random for use in the mating trials. By doing so, we intended to test if artificially selecting for the most active (and potentially least thermally sensitive) individuals in the population resulted in an inaccurate estimate of the thermal tolerance and sensitivity of copulation likelihood. Thus, we ran two analyses each for comparing the mating rates curves between years and comparing the 2018 mating rates curve to the 2018 male and female courtship activity curves. In one set of analyses, the 2018 mating rates data included only responsive males and females (see Results). The other included both non-responsive and responsive males and females in the 2018 mating rates data (see Tables S1 and S2). Overall, selecting individuals for the 2018 mating trials at random with respect to previous responsiveness did not affect the outcomes of the analyses comparing curves between or within years. There was no difference between the shape of the mating rates curves in 2017 and 2018 for either analysis (Year, Temperature  $\times$  Year, and Temperature  $\times$  Temperature  $\times$  Year terms; Tables 1 and S1). Furthermore, the outcomes of the analyses comparing the quadratic shape of the mating rates and male courtship activity curves (Mating activity versus Male activity: Temperature  $\times$  Temperature  $\times$  Trial Type term) and the linear and quadratic shape of the mating rates and female courtship activity curves (Mating activity versus Female activity: Temperature  $\times$  Trial Type and Temperature  $\times$  Temperature  $\times$  Trial Type terms) were the same for both

**TABLE 1** Yearly variation in the effects of temperature on male courtship activity, female courtship activity, and mating rates in *Enchenopa binotata* treehoppers

	$\chi^2$	df	p
<b>Male activity 2017 versus 2018</b>			
Temperature	10.5	1,5	<b>.0012</b>
Temperature $\times$ Temperature	80.0	1,5	<b>&lt;.0001</b>
Year	27.1	1,5	<b>&lt;.0001</b>
Temperature $\times$ Year	5.9	1,5	<b>.0151</b>
Temperature $\times$ Temperature $\times$ Year	1.1	1,5	.2941
<b>Female activity 2017 versus 2018</b>			
Temperature	18.8	1,5	<b>&lt;.0001</b>
Temperature $\times$ Temperature	1.7	1,5	.1917
Year	3.9	1,5	<b>.0486</b>
Temperature $\times$ Year	6.3	1,5	<b>.0121</b>
Temperature $\times$ Temperature $\times$ Year	0.0	1,5	.8517
<b>Mating rates 2017 versus 2018</b>			
Temperature	1.8	1,5	.1750
Temperature $\times$ Temperature	3.4	1,5	.0657
Year	0.2	1,5	.6822
Temperature $\times$ Year	1.1	1,5	.2948
Temperature $\times$ Temperature $\times$ Year	1.3	1,5	.2634

Note:  $\chi^2$  denotes the likelihood-ratio chi-squared value. Statistically significant terms indicated in boldface. Mating rates data only include individuals that were previously responsive during the male activity trials or female activity trials. For analyses including responsive and unresponsive males and females in the 2018 mating rates data, see Table S1.

sets of analyses (Tables 2 and S2). To avoid a potential confound of year and the selection process for mating trials, we only used mating rates data from previously responsive males and females in 2018 for our final models. We tested 8–9 pairs at each target temperature in 2017 ( $n = 57$ ) and 2–6 pairs at each temperature in 2018 ( $n = 25$ ). In 2018, mating trials at 36°C were not included in the final analyses because each pair tested at this target temperature included a male or a female that was non-responsive during the courtship activity trials. However, as stated above, including these pairs does not affect the shape of the mating curve across years or how the mating curve related to the shape of the male and female courtship activity curves (See Results, Tables S1 and S2).

## 2.5 | Visualizing thermal sensitivity using a function-valued approach

Thermally sensitive traits are function-valued in that the expression of the traits vary in response to a continuous environmental variable (i.e., temperature) (Hadjipantelis, Jones, Moriarty, Springate, & Knight, 2013; Kingsolver, Gomulkiewicz, & Carter, 2001; Meyer & Kirkpatrick, 2005; Stinchcombe & Kirkpatrick, 2012). Cubic splines and other nonparametric curves are useful for visualizing thermal

	2017			2018		
	$\chi^2$	df	p	$\chi^2$	df	p
<b>All curves</b>						
Temperature	1.2	1,8	.2802	24.9	1,8	<.0001
Temperature × Temperature	48.6	1,8	<.0001	9.4	1,8	.0021
Trial type	23.6	2,8	<.0001	16.2	2,8	.0003
Temperature × Trial type	0.8	2,8	.6656	1.5	2,8	.4635
Temperature × Temperature × Trial type	14.6	2,8	.0007	12.0	2,8	.0025
<b>Male activity versus Female activity</b>						
Temperature	2.0	1,5	.1624	37.0	1,5	<.0001
Temperature × Temperature	28.1	1,5	<.0001	20.0	1,5	<.0001
Trial type	5.9	1,5	.0156	15.1	1,5	.0001
Temperature × Trial type	0.0	1,5	.9483	0.2	1,5	.6210
Temperature × Temperature × Trial type	12.3	1,5	.0004	9.2	1,5	.0024
<b>Mating activity versus Male activity</b>						
Temperature	0.2	1,5	.6712	12.0	1,5	.0005
Temperature × Temperature	51.0	1,5	<.0001	9.0	1,5	.0028
Trial type	6.5	1,5	.0106	6.6	1,5	.0100
Temperature × Trial type	0.3	1,5	.6136	1.1	1,5	.2882
Temperature × Temperature × Trial type	0.9	1,5	.3335	6.1	1,5	.0132
<b>Mating activity versus Female activity</b>						
Temperature	0.7	1,5	.4035	13.1	1,5	.0003
Temperature × Temperature	20.1	1,5	<.0001	0.6	1,5	.4223
Trial type	23.1	1,5	<.0001	1.7	1,5	.1972
Temperature × Trial type	0.9	1,5	.3507	0.5	1,5	.4618
Temperature × Temperature × Trial type	7.1	1,5	.0075	0.0	1,5	.9804

Note:  $\chi^2$  denotes likelihood-ratio chi-squared value. Statistically significant terms indicated in boldface. Mating rates data only include individuals that were previously responsive during the male activity trials or female activity trials. For analyses including responsive and unresponsive males and females in the 2018 mating rates data, see Table S2.

responses because they describe patterns of behavior with some smoothness without assuming general function shapes (Kilmer et al., 2017; Schluter, 1988). We used cubic splines to visualize binomial behavioral responses to temperature (e.g., Figure 1: male courtship activity, female courtship activity, and mating activity across temperatures). We produced the splines using the Pfunc module in the coding language R (with “mgcv” package) executed in Python (Kilmer et al., 2017; <https://github.com/Joccalor/PFunc>).

## 2.6 | Yearly variation in courtship activity and mating rates

We first tested whether the effects of temperature on activity within each trial type differed between years using logistic regressions. For

**TABLE 2** Comparisons between the effects of temperature on activity during different trial types in *Enchenopa binotata* treehoppers (trial types include male courtship activity, female courtship activity, and mating)

male and female courtship trials, the response variable was whether an individual courted. For mating trials, the response variable was whether a pair mated. The fixed effects were temperature, a temperature × temperature interaction (the quadratic term reflects the shape of a typical thermal performance curve that peaks at intermediate temperatures), year, a temperature × year interaction, and a temperature × temperature × year interaction. A significant year term indicates that activity is overall higher in one year than the other. A significant temperature × year term indicates that the linear relationship between temperature and responses differs by year, and a significant temperature × temperature × year term indicates that the quadratic shape of the curve differs by year. We originally included whether males had previously mated in the logistic regression for mating trials, but it did not affect the likelihood to mate (Table S3) and so we excluded this variable from our final models.

## 2.7 | Comparing thermal sensitivity of male, female, and mating activity

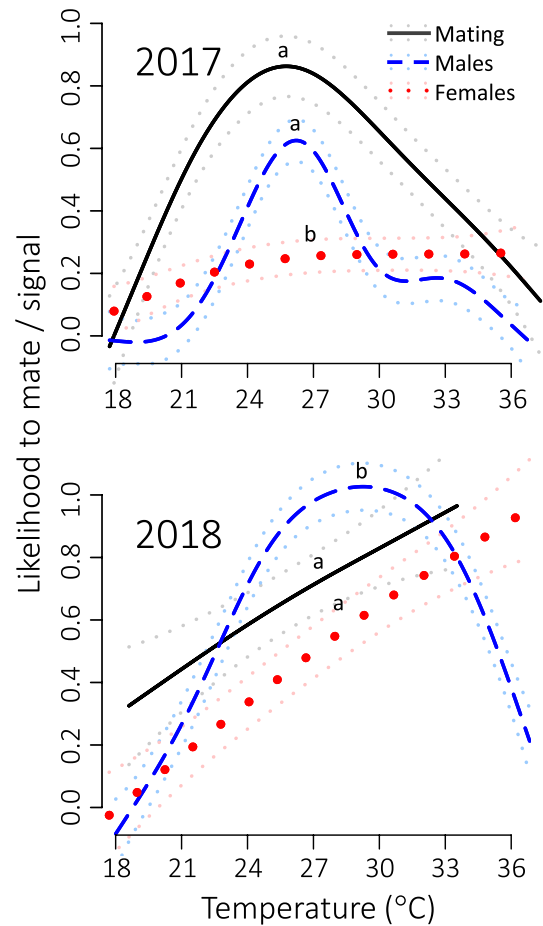
We used a nominal logistic regression in JMP (version 14.1.0) to statistically compare courtship activity of males, courtship activity of females, and mating activity across the temperature range. We ran separate models for 2017 and 2018 data. We pooled the data from all three trials into a single data set, with the response variable being either whether an individual courted (for male and female courtship trials) or a pair mated (for mating trials). The fixed effects were temperature, a temperature  $\times$  temperature interaction, trial type (i.e., male courtship, female courtship, mating), a temperature  $\times$  trial type interaction, and a temperature  $\times$  temperature  $\times$  trial type interaction. A significant temperature  $\times$  trial type interaction indicates a difference in the linear relationship between activity and temperature, and a significant temperature  $\times$  temperature  $\times$  trial type interaction indicates a difference in the quadratic relationship between activity and temperature. Given that our interaction terms indicated overall variation across trial types (see Results), we ran post hoc pairwise models to determine which activity curves differed from one another. These models were constructed the same as above, but with only two of the trial types included in each of three pairwise comparisons.

## 2.8 | Thermal sensitivity of success in each stage of mating

We ran nominal logistic regressions to test the effects of temperature on the success of each stage of mating measured in the mating trials from 2017. The response variables were likelihood to duet, likelihood to attempt copulation (if duetting occurred), likelihood of copulation (if the male attempted), and the likelihood to mate on the first attempt (if the male attempted). Fixed effects included a temperature term and a temperature  $\times$  temperature interaction term.

## 2.9 | Thermal sensitivity in the timing of mating-related behaviors

We ran parametric survival models to test the effects of temperature on the timing of mating-related behaviors measured in the mating trials from 2017. The response variables were latency of the first duet, latency of the first copulatory attempt, latency of copulation, and the duration of copulation for pairs that mated. Fixed effects included a temperature term and a temperature  $\times$  temperature interaction term. The distributions for the response variables were not normal. Thus, we used a Weibull distribution for the latency of the first duet, the latency to attempt copulation, and latency to copulate, and a lognormal distribution for the duration of copulation. Whether males had previously mated did not affect the timing of mating behaviors (Table S3) and so this term was excluded from the final models.



**FIGURE 2** Curves illustrating the likelihood for male and female *Enchenopa binotata* to signal as a function of temperature, compared to the likelihood of mating across the same temperatures. The top and bottom graphs show data collected in 2017 and 2018, respectively. The peak of each curve indicates the thermal optima for signaling and mating. Different letters (a, b) indicate curves that significantly differed in shape when we compared curves from the same year. Light dotted lines show standard error for each curve

## 3 | RESULTS

### 3.1 | Yearly variation in courtship activity and mating rates

Both male and female courtship activity was overall higher in 2018 than in 2017 (Table 1; Figure 2). In 2017, male courtship activity peaked around 27°C and female courtship activity leveled off and remained high at temperatures above 27°C (Figure 2). In 2018, male and female courtship activity peaked around 30 and 36°C, respectively, with 36°C being the highest temperature tested for courtship activity trials (Figure 2). While the quadratic shape of the male and female curves did not differ between years (Temperature  $\times$  Temperature  $\times$  Year terms; Table 1, Figure 2) males and females were more active at the warmest temperatures tested in 2018 and in 2017 (Temperature  $\times$  Year terms; Table 1, Figure 2). Mating activity curves did not significantly differ between years, but appeared to

peak around 27°C in 2017 and at the warmest temperatures tested in 2018 (Table 1; Figure 2).

### 3.2 | Comparing thermal sensitivity of male, female, and mating activity

Male courtship activity was overall greater than female courtship activity in both years (Figure 2, Table 2). Male and female courtship activity differed in the quadratic response to temperature in both years (Figure 2, Table 2). Mating activity matched male courtship activity in 2017. In 2018, mating activity differed from male courtship activity in the linear and quadratic components of the curves, though both peaked at similar intermediate temperatures (Figure 2, Table 2). In contrast, mating activity differed from female courtship activity in the quadratic component in 2017, but did not statistically differ from female courtship activity in 2018 (Figure 2, Table 2).

### 3.3 | Thermal sensitivity of success in each stage of mating

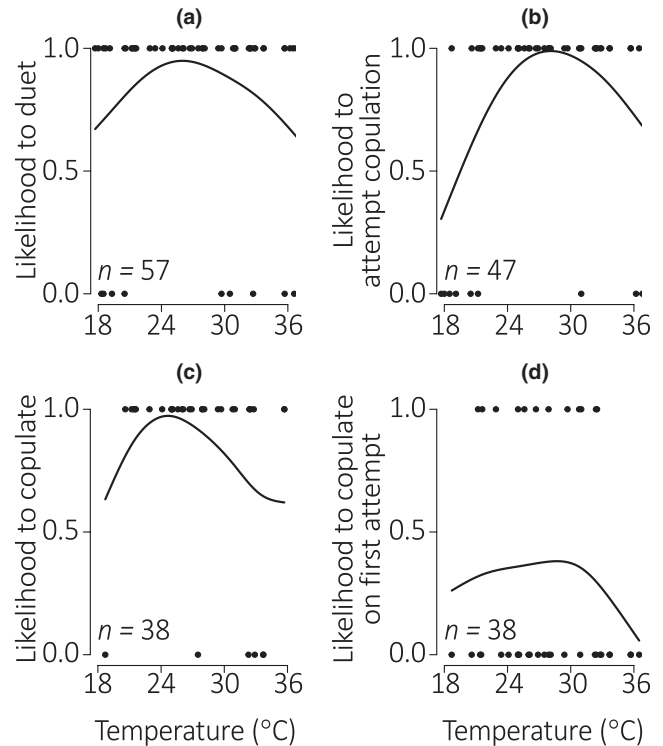
The likelihood for duetting to occur and for males to attempt copulation decreased at thermal extremes (Figure 3, Table 3). However, the success of copulatory attempts did not change across temperatures: The likelihood for the male to be successful on his first attempt to copulate and after multiple attempts did not vary with temperature (Figure 3, Table 3).

### 3.4 | Thermal sensitivity in the timing of mating-related behaviors

As mating temperature increased, latency to duet decreased and copulations became shorter (Figure 4, Table 4). A significant temperature × temperature term for copulation duration reflects that decreases in duration began to level off at higher temperatures (Figure 4, Table 4). Temperature did not affect the latency of the first copulatory attempt nor the onset of copulation (Figure 4, Table 4).

## 4 | DISCUSSION

We investigated how thermal constraints on male and female courtship activity culminate into overall mating rates and compared the thermal sensitivity of behaviors exhibited at different stages of male–female mating interactions. Mating activity matched male courtship activity in the first year of the study, and female courtship activity in the second year of the study. While these results suggest that mating rates are constrained by thermal effects on one sex, the sex that limits mating may be variable. Temperature did not influence all behaviors involved with the coordination of mating, but did affect



**FIGURE 3** The likelihood for paired males and females to (a) duet, (b) attempt copulation (only if duetting occurred), (c) copulate (only if the male attempted copulation), and (d) copulate on the first attempt (only if the male attempted copulation) across temperatures in *Enchenopa binotata* treehoppers. For each behavior, solid black lines are cubic splines

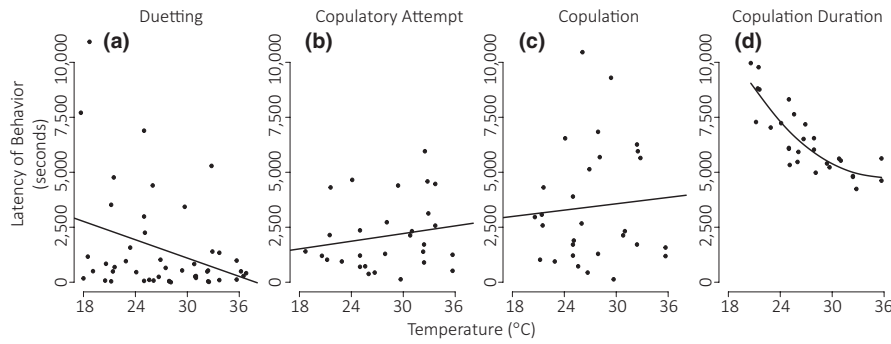
**TABLE 3** Effects of temperature on the likelihood to duet, likelihood to attempt copulation (if duetting occurred), the likelihood to copulate (if the male attempted copulation), and the likelihood to copulate on the first attempt (if the male attempted copulation) in *Enchenopa binotata*

	$\chi^2$	df	p
<b>Likelihood to duet</b>			
Temperature	0.1	1,2	.7659
Temperature × Temperature	5.8	1,2	<b>.0164</b>
<b>Likelihood to attempt copulation</b>			
Temperature	3.0	1,2	.0816
Temperature × Temperature	15.4	1,2	<b>&lt;.0001</b>
<b>Likelihood to copulate</b>			
Temperature	2.0	1,2	.1562
Temperature × Temperature	2.6	1,2	.1101
<b>Likelihood to copulate on first attempt</b>			
Temperature	0.4	1,2	.5265
Temperature × Temperature	1.7	1,2	.1882

Note:  $\chi^2$  denotes likelihood-ratio chi-squared value. Statistically significant terms indicated in boldface.

the likelihood of duetting, the likelihood of copulatory attempts, and copulation duration. Overall, temperature influenced the coordination of mating, but thermal effects were complex and sex-specific.





**FIGURE 4** The timing of courtship and copulatory behaviors (including latency of the first duet, first copulatory attempt, successful copulation, and copulation duration) across temperatures in *Enchenopa binotata* treehoppers. Solid black lines (a–c) are least-squares regression lines for latency to behaviors, and a cubic spline (d) illustrates the quadratic effect of temperature on copulation duration. Latency of the first duet, first copulatory attempt, and the onset of copulation were transformed into Weibull distribution and copulation duration was transformed into lognormal distribution for statistical analyses but are left untransformed in these visualizations

**TABLE 4** Effects of temperature on the latency of duetting, latency of copulatory attempts, latency of copulation, and duration of copulation in *Enchenopa binotata*

	$\chi^2$	df	p
Latency of first duet			
Temperature	5.2	1,2	<b>.0319</b>
Temperature × Temperature	0.3	1,2	.6170
Latency of first copulatory attempt			
Temperature	1.1	1,2	.3055
Temperature × Temperature	0.0	1,2	.9839
Latency of copulation			
Temperature	0.5	1,2	.4881
Temperature × Temperature	2.5	1,2	.1128
Duration of copulation			
Temperature	37.5	1,2	<b>&lt;.0001</b>
Temperature × Temperature	6.2	1,2	<b>.0125</b>

Note:  $\chi^2$  denotes likelihood-ratio chi-squared value. Statistically significant terms indicated in boldface.

Males and females consistently differed in how their courtship activity changed with temperature across years. While males showed higher activity at intermediate temperatures, females were most active at the hottest temperatures tested. Higher thermal sensitivity of courtship behavior in males may result from the overall greater energetic costs for males to find mates, as male *E. binotata* actively search for stationary females (Cocroft et al., 2008; Gibson & Cocroft, 2018; Hunt, 1994). Males also produce longer bouts of signals during duetting than females (personal observation). If signaling further increases body temperature, males may be more sensitive to thermal variation (Block, 1994). For example, male fiddler crabs are more thermally sensitive and active during courtship, and higher male activity has been hypothesized to result in greater physiological stress in extreme thermal conditions (Darnell et al., 2013). Also, males may be more sensitive to thermal variation if increasing activity at physiologically optimal temperatures could lead to a greater increase

in mating opportunities for males, which are polygynous in *E. binotata*. Given that *E. binotata* females only mate once (Sullivan-Beckers & Cocroft, 2010; Wood, 1993; Wood & Guttman, 1982), females may not benefit as much from altering activity across temperatures. However, this explanation does not clarify why female courtship activity increased so dramatically at the hot extreme in 2018.

We found support for one sex imposing greater constraints on mating, though the more constraining sex switched between years. In 2017, mating rates did not differ from patterns in male courtship activity and had the same peak of activity. In 2018, mating rates did not significantly differ from patterns in female courtship activity. This shift in which sex constrains mating rates between years may be due to the overall increase in courtship activity from 2017 to 2018. When overall activity is low across all temperatures, thermal sensitivity in male courtship may constrain mating if signal duets only occur at temperatures that are optimal for male activity. As overall activity increases for both sexes, females may constrain mating by limiting the range of temperatures at which mating occurs after males have located females. Differences in overall activity patterns between years may also be due, in some part, to differences in field conditions prior to the collection of nymphs. We suspect these effects were minimal given that all individuals experienced the same thermal conditions during their final two (of five) instars and through early adulthood. Overall, our data suggest the relationship between male and female courtship activity and mating may shift with variation in overall activity levels, context, or thermal constraints of each sex.

Both male and female courtship activity were less constrained at the hotter versus lower extreme. As a result, overall patterns of activity deviate from typically left-skewed thermal performance curves of traits dictated by physiological rates (Dell, Pawar, & Savage, 2011; Dewitt & Friedman, 1979; Gunderson & Leal, 2015; Huey & Stevenson, 1979; Kingsolver, 2009). Thus, it is likely that mating-related behaviors are influenced by more than basic physiological thermal constraints (Abram et al., 2017). The shape of thermal activity curves for courtship and mating behaviors may differ considerably from other traits and may be difficult to predict from thermal performance curves of physiological rates. For example,

female *Habronattus clypeatus* jumping spiders are most likely to mate at hot temperatures, even though male courtship activity does not appear constrained at much lower temperatures in a laboratory environment and general activity is highest for both sexes at cooler temperatures in the field (Brandt et al., 2018).

Pairs were less likely to duet and males were less likely to attempt copulation at thermal extremes, indicating that temperature-related breakdowns in reproduction occurred in at least two stages of mating: first by constraining courtship activity, and second by affecting female receptivity or other processes involved in generating male copulatory attempts. In contrast, of males that did attempt copulation, temperature variation did not affect whether successful copulation occurred. The likelihood to mate on the first attempt was also unaffected across temperatures. However, very few males attempted to copulate at the lower temperature extremes, potentially making it difficult to detect a significant pattern. Interestingly, when duetting occurred, it occurred earlier at hotter temperatures, which could be a result of overall higher activity or motivation to mate (Jiao, Wu, Chen, Chen, & Liu, 2009; Schäfer & Uhl, 2004). These results suggest that thermal constraints on mating rates likely arise at two points: prior to initiating courtship and after males have located females on the plant stem. Finally, copulation duration was shorter at hotter temperatures, which could also affect fertilization if longer copulations increase sperm transmission (Dickinson, 1986; Laird et al., 2004; Sato et al., 2017).

With imminent increases in temperature means and fluctuations due to global climate change (Collins et al., 2013; Meehl et al., 2007), understanding the thermal sensitivity of mating behaviors can shed light on a potential threat to the persistence of insect populations. Because physiological processes studied to assess thermal sensitivity are often less sensitive to temperature than the behavioral traits they underlie (Gunderson & Leal, 2015), quantifying the thermal sensitivity of fitness-related behaviors may be an important part of this task. Here, we found that patterns of thermal variation in courtship activity relate to overall mating rates across temperatures. However, the patterns of sex specificity in thermal sensitivity we found as well as the potential for one sex to constrain mating rates more than the other suggest that courtship activity alone may not be a consistent predictor of thermal sensitivity in mating (see also Brandt et al., 2018). Alternatively, temperature-related breakdowns in reproduction may emerge from a combination of physiological and behavioral processes that occur throughout mating interactions. As a result, thermal sensitivity in a variety of mating processes could have potentially important implications for ectotherm reproduction in a changing climate.

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#### REFERENCES

- Abram, P. K., Boivin, G., Moiroux, J., & Brodeur, J. (2017). Behavioural effects of temperature on ectothermic animals: Unifying thermal physiology and behavioural plasticity. *Biological Reviews*, 92(4), 1859–1876. <https://doi.org/10.1111/brv.12312>
- Andrés, J. A., & Cordero Rivera, A. (2000). Copulation duration and fertilization success in a damselfly: An example of cryptic female choice? *Animal Behaviour*, 59(4), 695–703. <https://doi.org/10.1006/anbe.1999.1372>
- Andrew, N. R., Hill, S. J., Binns, M., Bahar, M. H., Ridley, E. V., Jung, M., ... Khusro, M. (2013). Assessing insect responses to climate change: What are we testing for? Where should we be heading? *PeerJ*, 1, e11. <https://doi.org/10.7717/peerj.11>
- Angilletta, M. J., Bennett, A. F., Guderley, H., Navas, C. A., Seebacher, F., & Wilson, R. S. (2006). Coadaptation: A unifying principle in evolutionary thermal biology. *Physiological and Biochemical Zoology*, 79(2), 282–294. <https://doi.org/10.1086/499990>
- Angilletta, M. J., Niewiarowski, P. H., & Navas, C. A. (2002). The evolution of thermal physiology in ectotherms. *Journal of Thermal Biology*, 27(4), 249–268. <https://doi.org/10.2741/E148>
- Angilletta, M. J., Steury, T. D., & Sears, M. W. (2004). Temperature, growth rate, and body size in ectotherms: Fitting pieces of a life-history puzzle. *Integrative and Comparative Biology*, 44(August), 498–509. <https://doi.org/10.1093/icb/44.6.498>
- Beaupre, S. J., & Duvall, D. (1998). Variation in oxygen consumption of the western diamondback rattlesnake (*Crotalus atrox*): Implications for sexual size dimorphism. *Journal of Comparative Physiology - B Biochemical, Systemic, and Environmental Physiology*, 168(7), 497–506. <https://doi.org/10.1007/s003600050170>
- Bennett, A. F. (1980). The thermal dependence of lizard behaviour. *Animal Behaviour*, 28(3), 752–762. [https://doi.org/10.1016/S0003-3472\(80\)80135-7](https://doi.org/10.1016/S0003-3472(80)80135-7)
- Bennett, A. F. (1984). Thermal dependence of muscle function. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 247(2), R217–R229. <https://doi.org/10.1152/ajpregu.1984.247.2.R217>
- Bennett, A. F. (1990). Thermal dependence of locomotor capacity. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 259, 253–258.
- Birch, L. C. (1953). Experimental Background to the study of the distribution and abundance of insects: II. the relation between innate capacity for increase in numbers and the abundance of three grain beetles in experimental populations. *Ecology*, 34(4), 712–726.
- Block, B. A. (1994). Thermogenesis in muscle. *Annual Review of Physiology*, 56(1), 535–577. <https://doi.org/10.1146/annurev.ev.ph.56.030194.002535>
- Both, C., Artemyev, A. V., Blaauw, B., Cowie, R. J., Dekhuijzen, A. J., Eeva, T., ... Visser, M. E. (2004). Large-scale geographical variation confirms that climate change causes birds to lay earlier. *Proceedings of the Royal Society B: Biological Sciences*, 271(1549), 1657–1662. <https://doi.org/10.1098/rspb.2004.2770>
- Bowler, K., & Terblanche, J. S. (2008). Insect thermal tolerance: What is the role of ontogeny, ageing and senescence? *Biological Reviews*, 83(3), 339–355. <https://doi.org/10.1111/j.1469-185X.2008.00046.x>

- Brandt, E. E., Kelley, J. P., & Elias, D. O. (2018). Temperature alters multimodal signaling and mating success in an ectotherm. *Behavioral Ecology and Sociobiology*, 72(191).
- Brown, G. P., & Weatherhead, P. J. (2000). Thermal ecology and sexual size dimorphism in Northern water snakes, *Nerodia sipedon*. *Ecological Monographs*, 70(2), 311–330.
- Caillon, R., Suppo, C., Casas, J., Arthur Woods, H., & Pincebourde, S. (2014). Warming decreases thermal heterogeneity of leaf surfaces: Implications for behavioural thermoregulation by arthropods. *Functional Ecology*, 28(6), 1449–1458. <https://doi.org/10.1111/1365-2435.12288>
- Candolin, U. (2019). Mate choice in a changing world. *Biological Reviews*, 94, 1246–1260. <https://doi.org/10.1111/brv.12501>
- Clusella-Trullas, S., Blackburn, T. M., & Chown, S. L. (2011). Climatic predictors of temperature performance curve parameters in ectotherms imply complex responses to climate change. *The American Naturalist*, 177(6), 738–751. <https://doi.org/10.1086/660021>
- Cocroft, R. B., Rodríguez, R. L., & Hunt, R. E. (2008). Host shifts, the evolution of communication, and speciation in the *Enchenopa binotata* species complex of treehoppers. In K. J. Tilmon (Ed.), *Specialization, Speciation and Radiation: The Evolutionary Biology of Herbivorous Insects* (pp. 88–100). Berkeley, CA: University of California Press.
- Cocroft, R. B., Rodríguez, R. L., & Hunt, R. E. (2010). Host shifts and signal divergence: Mating signals covary with host use in a complex of specialized plant-feeding insects. *Biological Journal of the Linnean Society*, 99, 60–72.
- Collins, M., Knutti, R., Arblaster, J., Dufresne, J.-L., Fichetef, T., Friedlingstein, P., Wehner, M. (2013). Long-term climate change: projections, commitments and irreversibility. In T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, & J. Boschung ... P. M. Midgley (Eds.), *Climate Change 2013: The Physical Science Basis*. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom and New York, NY: Cambridge University Press.
- Condon, C., Acharya, A., Adrian, G. J., Hurliman, A. M., Malekooti, D., Nguyen, P., ... Angilletta, M. J. (2015). Indirect selection of thermal tolerance during experimental evolution of *Drosophila melanogaster*. *Ecology and Evolution*, 5(9), 1873–1880. <https://doi.org/10.1002/ece3.1472>
- Conrad, T., Stöcker, C., & Ayasse, M. (2017). The effect of temperature on male mating signals and female choice in the red mason bee, *Osmia bicornis* (L.). *Ecology and Evolution*, 7(21), 8966–8975. <https://doi.org/10.1002/ece3.3331>
- Costa, F. G., & Sotelo, J. R. J. (1984). Influence of temperature on the copulation duration of *Lycosa malitiosa* Tullgren (Araneae, Lycosidae). *Journal of Arachnology*, 12(3), 273–277.
- Cullum, A. J. (1998). Sexual dimorphism in physiological performance of whiptail lizards (genus *Cnemidophorus*). *Physiological Zoology*, 71(5), 541–552. <https://doi.org/10.1086/515961>
- Darnell, M. Z., Fowler, K. K., & Munguia, P. (2013). Sex-specific thermal constraints on fiddler crab behavior. *Behavioral Ecology*, 24(4), 997–1003. <https://doi.org/10.1093/beheco/art006>
- Dell, A. I., Pawar, S., & Savage, V. M. (2011). Systematic variation in the temperature dependence of physiological and ecological traits. *Proceedings of the National Academy of Sciences*, 108(26), 10591–10596. <https://doi.org/10.1073/pnas.1015178108>
- Dewitt, C. B., & Friedman, R. M. (1979). Significance of skewness in ectotherm thermoregulation. *American Zoologist*, 19, 195–209. <https://doi.org/10.1093/icb/19.1.195>
- Dickinson, J. L. (1986). Prolonged mating in the milkweed leaf beetle *Labidomera clivicollis clivicollis* (Coleoptera: Chrysomelidae): A test of the “sperm-loading” hypothesis. *Behavioral Ecology and Sociobiology*, 18(5), 331–338. <https://doi.org/10.1007/BF00299664>
- Flanagan, S. P., & Bevier, C. R. (2014). Do male activity level and territory quality affect female association time in the brown anole, *Anolis sagrei*? *Ethology*, 120(4), 365–374. <https://doi.org/10.1111/eth.12213>
- Gerhardt, H. C. (1978). Temperature coupling in the vocal communication system of the gray tree frog, *Hyla versicolor*. *Science*, 199(4332), 992–994.
- Gibson, J. S., & Cocroft, R. B. (2018). Vibration-guided mate searching in treehoppers: Directional accuracy and sampling strategies in a complex sensory environment. *The Journal of Experimental Biology*, 221(6), jeb175083. <https://doi.org/10.1242/jeb.175083>
- Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., & Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science*, 293(5538), 2248–2251. <https://doi.org/10.1126/science.1061967>
- Gotoh, T., Yamaguchi, K., & Mori, K. (2004). Effect of temperature on life history of the predatory mite *Amblyseius (Neoseiulus) californicus* (Acari: Phytoseiidae). *Experimental and Applied Acarology*, 32(1–2), 15–30. <https://doi.org/10.1023/B:APPA.0000018192.91930.49>
- Green, B. S., & Fisher, R. (2004). Temperature influences swimming speed, growth and larval duration in coral reef fish larvae. *Journal of Experimental Marine Biology and Ecology*, 299(1), 115–132. <https://doi.org/10.1016/j.jembe.2003.09.001>
- Greenfield, M. D., & Medlock, C. (2007). Temperature coupling as an emergent property: Parallel thermal effects on male song and female response do not contribute to species recognition in an acoustic moth. *Evolution*, 61(7), 1590–1599. <https://doi.org/10.1111/j.1558-5646.2007.00140.x>
- Groot, M. D., Čokl, A., & Virant-Doberlet, M. (2011). Search behaviour of two hemipteran species using vibrational communication. *Central European Journal of Biology*, 6(5), 756–769. <https://doi.org/10.2478/s11535-011-0056-2>
- Gunderson, A. R., & Leal, M. (2015). Patterns of thermal constraint on ectotherm activity. *The American Naturalist*, 185(5), 653–664. <https://doi.org/10.1086/680849>
- Hadjipantelis, P. Z., Jones, N. S., Moriarty, J., Springate, D. A., & Knight, C. G. (2013). Function-valued traits in evolution. *Journal of the Royal Society Interface*, 10(82), 20121032. <https://doi.org/10.1098/rsif.2012.1032>
- Hamilton, K. G. A., & Cocroft, R. B. (2009). Establishing the identity of existing names in the North American *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). *Entomological News*, 120(5), 554–565. <https://doi.org/10.3157/021.120.0513>
- Hertz, P. E., Huey, R. B., & Nevo, E. (1983). Homage to Santa Anita: Thermal sensitivity of sprint speed in agamid lizards. *Evolution*, 37(5), 1075. <https://doi.org/10.2307/2408420>
- Huey, R. B., & Berrigan, D. (2001). Temperature, demography, and ectotherm fitness. *American Society of Naturalists*, 158(2), 204–210.
- Huey, R. B., & Stevenson, R. D. (1979). Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. *American Zoologist*, 19(1), 357–366. <https://doi.org/10.1093/icb/19.1.357>
- Hunt, R. E. (1994). Vibrational signals associated with mating behavior in the treehopper, *Enchenopa binotata* Say (Hemiptera: Homoptera: Membracidae). *Journal of the New York Entomological Society*, 102(2), 266–270.
- Irllich, U. M., Terblanche, J. S., Blackburn, T. M., & Chown, S. L. (2009). Insect rate-temperature relationships: Environmental variation and the metabolic theory of ecology. *The American Naturalist*, 174(6), 819–835. <https://doi.org/10.1086/647904>
- Jiao, X., Wu, J., Chen, Z., Chen, J., & Liu, F. (2009). Effects of temperature on courtship and copulatory behaviours of a wolf spider *Pardosa astrigera* (Araneae: Lycosidae). *Journal of Thermal Biology*, 34(7), 348–352. <https://doi.org/10.1016/j.jtherbio.2009.06.004>
- Jocson, D. M. I., Smeester, M. E., Leith, N. T., Macchiano, A., & Fowler-Finn, K. D. (2019). Temperature coupling of mate attraction signals

- and female mate preferences in four populations of *Enchenopa* treehopper (Hemiptera : Membracidae). *Journal of Evolutionary Biology*, 00, 1–11. <https://doi.org/10.1111/jeb.13506>
- Katsuki, M., & Miyatake, T. (2009). Effects of temperature on mating duration, sperm transfer and remating frequency in *Callosobruchus chinensis*. *Journal of Insect Physiology*, 55(2), 113–116. <https://doi.org/10.1016/j.jinsphys.2008.10.012>
- Kilmer, J. T., Fowler-Finn, K. D., Gray, D. A., Höbel, G., Rebar, D., Reichert, M. S., & Rodríguez, R. L. (2017). Describing mate preference functions and other function-valued traits. *Journal of Evolutionary Biology*, 30(9), 1658–1673. <https://doi.org/10.1111/jeb.13122>
- Kingsolver, J. G. (2009). The well-temperated biologist. *The American Naturalist*, 174(6), 755–768. <https://doi.org/10.1086/648310>
- Kingsolver, J. G., Gomulkiewicz, R., & Carter, P. A. (2001). Variation, selection, and evolution of function-valued traits. *Microevolution Rate, Pattern, Process*, 8(December), 87–104. <https://doi.org/10.1007/978-94-010-0585-2>
- Kingsolver, J. G., & Huey, R. B. (2008). Size, temperature, and fitness: Three rules. *Evolutionary Ecology Research*, 10(2), 251–268. <https://doi.org/10.1111/j.1525-142X.2006.00090.x>
- Kvarnemo, C. (1994). Temperature differentially affects male and female reproductive rates in the sand goby: Consequences for operational sex ratio. *Proceedings of the Royal Society B: Biological Sciences*, 256(1346), 151–156. <https://doi.org/10.1098/rspb.1994.0063>
- Lailvaux, S. P., Alexander, G., & Whiting, M. (2003). Sex-based differences and similarities in locomotor performance, thermal preferences, and escape behaviour in the lizard *Platysaurus intermedius* wilhelmi. *Physiological and Biochemical Zoology*, 76(4), 511–521. <https://doi.org/10.1086/376423>
- Laird, G., Gwynne, D. T., & Andrade, M. C. B. (2004). Extreme repeated mating as a counter-adaptation to sexual conflict? *Proceedings of the Royal Society B: Biological Sciences*, 271, S402–S404. <https://doi.org/10.1098/rsbl.2004.0198>
- Legendre, F., Marting, P. R., & Coccoft, R. B. (2012). Competitive masking of vibrational signals during mate searching in a treehopper. *Animal Behaviour*, 83(2), 361–368. <https://doi.org/10.1016/j.anbehav.2011.11.003>
- Macchiano, A., Sasson, D. A., Leith, N. T., & Fowler-Finn, K. D. (2019). Patterns of thermal sensitivity and sex-specificity of courtship behavior differs between two sympatric species of *Enchenopa* Treehopper. *Frontiers in Ecology and Evolution*, 7(September), 1–11. <https://doi.org/10.3389/fevo.2019.00361>
- Magnuson, J. J., Crowder, L. B., & Medvick, P. A. (1979). Temperature as an ecological resource. *American Zoologist*, 19(1), 331–343. <https://doi.org/10.1086/280210>
- Meehl, G. A., Stocker, T. F., Collins, W. D., Friedlingstein, P., Gaye, A. T., Gregory, J. M., Zhao, Z.-C. (2007). 2007: Global climate projections. In S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, & K. B. Averyt, ... H. L. Miller (Eds.), *Climate Change 2007: The Physical Science Basis*. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (pp. 747–846). <https://doi.org/10.1080/07341510601092191>
- Meyer, K., & Kirkpatrick, M. (2005). Up hill, down dale: Quantitative genetics of curvaceous traits. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 360(1459), 1443–1455. <https://doi.org/10.1098/rstb.2005.1681>
- Michiels, N. K. (1992). Consequences and adaptive significance of variation in copulation duration in the dragonfly *Sympetrum danae*. *Behavioral Ecology and Sociobiology*, 29(6), 429–435.
- Parmesan, C. (2007). Influences of species, latitudes and methodologies on estimates of phenological response to global warming. *Global Change Biology*, 13(9), 1860–1872. <https://doi.org/10.1111/j.1365-2486.2007.01404.x>
- Pörtner, H. O. (2002a). Climate variations and the physiological basis of temperature dependent biogeography: Systemic to molecular hierarchy of thermal tolerance in animals. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, 132(4), 739–761. [https://doi.org/10.1016/S1095-6433\(02\)00045-4](https://doi.org/10.1016/S1095-6433(02)00045-4)
- Pörtner, H. O. (2002b). Environmental and functional limits to muscular exercise and body size in marine invertebrate athletes. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, 133(2), 303–321. [https://doi.org/10.1016/S1095-6433\(02\)00162-9](https://doi.org/10.1016/S1095-6433(02)00162-9)
- Régnière, J., Powell, J., Bentz, B., & Nealis, V. (2012). Effects of temperature on development, survival and reproduction of insects: Experimental design, data analysis and modeling. *Journal of Insect Physiology*, 58(5), 634–647. <https://doi.org/10.1016/j.jinsphys.2012.01.010>
- Rodríguez, R. L., & Coccoft, R. B. (2006). Divergence in female duetting signals in the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). *Ethology*, 112(12), 1231–1238. <https://doi.org/10.1111/j.1439-0310.2006.01285.x>
- Rodríguez, R. L., Haen, C., Coccoft, R. B., & Fowler-Finn, K. D. (2012). Males adjust signaling effort based on female mate-preference cues. *Behavioral Ecology*, 23(6), 1218–1225. <https://doi.org/10.1093/behec/ars105>
- Rodríguez, R. L., Sullivan, L. E., & Coccoft, R. B. (2004). Vibrational communication and reproductive isolation in the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). *Evolution*, 58(3), 571–578. <https://doi.org/10.1111/j.0014-3820.2004.tb01679.x>
- Rosenthal, M. F., & Elias, D. O. (2019). Nonlinear changes in selection on a mating display across a continuous thermal gradient. *Proceedings of the Royal Society B: Biological Sciences*, 286(1907), 20191450. <https://doi.org/10.1098/rspb.2019.1450>
- Sato, N., Yoshida, M. A., & Kasugai, T. (2017). Impact of cryptic female choice on insemination success: Larger sized and longer copulating male squid ejaculate more, but females influence insemination success by removing spermatangia. *Evolution*, 71(1), 111–120. <https://doi.org/10.1111/evo.13108>
- Sattman, D. A., & Coccoft, R. B. (2003). Phenotypic plasticity and repeatability in the mating signals of *Enchenopa* Treehoppers, with implications for reduced gene flow among host-shifted populations. *Ethology*, 109(12), 981–994. <https://doi.org/10.1046/j.1439-0310.2003.00940.x>
- Schäfer, M. A., & Uhl, G. (2004). Sequential mate encounters: Female but not male body size influences female remating behavior. *Behavioral Ecology*, 16(2), 461–466. <https://doi.org/10.1093/behec/ari013>
- Schluter, D. (1988). Estimating the form of natural selection on a quantitative trait. *Evolution; International Journal of Organic Evolution*, 42(5), 849–861. <https://doi.org/10.2307/2408904>
- Sinclair, B. J., Williams, C. M., & Terblanche, J. S. (2012). Variation in thermal performance among insect populations. *Physiological and Biochemical Zoology*, 85(6), 594–606. <https://doi.org/10.1086/665388>
- Snow, L. S. E., & Andrade, M. C. B. (2004). Pattern of sperm transfer in redback spiders: Implications for sperm competition and male sacrifice. *Behavioral Ecology*, 15(5), 785–792. <https://doi.org/10.1093/behec/arl080>
- Stinchcombe, J. R., & Kirkpatrick, M. (2012). Genetics and evolution of function-valued traits: Understanding environmentally responsive phenotypes. *Trends in Ecology and Evolution*, 27(11), 637–647. <https://doi.org/10.1016/j.tree.2012.07.002>
- Sullivan-Beckers, L., & Coccoft, R. B. (2010). The importance of female choice, male-male competition, and signal transmission as causes of selection on male mating signals. *Evolution*, 64(11), 3158–3171. <https://doi.org/10.1111/j.1558-5646.2010.01073.x>
- Tattersall, G. J., Sinclair, B. J., Withers, P. C., Fields, P. A., Seebacher, F., Cooper, C. E., & Maloney, S. K. (2012). Coping with thermal challenges: Physiological adaptations to environmental temperatures. *Comprehensive Physiology*, 2(3), 2151–2202. <https://doi.org/10.1002/cphy.c110055>
- Terrien, J., Perret, M., & Aujard, F. (2011). Behavioral thermoregulation in mammals: A review. *Frontiers in Bioscience*, 16, 1428–1444.

- Visser, M. E. (2008). Keeping up with a warming world; assessing the rate of adaptation to climate change. *Proceedings of the Royal Society B: Biological Sciences*, 275(1635), 649–659. <https://doi.org/10.1098/rspb.2007.0997>
- Wood, T. K. (1993). Speciation of the *Enchenopa binotata* complex (Insecta: Homoptera: Membracidae). In D. R. Lees, & D. Edwards (Eds.), *Evolutionary patterns and processes* (pp. 299–317). London: Academic Press.
- Wood, T. K., & Guttman, S. I. (1982). Ecological and behavioral basis for reproductive isolation in the sympatric *Enchenopa binotata* complex (Homoptera: Membracidae). *Evolution*, 36(2), 233. <https://doi.org/10.2307/2408041>
- Yamagishi, M., & Tsubaki, Y. (1990). Copulation duration and sperm transfer in the melon fly, *Dacus cucurbitae* coquillett (Diptera: Tephritidae). *Applied Entomology and Zoology*, 25(4), 517–519. <https://doi.org/10.1248/cpb.37.3229>
- Yenisetti, S. C., Hegde, S. N., Venkateswarlu, M., & Krishna, M. S. (2006). Phenotypic plasticity of sexual behavior at different temperatures in a Drosophilid: *Phorticella striata*. *Korean Journal of Genetics*, 28(4), 395–401.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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