



Female chorus frogs delay mate choice under suboptimal environmental conditions

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Many traits important for reproductive isolation are environmentally responsive. However, most studies examining reproductive isolation do not explicitly take into consideration environmental variation. Temperature can have a particularly large effect on reproductive behaviours, especially in ectotherms. Here, we tested whether temperature affects the degree of reproductive isolation between the upland chorus frog, *Pseudacris feriarum*, and its congener *Pseudacris nigrita*. These two species engage in costly hybridization in nature, leading to reinforcement of male signals and female preferences in sympatry. However, male advertisement calls vary with temperature such that the difference between the advertisement calls of the two species is narrower at cold temperatures than at warm temperatures. To examine the effect of temperature on reproductive isolation in this system, we performed six binary choice preference trials using sympatric female *P. feriarum*. In these experiments, we acclimated females to either warm (20 °C) or cool (10 °C) temperatures and gave them a choice between heterospecific and conspecific advertisement calls appropriate for the temperature. We also conducted experiments where we gave females a choice between more similar stimuli at warm temperatures to test whether females have narrower preferences at cold temperatures. We found that females always had a significant preference for the conspecific advertisement call, regardless of temperature or signal similarity. However, females took twice as long to make a choice in suboptimal conditions. This time delay may lead to increased risk of hybridization, despite females' ability to discriminate between calls. Our results highlight the importance of considering environmental context when examining traits involved in reproductive isolation.

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Sexual communication is ubiquitous in nature, but the efficacy of signal transmission and reception can be affected by the environment. Factors such as temperature (Edmunds, 1963; Gerhardt, 1978; Heath & Josephson, 1970; Shimizu & Barth, 1996), humidity (Bossert & Wilson, 1963; Chaverri & Quirós, 2017; Regnier & Goodwin, 1977; YuYong et al., 2018), vegetation type (Brenowitz, 1986; Richards & Wiley, 1980) and other variables can affect visual, acoustic and chemical communication (Boughman, 2002; Cummings & Endler, 2018; Endler, 1992; Endler & Basolo, 1998; Endler et al., 1993). Consequently, under suboptimal or variable ecological conditions, individuals may choose lower-quality mates (Heuschele et al., 2009; Higginson & Reader, 2009; Kelly, 2018), hybridize with other species (Chunco, 2014; Pfennig, 2007; Tobler & Carson, 2010) or increase their risk of predation by requiring additional time to make mating

decisions (Cronin et al., 2019; Heuschele et al., 2009). Thus, environmental factors can exert strong selection on mate choice within and across species, potentially influencing the fitness of offspring and the stability of species boundaries.

Environmental conditions may also influence the type and intensity of interactions among species, particularly when a given factor affects species differently. In Darwin's finches (*Geospiza* sp.) optimal beak size evolves within and among species in response to availability of food resources. Thus, in drought conditions, increased competition can lead to strong divergent selection on beak size (Grant, 2006; Schluter et al., 1985). In the Ambon damselfish, *Pomacentrus amboinensis*, anthropogenic noise elevates predation risk as a result of increased stress and reduced response by the fish to predators (Simpson et al., 2016). Effects of the environment on predator–prey interactions have been widely documented in both aquatic and terrestrial systems (Chan et al., 2010; Kern & Radford, 2016; Morris-Drake et al., 2016; Spiga et al., 2017). Collectively, these studies suggest that as species

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respond differentially to ecological variables, the degree and nature of species interactions can shift as a direct effect.

For taxa undergoing speciation via reinforcement, the process through which prezygotic isolation between species is increased due to selection against maladaptive hybridization (Dobzhansky, 1940; Servedio & Noor, 2003), environmental factors have the potential to inhibit or enhance the evolution of reproductive barriers (Boughman & Servedio, 2022; Chunco, 2014; Owens & Samuk, 2020; Pfennig, 2007; Servedio & Hermisson, 2020). In spadefoot toads experiencing reinforcement, two interacting species (*Spea bombifrons* and *Spea multiplicata*) are unlikely to hybridize when water levels in breeding ponds are high (Pfennig, 2007). In shallower ponds, however, *S. bombifrons* frequently mate with heterospecifics (Pfennig, 2007). Ecological experiments show that hybrid offspring metamorphose more rapidly than pure *S. bombifrons* tadpoles, leading to adaptive hybridization by this species during drought conditions (Pfennig, 2007). Similar phenomena have been described in brown hares, *Lepus europaeus*, where such hybridization facilitates range expansions in unfavorable habitat at the edges of their distribution (Pohjoismäki et al., 2021), and in introduced African honey bees, *Apis mellifera scutellata*, where hybridization facilitates local adaptation in the novel environment of their introduced range (Calfee et al., 2020). In this way, environmental variation may slow species divergence or reverse existing genetic divergence in some systems. However, environmental variation may instead increase rates of maladaptive hybridization. Although some studies have investigated the interaction between environmental conditions and speciation, few have focused on how these factors directly affect the targets of selection during speciation by reinforcement: reproductive behaviours. Here, we quantify the effect of a particular environmental factor, temperature, on male acoustic mating signals and female preferences for these signals.

Studies in both ectotherms and endotherms have found that temperature can strongly affect intra- and interspecific communication (Brandt et al., 2020; Coomes et al., 2019; Doherty & Hoy, 1985; Gerhardt, 1978; Gerhardt & Mudry, 1980; Wu et al., 2021). Temperature influences the physiology of an organism, which in turn can affect its behaviour (Angilletta, 2009; Gillooly et al., 2001). Sexual signals in ectotherms have long been known to be influenced by temperature. One of the first examples to be discovered was the ‘thermometer cricket’, whose call is so tightly correlated to temperature that the ambient environmental temperature could be estimated from the cricket’s chirp pattern (Dolbear, 1897; Pires & Hoy, 1992). Temperature has a particularly strong influence on taxa that communicate through acoustic signals, since both spectral and temporal properties of acoustic signals can be affected. Additionally, the phenomenon of ‘temperature coupling’, whereby receiver preferences for a signal change in coordination with changes in the calls of a signaller across temperatures, has been documented in numerous taxa including frogs (Gerhardt, 1978; Gerhardt & Mudry, 1980) and insects (Brandt et al., 2020; Conrad et al., 2017; Doherty & Hoy, 1985; Pires & Hoy, 1992).

The rate at which signals shift across temperatures may vary among species (Jang & Gerhardt, 2006; Walker, 1957), possibly leading to variation in the degree of behavioural isolation between species across environmental temperatures. This phenomenon may play an important role in trait evolution within multispecies assemblages (Symes et al., 2017). For example, mating interactions and hybridization may be rare at some temperatures but common in others, depending upon how reproductive characters shift with temperature in different species. Little direct work has been done, however, to investigate how temperature affects the degree of reproductive isolation among members of species assemblages.

Anurans (frogs and toads) are an excellent system for addressing the effect of temperature on reproductive isolation, as acoustic signals are sensitive to temperature and key to species recognition in many taxa. Two species, the upland chorus frog, *Pseudacris feriarum*, and the southern chorus frog, *Pseudacris nigrita*, experience costly hybridization in the coastal plain of the southeastern United States where they form several distinct hybrid zones (Lemmon & Lemmon, 2010; Fig. 1). These species, like many other frog species, are explosive breeders, where males gather in large breeding aggregations (Vitt & Caldwell, 2013; Wells, 1977). Males produce a series of pulsed advertisement calls (Fig. 1b) that females use to make mating decisions (Lemmon, 2009). The calls increase in frequency (pitch) across the call as pulses become closer together. The number of pulses and the pulse rate within a call vary by species and population (Lemmon, 2009). Although males will call regardless of whether a female is present, they do orient towards an approaching female. Interestingly, *P. feriarum* displays a pattern of reproductive character displacement in advertisement calls, where sympatric males produce a higher pulse rate (pulses/s) signal in comparison to allopatric individuals (Lemmon, 2009; Fig. 1). Female *P. feriarum* also show divergent preferences for variation in advertisement calls, particularly in pulse rate (Lemmon, 2009). These patterns have been shown to be driven by reinforcement due to costly hybridization (Lemmon & Lemmon, 2010).

Additionally, allopatric and sympatric *P. feriarum* populations have different responses to temperature with respect to the pulse rate of male advertisement calls (Lemmon, 2009). Specifically, pulse rate changes more rapidly with increasing temperature in sympatric frogs than in allopatric frogs and in *P. nigrita* (Lemmon, 2009), leading to greater similarity between calls of the two species at low temperatures (close to 10 °C) than at high temperatures (close to 20 °C), which represents the range of temperatures at which they breed. Previous studies found that at a mid-range temperature (14 °C), the difference between mating calls of the two species in sympatry was sufficient for females to discriminate between species, but the difference between the allopatric *P. feriarum* call and the *P. nigrita* call were insufficient (Lemmon, 2009). However, since hybrids are found at rates of 1–2% in nature (Anderson et al., 2023; Banker et al., 2020), we hypothesized that temperature variation across different breeding events may explain the occurrence of hybrids in nature, despite strong discrimination against the heterospecific call by females in laboratory mate choice experiments. We chose to focus solely on the variable of pulse rate three reasons. First, because it is critical for species recognition and mate choice in this and other frog systems (Klump & Gerhardt, 1987; Lemmon, 2009; Lemmon & Lemmon, 2010; Ryan, 1991). Second, pulse rate is strongly affected by the body temperature of the male frog, which closely follows the environmental temperature (Gerhardt & Huber, 2002). Third, pulse rate shows reproductive character displacement in *P. feriarum* where it is sympatric with *P. nigrita* (Lemmon, 2009), suggesting that environmentally induced plasticity could influence speciation processes in this system. In contrast, dominant frequency is influenced to a lesser degree by temperature, but it does not differ between species; moreover, pulse number does differ between populations/species, but it is unaffected by temperature (Lemmon, 2009).

Here, we focus on two aims to disentangle the effects of temperature on mate choice and reproductive isolation in the chorus frog system. We conducted four experiments, as summarized in Table 1 and Fig. 2. An experiment is defined as the comparison of the results between two trials. First, we determined whether temperature affects species recognition (experiments A1 and A2). We tested the prediction that females would choose a heterospecific signal more frequently at low temperatures when male

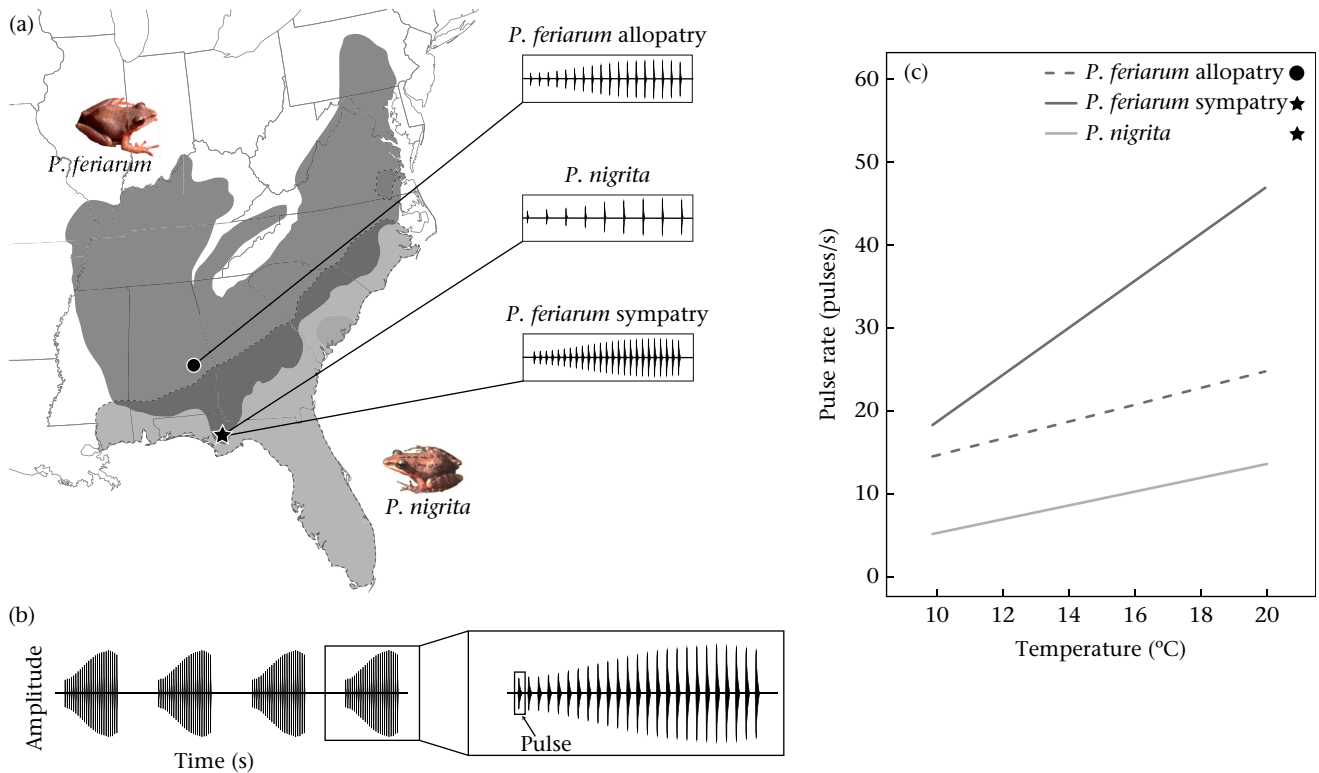


Figure 1. (a) Range map of *P. feriarum* and *P. nigrita*. The dark grey area shows the narrow region of range overlap between species; medium and light grey regions indicate allopatric *P. feriarum* and *P. nigrita* ranges, respectively. The sympatric site where females were tested is marked with a star and the reference allopatric site with a circle. Oscillograms of male advertisement calls for both species at approximately 16 °C are presented, demonstrating increased pulse rate for *P. feriarum* in sympatry relative to allopatry. (b) Example of an oscillogram of a *Pseudacris* calling bout. A single call is highlighted with a pulse identified. Pulse rate (pulses/s) was the trait of interest in the present study. (c) Temperature-corrected slopes for male advertisement call pulse rate versus temperature for allopatric and sympatric populations of *P. feriarum* and for *P. nigrita* taken from call data collected during 2009–2018 (E. M. Lemmon, personal communication). Advertisement call pulse rates between sympatric *P. feriarum* and *P. nigrita* are more similar at cold temperatures than at warm temperatures. Symbols correspond to localities on the map in (a).

advertisement calls are the most similar between the two species. Second, we determined whether females' ability to discriminate between fixed signal distance advertisement calls changes with temperature (experiments B1 and B2). To do so, we generated scaled advertisement calls for warm temperatures that created a fixed distance between the pulse rates of stimuli that matched the narrow difference in pulse rate at cold temperatures. We tested the prediction that females would make more mating errors between equidistant calls at warm temperatures than at cold temperatures because the true difference between mating calls is greater at warm temperatures. Additionally, for all experiments, we quantified the time required for a female to make a decision, as the time to choose may impact mating outcomes in large breeding choruses.

METHODS

Ethical Note

All procedures described here were approved by the Florida State University Animal Care and Use Committee (IACUC number PROTO202000029) and met published guidelines for ethical treatment of animals. Research permits to collect frogs from publicly owned lands were issued by the Florida Department of Fish and Wildlife. Females used in this study were toe-clipped and subsequently released at the site of capture.

Field Collection

We captured amplexed pairs of *P. feriarum* by hand during the breeding seasons of 2020 and 2021 in natural breeding ponds

within the Apalachicola National Forest (Liberty Co., FL, U.S.A.; [Supplementary Table S1](#)). We conducted all experiments on *P. feriarum* females from areas of sympatry with *P. nigrita*. We transported pairs from the field sites to our experimental testing trailer in Tallahassee, Florida, where we promptly separated females from the males and transferred them to small plastic 0.7-litre square tubs filled with water and debris and set to acclimate to the target temperature for at least 2 h prior to testing.

Male Acoustic Stimuli Synthesis

We digitally generated a total of 10 acoustic stimuli representing male mating calls that we used across these trials. Five stimuli represented the conspecific (*P. feriarum*), four of which approximated the population average pulse rate and one of which represented a more extreme *P. feriarum* pulse rate. Five stimuli represented the heterospecific (*P. nigrita*), four of which approximated the population average and one of which represented a more extreme *P. nigrita* pulse rate. We used the extreme pulse rate stimuli in the fixed signal distance discrimination experiment to represent calls where the absolute difference in pulse rate is constant across temperatures, as explained below. All stimuli are described in the [Appendix, Table A1](#).

For each stimulus, we synthesized each pulse in the call individually, because pulses vary in structure across a given call. We synthesized pulses using custom-made software (J. J. Schwartz, jschwartz2@pace.edu) with the following information: pulse rise time, pulse fall time, pulse rise time to half maximum amplitude, pulse fall time to half maximum amplitude, pulse duration,

Table 1
Summary of all binary choice trials conducted and their respective experiments

Test	Experiment	Trial	Description	Stimulus creation method	Stimulus 1 ‘Correct’		Stimulus 2 ‘Mistake’		Absolute difference (ΔI)	Proportional difference (ΔI/I)
					<i>P. feriarum</i>	Pulse rate	<i>P. nigrita</i>	Pulse rate		
Species discrimination	A1	1	Cold	Mode pulse number	ColdA	25.3	ColdA	7.0	18.3	0.72/2.6-fold
		2	Warm	Mode pulse number	WarmA	42.6	WarmA	17.2	25.4	0.60/1.5-fold
	A2	3	Cold	Pulse rate regression	ColdB	17.8	ColdB	5.9	11.8	0.67/1.4-fold
		4	Warm	Pulse rate regression	WarmB	45.2	WarmB	14.7	30.5	0.67/1.4-fold
Fixed signal distance	B1	3	Cold	Pulse rate regression	ColdB	17.8	ColdB	5.9	11.8	0.67/1.4-fold
discrimination	B2	5	Warm high pulse rate	Pulse rate regression	WarmB	45.2	Fast	35.0	10.2	0.23/0.3-fold
		3	Cold	Pulse rate regression	ColdB	17.8	ColdB	5.9	11.8	0.67/1.4-fold
		6	Warm low pulse rate	Pulse rate regression	Slow	25.7	WarmB	14.7	11.0	0.43/0.7-fold

An experiment consisted of two trials for which the results would be compared. A trial consisted of two stimuli between which the females could choose. The proportional difference is shown first as the ratio of the absolute difference between stimuli divided by stimulus 1 (the difference between the calls relative to the correct choice), then as a fold difference calculated as the ratio of the absolute difference to the slower call, stimulus 2 (how much faster stimulus 1 is compared to stimulus 2). In experiments A1 and A2, we tested whether females make more mistakes at cold temperatures due to the narrower difference between advertisement call pulse rate. In experiments B1 and B2, we tested whether females discriminate between calls with a fixed signal distance comparable to the magnitude of pulse rate difference between calls at cold temperatures. To do so, we scaled the pulse rate of either *P. feriarum* or *P. nigrita* advertisement calls as described. Visual depictions of these experiments can be found in Fig. 2.

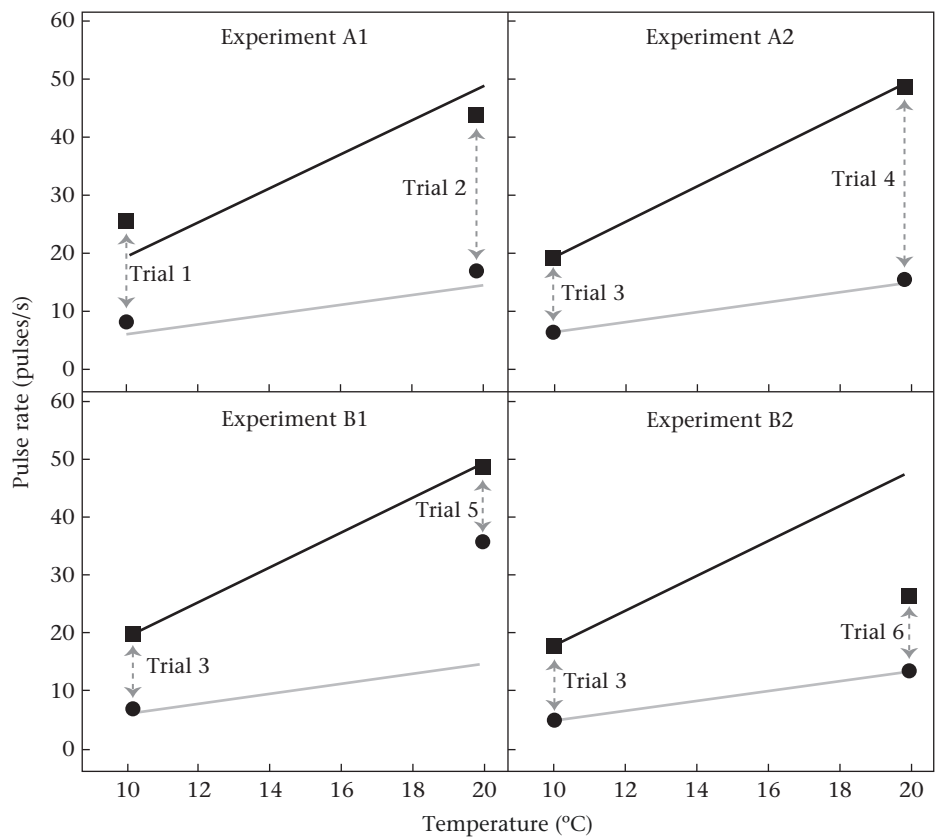


Figure 2. Stimulus pairs (1–6) used in binary choice trials plotted against the temperature regression slopes for sympatric *P. feriarum* and *P. nigrita* (as presented in Fig. 1c). Squares represent the conspecific stimulus (correct choice); circles represent the heterospecific stimulus (incorrect choice); dotted arrows connect stimuli used in a trial. Experiments A1 and A2 address the species recognition question. Experiment A1 consisted of a cold trial (trial 1) and a warm trial (trial 2). Experiment A2 consisted of a cold trial (trial 3) and a warm trial (trial 4). The difference between these experiments was the stimulus generation method (see Methods). Experiments B1 and B2 address the fixed signal distance discrimination question. Experiment B1 consisted of a cold trial (trial 3; from experiment A2) and an altered warm trial (trial 5), where the ‘*P. nigrita*’ pulse rate was artificially increased. Experiment B2 consisted of a cold trial (trial 3; from experiment A2) and an altered warm trial (trial 6), where the ‘*P. feriarum*’ pulse rate was artificially decreased. Further description can be found in Table 1.

fundamental frequency, dominant frequency, third harmonic frequency, relative amplitude of fundamental frequency, relative amplitude of third harmonic, maximum amplitude and relative amplitude of each pulse. To determine the values of these parameters to use for call synthesis, we identified the mode number of pulses for each species at each temperature using raw data from the

acoustic analysis software SoundRuler version 0.9.6.0 (<http://soundruler.sourceforge.net/>; reviewed by Bee, 2004), aligned homologous pulses of individuals with the mode number of pulses within each group and averaged each call parameter by pulse across individuals in order to generate the parameters typical of each population (following Lemmon, 2009). Additionally, we used

temperature- and species-appropriate dominant frequency for each pulse and generated a number of pulses that was species appropriate.

We assembled these pulses into calls using the appropriate interpulse intervals (spacing between pulses; IPI). The IPI is directly related to the pulse rate and call duration for a given stimulus and varies directly with temperature (Lemmon, 2009). We assembled the pulses into calls using two different methods of approximating the IPI: mode pulse number (used for trials 1–2 in experiment A1) and pulse rate regression (used for trials 3–6 in all other experiments described below). For the mode pulse number method, we used the pulse rate and associated IPI of the calls with the mode number of pulses for each target temperature derived from recordings available through 2009. For the pulse rate regression method, we used the pulse rate and associated IPI predicted by the temperature correction slope derived from call recordings through 2021. For stimuli with an ‘adjusted pulse rate’, we adjusted the IPI proportions to represent the target pulse rate as listed in Table 1. For all experiments, acoustic stimuli within a species only varied with respect to pulse rate; we held all other variables constant.

Female Preference Trial Protocol

In the female preference experiments, we tested females in binary choice trials at two different temperatures (Cold = 10 °C, Warm = 20 °C) following a protocol expanded from Lemmon (2009). We conducted a total of six trials, the results of which we compared to draw conclusions in our two experiments (Table 1, Fig. 2). During a trial, we placed a female in the centre of a 1.2 m diameter plastic pool containing a bamboo grid at the water's surface (to facilitate the frog's movement). We filled the pool approximately 7.6 cm deep with pond water and placed field speakers (Mineroff SME-AFS Amplified Field Speaker) on opposing sides. We played stimuli 1 s apart in an alternating manner from the speakers until a female responded with a choice (scored when she swam to and touched the front of the speaker), timed out at 30 min, or disqualified herself by climbing out of the pool. Across females, we randomized which call played first and which call played from the left speaker, to control for precedence effects and directional bias (Appendix, Table A2). We monitored temperature constantly and manually maintained the pool water at the target temperature by adding ice or hot water to the pool water between trials as needed. We did not begin a new trial until the target temperature was reached evenly throughout the pool and there were no more ice cubes remaining.

For the experiments, we randomly assigned females to the two temperature treatments and acclimated them to the target temperature for 2 h or longer in either a wine cooler (for 10 °C Cold trials) or a warmed cabinet (for 20 °C Warm trials). If a female successfully responded at her first assigned temperature, we reacclimated her to the second temperature treatment for at least 2 h and tested her again. For each trial, we recorded the temperature of the female's acclimation chamber, the length of the acclimation period, the temperature of the water in her testing chamber, the start time of her trial, the time she left her acclimation container once the trial began, the time at choice and the speaker she chose. From this information, we determined the response time (or time-to-choice) as the time at choice minus the start time and converted this time to seconds. Following testing, we toe-clipped females for a genetic sample and released them at their site of capture. In total, we conducted 318 trials on 125 females. Of these, 58 females responded in a total of 129 trials. For all trials, we report the percentage of correct choices (choosing the *P. feriarum* or *P. feriarum*-like call) compared to the percentage of mistakes (choosing the *P. nigrita* or *P. nigrita*-like call). Females that were used in repeat trials were either tested in

trials 1–2 or trials 3–6. Females repeated in trials 3–6 participated in up to all four trials if responsive.

Species Discrimination Experiments

To determine whether temperature affects discrimination between species, we conducted two experiments (A1 and A2), which consisted of binary choice trials with female *P. feriarum* at each of the two target temperatures (four trials total). The difference between these experiments is the method used to estimate the average call for each species at each target temperature, as described above. We used synthetic average calls for each species with pulse rate adjusted via regression analysis of field-recorded calls to the target temperature (Table 1). For Cold trials (trials 1, 3), we gave females a choice between the average conspecific (*P. feriarum*) call and the average heterospecific (*P. nigrita*) call at 10 °C. For the Warm trials (trials 2, 4), we offered females a choice between the average conspecific call and the average heterospecific call at 20 °C. We conducted trials 1 and 2 in 2020 and trials 3 and 4 in 2021.

Fixed Signal Distance Discrimination Experiment

We conducted two additional experiments to further disentangle how temperature may be driving signal divergence in this system. For experiments B1 and B2, we added trials at 20 °C, (Warm trials 5 and 6; Table 1) to determine whether temperature affects discrimination between pairs of acoustic stimuli that differ in pulse rate by the same magnitude. Here, we set a fixed signal distance in pulse rate between the Warm temperature stimuli to be equal to the difference between the Cold temperature stimuli in trial 3 (Table 1, Fig. 2). Thus, trials 5 and 6 employed more similar stimulus pairs than females would encounter in nature at 20 °C and correspond to cases where either the heterospecific has a higher pulse rate than normal (trial 5) or the conspecific has a lower pulse rate than normal (trial 6). Experiments B1 and B2 differed from one another in the species whose stimulus we adjusted to obtain this fixed signal difference (Table 1, Fig. 2). As experiment B2 did not include a preferred sympatric male *P. feriarum* call, we predicted that females would make more mistakes.

In trial 5, we gave females a choice between the average call of her species at 20 °C (45.2 pulses/s; *P. feriarum*) and the heterospecific call (*P. nigrita*) with pulse rate artificially adjusted upward to 35.0 pulses/s. All variables besides pulse rate of the *P. nigrita* call matched the average 20 °C values (same as in trial 4 above). In trial 6, we gave females a choice between the average heterospecific call (14.7 pulses/s; *P. nigrita*) and a conspecific call (*P. feriarum*) with pulse rate artificially adjusted downward to 25.7 pulses/s. All other variables of the *P. feriarum* call matched the normal 20 °C values (same as in trial 4). We conducted all trials for this experiment in 2021.

Statistical Analyses

We conducted all statistical tests in R 4.2.0 (R Core Team, 2022). To determine whether we would have enough power to detect our hypothesized effect size of temperature on choice, we conducted simulations using the standard method to estimate power for data that would be analysed with generalized linear mixed models (Green & MacLeod, 2016; Johnson et al., 2015). We hypothesized that females in the Warm condition would choose their own species 81% of the time and females in the Cold condition would choose their own species only 61% of the time, based on preliminary data and past female preference studies in *P. feriarum* (Lemmon, 2009). This gave us a hypothesized effect size of 2.73

based upon the odds ratio of correct to incorrect choices in the two conditions. We then estimated the power of our experiment using the simulation feature in the R package *simR* (Green & MacLeod, 2016). This package takes a given effect size and simulates data given that effect size. It then determines power by calculating the percentage of the time that the data correctly rejects the null hypothesis of the model given. To estimate power for our target sample size, we assumed that a total of 50 females would be tested at each temperature, with 30 of the females tested in both temperatures. In this scenario, half of the females would have been tested under one stimulus (corresponding to experiment A1) and half of the females would have been tested under a second stimulus (corresponding to experiment A2). This is approximately the number of females we tested across both sets of stimuli in the species recognition experiment (experiment A). In our model used for simulation, we set female identity as a random effect and stimulus and temperature as fixed effects. We set the effect size of temperature to 2.73 and estimated the power to be 96.10% (94.71–97.21), which is above the accepted threshold of 80% power set in most studies. Of the 1000 simulations, 68 of them produced warnings in which the simulated data fitted the model poorly.

To determine whether there was an effect of temperature on species recognition, we constructed generalized linear mixed models (GLMM) using the binomial distribution in the R package *lme4* (Bates et al., 2015). We examined the effects of temperature, stimuli and their interaction. We included female identity as a random intercept as we tested females in multiple trials. Next, we used a GLMM with a gamma distribution to determine whether temperature affected the response time. Similarly, we examined the effect of temperature and stimuli on the response time with female identity included as a random effect variable. Since chorus frogs breed in large aggregations where males engage in scramble competition, we also examined the effect of response time (in minutes) on choice using a GLMM with a binomial distribution and female identity included as a random intercept. We performed model selection for all tests using Akaike's information criterion for small samples (AICc) within the R package *AICcmodavg* (Mazerolle, 2016). If two models were equally fit to the data, we used the 'modavg' function in *AICcmodavg* (Mazerolle, 2016) to estimate model average effects.

To determine whether female signal discrimination between equidistant signals changed with temperature, we fitted additional GLMMs in *lme4* (Bates et al., 2015). We examined the effect of trial on choice, which encompassed the effects of temperature and direction of reduced difference between stimuli. We again included female identity as a random intercept. We then employed a GLMM with a gamma distribution to determine whether test affected the response time. Similarly, we examined the effect of test on the response time with female identity included as a random effect variable. We also included trial 4 in this analysis to compare the fixed signal distance data to the natural warm data. Again, we also examined the effect of response time (in minutes) on choice using a GLMM with a binomial distribution and female identity included as a random intercept. We performed model selection for all tests using Akaike's information criterion for small samples (AICc) within the R package *AICcmodavg* (Mazerolle, 2016). If two models were equally fitted to the data, we used the 'modavg' function in *AICcmodavg* (Mazerolle, 2016) to estimate model average effects.

RESULTS

Species Discrimination

The goal of the species discrimination experiments was to determine whether temperature affects the ability of females to

discern between conspecific and heterospecific signals. In experiment A1, 27 of 49 females responded in the Cold trial (trial 1) and 26 of 34 females responded in the Warm trial (trial 2). Of these, 25 females responded in both trials. In experiment A2, 27 of 42 females responded in the Cold trial (trial 3) and 26 of 29 females responded in the Warm trial (trial 4). Of these, only 11 females responded in both trials (Supplementary Table S2).

We did not find evidence that temperature reduces a female's ability to discern between the advertisement calls of her own species versus another species. The model with the lowest AICc score was the null model, but this was indistinguishable from models including solely the effect of temperature or stimulus (both $\Delta\text{AICc} < 2$) (Table 2, Fig. 3a). Therefore, we estimated the model-averaged effects. Neither the effect of temperature (95% confidence interval (CI) estimate: $-1.53, 0.93$; generalized linear mixed model: $N = 70$) or stimulus (95% CI estimate: $-1.61, 7.08$; generalized linear mixed model: $N = 70$) were significantly different from zero (Table 2). Females in the Cold trials chose the conspecific call 74% and 96% of the time (trials 1 and 3, respectively). Females in the Warm trials chose the conspecific call 81% and 96% of the time (trials 2 and 4, respectively).

We did, however, find that female response time was approximately double in cold temperature experiments compared to warm temperature experiments. The model with the lowest AICc score was the model including only an effect of temperature, but this was indistinguishable from the model including both temperature and stimulus ($\Delta\text{AICc} < 2$) (Table 2, Fig. 4). Therefore, we estimated the model-averaged effects. The effect of temperature (95% CI estimate: $-0.89, -0.43$; generalized linear mixed model: $N = 70$) was substantive, but the effect of stimulus (95% CI estimate: $-0.45, 0.19$; generalized linear mixed model: $N = 70$) was not. On average, we observed that females in the Cold trials took twice as long to make a choice compared to females in the Warm trials (Fig. 4). However, response time was not a strong predictor of choice (Appendix, Table A3).

Fixed Signal Distance Discrimination

The aim of the fixed signal discrimination experiments was to determine whether temperature affects the ability of females to distinguish between stimuli that differ by the same magnitude (signal distance). In these experiments (B1 and B2), 27 females responded in the Cold trial (trial 3) and 25 responded in both the Warm high pulse rate (trial 5) and Warm low pulse rate (trial 6) trials. Of these, 13 responded in all three tests and 11 responded in only the two Warm trials (Supplementary Table S2). For trial 5, we had a response rate of 25 responding of 26 females tested. For trial 6, we had a response rate of 25 responding of 46 females tested.

We did not find support for our hypothesis that females make a 'mistake' choosing the slower pulse rate call at warm temperatures when given a choice between two more similar stimuli. The model with the lowest AICc score included an effect of choice, but this was indistinguishable from the null model ($\Delta\text{AICc} < 2$) (Table 2, Fig. 3b). Therefore, we estimated the model-averaged effects. The effect size of the Warm high pulse rate trial (95% CI estimate: $-4.86, 2.6$; generalized linear mixed model: $N = 43$) and Warm low pulse rate trial (95% CI estimate: $-8.1, 0.48$; generalized linear mixed model: $N = 43$) were not significantly different from zero. Females in the Cold trials chose the conspecific call 96% of the time (trial 3), females in the Warm high pulse rate trial chose the correct call 90% of the time (trial 5) and females in the Warm low pulse rate trial chose the correct call 74% of the time (trial 6). Overall, there was a tendency for females to make more mistakes when given a narrower choice at warm temperatures, but this was not a significant effect.

Table 2
Species discrimination test results

Response	Model effects	K	AICc	ΔAICc	AICc weight	Log likelihood
Choice	(1 FieldID)	2	73.05	0.00	0.37	−34.47
	Stimulus + (1 FieldID)	3	73.44	0.39	0.3	−33.6
	Temperature + (1 FieldID)	3	74.78	1.73	0.16	−34.27
	Temperature + Stimulus + (1 FieldID)	4	75.23	2.18	0.12	−33.42
	Temperature × Stimulus + (1 FieldID)	5	77.39	4.34	0.04	−33.39
Response time	Temperature + (1 FieldID)	4	1481.37	0.00	0.61	−736.49
	Temperature + Stimulus + (1 FieldID)	5	1482.96	1.58	0.28	−736.18
	Temperature × Stimulus + (1 FieldID)	6	1484.82	3.45	0.11	−735.99
	(1 FieldID)	3	1505.85	24.47	0.00	−749.81
	Stimulus + (1 FieldID)	4	1507.69	26.32	0.00	−749.65

AICc model selection results for the effect of temperature and stimulus on choice and response time (time to choice) for experiments A1 and A2. Models are ranked from best (lowest ΔAICc) to worst (highest ΔAICc).

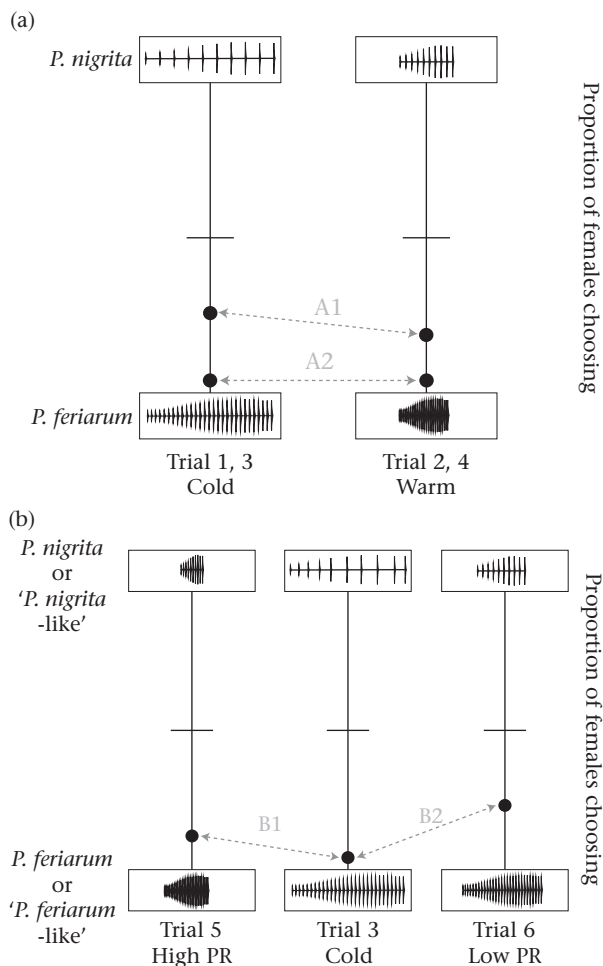


Figure 3. Results for both female preference experiments conducted in sympatric *P. feriarum* females. (a) Species recognition experiments A1 versus A2. (b) Fixed signal distance discrimination experiments B1 versus B2. The solid line denotes the point where 50% of females choose each stimulus. Shown are the stimuli used and the proportion of females choosing the conspecific stimulus for each trial (*P. feriarum* or *P. feriarum*-like). Dotted arrows connect trials compared for each experiment.

Additionally, we found that females took twice as long to make a decision when neither stimulus presented was an attractive call. The model with the lowest AICc score included the effect of trial (Table 2, Fig. 4). The Warm low pulse rate trials did not have a significant effect compared to the Cold trials (95% CI estimate: −0.35, 0.33; generalized linear mixed model: $N = 43$).

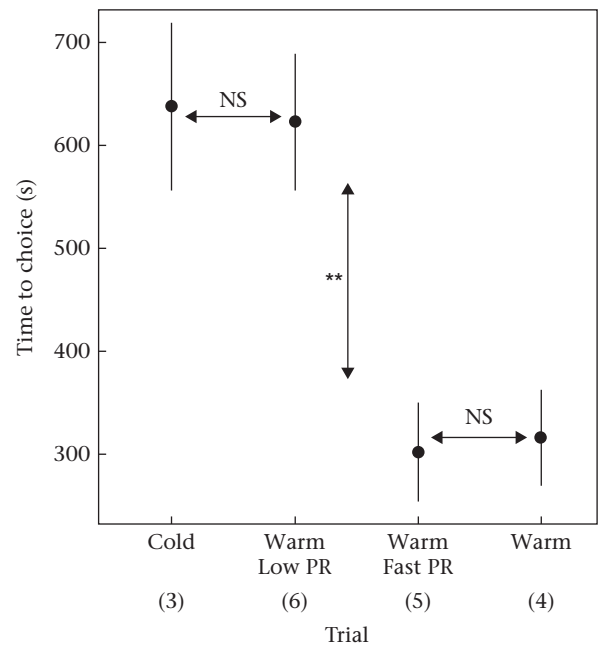


Figure 4. Mean $\pm$ SD time to choice for sympatric *P. feriarum* females in trials 3–6. Trials 1–2 are not shown because they did not differ from trials 3–4, respectively. Arrows show significance in time comparisons between trials. **ΔAICc > 2.

However, both the Warm trials (95% CI estimate: −1.14, −0.45; generalized linear mixed model: $N = 43$) and the Warm high pulse rate trials (trial 5) (95% CI estimate: −1.19, −0.49; generalized linear mixed model: $N = 43$) were significantly different from the Cold trial response time, although their estimates did not differ. Females in the Cold trials and in the Warm low pulse rate trials took longer to make a choice than females in the Warm trials and the high pulse rate trials. However, response time was not a strong predictor of choice (Appendix, Table A3).

DISCUSSION

We found no support for our prediction that females choose heterospecifics more frequently at low temperatures where male advertisement calls are more similar between species (Table 2, Fig. 3). Additionally, we found that females' ability to discriminate between calls that differed by the same magnitude did not significantly decline with increasing temperature (Table 3, Fig. 3). Furthermore, we demonstrated that females required significantly more time to choose between stimuli at cold temperatures than at

Table 3
Fixed signal distance discrimination test results

Response	Model effects	K	AICc	ΔAICc	AICc weight	Log likelihood
Choice	Trial + (1 FieldID)	4	56.36	0	0.7	−23.91
	(1 FieldID)	2	58.08	1.72	0.3	−26.96
Response time	Trial + (1 FieldID)	5	1468.61	0	1	−729
	(1 FieldID)	2	1488.6	19.99	0	−742.24

AICc model selection results for the effect of trial on choice and response time (time to choice) for experiments B1 and B2. Models are organized from best (lowest ΔAICc) to worst (highest ΔAICc). The time to choice analyses also include trial 4 for comparison.

warm temperatures or when neither stimulus was attractive. Our results suggest that females compensate for the similarities in calls at cold temperatures, requiring more time to make a choice. These results have important implications for mating outcomes in large breeding aggregations at different temperatures.

Potential explanations for how females compensate for temperature-dependent acoustic differences are derived from neurophysiological studies of how these signals are processed in the auditory midbrain. The ability of females to parse temporal properties of acoustic signals has been attributed to a class of interval-counting neurons in the anuran inferior colliculus. These neurons are tuned to specific pulse rates and only fire after a threshold number of pulses is transmitted at the appropriate pulse rate. Through this mechanism, females are able to discriminate between species- and population-specific pulse rates (Edwards et al., 2002; Rose et al., 2011, 2015). Neurons associated with auditory processing in anuran amphibians have been found to be temperature dependent in their selectivity for temporal properties, which correlates with the temperature-dependent shifts in male mating call traits (Brenowitz et al., 1985; Rose et al., 1985). Therefore, it is possible that the effect of temperature on neural processing matches the effect of temperature on male advertisement calls. Further investigation into the temperature dependency of temporal processing in populations experiencing different selection regimes is needed.

Our results also provide some support for Weber's law, which posits that the receiver response is determined by the proportional difference, defined as a fold difference, rather than the absolute difference between two signals (Ekman, 1959; Forrest, 1994). We focused on absolute differences in this study, but previous studies have found that proportional differences may be more salient to females (Gerhardt & Doherty, 1988; LaBarbera et al., 2020; Ryan & Cummings, 2013; Ryan et al., 2019). Based on a review of the literature, Littlejohn (2001) posited that anurans require an approximately two-fold difference in pulse rate to discriminate between calls. Gerhardt and Doherty (1988), however, found that grey treefrogs, *Hyla versicolor*, which belong to the same treefrog family as *Pseudacris*, need only a 1.2-fold difference to distinguish between calls (Gerhardt & Doherty, 1988). Table 1 details the proportional differences between our stimuli.

In our study, Weber's law or the effect of temperature on neural processing may explain why females did not make more mistakes at low temperatures, where the absolute difference was smaller than at high temperatures, because the proportional difference was equivalent (Table 1). Curiously, however, it does not explain why females made more mistakes in trial 6 than in trial 5, as the proportional difference between the calls in trial 6 was much larger than the proportional difference between the calls in trial 5. A more likely explanation for why females made more mistakes in trial 6 is that, in this trial, both calls were not desirable to the females. While Weber's law has been well studied in various taxa and for different signal modalities (Cheng et al., 1999; Dixit et al., 2021, 2022; LaBarbera et al., 2020; Nachev et al., 2013), our results are among the first to suggest a role for proportional processing in

reinforcement of reproductive characters to facilitate species recognition and strengthen reproductive isolation. Further exploration of how proportional processing facilitates species divergence under different environmental conditions could further our understanding of the role of Weber's law in speciation.

While females did not make more errors at low temperatures, they did spend twice as long sampling before making a choice (Table 3, Fig. 4). Chorus frogs are explosive breeders that gather in large densities at suitable breeding ponds in leks, similar to many other frog species (Vitt & Caldwell, 2013; Wells, 1977). Like other chorus frog species, males will gather at varying densities on nights following rain for several months each year, while females only frequent ponds in large numbers on a few nights per year (Caldwell, 1987; Whitaker, 1971). During large breeding events, males greatly outnumber females and thus can diminish the strength of female choice on mating outcomes, as males participate in scramble competition and can amplex a female before she can exert her choice (Caldwell, 1987; Whitaker, 1971). Therefore, the longer a female spends exploring the pond and sampling potential mates, the more likely she is to be monopolized by a male not of her own choosing. When considering sexual selection and mate choice, it is important to determine the likelihood of choice influencing mating outcomes and reproductive success. In situations such as large breeding choruses, female preferences and mate choice may be less important than male–male competition and, therefore, mating preferences may only matter for small choruses (Arak, 1983; Gerhardt, 1991; Telford et al., 1989; Wong & Candolin, 2005). Additionally, chorus noise can alter female discrimination of male signals as well as female mate choice, which may compound the effects of temperature (Coss et al., 2021; Gerhardt & Klump, 1988; Schwartz et al., 2001; Wollerman & Wiley, 2002). Our work suggests that more research is needed to investigate how cold environmental temperatures affect mating outcomes by weakening the effect of choice as opposed to altering choice itself.

Physiological limitations and adaptation are likely a partial explanation of our results. Frogs are ectotherms, and it is well established that environmental temperatures affect muscle movements, and that colder frogs move more slowly. Additionally, auditory neural processing occurs more slowly in colder individuals, likely increasing their time to respond to a stimulus (Rose et al., 1985; Stiebler & Narins, 1990), as found in our study. Females in the cold temperature trials, however, often took indirect paths towards their preferred stimuli (M. Dye, personal observation), which suggests difficulty in sound localization as well as delayed neural processing. In frogs, sound localization occurs via binaural cues and the perception of pressure differences, where moving towards a sound reduces the difference in time between sound arriving at the different ears, but moving away from the sound increases the difference in arrival at the different ears (Feng & Capranica, 1978; Feng & Shofner, 1981). Therefore, although females may be able to distinguish between species at cooler temperatures, their ability to localize sound sources may be negatively affected by temperature, which increases the time a female spends circling the breeding pond before making a choice.

As temperatures warm and seasonal weather becomes more variable, it is important to consider how changing environmental conditions may affect animal behaviour and evolutionary processes. Shifts in sexual signalling and acoustic communication as the climate warms have already been posited as an important outcome of climate change as well as a measure of ecosystem change (Krause & Farina, 2016; Sueur et al., 2019). Shifts in acoustic signals due to climate warming have been documented in frogs (Calabrese & Pfennig, 2022; Narins & Meenderink, 2014), marine organisms (Duarte et al., 2021), insects (Larson et al., 2019) and birds (Møller, 2011). This finding is not surprising, since attributes of male signals in frogs and other ectotherms are often positively correlated with environmental temperature (Gerhardt & Huber, 2002). These environmentally induced changes may affect interactions within communities, for example, by compressing, expanding or shifting the acoustic niche space available to species. In this way, environmental factors may significantly influence the assembly and long-term maintenance of species communities and the integrity of species boundaries.

Temperature has been implicated to play an important role in sexual selection across the tree of life, playing a role in both intersexual mate choice and intrasexual competition (García-Roa et al., 2020). Our work adds to a growing body of literature that suggests that temperature-dependent plasticity in key mating traits may affect levels of reproductive isolation in varying ways for both endotherms and ectotherms. Temperature may influence reproductive isolation by altering breeding phenology (Bründl et al., 2020; Forchhammer et al., 1998; Imlay et al., 2018; Shutt et al., 2019) and chemical signals (Groot & Zizzari, 2019) in addition to acoustic signals. Our findings also suggest that greater differences in reproductive traits are essential for species discrimination under warmer environmental conditions, as found in other systems (Coomes et al., 2019; Greenfield & Medlock, 2007; Jang & Gerhardt, 2007; Matute et al., 2009). For these reasons, the environmental context of reproductive isolation must be considered to gain greater insight into the processes driving speciation in a changing world.

Conclusions

Our results indicate that females are not more likely to choose heterospecific mates at low temperatures at which calls are more similar, but they do require substantially more time to make a choice at this end of the environmental spectrum. Extended decision time may in turn lead to undesirable mating outcomes such as increased hybridization, since a female that spends more time sampling potential mates in breeding aggregations is less likely to exert her choice (Arak, 1983; Telford et al., 1989; Wollerman & Wiley, 2002). We also found that females did not make more mating errors when given an equidistant choice test at warm temperatures than at cold temperatures, but they required significantly more time to choose between extreme calls that fell outside the trait distribution of their species compared to equidistant calls that included a stimulus with a species' average value. Collectively, these results suggest that environmental temperature plays a nontrivial role in mate choice decisions of chorus frogs undergoing speciation by reinforcement. Our findings emphasize the importance of uncovering the environmental context of species interactions to understand the evolutionary processes promoting behavioural divergence and speciation.

Author Contributions

Mysia Dye: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – Original

draft, Writing – Review & editing, Visualization, Project administration. **Alan R. Lemmon:** Methodology, Validation, Formal analysis, Investigation, Writing – Review & editing, Visualization. **Emily Moriarty Lemmon:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing – Review & editing, Funding acquisition.

Data Availability

Data for this study are available as [Supplementary Material](#).

Declaration of Interest

None.

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Supplementary Material

Supplementary material associated with this article is available in the online article at <https://doi.org/10.1016/j.anbehav.2023.11.012>.

References

- Anderson, C. B., Ospina, O., Beerli, P., Lemmon, A. R., Banker, S. E., Hassinger, A. B., Dye, M., Kortyna, M. L., & Lemmon, E. M. (2023). The population genetics of speciation by cascade reinforcement. *Ecology and Evolution*, 13(2), Article e9773. <https://doi.org/10.1002/ece3.9773>
- Angilletta, M. J., Jr. (2009). *Thermal adaptation: A theoretical and empirical synthesis*. Oxford University Press.
- Arak, A. (1983). Sexual selection by male–male competition in natterjack toad choruses. *Nature*, 306(5940), Article 5940. <https://doi.org/10.1038/306261a0>
- Banker, S. E., Lemmon, A. R., Hassinger, A. B., Dye, M., Holland, S. D., Kortyna, M. L., Ospina, O. E., Ralicki, H., & Lemmon, E. M. (2020). Hierarchical hybrid enrichment: Multitiered genomic data collection across evolutionary scales, with application to chorus frogs (*Pseudacris*). *Systematic Biology*, 69(4), 756–773. <https://doi.org/10.1093/sysbio/syaz074>
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1), 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Bee, M. A. (2004). Equipment Review: Sound Ruler: A free, open code, multi-platform sound analysis and graphing package. *Bioacoustics*, 14(2), 171–178. <https://doi.org/10.1111/ele.13970>
- Bossert, W. H., & Wilson, E. O. (1963). The analysis of olfactory communication among animals. *Journal of Theoretical Biology*, 5(3), 443–469. [https://doi.org/10.1016/0022-5193\(63\)90089-4](https://doi.org/10.1016/0022-5193(63)90089-4)
- Boughman, J. W. (2002). How sensory drive can promote speciation. *Trends in Ecology & Evolution*, 17(12), 571–577. [https://doi.org/10.1016/S0169-5347\(02\)02595-8](https://doi.org/10.1016/S0169-5347(02)02595-8)
- Boughman, J. W., & Servedio, M. R. (2022). The ecological stage maintains preference differentiation and promotes speciation. *Ecology Letters*, 25(4), 926–938. <https://doi.org/10.1111/ele.13970>
- Brandt, E. E., Rosenthal, M. F., & Elias, D. O. (2020). Complex interactions between temperature, sexual signals and mate choice in a desert-dwelling jumping spider. *Animal Behaviour*, 170, 81–87. <https://doi.org/10.1016/j.anbehav.2020.10.010>
- Brenowitz, E. A. (1986). Environmental influences on acoustic and electric animal communication. *Brain, Behavior and Evolution*, 28(1–3), 32–42. <https://doi.org/10.1159/000118690>
- Brenowitz, E. A., Rose, G., & Capranica, R. R. (1985). Neural correlates of temperature coupling in the vocal communication system of the gray treefrog (*Hyla versicolor*). *Brain Research*, 359(1), 364–367. [https://doi.org/10.1016/0006-8993\(85\)91452-0](https://doi.org/10.1016/0006-8993(85)91452-0)

- Bründl, A. C., Sallé, L., Lejeune, L. A., Sorato, E., Thiney, A. C., Chaine, A. S., & Russell, A. F. (2020). Elevational gradients as a model for understanding associations among temperature, breeding phenology and success. *Frontiers in Ecology and Evolution*, 8, Article 563377. <https://doi.org/10.3389/fevo.2020.563377>
- Calabrese, G., & Pfennig, K. S. (2022). Climate change alters sexual signaling in a desert-adapted frog. *American Naturalist*, 201(1), 91–105. <https://doi.org/10.1086/722174>
- Caldwell, J. P. (1987). Demography and life history of two species of chorus frogs (Anura: Hylidae) in South Carolina. *Copeia*, 1987(1), 114–127.
- Calfee, E., Agra, M. N., Palacio, M. A., Ramírez, S. R., & Coop, G. (2020). Selection and hybridization shaped the rapid spread of African honey bee ancestry in the Americas. *PLoS Genetics*, 16(10), Article e1009038. <https://doi.org/10.1371/journal.pgen.1009038>
- Chan, A. A. Y.-H., Giraldo-Perez, P., Smith, S., & Blumstein, D. T. (2010). Anthropogenic noise affects risk assessment and attention: The distracted prey hypothesis. *Biology Letters*, 6(4), 458–461. <https://doi.org/10.1098/rsbl.2009.1081>
- Chaverri, G., & Quirós, O. E. (2017). Variation in echolocation call frequencies in two species of free-tailed bats according to temperature and humidity. *Journal of the Acoustical Society of America*, 142(1), 146–150. <https://doi.org/10.1121/1.4992029>
- Cheng, K., Srinivasan, M. V., & Zhang, S. W. (1999). Error is proportional to distance measured by honeybees: Weber's law in the odometer. *Animal Cognition*, 2(1), 11–16. <https://doi.org/10.1007/s100710050020>
- Chunco, A. J. (2014). Hybridization in a warmer world. *Ecology and Evolution*, 4(10), 2019–2031. <https://doi.org/10.1002/ece3.1052>
- 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>
- Coomes, C. M., Danner, R. M., & Derryberry, E. P. (2019). Elevated temperatures reduce discrimination between conspecific and heterospecific sexual signals. *Animal Behaviour*, 147, 9–15. <https://doi.org/10.1016/j.anbehav.2018.10.024>
- Coss, D. A., Hunter, K. L., & Taylor, R. C. (2021). Silence is sexy: Soundscape complexity alters mate choice in túngara frogs. *Behavioral Ecology*, 32(1), 49–59. <https://doi.org/10.1093/beheco/araa091>
- Cronin, A. D., Ryan, M. J., Page, R. A., Hunter, K. L., & Taylor, R. C. (2019). Environmental heterogeneity alters mate choice behavior for multimodal signals. *Behavioral Ecology and Sociobiology*, 73(4), Article 43. <https://doi.org/10.1007/s00265-019-2654-3>
- Cummings, M. E., & Endler, J. A. (2018). 25 Years of sensory drive: The evidence and its watery bias. *Current Zoology*, 64(4), 471–484. <https://doi.org/10.1093/cz/zoy043>
- Dixit, T., Apostol, A. L., Chen, K.-C., Fulford, A. J. C., Town, C. P., & Spottiswoode, C. N. (2022). Visual complexity of egg patterns predicts egg rejection according to Weber's law. *Proceedings of the Royal Society B: Biological Sciences*, 289(1978), Article 20220710. <https://doi.org/10.1098/rspb.2022.0710>
- Dixit, T., Caves, E. M., Spottiswoode, C. N., & Horrocks, N. P. C. (2021). Why and how to apply Weber's law to coevolution and mimicry. *Evolution*, 75(8), 1906–1919. <https://doi.org/10.1111/evo.14290>
- Dobzhansky, T. (1940). Speciation as a stage in evolutionary divergence. *American Naturalist*, 74(753), 312–321. <https://doi.org/10.1086/280899>
- Doherty, J., & Hoy, R. (1985). The auditory behavior of crickets: Some views of genetic coupling, song recognition, and predator detection. *Quarterly Review of Biology*, 60(4), 457–472. <https://doi.org/10.1086/414566>
- Dolbear, A. E. (1897). The cricket as a thermometer. *American Naturalist*, 31(371), 970–971. <https://doi.org/10.1086/276739>
- Duarte, C. M., Chapuis, L., Collin, S. P., Costa, D. P., Devassy, R. P., Eguiluz, V. M., Erbe, C., Gordon, T. A. C., Halpern, B. S., Harding, H. R., Havlik, M. N., Meekan, M., Merchant, N. D., Miksis-Olds, J. L., Parsons, M., Predragovic, M., Radford, A. N., Radford, C. A., Simpson, S. D., ... Juanes, F. (2021). The soundscape of the Anthropocene ocean. *Science*, 371(6529), Article eaba4658. <https://doi.org/10.1126/science.aba4658>
- Edmunds, L. N., Jr. (1963). The relation between temperature and flashing intervals in adult male fireflies, *Photinus pyralis*. *Annals of the Entomological Society of America*, 56(5), 716–718. <https://doi.org/10.1093/aesa/56.5.716>
- Edwards, C. J., Alder, T. B., & Rose, G. J. (2002). Auditory midbrain neurons that count. *Nature Neuroscience*, 5(10), 934–936. <https://doi.org/10.1038/nn916>
- Ekman, G. (1959). Weber's law and related functions. *Journal of Psychology*, 47(2), 343–352. <https://doi.org/10.1080/00223980.1959.9916336>
- Endler, J. A. (1992). Signals, signal conditions, and the direction of evolution. *American Naturalist*, 139(Suppl.), S125–S153. <https://doi.org/10.1086/285308>
- Endler, J. A., & Basolo, A. L. (1998). Sensory ecology, receiver biases and sexual selection. *Trends in Ecology & Evolution*, 13(10), 415–420. [https://doi.org/10.1016/S0169-5347\(98\)01471-2](https://doi.org/10.1016/S0169-5347(98)01471-2)
- Endler, J. A., Butlin, R. K., Guilford, T., & Krebs, J. R. (1993). Some general comments on the evolution and design of animal communication systems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 340(1292), 215–225. <https://doi.org/10.1098/rstb.1993.0060>
- Feng, A. S., & Capranica, R. R. (1978). Sound localization in anurans. II. Binaural interaction in superior olivary nucleus of the green tree frog (*Hyla cinerea*). *Journal of Neurophysiology*, 41(1), 43–54. <https://doi.org/10.1152/jn.1978.41.1.43>
- Feng, A. S., & Shofner, W. P. (1981). Peripheral basis of sound localization in anurans. Acoustic properties of the frog's ear. *Hearing Research*, 5(2), 201–216. [https://doi.org/10.1016/0378-5955\(81\)90046-0](https://doi.org/10.1016/0378-5955(81)90046-0)
- Forchhammer, M. C., Post, E., & Stenseth, N. C. (1998). Breeding phenology and climate. *Nature*, 391(6662), Article 6662. <https://doi.org/10.1038/34070>
- Forrest, T. G. (1994). From sender to receiver: Propagation and environmental effects on acoustic signals. *American Zoologist*, 34(6), 644–654. <https://doi.org/10.1093/icb/34.6.644>
- García-Roa, R., García-González, F., Noble, D. W. A., & Carazo, P. (2020). Temperature as a modulator of sexual selection. *Biological Reviews*, 95(6), 1607–1629. <https://doi.org/10.1111/brv.12632>
- Gerhardt, H. C. (1978). Temperature coupling in the vocal communication system of the gray tree frog, *Hyla versicolor*. *Science*, 199(4332), 992–994. <https://doi.org/10.1126/science.199.4332.992>
- Gerhardt, H. C. (1991). Female mate choice in treefrogs: Static and dynamic acoustic criteria. *Animal Behaviour*, 42(4), 615–635. [https://doi.org/10.1016/S0003-3472\(05\)80245-3](https://doi.org/10.1016/S0003-3472(05)80245-3)
- Gerhardt, H. C., & Doherty, J. A. (1988). Acoustic communication in the gray treefrog, *Hyla versicolor*: Evolutionary and neurobiological implications. *Journal of Comparative Physiology A*, 162(2), 261–278. <https://doi.org/10.1007/BF00606090>
- Gerhardt, H. C., & Huber, F. (2002). *Acoustic communication in insects and anurans: Common problems and diverse solutions*. University of Chicago Press.
- Gerhardt, H. C., & Klump, G. M. (1988). Masking of acoustic signals by the chorus background noise in the green tree frog: A limitation on mate choice. *Animal Behaviour*, 36(4), 1247–1249. [https://doi.org/10.1016/S0003-3472\(88\)80090-3](https://doi.org/10.1016/S0003-3472(88)80090-3)
- Gerhardt, H. C., & Mudry, K. M. (1980). Temperature effects on frequency preferences and mating call frequencies in the green treefrog, *Hyla cinerea* (Anura: Hylidae). *Journal of Comparative Physiology A*, 137(1), 1–6. <https://doi.org/10.1007/BF00656911>
- 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>
- Grant, P. R. (2006). Evolution of character displacement in Darwin's finches. *Science*, 313(5784), 224–226. <https://doi.org/10.1126/science.1128374>
- 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>
- Green, P., & MacLeod, C. J. (2016). SIMR: An R package for power analysis of generalized linear mixed models by simulation. *Methods in Ecology and Evolution*, 7(4), 493–498. <https://doi.org/10.1111/2041-210X.12504>
- Groot, A. T., & Zizzari, Z. V. (2019). Does climate warming influence sexual chemical signaling? *Animal Biology*, 69(1), 83–93. <https://doi.org/10.1163/15707563-20191103>
- Heath, J. E., & Josephson, R. K. (1970). Body temperature and singing in the katydid, *Neoconocephalus robustus* (Orthoptera: Tettigoniidae). *Biological Bulletin*, 138(3), 272–285. <https://doi.org/10.2307/1540212>
- Heuschele, J., Mannerla, M., Gienapp, P., & Candolin, U. (2009). Environment-dependent use of mate choice cues in sticklebacks. *Behavioral Ecology*, 20(6), 1223–1227. <https://doi.org/10.1093/beheco/arp123>
- Higginson, A. D., & Reader, T. (2009). Environmental heterogeneity, genotype-by-environment interactions and the reliability of sexual traits as indicators of mate quality. *Proceedings of the Royal Society B: Biological Sciences*, 276(1659), 1153–1159. <https://doi.org/10.1098/rspb.2008.1592>
- Imlay, T. L., Mills Flemming, J., Saldanha, S., Wheelwright, N. T., & Leonard, M. L. (2018). Breeding phenology and performance for four swallows over 57 years: Relationships with temperature and precipitation. *Ecosphere*, 9(4), Article e02166. <https://doi.org/10.1002/ecs2.2166>
- Jang, Y., & Gerhardt, H. C. (2006). Divergence in female calling song discrimination between sympatric and allopatric populations of the southern wood cricket *Gryllus fultoni* (Orthoptera: Gryllidae). *Behavioral Ecology and Sociobiology*, 60(2), 150–158. <https://doi.org/10.1007/s00265-005-0151-3>
- Jang, Y., & Gerhardt, H. C. (2007). Temperature effects on the temporal properties of calling songs in the crickets *Gryllus fultoni* and *G. vernalis*: Implications for reproductive isolation in sympatric populations. *Journal of Insect Behavior*, 20(1), 33–52. <https://doi.org/10.1007/s10905-006-9061-0>
- Johnson, P. C. D., Barry, S. J. E., Ferguson, H. M., & Müller, P. (2015). Power analysis for generalized linear mixed models in ecology and evolution. *Methods in Ecology and Evolution*, 6(2), 133–142. <https://doi.org/10.1111/2041-210X.12306>
- Kelly, C. D. (2018). The causes and evolutionary consequences of variation in female mate choice in insects: The effects of individual state, genotypes and environments. *Current Opinion in Insect Science*, 27, 1–8. <https://doi.org/10.1016/j.cois.2018.01.010>
- Kern, J. M., & Radford, A. N. (2016). Anthropogenic noise disrupts use of vocal information about predation risk. *Environmental Pollution*, 218, 988–995. <https://doi.org/10.1016/j.envpol.2016.08.049>
- Klump, G. M., & Gerhardt, H. C. (1987). Use of non-arbitrary acoustic criteria in mate choice by female gray tree frogs. *Nature*, 326(6110), Article 6110. <https://doi.org/10.1038/326286a0>
- Krause, B., & Farina, A. (2016). Using ecoacoustic methods to survey the impacts of climate change on biodiversity. *Biological Conservation*, 195, 245–254. <https://doi.org/10.1016/j.biocon.2016.01.013>
- LaBarbera, K., Nelson, P. B., & Bee, M. A. (2020). Mate choice and the 'opposite miss' to Weber's law: Proportional processing governs signal preferences in a treefrog. *Animal Behaviour*, 168, 199–209. <https://doi.org/10.1016/j.anbehav.2020.08.014>
- Larson, E. L., Tinghitella, R. M., & Taylor, S. A. (2019). Insect hybridization and climate change. *Frontiers in Ecology and Evolution*, 7, Article 348. <https://www.frontiersin.org/articles/10.3389/fevo.2019.00348>

- Lemmon, E. M. (2009). Diversification of conspecific signals in sympatry: Geographic overlap drives multidimensional reproductive character displacement in frogs. *Evolution*, 63(5), 1155–1170. <https://doi.org/10.1111/j.1558-5646.2009.00650.x>
- Lemmon, E. M., & Lemmon, A. R. (2010). Reinforcement in chorus frogs: Lifetime fitness estimates including intrinsic natural selection and sexual selection against hybrids. *Evolution*, 64(6), 1748–1761. <https://doi.org/10.1111/j.1558-5646.2010.00955.x>
- Littlejohn, M. J. (2001). Patterns of differentiation in temporal properties of acoustic signals of anurans. In M. J. Ryan (Ed.), *Anuran communication* (pp. 102–120). Smithsonian Institution Press.
- Matute, D. R., Novak, C. J., & Coyne, J. A. (2009). Temperature-based extrinsic reproductive isolation in two species of *Drosophila*. *Evolution*, 63(3), 595–612. <https://doi.org/10.1111/j.1558-5646.2008.00588.x>
- Mazerolle, M. J. (2016). *AIcmodavg: Model selection and multimodel inference based on Q(AICc)*. R (R package Version 2.2-0) <https://cran.r-project.org/package=AIcmodavg>.
- Møller, A. P. (2011). When climate change affects where birds sing. *Behavioral Ecology*, 22(1), 212–217. <https://doi.org/10.1093/beheco/arg200>
- Morris-Drake, A., Kern, J. M., & Radford, A. N. (2016). Cross-modal impacts of anthropogenic noise on information use. *Current Biology*, 26(20), R911–R912. <https://doi.org/10.1016/j.cub.2016.08.064>
- Nachev, V., Stich, K. P., & Winter, Y. (2013). Weber's law, the magnitude effect and discrimination of sugar concentrations in nectar-feeding animals. *PLoS One*, 8(9), Article e74144. <https://doi.org/10.1371/journal.pone.0074144>
- Narins, P. M., & Meenderink, S. W. F. (2014). Climate change and frog calls: Long-term correlations along a tropical altitudinal gradient. *Proceedings of the Royal Society B: Biological Sciences*, 281(1783), Article 20140401. <https://doi.org/10.1098/rspb.2014.0401>
- Owens, G. L., & Samuk, K. (2020). Adaptive introgression during environmental change can weaken reproductive isolation. *Nature Climate Change*, 10(1), Article 1. <https://doi.org/10.1038/s41558-019-0628-0>
- Pfennig, K. S. (2007). Facultative mate choice drives adaptive hybridization. *Science*, 318(5852), 965–967. <https://doi.org/10.1126/science.1146035>
- Pires, A., & Hoy, R. R. (1992). Temperature coupling in cricket acoustic communication. *Journal of Comparative Physiology A*, 171(1), 79–92. <https://doi.org/10.1007/BF00195963>
- Pohjoismäki, J. L. O., Michell, C., Levänen, R., & Smith, S. (2021). Hybridization with mountain hares increases the functional allelic repertoire in brown hares. *Scientific Reports*, 11(1), Article 1. <https://doi.org/10.1038/s41598-021-95357-0>
- R Core Team. (2022). *R: A language and environment for statistical computing* (4.2.0). R Foundation for Statistical Computing <https://www.R-project.org>.
- Regnier, F. E., & Goodwin, M. (1977). On the chemical and environmental modulation of pheromone release from vertebrate scent marks. In D. Müller-Schwarze, & M. M. Mozell (Eds.), *Chemical signals in vertebrates* (pp. 115–133). Springer. https://doi.org/10.1007/978-1-4684-2364-8_8
- Richards, D. G., & Wiley, R. H. (1980). Reverberations and amplitude fluctuations in the propagation of sound in a forest: Implications for animal communication. *American Naturalist*, 115(3), 381–399. <https://doi.org/10.1086/283568>
- Rose, G. J., Brenowitz, E. A., & Capranica, R. R. (1985). Species specificity and temperature dependency of temporal processing by the auditory midbrain of two species of treefrogs. *Journal of Comparative Physiology A*, 157(6), 763–769. <https://doi.org/10.1007/BF01350073>
- Rose, G. J., Hanson, J. L., Leary, C. J., Graham, J. A., Alluri, R. K., & Vasquez-Opazo, G. A. (2015). Species-specificity of temporal processing in the auditory midbrain of gray treefrogs: Interval-counting neurons. *Journal of Comparative Physiology A*, 201(5), 485–503. <https://doi.org/10.1007/s00359-015-0997-4>
- Rose, G. J., Leary, C. J., & Edwards, C. J. (2011). Interval-counting neurons in the anuran auditory midbrain: Factors underlying diversity of interval tuning. *Journal of Comparative Physiology A*, 197(1), 97–108. <https://doi.org/10.1007/s00359-010-0591-8>
- Ryan, M. J. (1991). Sexual selection and communication in frogs. *Trends in Ecology & Evolution*, 6(11), 351–355. [https://doi.org/10.1016/0169-5347\(91\)90224-L](https://doi.org/10.1016/0169-5347(91)90224-L)
- Ryan, M. J., & Cummings, M. E. (2013). Perceptual biases and mate choice. *Annual Review of Ecology, Evolution, and Systematics*, 44(1), 437–459. <https://doi.org/10.1146/annurev-ecolsys-110512-135901>
- Ryan, M. J., Page, R. A., Hunter, K. L., & Taylor, R. C. (2019). 'Crazy love': Nonlinearity and irrationality in mate choice. *Animal Behaviour*, 147, 189–198. <https://doi.org/10.1016/j.anbehav.2018.04.004>
- Schluter, D., Price, T. D., & Grant, P. R. (1985). Ecological character displacement in Darwin's finches. *Science*, 227(4690), 1056–1059. <https://doi.org/10.1126/science.227.4690.1056>
- Schwartz, J. J., Buchanan, B. W., & Gerhardt, H. C. (2001). Female mate choice in the gray treefrog (*Hyla versicolor*) in three experimental environments. *Behavioral Ecology and Sociobiology*, 49(6), 443–455. <https://doi.org/10.1007/s002650100317>
- Servedio, M. R., & Hermisson, J. (2020). The evolution of partial reproductive isolation as an adaptive optimum. *Evolution*, 74(1), 4–14. <https://doi.org/10.1111/evo.13880>
- Servedio, M. R., & Noor, M. A. F. (2003). The role of reinforcement in speciation: Theory and data. *Annual Review of Ecology, Evolution, and Systematics*, 34, 339–364. <https://doi.org/10.1146/annurev.ecolsys.34.011802.132412>
- Shimizu, I., & Barth, F. G. (1996). The effect of temperature on the temporal structure of the vibratory courtship signals of a spider (*Cupiennius salei* Keys.). *Journal of Comparative Physiology A*, 179(3), 363–370. <https://doi.org/10.1007/BF00194990>
- Shutt, J. D., Cabello, I. B., Keogan, K., Leech, D. I., Samplonius, J. M., Whittle, L., Burgess, M. D., & Phillimore, A. B. (2019). The environmental predictors of spatio-temporal variation in the breeding phenology of a passerine bird. *Proceedings of the Royal Society B: Biological Sciences*, 286(1908), Article 20190952. <https://doi.org/10.1098/rspb.2019.0952>
- Simpson, S. D., Radford, A. N., Nedelec, S. L., Ferrari, M. C. O., Chivers, D. P., McCormick, M. I., & Meekan, M. G. (2016). Anthropogenic noise increases fish mortality by predation. *Nature Communications*, 7(1), Article 10544. <https://doi.org/10.1038/ncomms10544>
- Spiga, I., Aldred, N., & Caldwell, G. S. (2017). Anthropogenic noise compromises the anti-predator behaviour of the European seabass, *Dicentrarchus labrax* (L.). *Marine Pollution Bulletin*, 122(1), 297–305. <https://doi.org/10.1016/j.marpolbul.2017.06.067>
- Stiebler, I. B., & Narins, P. M. (1990). Temperature-dependence of auditory nerve response properties in the frog. *Hearing Research*, 46(1), 63–81. [https://doi.org/10.1016/0378-5955\(90\)90140-K](https://doi.org/10.1016/0378-5955(90)90140-K)
- Sueur, J., Krause, B., & Farina, A. (2019). Climate change is breaking Earth's beat. *Trends in Ecology & Evolution*, 34(11), 971–973. <https://doi.org/10.1016/j.tree.2019.07.014>
- Symes, L. B., Rodríguez, R. L., & Höbel, G. (2017). Beyond temperature coupling: Effects of temperature on ectotherm signaling and mate choice and the implications for communication in multispecies assemblages. *Ecology and Evolution*, 7(15), 5992–6002. <https://doi.org/10.1002/ece3.3059>
- Telford, S. R., Dyson, M. L., & Passmore, N. I. (1989). Mate choice occurs only in small choruses of painted reed frogs *Hyperolius marmoratus*. *Bioacoustics*, 2(1), 47–53. <https://doi.org/10.1080/09524622.1989.9753113>
- Tobler, M., & Carson, E. W. (2010). Environmental variation, hybridization, and phenotypic diversification in Cuatro Ciénegas pupfishes. *Journal of Evolutionary Biology*, 23(7), 1475–1489. <https://doi.org/10.1111/j.1420-9101.2010.02014.x>
- Vitt, L. J., & Caldwell, J. P. (2013). *Herpetology: An introductory biology of amphibians and reptiles*. Academic Press.
- Walker, T. J., Jr. (1957). Specificity in the response of female tree crickets (Orthoptera, Gryllidae, Oecanthinae) to calling songs of the males. *Annals of the Entomological Society of America*, 50(6), 626–636. <https://doi.org/10.1093/aesa/50.6.626>
- Wells, K. D. (1977). The social behaviour of anuran amphibians. *Animal Behaviour*, 25, 666–693. [https://doi.org/10.1016/0003-3472\(77\)90118-X](https://doi.org/10.1016/0003-3472(77)90118-X)
- Whitaker, J. O. (1971). A study of the western chorus frog, *Pseudacris triseriata*, in Vigo County, Indiana. *Journal of Herpetology*, 5(3/4), 127–150. <https://doi.org/10.2307/1562735>
- Wollerman, L., & Wiley, R. H. (2002). Background noise from a natural chorus alters female discrimination of male calls in a Neotropical frog. *Animal Behaviour*, 63(1), 15–22. <https://doi.org/10.1006/anbe.2001.1885>
- Wong, B. B. M., & Candolin, U. (2005). How is female mate choice affected by male competition? *Biological Reviews*, 80(4), 559–571. <https://doi.org/10.1017/S1464793105006809>
- Wu, H., Gong, L., Jiang, T., Feng, J., & Lin, A. (2021). Echolocation call frequencies of bats vary with body temperature and weather conditions. *Animal Behaviour*, 180, 51–61. <https://doi.org/10.1016/j.anbehav.2021.08.005>
- YuYong, X., TongXian, L., & ShiZe, Z. (2018). Effects of temperature, humidity, photoperiod and host plants on the calling and mating behavior of female moths of black cutworm *Agrotis ipsilon* (Hüfnagel). *Journal of Plant Protection*, 45(2), 235–242.

Appendix

Table A1
Details for each stimulus used in female preference trials

Stimulus	Target temperature (°C)	Species	Type	Pulse number	Call duration (s)	Pulse rate (pulses/s)
ColdA	10	<i>P. feriarum</i>	Average (mode pulse number)	29	1.146	25.3
ColdA	10	<i>P. nigrita</i>	Average (mode pulse number)	10	1.425	7.0
WarmA	20	<i>P. feriarum</i>	Average (mode pulse number)	29	0.681	42.6
WarmA	20	<i>P. nigrita</i>	Average (mode pulse number)	10	0.582	17.2
ColdB	10	<i>P. feriarum</i>	Average (pulse rate regression)	29	1.631	17.8
ColdB	10	<i>P. nigrita</i>	Average (pulse rate regression)	10	1.681	5.9
WarmB	20	<i>P. feriarum</i>	Average (pulse rate regression)	29	0.642	45.2
WarmB	20	<i>P. nigrita</i>	Average (pulse rate regression)	10	0.681	14.7
Fast	20	' <i>P. nigrita</i> -like'	Increased	10	0.286	35.0
Slow	20	' <i>P. feriarum</i> -like'	Decreased	29	1.127	25.7

Table A2
Sets of stimuli used in each trial

Call set	Stimuli	Left speaker	Right speaker
1_A	ColdA	<i>P. nigrita</i>	<i>P. feriarum</i>
1_B	ColdA	<i>P. feriarum</i>	<i>P. nigrita</i>
1_C	ColdA	<i>P. nigrita</i>	<i>P. feriarum</i>
1_D	ColdA	<i>P. feriarum</i>	<i>P. nigrita</i>
2_A	WarmA	<i>P. nigrita</i>	<i>P. feriarum</i>
2_B	WarmA	<i>P. feriarum</i>	<i>P. nigrita</i>
2_C	WarmA	<i>P. nigrita</i>	<i>P. feriarum</i>
2_D	WarmA	<i>P. feriarum</i>	<i>P. nigrita</i>
3_A	ColdB	<i>P. nigrita</i>	<i>P. feriarum</i>
3_B	ColdB	<i>P. feriarum</i>	<i>P. nigrita</i>
3_C	ColdB	<i>P. nigrita</i>	<i>P. feriarum</i>
3_D	ColdB	<i>P. feriarum</i>	<i>P. nigrita</i>
4_A	WarmB	<i>P. nigrita</i>	<i>P. feriarum</i>
4_B	WarmB	<i>P. feriarum</i>	<i>P. nigrita</i>
4_C	WarmB	<i>P. nigrita</i>	<i>P. feriarum</i>
4_D	WarmB	<i>P. feriarum</i>	<i>P. nigrita</i>
5_A	Fast_WarmB	<i>P. nigrita</i>-like	<i>P. feriarum</i>
5_B	WarmB_Fast	<i>P. feriarum</i>	<i>P. nigrita</i>-like
5_C	Fast_WarmB	<i>P. nigrita</i> -like	<i>P. feriarum</i>
5_D	WarmB_Fast	<i>P. feriarum</i>	<i>P. nigrita</i> -like
6_A	WarmB_Slow	<i>P. nigrita</i>	<i>P. feriarum</i> -like
6_B	Slow_WarmB	<i>P. feriarum</i> -like	<i>P. nigrita</i>
6_C	WarmB_Slow	<i>P. nigrita</i>	<i>P. feriarum</i>-like
6_D	Slow_WarmB	<i>P. feriarum</i>-like	<i>P. nigrita</i>

The species stimulus played first (from the left or right speaker) is shown in bold. See Table A1 for parameters for each call.

Table A3
Results for the effect of response time on choice for both experiments

Experiment	Response	Model effects	K	AICc	ΔAICc	AICc weight	Log likelihood	Response time estimate (model average)
A	Choice	(1 FieldID)	2	73.05	0	0.73	−34.47	0.05 (±0.12)
		Response time + (1 FieldID)	3	74.99	1.95	0.27	−34.38	
B	Choice	(1 FieldID)	2	58.08	0	0.75	−26.96	−0.01 (±0.08)
		Response time + (1 FieldID)	3	60.23	2.15	0.25	−26.96	

AICc model selection results and model average parameter estimates for the effect of response time on choice and response time. Models are organized from best (lowest ΔAICc) to worst (highest ΔAICc). There was no effect of response time on choice for either experiment.