

Temperature coupling of mate attraction signals and female mate preferences in four populations of *Enchenopa* treehopper (Hemiptera: Membracidae)

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Abstract

Variation in temperature can affect the expression of a variety of important fitness-related behaviours, including those involved with mate attraction and selection, with consequences for the coordination of mating across variable environments. We examined how temperature influences the expression of male mating signals and female mate preferences—as well as the relationship between how male signals and female mate preferences change across temperatures (signal–preference temperature coupling)—in *Enchenopa binotata* treehoppers. These small plant-feeding insects communicate using plantborne vibrations, and our field surveys indicate they experience significant natural variation in temperature during the mating season. We tested for signal–preference temperature coupling in four populations of *E. binotata* by manipulating temperature in a controlled laboratory environment. We measured the frequency of male signals—the trait for which females show strongest preference—and female peak preference—the signal frequency most preferred by females—across a range of biologically relevant temperatures (18°C–36°C). We found a strong effect of temperature on both male signals and female preferences, which generated signal–preference temperature coupling within each population. Even in a population in which male signals mismatched female preferences, the temperature coupling reinforces predicted directional selection across all temperatures. Additionally, we found similar thermal sensitivity in signals and preferences across populations even though populations varied in the mean frequency of male signals and female peak preference. Together, these results suggest that temperature variation should not affect the action of sexual selection via female choice, but rather should reinforce stabilizing selection in populations with signal–preference matches, and directional selection in those with signal–preference mismatches. Finally, we do not predict that thermal variation will disrupt the coordination of mating in this species by generating signal–preference mismatches at thermal extremes.

KEYWORDS

insects, sexual selection & conflicts, vibrational communication, animal behavior, mating patterns

1 | INTRODUCTION

Temperature affects nearly all biological processes (Kingsolver, 2009) and thus can have wide-reaching effects on the expression of a multitude of fitness-related traits (Andrewartha & Birch, 1954; Bennett, 1990; Birch, 1953; Kingsolver, 2009). Research examining the effects of temperature on trait expression has primarily focused on physiological and performance-related traits like growth rates, body size, development and general locomotion (Angilletta, 2009; Deutsch et al., 2008; Gillooly, Brown, West, Savage, & Charnov, 2001; Gunn, 1934; Irlich, Terblanche, Blackburn, & Chown, 2009), along with life history-related traits like the timing and location of breeding or clutch size (Both et al., 2004; Charmantier et al., 2008; Parmesan, 2007; Régnière, Powell, Bentz, & Nealis, 2012). However, temperature can affect the expression of a wide range of reproductive-related traits involved in sexual communication (Al, Pawar, & Savage, 2011; Schulte, Healy, & Fangue, 2011). Limited studies have examined the effects of temperature fluctuations on mating signals and mate preference traits involved in coordinating mating interactions (Doherty, 1985; Greenfield & Medlock, 2007). Understanding the effects of temperature variation on the expression of signals and preferences is key for understanding how short-term fluctuations in temperature may (or may not) alter sexual selection on male signals and/or change or disrupt patterns of mating.

Due to the underlying temperature-dependent rhythmicity of signal production, male signal traits used to attract mates typically covary strongly with temperature (Dolbear, 1897; Gayou, 1984; Gerhardt, 1994; Martin, Gray, & Cade, 2000; Narins & Meenderink, 2014; Pires & Hoy, 1992; Symes, Rodríguez, & Höbel, 2017; Walker, 1975). This phenomenon occurs across a wide range of taxonomic groups and signal modalities (e.g. airborne acoustics of orthopterans and anurans (Walker, 1975; Gerhardt & Mudry, 1980); bioluminescent flashes of fireflies (Edmunds & Leland, 1963); electric discharges in fish (Dunlap, Smith, & Yekta, 2000; Feng, 1976)). Females often identify conspecifics and select among potential mates using the same signal traits that covary with temperature (Gerhardt, 1978; Von Helversen, 1981); thus, variation in male signals across thermal conditions could pose a problem for the coordination of mating if female preferences do not track changes in male signals across temperatures (Beckers & Schul, 2008).

Female mate preferences are sometimes thermally sensitive (Conrad, Stöcker, & Ayasse, 2017; Gerhardt, 1991; Reaney & Backwell, 2007), but not always (Gerhardt & Mudry, 1980; Ritchie, Saarikettu, Livingstone, & Hoikkala, 2001). When female mate preferences are thermally sensitive in the same direction and to a similar degree as male signals, signal–preference temperature coupling occurs (Doherty, 1985; Gerhardt, 1978). In other words, changes in male signals across temperatures are matched by changes in female preferences. This coupling reinforces stabilizing selection on signals when mean signals and preferences match and reinforces directional selection when mean signals and preferences are shifted relative to one another. When female preferences are thermally sensitive to a lesser degree than male signals, or not at all (Gerhardt &

Mudry, 1980; Ritchie et al., 2001), thermal coupling is incomplete or absent, generating mismatches between signals and preferences at thermal edges (Grace & Shaw, 2004; Humfeld & Grunert, 2015). When temperature coupling is incomplete or absent, selection on male signals via female choice will depend on the thermal environment, and breakdowns in mating can occur at thermal extremes. The effects of short-term temperature fluctuations on sexual selection and the coordination of mating may be particularly significant for ectotherms because they are unable to regulate internal temperature independently of their external environment. However, surprisingly few studies have tested the temperature coupling hypothesis by analysing thermal sensitivity of male signals and female preferences simultaneously.

Here, we test the signal–preference temperature coupling hypothesis in *E. binotata* (Hemiptera: Membracidae) treehoppers, which occur throughout much of Eastern North America. We specifically test the prediction that changes in female preferences will parallel changes in male signals across temperatures in four populations of the insect. Comparisons across populations allow us to test whether populations that have experienced different historical thermal conditions differ in thermal sensitivity and thus the potential effects of thermal variation on sexual selection and the coordination of mating. We additionally test the hypothesis across two years in two of the populations in order to test for inter-annual variation in signal and preference thermal sensitivity.

Enchenopa binotata treehoppers are small (~0.5 cm) plant-feeding insects that coordinate mating through species-specific substrate-borne vibrational signals that travel as bending waves through plant stems (Cocroft & McNett, 2006; McNett & Cocroft, 2008). Male *E. binotata* fly from stem to stem in search of mates and produce vibrational advertisement signals when they land; females respond with their own response signal if they find the male signal attractive, thus initiating a male–female duet that facilitates pair formation (Cocroft, Rodríguez, & Hunt, 2008; Rodríguez & Cocroft, 2006). Female *E. binotata* exhibit strong preference for dominant signal frequency, which is the most divergent signal trait across species in the *E. binotata* complex (McNett & Cocroft, 2008; Rodríguez, Ramaswamy, & Cocroft, 2006; Rodríguez, Sullivan, & Cocroft, 2004; Sullivan-Beckers & Cocroft, 2010). Variation in mating signals and preferences have played a major role in diversification and speciation in the *E. binotata* species complex (McNett & Cocroft, 2008; Rodríguez et al., 2004), highlighting the importance of understanding how environmental variables like temperature affect the expression of mating signals and preferences and potentially the selection of mates and coordination of mating.

We also provide the first comprehensive test of the temperature coupling hypothesis in substrate-borne vibrational signalers. Research on acoustic communication—not just in the context of temperature variation—is almost exclusively focused on airborne acoustic signalers despite substrate-borne vibrations representing the most ubiquitous form of acoustic communication in the animal kingdom (Cocroft, 2001, 2010a; Cocroft & McNett, 2006; Virant-Doberlet & Cokl, 2004). Substrate-borne vibrations are produced

by many arthropods (spiders, crabs, insects), mammals (rodents, elephants), and frogs and reptiles (Cocroft, 2010a; Payne, Starks, & Liebert, 2010). Conservatively, over 70% of acoustic insects use *exclusively* substrate-borne vibrations to communicate and over 90% use substrate-borne vibrations in conjunction with airborne acoustics (Cocroft & Rodríguez, 2005). Vibrational signallers may differ from airborne signallers in thermal sensitivity for two reasons. First, vibrational signaller lacks the same physical constraints as airborne signaller, for which body size constrains the lower end of frequencies an individual can produce (Cocroft & De Luca, 2006). Second, airborne signallers rely heavily on temporal signal features (e.g. call rate or duty cycle) for mate choice, whereas many vibrational signallers use, wholly or partially, signal frequency (Hz) (Cocroft & Rodríguez, 2005; Hill, 2001). Thus, in addition to providing a geographic study of temperature coupling, we generate novel and important perspective for how temperature affects sexual selection and the coordination of mating by exploring the effects of temperature fluctuations on the most common form of acoustic communication: vibrational signalling.

2 | MATERIALS AND METHODS

2.1 | Study system

We studied the species of *E. binotata* that lives on the host plant *Ptelea trifoliata* (Rutaceae) (Figure 1). Although species in the *E. binotata* complex have not yet been formally described (Hamilton & Cocroft, 2009), they are highly host plant specific and distinguished primarily by host plant use and the species-specific dominant male signal frequency, which ranges from ~185 Hz to 485 Hz across the clade (Mcnett & Cocroft, 2008; Rodríguez et al., 2004, 2006; Sullivan-Beckers & Cocroft, 2010).

Ptelea trifoliata is patchily distributed throughout the range of the treehopper, from Texas to Michigan (Deitz & Wallace, 2012). We collected insects from four locations that varied in local thermal conditions (Table S1): Shaw Nature Reserve in Gray Summit, Missouri (Latitude, Longitude: 38.473464, -90.801859), Emmenegger Nature Park in St. Louis, Missouri (Latitude, Longitude: 38.549482,

-90.435113), Grindstone Nature Area in Columbia, Missouri (Latitude, Longitude: 38.927133, -92.320419) and St. Charles, Illinois (Latitude, Longitude: 42.125166, -89.253631). For the St. Charles, IL populations, we collected insects as eggs laid in the field by multiple females. For the Gray Summit, MO, St. Louis, MO, and Columbia, MO, populations, we collected insects as second to third-instar nymphs. We collected data over two years for the population in St. Louis, MO (females only), Gray Summit, MO (females and males), and Columbia, MO (females and males), and in a single year for St. Charles, IL. The second year of data collection from the Gray Summit, MO, population came from offspring laid as eggs in the fall from field-mated females as part of a larger quantitative genetics project; for the current study, we used data from only a single offspring per family. For a summary of the life stage and years of collection, see Table S1. The diversity in collection methods allows for a valuable opportunity to test for the consistency of thermal effects on signals and preferences across different developmental environments and years.

Nymphs were placed on host plant exemplars in the Saint Louis University greenhouse, on which they were reared until adulthood. After the last moult to adulthood, we separated males and females and placed them on new sex-specific plants in order to ensure all insects were virgins. Males reach sexual maturity one to two weeks after the adult moult, at which point we assayed for signal frequency (Hz) across a range of biologically relevant temperatures (see methods2 below). Females become sexually receptive three to four weeks after the adult moult (Wood & Guttman, 1982), and so we assayed female preferences across the same range of temperatures one to two weeks after males (e.g. Fowler-Finn, Kilmer, Cruz, & Rodríguez, 2018; Fowler-Finn, Kilmer, Hallett, & Rodríguez, 2015; Fowler-Finn & Rodríguez, 2012; Fowler-Finn & Rodríguez, 2013; Kilmer et al., 2017; Rodríguez, Haen, Cocroft, & Fowler-Finn, 2012).

2.2 | Temperature range selection

We wanted to test the temperature coupling hypothesis across a biologically relevant temperature range. We therefore surveyed temperatures on *P. trifoliata* at various times of day and on multiple

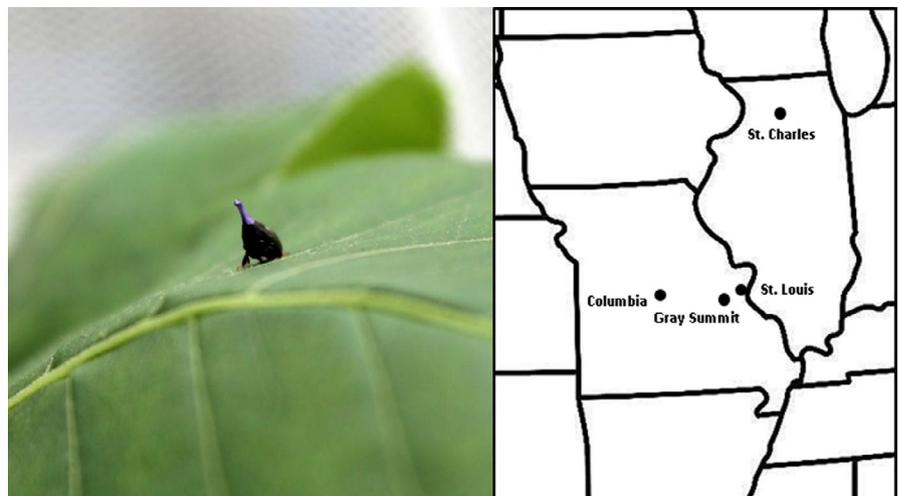


FIGURE 1 (left) *Enchenopa binotata* on a host plant leaf with its pronotum painted with a population-specific colour from a mixed-population rearing plant. (right) A map showing Illinois and Missouri populations where we collected treehoppers

plants at a focal field site in June to August of 2015 (Shaw Nature Reserve: Gray Summit, MO) during the insect's primary mating season. Using an infrared thermometer (Fluke IR thermometer), we recorded leaf temperature for six plants at four different locations in Shaw Nature Reserve where the host plant *P. trifoliata* occurs, noting when treehoppers were present. We supplemented our sampling with weather data from the site to capture temperature variation in the early mornings when insects initiate mating activity (Table S1). Our sampling indicates that treehoppers have the potential to experience a temperature range from 18°C to 38°C, with up to 10°C variation within a single plant at a single time of the day (Figure S1). In the laboratory, we found an increased risk of mortality at temperatures above 36°C and very little activity above 33°C or below 21°C (Jocson and Fowler-Finn unpubl. data). Thus, we used a range of 18°C–36°C for testing.

2.3 | Temperature manipulations and recording and playback setup

Prior to testing male signals and female preferences, we randomly assigned each insect to a testing temperature between 18°C and 36°C at intervals of 3°C. We acclimated each insect at its assigned testing temperature in an incubator for a minimum of 20 min (Greenfield & Medlock, 2007). We then placed the insect on a host plant exemplar in a testing incubator (Fisher Scientific Isotemp Incubator Model 550D) set to the assigned testing temperature. We monitored the insects through the glass doors of the incubators (see for diagrammed setup, Figure S2). We also monitored temperature throughout the trials; temperature was uniform within the incubator and deviated < 1°C from bottom to top. We measured the temperature at the spot in the incubator at which the insect was sitting during testing as a precaution. In 2015, we did so by using an infrared thermometer directed onto a piece of cardboard hanging directly next to the testing plant. In 2016 and 2017, we used a custom-built temperature sensor constructed using Bosch digital sensors (BME280) that hung right adjacent to the insect; the environmental data integrated by the sensors were transmitted over a 'I2c bus' to an Arduino (with ATmega168 chipset) that displayed temperature on an oLED display (SSD1306) (custom program for the system integration is available upon request). The custom temperature sensors deviated < 0.1°C from the infrared thermometer readings.

To playback vibrational signals to encourage male signalling and test female preferences, we used surface transducers (Model No. LB07 4Ω5W) coupled to the stem with a nylon tube and beeswax (2015 and 2016) and linear resonant actuators (LRA coin type z-axis model G0832012) coupled to the stem with beeswax (2017 and 2018). We synthesized and delivered playback signals using a custom script (available upon request) in MATLAB (v. 8.3 2014) at 0.2 mm/s. To record vibrational signals of males and monitor responses from females, we used a laser Doppler vibrometer (Polytec: PDV-100 portable digital vibrometer) and accelerometers (Vibra Metrics Model No. 9002A with signal conditioner and power supply Model P5000). The laser and accelerometers were connected to an audio interface

(Roland Duo Capture; Roland Corporation U.S.) and then to a computer. Signals were recorded and saved with the sound analysis program Audacity (v. 2.1.1; Audacity Team). We isolated our testing setup from building vibrations using the following steps. We placed the testing plant on a sorbothane pad inside the incubator and then isolated the entire testing setup using a heavy slab (either a steel plank or concrete cement blocks) floated on top of partially inflated bicycle inner tubes on a heavy (~75 kg) table with rubber casters.

2.4 | Behavioural assay: Measuring male signal frequency

After placing a male on a testing plant, we allowed him a two-minute acclimation period and then played back a primer to encourage signalling (Fowler-Finn, Cruz, & Rodríguez, 2017). The primer consisted of a recording of a live male–female duet, followed by two minutes of silence and another male–female duet. For males that commenced signalling, we recorded their signals for a 10-min period, playing the primer every two minutes for a total of 4 playbacks each trial. If a male did not signal after the first two primer playbacks, we marked him with nontoxic, acrylic, weather-resistant paint (Apple Barrel®) on his pronotum (Figure 1) and returned him to his rearing plant. Males that did not respond were tested up to three times total, with a week between each test, before being marked as unresponsive.

We used the sound analysis program Audacity (v. 2.1.1) to measure dominant signal frequency of the male signals. Male *E. binotata* signal in successive signal bouts, which comprise six signals on average, that systematically change in amplitude across signals and whine portion of the signal (Rodríguez et al., 2006). Thus, we used a standard landmark signal and position within the signal to measure signal frequency: the end of the whine portion of the third signal in the second signal bout (Figure 2), or the closest to that signal if fewer were produced (Cocroft, Rafael, & Hunt Randy, 2010b; Fowler-Finn, Al-Wathiqui, Cruz, Al-Wathiqui, & Rodríguez, 2014; Fowler-Finn & Rodríguez, 2012; Fowler-Finn & Rodríguez, 2016; Sattman & Cocroft, 2003).

2.5 | Behavioural assay: Measuring female preference

After placing a female on a testing plant, we allowed her two minutes to acclimate and then played back a series of primers to determine whether she was sexually receptive. Tests of receptivity can be confounded by variation in the likelihood of a female responding due to varying preference across temperatures. Thus, we designed the series of primers to account for variation in female preference. The primers comprised recordings of live males signalling at three different temperatures (21°C, 25°C and 33°C). Primers recorded from males at ~25°C matched the mean signal frequency of the species (~350 Hz). We played back the primer closest to the testing temperature first and then randomly ordered the other two (see Table S3). If a female responded to at least one of the primers, they were deemed sexually receptive.

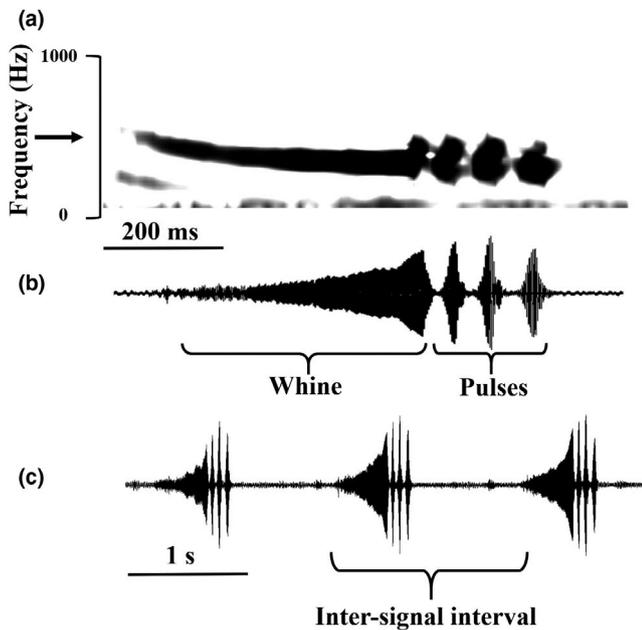


FIGURE 2 (a) a spectrogram of a male signal. The arrow shows the dominant frequency, which is 360 Hz for the pictured signal. (b) A waveform of the same male signal from above showing the two main parts of the call, the whine and the pulses. (c) A single signalling bout containing three signals including the signal pictured above

We next determined the mate preference of sexually receptive females by testing the responses of each female to a range of signal frequencies and then constructing a full mate preference function. From the full preference function for each female, we extracted the peak preference (Figure 3). We took advantage of the duetting system in *E. binotata* treehoppers to construct the mate preference function: males produce advertisement signals and females respond with their own duetting signal if they find the male signal attractive. We played back a series of signal bouts and recorded female responses as follows. We used the same set of stimuli for all populations: synthetic signals designed with all signal parameters set to the species means, except for signal frequency, which varied across bouts (see Fowler-Finn & Rodríguez, 2013; Mcnett & Coccoft, 2008; Rodríguez et al., 2004, 2006; Sattman & Coccoft, 2003). We generated bouts of six signals for 19 signal frequencies that varied from 215 to 485 Hz in increments of 15 Hz using a custom-written script (available upon request) in MATLAB (v. 8.3 2014) (Fowler-Finn & Rodríguez, 2012; Rodríguez et al., 2006). The signal bouts were randomly ordered and played back with 15 s of silence in between each signal bout. Females could respond zero to six times to each signal frequency, with the number of times she responded with a duetting signal indicating her level of preference for that signal frequency (Fowler-Finn & Rodríguez, 2013; Rodríguez et al., 2006) (Figure 3).

We next derived individual female mate preference functions from the raw response data using cubic spline regressions (Fowler-Finn & Rodríguez, 2013; Ritchie, 1996, 2000; Ritchie et al., 2001; Rodríguez, Hallett, Kilmer, & Fowler-Finn, 2013; Rodríguez et al.,

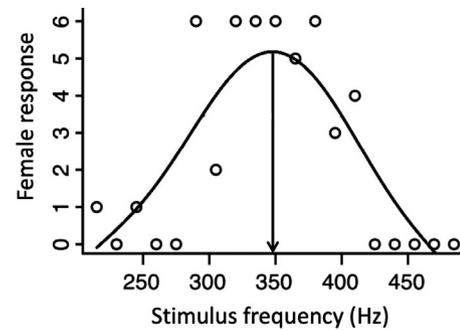


FIGURE 3 An example of a preference function where the data points represent whether or not the female responses across the 19 frequencies played back. A cubic spline is fitted over the responses with no assumption of shape and forms the female's preference function. The female's peak preference is extracted from the peak of the spline

2006; Simmons, Zuk, & Rotenberry, 2001; Symes et al., 2017). Cubic spline regressions make no assumptions about the shape of the curve other than that it is smooth (Schluter, 1988). To generate the splines, we used the Pfunc module executed with a Python GUI (Kilmer et al., 2017). The Pfunc module derives the spline from the raw data and measures peak preference from the curve, which is equivalent to the stimulus frequency at which females are most responsive to male signals (Fowler-Finn & Rodríguez, 2012; Fowler-Finn & Rodríguez, 2013; Rodríguez et al., 2012).

2.6 | Statistical analyses

Our analyses included the following steps: (a) testing for the effects of temperature on male signal frequency across populations, (b) testing for the effects of temperature on the frequency of female peak preference across populations and (c) testing for signal-preference temperature coupling, as well as predicted selection on male signals, within each population. We tested the effects of replicate plant on our analyses from 2015 and found it did not affect the results, so this factor was removed from the final analyses (see Table S4). For (a) and (b), we also tested for the effects of year on thermal sensitivity of male signals and female peak preference in the two populations in which we sampled across multiple years.

2.6.1 | Effects of temperature on male signal frequency across populations

We ran a multiple linear regression in JMP (JMP®, version 13. SAS Institute Inc., 1989–2018) with dominant signal frequency (Hz) as the response variable. The independent variables were temperature, population and a temperature \times population interaction. A significant temperature \times population interaction term indicates that temperature affects the frequency of male signals in a population-specific manner. We tested for the effect of year on male signal thermal reaction norms for the two populations for which

we tested males in more than one year (Gray Summit, MO, and Columbia MO) using a linear regression with temperature, year and year \times temperature interaction as independent variables; a significant interaction term would indicate the slope of the reaction norm differs between years.

2.6.2 | Effects of temperature on female peak preference across populations

We ran a multiple linear regression in JMP as above, except the response variable was the frequency of the female peak preference. Again, a significant temperature \times population interaction term indicates a population-specific effect of temperature on female peak preference. We tested for the effect of year on female peak preference thermal reaction norms for the three populations for which we tested females in more than one year (Gray Summit, MO, Columbia MO, and St. Louis, MO) using a linear regression with temperature, year and year \times temperature interaction as independent variables; a significant interaction term would indicate the slope of the reaction norm differs between years.

2.6.3 | Signal-preference temperature coupling

We tested the signal-preference temperature coupling hypothesis for each population independently by running multiple linear regressions in JMP with the frequency (of male signals and female peak preference) as the response variable. The independent variables were temperature, sex and the temperature \times sex interaction. The interaction term is the key variable for testing the signal-preference temperature coupling hypothesis: a significant temperature-by-sex interaction term would indicate nonparallel changes in the frequency of male signals and female peak preference across temperature and would reject the hypothesis. We

tested whether temperature coupling varied across years—for the two populations in which we tested both males and females in more than one year (Gray Summit, MO, and Columbia MO)—with two approaches. First, we tested whether the temperature \times sex interaction varied across years by building a linear regression with temperature, sex, year, temperature \times sex interaction and a temperature \times sex \times year interaction as the independent variables; the temperature \times sex \times year interaction term indicates that the degree of coupling differs across years. We then tested for temperature coupling within each year for each of the two populations by running a linear regression with data from both years included. The dependent variables were temperature, sex, year, temperature \times sex interaction. We note that the 2015 female sample from Gray Summit, MO, was small.

Finally, we calculated the effect size of the temperature \times sex interaction for each analysis for each population using the following equation $r = \sqrt{\frac{F}{F + df_{error}}}$.

3 | RESULTS

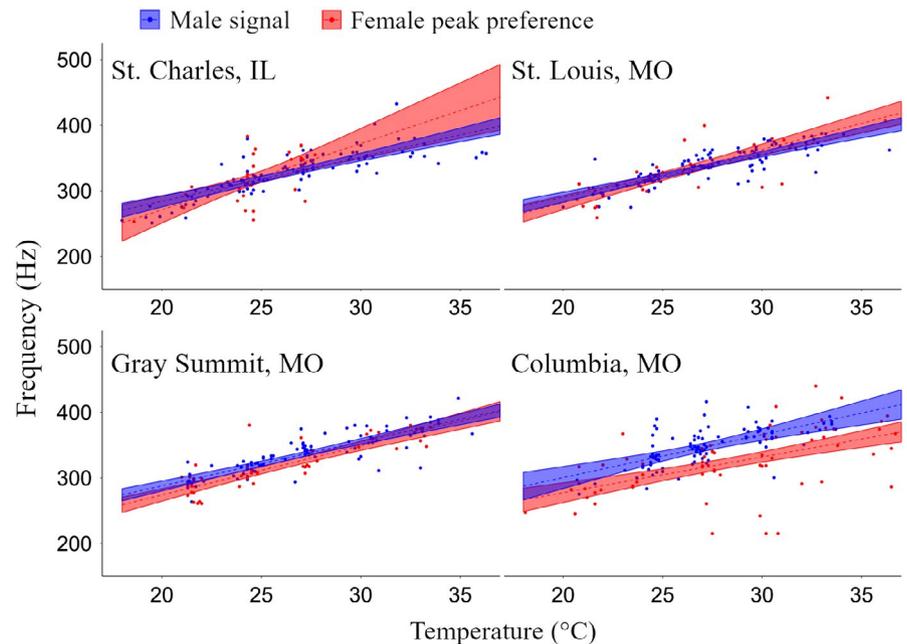
3.1 | Effects of temperature on male signal frequency across populations

Male signal frequency was strongly positively correlated to temperature in all four populations (Table 1; Figure 4). Populations varied in the mean signal frequency, indicated by a significant population term (Table 1; Figure 4). However, the thermal sensitivity of male signals did not vary across populations, as indicated by the non-significant temperature \times population interaction term (Table 1; Figure 4). Nonsignificant temperature \times year terms for the two populations for which we sampled male signals across multiple years indicate that the slope of the reaction norm did not change across years (Table S5).

A. Linear regression					
	Factor	F ratio	(df)	p-values	
Males N = 466	Temp	799.7	(1,458)	<.0001	
	Population	12.3	(3,458)	<.0001	
	Temp \times Population	0.3	(3,458)	.8145	
Females N = 203	Temp	80.3	(1,195)	<.0001	
	Population	3.2	(3,195)	.0240	
	Temp \times Population	2.8	(3,195)	.1098	
B. Post hoc analyses					
Population	LSM males	Tukey HSD	LSM females	Tukey HSD	Pairwise comparisons for females
Columbia, MO	344.9 Hz	B	321.7 Hz	A	B
St Louis, MO	336.0 Hz	A	339.1 Hz	A	A
Gray Summit, MO	332.3 Hz	A	326.4 Hz	A	AB
St Charles, IL	336.1 Hz	A	342.3 Hz	A	A

TABLE 1 A. Linear regressions testing the effects of temperature and population on the frequency of male signals and female peak preference in the four *Enchenopa binotata* populations studied. B. Least-square means (LSM) for the mean frequency of signals and preferences in each of the four populations. Different letters indicate significant differences between populations. For females, pairwise comparisons revealed differences between the Columbia, MO, populations and two other populations; St. Louis, MO, and St. Charles, IL

FIGURE 4 The y-axis shows both the dominant frequency of male signals (blue) and peak preference of females (red) as the x-axis for the four populations as a function of temperature: A. St. Charles, IL; B. St. Louis, MO; C. Gray Summit, MO; and D. Columbia, MO. Dashed lines indicate the mean frequency at a temperature, and shading encompasses the 95% confidence interval



3.2 | Effects of temperature on female peak preference across populations

Female peak preference was strongly positively correlated to temperature, with the mean frequency of peak preference varying across populations; thermal sensitivity of preference did not vary across populations, as indicated by a nonsignificant temperature \times population interaction term (Table 1; Figure 4). Nonsignificant temperature \times year terms for the three populations for which we tested female mate preferences across multiple years indicate that the slope of the reaction norm did not change across years (Table S5).

3.3 | Signal-preference temperature coupling

We found support for signal-preference temperature coupling in all four populations; the temperature \times sex interaction terms were not only nonsignificant; they were also of small effect for every population tested (Table 2; Figure 4). Mean male signal frequency matched the mean female peak preference in all but one population (Columbia MO: Table 2; Figure 4). In Columbia, MO, the significant sex term—coupled with a nonsignificant temperature \times sex interaction of small effect size ($r = 0.07$)—indicates that females consistently preferred signal frequencies that were lower than the male mean signal frequency across all temperatures (Table 2; Figure 4; Figure S3).

The nonsignificant temperature \times sex \times year term for both Gray Summit, MO, and Columbia, MO, indicates that temperature

coupling did not vary significantly across years (Table S6). However, when we analysed each year separately, our analyses revealed that Gray Summit, MO, shows some variation in the degree of temperature coupling across years: the 2016 data reject the hypothesis, with a significant temperature \times sex term of small-medium effect size (Table S7, Figure S3).

4 | DISCUSSION

We tested for signal-preference temperature coupling in *E. binotata* treehoppers collected from four populations. We found high thermal sensitivity of both male signals and female preferences, with a similar degree of thermal sensitivity across all populations despite inter-population variation in the mean frequency of signals and peak preferences. We also found overall strong support for the signal-preference temperature coupling hypothesis in all four populations even though signals were shifted lower than preferences in one population, with the exception of a slight decoupling in one population in one year. Our results suggest constraints on the thermal sensitivity of both signals and preferences. Additionally, our results suggest that temperature fluctuations should not affect the action of sexual selection, nor disrupt the coordination of mating, by generating signal-preference mismatches at thermal extremes.

A major consequence of signal-preference temperature coupling is the preservation of how sexual selection is predicted to act on male

TABLE 2 Linear regressions used to test the signal–preference temperature coupling hypothesis by testing the effects of temperature and sex on frequency (of signals for males and of peak preference for females) for each of the four populations of *Enchenopa binotata*. Significant Temp × Sex terms would reject the signal–preference temperature coupling hypothesis

Population	Columbia, MO (N = 276)		St Louis, MO (N = 131)		Gray Summit, MO (N = 133)		St Charles, IL (N = 129)	
Factor	$F_{(df)}$	<i>p</i>	$F_{(df)}$	<i>p</i>	$F_{(df)}$	<i>p</i>	$F_{(df)}$	<i>p</i>
Temp	227.2 _(1,272)	<.0001	226.2 _(1,127)	<.0001	307.8 _(1,129)	<.0001	65.0 _(1,125)	<.0001
Sex	46.9 _(1,272)	<.0001	0.8 _(1,127)	.3719	8.0 _(1,129)	.0054	1.8 _(1,125)	.1771
Temp × Sex	1.2 _(1,272)	.2760	2.8 _(1,127)	.0939	0.6 _(1,129)	.4278	2.5 _(1,125)	.1158
Effect size of Temp × Sex		.07		.15		.07		.14

Note: Bolded text indicate values that are statistically significant

signals in a thermally variable environment. In populations in which the mean frequency of signals and preferences match—the case for three of the four populations we sampled—temperature coupling is predicted to reinforce stabilizing selection on signals, such as that occurring through Fisherian selection (Fisher, 1930). In populations in which the mean male signal and female preference mismatch, temperature coupling would reinforce directional selection across temperatures—which would be expected in Columbia, MO, where the mean female peak preference averages ~30 Hz lower than the mean signal frequency. Sexual selection via female choice is important in shaping male signal variation in this clade (Rodriguez & Cocroft, 2006; Sullivan-Beckers & Cocroft, 2010), and male *E. binotata* signals exhibit broad-sense heritability (Fowler-Finn et al., 2015, 2018). Thus, if populations were to vary in the mean female preference, sexual selection across variable thermal environments could reinforce differential selection on male signals across populations, potentially contributing to rapid divergence across population (e.g. Bailey & Zuk, 2012).

Signal–preference temperature coupling could be present as a result of two potential mechanisms. First, selection could favour female preferences to match signals across temperatures (Beckers & Schul, 2008), for example to avoid mismating in regions of high sympatry with other species (Simmons et al., 2001) or to retain the ability to identify high-quality mates across variable environmental conditions (Beckers & Schul, 2008). Second, underlying physiological underpinnings that regulate signal production could similarly affect signal perception (Gerhardt, 1978; Greenfield & Medlock, 2007; Pires & Hoy, 1992; Ritchie et al., 2001). These two potential mechanisms for temperature coupling are difficult to distinguish (Greenfield & Medlock, 2007). However, we suggest physiological constraints likely play a role in temperature coupling in *E. binotata* because thermal sensitivity of signals and preferences was conserved across all populations despite variation in: patterns of sympatry with conspecifics, thermal variation, and shifts in mean signal and preference frequency relative to one another within one population. Furthermore, rearing conditions varied across some of the populations—including insects reared from wild-caught nymphs, eggs collected in dormant trees and mated females the fall prior—with little effect on the signal and preference reaction norms, with the exception of a lack of complete temperature coupling in the Gray Summit, MO, population in 2016.

Signals and preferences had higher variance at thermal extremes and female preferences demonstrated a much greater range of variation than male signals across all temperatures (females ~225 Hz vs. males ~185 Hz), with the slight deviation from temperature coupling in the Gray Summit, MO, 2016 being driven primarily by a mismatch between signals and preferences at the lower temperature extreme. Two major consequences may arise as a result of these patterns. First, the strength of selection could vary across temperatures, being weakest where females are most variable in their preferences (Cotton, Small, & Pomiankowski, 2006), that is, at thermal extremes. Second, depending on patterns of thermal sensitivity in sympatric heterospecifics, females may be more prone to making mistakes in mating decisions. The potential to prefer heterospecific signals in certain temperature ranges comes in part from high overlap with the mean signal frequencies of multiple species in the complex (Rodriguez et al., 2006).

Heterogeneity in the thermal environment and the thermal niches that insects occupy will be critical in determining how variation in temperature influences sexual selection and the coordination of mating. For example, behavioural thermoregulation (Bogert, 1949; Cowles & Bogert, 1944; Forsman, 2000; Huey, Peterson, Arnold, & Porter, 1989; Lillywhite, Licht, & Chelgren, 1973) could potentially affect male signals and how females evaluate those males (Willmer, 1991). We documented considerable variation in temperature in the field, with as much as 10°C difference from one branch to another on the same tree. Despite the wide range of potential temperatures document, we found few animals in the field at lower temperatures. As a result, any variation in signals or preferences generated at lower temperatures may be relatively unimportant. Second, variation in the temperature experienced by females at perch sites could generate greater variation in preferences, which could in turn weaken selection on male signals (Cotton et al., 2006). Equally important would be the thermal niche occupied by singing males relative to choosing females. For example, in Columbia, MO, females may consistently prefer males that sing from cooler parts of a tree, because females prefer signal frequencies below the mean signal frequency of the population. Further investigation of thermal niche use will be vital in understanding the full picture of how thermal variation affects sexual selection and the coordination of mating.

Here, we provide the first test of how temperature variation affects signals, preferences and signal–preference relationship in the most dominant form of acoustic communication—vibrational signalling. Further investigation into other vibrational signalers will help determine whether the patterns we observe are representative of how temperature affects acoustic communication more generally. Our population-comparative approach—in combination with variable rearing conditions within and across populations—lends support for physiological constraints underlying the strong temperature coupling we observed across four populations of treehoppers. As a result of signal–preference temperature coupling, we predict that changes in temperature means or variability would not significantly influence how sexual selection from female mate choice acts on male signals within a population, nor disrupt the coordination of mating. In an era where there are increasing concerns about how global warming will influence animals in a variety of contexts, these results provide some hope that animal populations will persist in the face of change.

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AUTHOR CONTRIBUTIONS

D.I.J. conceived and designed the project; acquired, analysed and interpreted the data; and wrote the manuscript. M.E.S., N.T.L. and A.M. acquired the data. K.D.F.F. conceived and designed the project; analysed and interpreted the data; and wrote the manuscript.

DATA ACCESSIBILITY

Data are made available at the Dryad Digital Repository <https://doi.org/10.5061/dryad.7m67bg9>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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