

RESEARCH ARTICLE

Thermal Plasticity Changes Competitive Ability Across a Woodland Salamander Hybrid System

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ABSTRACT

Temperature mediates performance in ectotherms, affecting their ability to grow, survive, and reproduce. Aggression and evasion are key examples of thermally dependent behaviors that can impact fitness. However, we know relatively little about how the thermal plasticity of such behaviors varies among close relatives and impacts competitive outcomes. Woodland salamanders (Genus: *Plethodon*) from the Appalachian Mountains are distributed across wide thermal gradients in accordance with latitude or elevation. These plethodontid (lungless) salamanders compete for space and develop hybrid zones where territories overlap among species. Plethodontids tend to exhibit increased aggression at warmer temperatures, suggesting that as temperatures rise, behavioral interactions may be altered in ways that impact hybrid zone dynamics. It is thus far unclear, however, how salamander hybrids, which may encroach on their parent populations and drive competitive exclusion, respond behaviorally to warming. Here, we used staged bouts to examine the effects of temperature on aggression and evasion in the *Plethodon shermani* and *Plethodon tayahalee* hybrid system from the southern Appalachians. The behavior of salamanders from parent populations, particularly *P. shermani*, appears to be more sensitive to thermal changes than that of hybrid individuals. Additionally, evasive behavior was significantly more plastic than aggressive behavior in response to warming. Our results suggest that rising temperatures may increase competition for preferable microhabitats, but the effects on behavior among parental and hybrid salamanders will be asymmetric. Temperature may therefore alter the outcomes of competition, determining which populations can persist under rapid warming.

1 | Introduction

Ambient temperature is a key determinant of an ectotherm's ability to perform fitness-related activities, such as foraging and competing for resources (Angilletta Jr. 2009). The sensitivity of performance follows a unimodal curve: performance is optimized over a relatively narrow range of temperatures, and then declines at higher and lower temperatures until organismal function is impaired (Huey and Stevenson 1979;

Angilletta Jr. 2009; Meza-Parral et al. 2020). Given this tight relationship between temperature and fitness-based activities, temperature correspondingly exerts a strong influence on how species are distributed across space and time. Species' thermal limits (maximum and minimum temperatures for activity), for example, are strongly associated with the thermal features of their geographic ranges (Sexton et al. 2009; Gaston 2009; Bozinovic, Calosi, and Spicer 2011). As temperatures rise or fall, mobile ectotherms alter their activity patterns and

microhabitat use to track their preferred thermal microclimates (Parmesan and Yohe 2003; Kearney and Porter 2009; Sears, Raskin, and Angilletta Jr. 2011; Sears et al. 2019; Taylor et al. 2014; Dallalio, Brand, and Grant 2017; Muñoz and Losos 2018; Feldmeier et al. 2020; Hedrick et al. 2023). Concurrently, temperature fluctuations may increase or decrease an organism's metabolic rate; as metabolism mediates energy availability, temperature fluctuations may therefore affect the performance of behaviors such as sprinting or foraging (Huey and Stevenson 1979; Hertz, Huey, and Nevo 1982; Huey and Kingsolver 1989; Ratnasabapathi, Burns, and Souček 1992; Herrel, James, and Van Damme 2007; Havird et al. 2020). Rapidly warming temperatures thus impose ecological constraints that demand an organism to respond behaviorally (Bogert 1949; Huey, Hertz, and Sinervo 2003; Muñoz 2022), while also mediating an individual's ability to perform these behaviors. These effects reflect the thermal plasticity of behavior, or predictable changes in behavior in response to changes in ambient temperature.

Prior studies have demonstrated that temperature changes can alter the outcomes of competition among ectotherms; if some taxa exhibit more thermal plasticity than their competitors, then they may gain or lose a competitive edge when the ambient temperature fluctuates (Gvoždík 2022; Carvalho et al. 2022; Costaz et al. 2023). In systems with intense competition for space, even subtle variations in plasticity may improve or reduce the competitive ability of an individual, affecting whether and how populations persist over time. Thus, temperature fluctuation may mediate the outcomes of bouts by affecting individuals' competitive ability.

Interactions between temperature and competitive ability may be particularly relevant for predicting hybrid zone dynamics (Engler et al. 2013; Hunter et al. 2017; Burger et al. 2024). Hybridization has been observed extensively across ectothermic taxa (Hubbs 1955; Montanucci 1983; Parris 1999; Walls 2009; Scott, Glenn, and Rissler 2019), often occurring between two adjacent populations. This phenomenon can lead to persistent hybrid zones, characterized by regular interbreeding between hybrid and parent populations over multiple generations. As such, hybrid individuals may compete for space with other hybrids, with individuals from parent populations, or both. Several recent studies have found that hybrid morphology and physiology are more sensitive to environmental parameters, including temperature, than their parent taxa (Trotta et al. 2010; Bernatowicz, Dawidowicz, and Pijanowska 2021; Singh et al. 2022). Under rising temperatures, then, hybrids may experience altered fitness compared to their parent taxa. The thermal plasticity of hybrid behavior, however, which may mediate competitive ability in densely packed populations, has been comparatively less well studied.

Lungless salamanders (Family: Plethodontidae) offer a compelling system with which to examine the effects of temperature on agonistic behavior across a hybrid zone. As ectotherms, plethodontids exhibit thermally-plastic performance. Moreover, as lungless amphibians, they rely on cutaneous respiration, so the thermal plasticity of behavior is also relevant for hydric balance and gas exchange (Tattersall 2007; Riddell et al. 2024), in turn influencing

activity patterns (Farallo et al. 2020). With more than 500 species (AmphibiaWeb 2024), plethodontids comprise the largest family of Caudata. The Appalachian Mountains, particularly the southern Appalachians, are a global biodiversity hotspot for lungless salamanders (AmphibiaWeb 2024), where they form adjacent populations along elevational gradients, in turn generating hybrid zones that can persist for decades and blur the lines between previously distinct taxa. Previous research has shown clear examples of thermal plasticity in social behavior in lungless salamanders; specifically, warmer temperatures are associated with heightened aggression in competitive bouts (Clay and Gifford 2016; Bissell and Cecala 2019). Hybrid zones exist between populations competing for resources, but to our knowledge, no research has compared the thermal plasticity of hybrid salamanders to that of their parent taxa. In the Nantahala Mountains of North Carolina, a stable hybrid zone of woodland salamanders (Genus: *Plethodon*) comprised of low-elevation *Plethodon teyahalee* and high-elevation *Plethodon shermani* has persisted for decades (Walls 2009; Gade, Zhao, and Peterman 2022). As ambient temperatures have risen in response to anthropogenic climate change, the hybrid zone has gradually expanded upwards, shrinking the size of the *P. shermani* population (Hairston et al. 1992; Walls 2009). The geographic limits on this hybrid zone are fluctuating with warming temperatures, affecting the space available for neighboring populations. Variation in the thermal plasticity of agonistic behavior may be influencing hybrid zone dynamics in response to changing temperatures, but this idea remains unexplored.

Here, we investigate the thermal plasticity of behavior in hybrid and parental populations of *P. teyahalee* and *P. shermani*. We used staged bouts to simulate interspecific competition under two different temperature regimes, observing the behavior of individuals from different populations at both warm and cool temperatures. We scored social behaviors, namely aggression and evasion, as these are key determinants of the outcome of competition for salamander bouts (Wrobell, Gergits, and Jaeger 1980; Tanner 2006; Rillich and Stevenson 2019). We measured thermal plasticity by quantifying an individual's aggressive and evasive behavior at warm and cool temperatures. Following prior studies on hybrid thermal plasticity (Trotta et al. 2010; Bernatowicz, Dawidowicz, and Pijanowska 2021; Singh et al. 2022), we hypothesized that hybrid social behavior may be more plastic to temperature change than that of parent populations; aggression and evasion of hybrids should be much higher in the warmer treatment, while parent individuals should show minimal change across treatments. Given that aggressive intensity is correlated with competitive ability in salamanders (Wrobell, Gergits, and Jaeger 1980), individuals that increase aggression at warmer temperatures may win more bouts for food or territory and experience greater fitness than their competitors. Conversely, some populations may exhibit little to no thermal plasticity; aggression and evasion would not be affected by temperature. Individuals that are less thermally plastic may be better equipped to maintain homeostasis when experiencing warmer temperatures, though they may be poorly equipped to acquire food and maintain territory (Biro and Stamps 2010; Metcalfe, Van Leeuwen, and Killen 2016). The competitive landscape is subject to change with increasing temperature, and identifying population-level

variation in thermal plasticity is crucial for understanding population persistence or extinction.

2 | Methods

2.1 | Salamander Collection and Husbandry

All data were collected by observing a colony of 27 lungless salamanders housed at Yale University. This sample included ten individuals of *P. shermani*, seven individuals of *P. teyahalee*, and ten individuals of *P. shermani*-*P. teyahalee* hybrids. We collected salamanders by hand from Macon County, North Carolina between May 10–12, 2022, from the hours of 9:00 pm to 12:00 am (Figure 1). Individuals of *P. shermani* were identified visually by the red pigmentation of their legs and comparatively smaller body size, while individuals of *P. teyahalee* were identified by white spots along their backs and sides, as well as a comparatively larger body size. Hybrids exhibited intermediary

characteristics of both, with reduced red pigmentation along the legs and limited white spots (Hairston et al. 1992). Prior to experiments, we held individuals in Darwin Environmental Chambers over a period of four months at approximately 15°C and 75% relative humidity. Chambers were set to a 12:12 h light schedule. Each salamander was individually housed in its own plastic translucent enclosure (Tupperware brand), in which they were provided with wetted paper towels so they could stay moist and hide. Enclosures were misted once daily with spring water and changed weekly for cleanliness. We fed each salamander 5–10 live *Drosophila melanogaster* flies twice weekly. Individuals were isolated and did not encounter another individual prior to behavioral experiments.

To analyze how behavior changes with temperature, we observed interspecific interactions at 15°C and 23°C. When individuals were first collected, air temperature at collection points ranged from 14.8°C to 21.0°C. As salamanders were acclimated to 15°C and as this temperature fell within the range observed

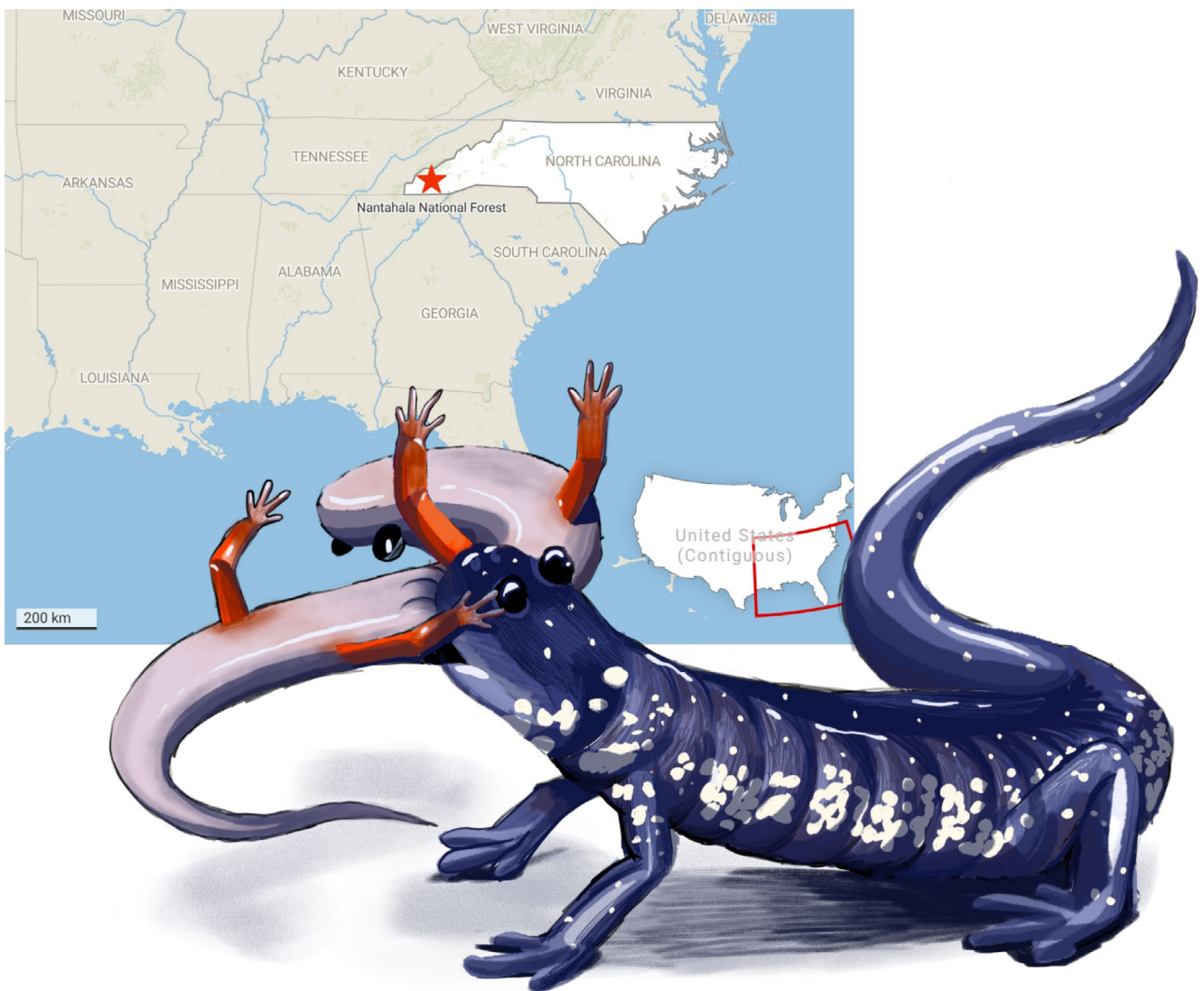


FIGURE 1 | Illustration of the “bite with hold” aggressive behavior and a map indicating the collection site. The map shows the location of the Nantahala National Forest (indicated by the star) in the Southeastern United States, from which all study individuals were collected. Salamanders illustrated by Emmy James in Procreate, map created in Datawrapper, and images compiled using BioRender.

while collecting individuals in the wild, we elected to use 15°C as our control temperature. Under the most extreme Shared Socioeconomic Pathway (SSP) outlined by the Intergovernmental Panel on Climate Change (IPCC), we expect 4.4°C of warming by 2100 (IPCC 2021). The average air temperature recorded at collection sites was 18.3°C, so we added 4.4°C to this value to obtain our high temperature, approximately 23°C. Higher temperatures are known to increase water loss rate, even during relatively short experimental periods, introducing potential confounding effects of moisture loss on behavior (Spotila 1972). Competitive outcomes among lungless salamanders are not sensitive to changes in ambient humidity (at least within the range of 60%–100% relative humidity) (Dallalio, Brand, and Grant 2017). Therefore, to prevent desiccation due to higher temperatures and rates of water loss, we increased the relative humidity setpoint of the Darwin Environmental Chambers from 75% to 90% when individuals were placed in the 23°C chamber. Likewise, we misted salamanders twice daily when moved to the warmer chamber. Individuals received a seven-day break between trials, as well as seven days to acclimate to the increase from 15°C to 23°C before trials began. We kept competitors on the same feeding schedule, feeding them five days before their trial, to ensure that differences in feeding state would not affect behavior. To monitor health, we weighed salamanders periodically between trials. Body mass did not significantly vary during 15°C trials (~2.71% decrease in mass) but mass decreased slightly during 23°C trials (~7.38% decrease in body mass), likely due to increased water loss at warmer temperatures (Spotila 1972).

2.2 | Behavioral Experiments

We conducted all trials between September 12, 2022 and October 17, 2022. We constructed arenas using square Tupperware containers of approximately 23.5 cm L by 23.5 cm W by 11.5 cm H. We paired salamanders randomly for trials. Opponent identity does not affect competitive behavior in this system (Drummond Jr 2015). Given that interspecific encounters are integral to range shifts and the creation of hybrid zones, we elected to focus on interspecific bouts; all pairings included an individual of *P. teyahalee* and an individual of *P. shermani*, *P. teyahalee* and a hybrid of both parent species, or *P. shermani* and a hybrid of both parent species. Notably, the extent of hybridization in sampled individuals is unclear. The population likely experiences backcrossing, making it difficult to determine the extent of the genomic contribution of either parent species to the hybrid population. Therefore, until further genetic testing may be done, we classified all hybrid individuals into a single category.

We conducted 52 trials, with 26 trials occurring at 15°C and 26 occurring at 23°C. All trials were conducted in Darwin Environmental Chambers (total area 2.5 m²), in a dark space lit minimally by a red LED bulb that was suspended approximately 1 m above the arena. The trials occurred between the hours of 20:00 to 00:00, taking place within the first 4 h of the scotophase. We placed individuals on opposite sides of the arena and separated them with an opaque divider. We covered the arena with a thin sheet of glass to both prevent escape and permit observation. After a 10-min habituation period, the divider was removed. Recording began at the onset of the first aggressive behavior demonstrated by one individual toward another. This occurred

anywhere from 1 to 10 min after removing the barrier, and usually occurred by physical touch or by one individual rapidly approaching the other. If no aggressive behaviors occurred after 10 min (which occurred four times during trials at 15°C and not at all at 23°C), then trials were ended. These trials were scored as containing no social behaviors and were included in the analysis.

We recorded encounters with a Canon T6i Rebel DSLR camera for 15 min. Following each trial, salamanders were returned to their original enclosures. In total, trials took place on 12 nights over 4 weeks, with a week-long pause in the middle to allow acclimation to the warmer temperature treatment. We conducted all low temperature trials followed by high temperature trials. We did not randomize the order of temperature conditions because of the extended time needed to acclimate the large walk-in chambers to different temperature settings. Individuals competed two to four times throughout the experiment. Individuals were never paired with the same partner twice to avoid the confounding effects of prior encounter outcomes. During the experiment, three individuals in separate trials crossed the barrier before the 10 min acclimation period elapsed, and all instances occurred across different temperatures and by individuals from different populations. When this occurred, recording began immediately upon the first sign of acknowledgement. All procedures were reviewed and approved by Yale University's IACUC, Protocol 2022–20,297.

2.3 | Behavior Scoring

Aggressive and evasive behaviors were categorized and defined with reference to established plethodontid ethograms (Jaeger 1984; Davis 2002; Tornick 2010; Clay and Gifford 2016), and personal observation. Continuous focal sampling was done for both individuals in each trial. Each observed social behavior was noted. We described the behaviors observed throughout the experiment, ranking them according to their relative level of intensity (S1). Ranking behaviors to generate an aggressive index is a well-established technique in ectotherm literature (Losey 1982; Hoffmaster 1986; Grover et al. 2007; Abhinandini and Venkatesha 2015) and was previously applied to lungless salamanders by Clay and Gifford (2016). Here, we extend this metric to include evasion. By multiplying the frequency of each behavior by its rank (its relative intensity) and then summing the behaviors together, we generated weighted sums of aggression (Total Aggressive Index, TAI) and evasion (Total Evasive Index, TEI). Thus, each data point reports the behavior of one individual in a single trial.

2.4 | Statistical Analysis

We employed linear mixed effects models to investigate the effects of temperature and taxon on performance. Hybrid status (which classified individuals as “hybrid” or “parental”), body mass, and temperature were used as predictor variables and TAI or TEI (log-transformed to meet model assumptions) were response variables. As repeated measures were used, bout order (the number of bouts in which an individual had previously been involved) was included as a fixed effect. Likewise, individual identity was included as a random effect. Combining *P. shermani* and *P. teyahalee* into the single “parental” category may

mask any taxon-specific trends. Therefore, we also examined the thermal plasticity of each population separately.

First, we investigated the relationship between hybrid status, temperature, body size, and performance (TAI or TEI) across all individuals. These models included our full sample size, with the variable of “group” denoting whether an individual was from the hybrid population (“hybrid”), *P. shermani* (“parental”), or *P. teyahalee* (“parental”). These models tested for the interaction between temperature and hybrid status; if thermal plasticity differs between hybrids and non-hybrids, then we expect to find a significant interaction between temperature and hybrid status. We ranked models according to AICc, Akaike’s Information Criterion for small sample sizes ($\Delta\text{AICc} < 2$) (Kuznetsova, Brockhoff, and Christensen 2017; Mazerolle 2023). We also calculated the model-averaged estimate of temperature on TAI or TEI.

It is possible that combining *P. shermani* and *P. teyahalee* into the single category of “parental” may mask any trends in thermal plasticity. To account for this possibility, we built linear models for each population, thus subdividing our dataset. With these models, we compared the effects of temperature on behavior within each taxon.

We used linear mixed-effect models to compare performance across populations within the 15°C treatment. Additionally, we used chi-square analysis to examine the relationship between experiment temperature and boldness, or the proportion of encounters initiated by an individual. We used R version 4.2.2 (R Core Team 2022) and the following R packages: AICcmodavg v. 2.3.3 (Mazerolle 2023), car v. 3.1.2 (Fox and Weisberg 2019), ggplot2 v. 3.5.1 (Wickham 2016), ggpubr v. 0.6.0 (Kassambar 2023), ggtext v. 0.1.2 (Wilke and Wiernik 2022), grateful (Rodriguez-Sanchez and Jackson 2023), kableExtra v. 1.4.0 (Zhu 2017), knitr v. 1.45 (Xie 2014, 2015, 2024), lmerTest v. 3.1.3 (Kuznetsova, Brockhoff, and Christensen 2017), rmarkdown v. 2.25 (Xie, Allaire, and Golemund 2018; Xie, Dervieux, and Riederer 2020; Allaire et al. 2024), systemfonts v. 1.1.0 (Pedersen, Ooms, and Govett 2024), and xtable v. 1.8.4 (Dahl et al. 2019). All data and code are available on GitHub at https://github.com/emmylia18/salamander_hybrid_behavior.

3 | Results

3.1 | Evasive Behavior Was More Thermally Variable Than Aggressive Behavior

Evasive behavior exhibited greater variation in response to temperature than aggressive behavior in the models that included all individuals (Figure 2). The best model for TAI included bout-order and mass as predictors (S2). Four other models for TAI had sufficiently small AICc to be considered ($\Delta\text{AICc} < 2$), including the temperature-only model. However, temperature was not a significant predictor in the temperature-only model, suggesting that aggression was not sensitive to warming (Table 1). The best model for TEI included temperature as the only predictor, followed by the interaction of hybrid status and temperature (S3). After model averaging, the estimated effect size of temperature was 0.68 ± 0.3 , meaning that for each 1°C increase, an individual’s TEI (or intensity of evasion behavior) increased by 0.68. Significance testing on

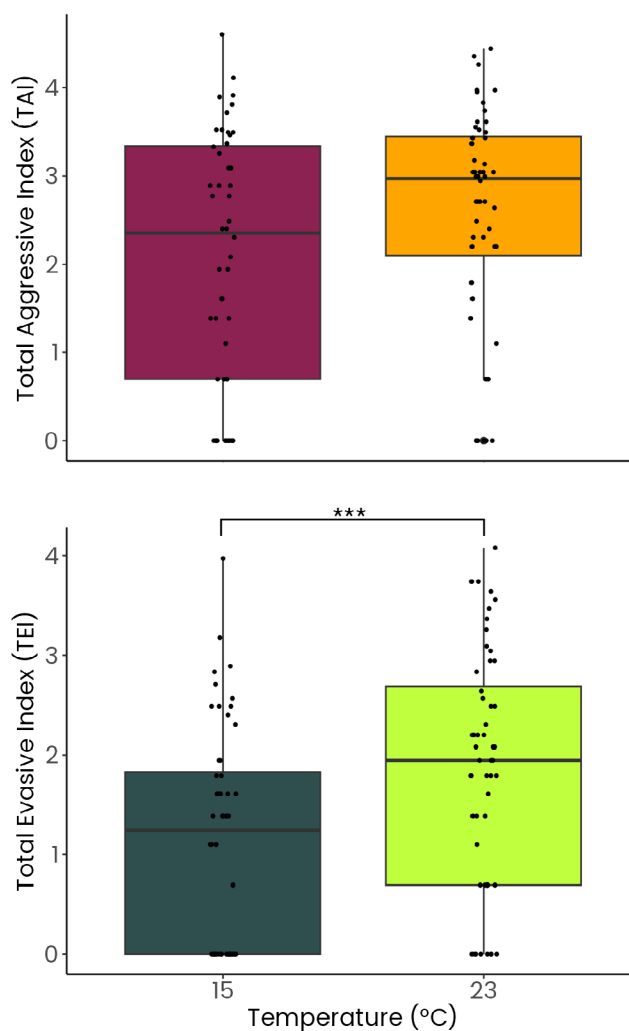


FIGURE 2 | The relationship between temperature and aggression (TAI) (top panel) and evasion (TEI) (lower panel) for all individuals. (***) indicate $p < 0.001$. Each point denotes the TAI or TEI of a single individual in a single trial. Data were collected across 52 trials, with 26 trials at each temperature treatment. We scored the behavior of 27 individuals, of which 26 were exposed to both temperature treatments. As each trial required two participants, most salamanders underwent four trials (two at each temperature treatment) (See section 2). Figure constructed in BioRender.

the best-fit model showed a significantly higher intensity of evasion at the warmer temperature, regardless of taxon.

3.2 | Predictors of Behavioral Variation Differed Between Models

In our models that included all individuals, we found no evidence that thermal plasticity varies with hybrid status. The interaction between temperature and hybrid status was not included in either of our best-fitting models for TEI or TAI, though it was included in the second-best model for TEI. Next, we subdivided the dataset to assess the thermal plasticity of each population separately (Figure 3).

We found evidence suggesting that evasion increased in intensity in response to warming in *P. shermani*. The best-fit model included

TABLE 1 | (A) Outputs for models that included temperature as the only predictor variable, for models including all individuals as well as population-specific models. (B) Outputs for model-averaged estimate of temperature on behavior, for models including all individuals as well as population-specific models.

(A) Response	Population	Model estimate	Model SE	DF	<i>t</i>	<i>p</i>
TAI	All	0.51	0.27	104.00	1.90	0.06
TEI	All	0.71	0.21	80.79	3.43	0.00
TAI	<i>P. shermani</i>	0.75	0.43	28.94	1.76	0.09
TEI	<i>P. shermani</i>	1.10	0.38	29.81	2.92	0.01
TAI	<i>P. teyahalee</i>	-0.19	0.47	28.00	-0.41	0.69
TEI	<i>P. teyahalee</i>	0.81	0.40	21.00	2.05	0.05
TAI	Hybrids	0.81	0.43	40.00	1.89	0.07
TEI	Hybrids	0.29	0.29	30.00	0.99	0.33

(B) Response	Population	Model-averaged estimate	Model-averaged SE	Confidence level	Lower CI	Upper CI
TAI	All	-0.04	0.93	0.95	-1.85	1.77
TEI	All	0.68	0.30	0.95	0.10	1.27
TAI	<i>P. shermani</i>	0.56	0.68	0.95	-0.77	1.89
TEI	<i>P. shermani</i>	1.08	0.42	0.95	0.25	1.91
TAI	<i>P. teyahalee</i>	-0.15	0.51	0.95	-1.15	0.85
TEI	<i>P. teyahalee</i>	0.80	0.42	0.95	-0.01	1.61
TAI	Hybrids	0.85	0.46	0.95	-0.05	1.76
TEI	Hybrids	0.34	0.33	0.95	-0.30	0.98

temperature as the only predictor and no other models had sufficiently small AICc to be considered. In the best-fit model, temperature was a statistically significant predictor (Table 1). For TAI in *P. shermani*, temperature was only marginally correlated with aggression. The temperature-only model was the best-fit model. However, the null model, the mass-only model, and the model including temperature and mass were also considered ($\Delta\text{AICc} < 2$). In the temperature-only model, temperature was not correlated with TAI. Thus, in *P. shermani*, evasive behavior varied more with temperature than did aggressive behavior.

In *P. teyahalee*, evasion marginally increased with temperature and aggression was not correlated with temperature. The best-fit model predicting TEI was the temperature-only model followed by the null model. In the best-fit model, a warmer temperature corresponded with a marginal increase in evasion in *P. teyahalee*, but this relationship was not significant (Table 1). Additionally, temperature was not associated with TAI. The best-fit model was the null model. Therefore, TAI does not appear to be sensitive to temperature in *P. teyahalee*.

In the hybrids of *P. teyahalee* and *P. shermani*, aggressive and evasive behavior were largely unaffected by temperature change. For TEI, the best-fit model was the null model followed by the mass-only and temperature-only models. In the temperature-only model, temperature was not a significant predictor of TEI (Table 1). Therefore, temperature was not related to evasive behavior. The best-fit model for TAI was the temperature-only model, followed by the null model, but the relationship between

TAI and temperature was not significant. Therefore, we are unable to reject the null hypothesis that temperature is unrelated to aggressive or evasive behavior.

3.3 | Performance Was Relatively Similar Across Taxa

We found no differences in performance across taxa in the models ($p > 0.05$) (Figure 3).

3.4 | Temperature Marginally Affected Hybrid Boldness

Across the three taxa, hybrid salamanders exhibited the greatest increase in boldness following a temperature increase, measured by the proportion of encounters initiated by the individual (an increase from 40% of encounters initiated to 60%) (Figure 4). However, this increase was not statistically significant ($p = 0.343$). *P. shermani* and *P. teyahalee* showed negligible changes in boldness following temperature increase.

4 | Discussion

In ectotherms like the salamanders studied here, temperature is a key determinant of fitness-based processes, affecting behavior (Liles et al. 2017; Bissell and Cechala 2019; Giacometti and

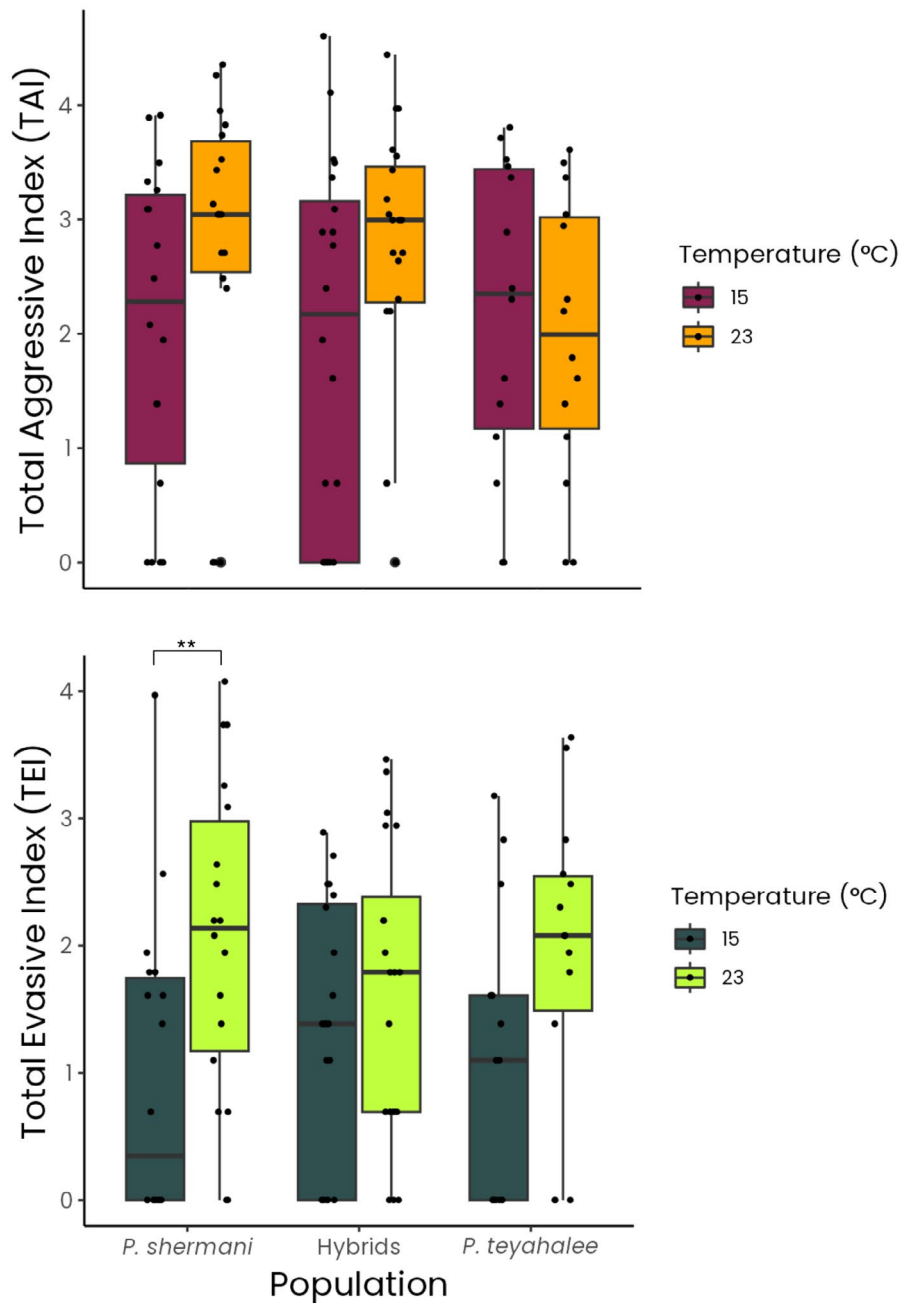


FIGURE 3 | The relationship between temperature and aggression (TAI) (top panel) and evasion (TEI) (lower panel), separated by population and temperature treatment (see legend). (**) indicate $p < 0.01$. Each point denotes the TAI or TEI of a single individual in a single trial. Data were collected across 52 trials, with 26 trials at each temperature treatment. We scored the behavior of 27 individuals, of which 26 were exposed to both temperature treatments. As each trial required two participants, most salamanders underwent four trials (two at each temperature treatment) (See section 2). Figure constructed in BioRender.

Tattersall 2024) and physiology (Muñoz et al. 2022; Moldowan, Tattersall, and Rollinson 2022; Messerman, Turrell, and Leal 2022; Burger et al. 2024). While we did observe increases in certain behaviors, the magnitude of behavioral plasticity to temperature varied among populations. The high-elevation species, *P. shermani*, exhibited the clearest evidence of thermal plasticity; warming significantly increased evasive behavior and marginally increased aggressive behavior. The low-elevation species, *P. teyahalee*, also exhibited some variation in evasive behavior among temperature treatments, though this trend was not significant. The behavior of hybrid salamanders, however, showed no thermal

plasticity; we observed no variation in evasive behavior and only marginal evidence of changes in aggression among temperature treatments. Given that the *P. shermani*–*P. teyahalee* hybrid zone occupies an intermediate temperature range to both parent populations, this difference is probably not due to a different preferred thermal range. Thus, *P. teyahalee* and *P. shermani* appear to be more similar to each other in the thermal plasticity of their aggressive and evasive behaviors than either is to the hybrids.

Our full-sample models and our population-specific models gave mixed evidence for behavioral differences among temperature

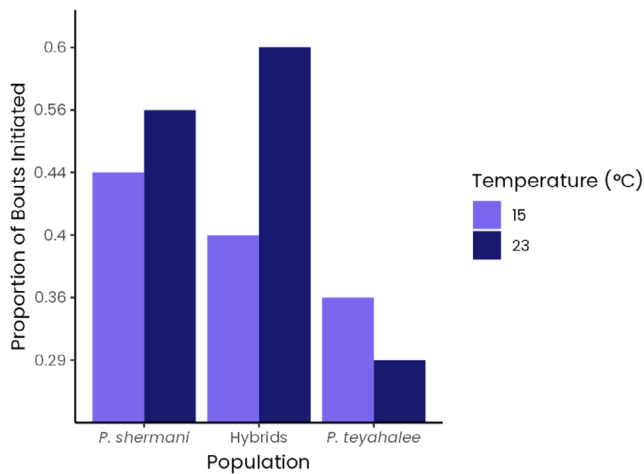


FIGURE 4 | The relative effect of temperature on boldness, or initiation frequency, separated by population and temperature treatment (see legend). Data were collected across 52 trials, with 26 trials at each temperature treatment. We scored the behavior of 27 individuals, of which 26 were exposed to both temperature treatments. As each trial required two participants, most salamanders underwent four trials (two at each temperature treatment) (See section 2). Figure constructed in BioRender.

treatments. In our full-sample models, the interaction between temperature and hybrid status was not significant, suggesting that hybrid status did not affect behavior between cooler and warmer temperature treatments. However, our population-specific models revealed across-population differences in thermal plasticity that might vary with hybridization. Interestingly, our hybrid-only model provided little evidence for behavioral variation, except for a slight correlation between temperature and aggression; this contradicts the pattern observed in both parent taxa, which exhibited thermal plasticity in evasive behavior. These findings conflict with previous studies that observed greater morphological or physiological plasticity in hybrids than in parent taxa (Trotta et al. 2010; Bernatowicz, Dawidowicz, and Pijanowska 2021; Singh et al. 2022). Our findings may be due to a high degree of variation in the category of “hybrid”, with different degrees of backcrossing potentially affecting the extent of plasticity. Alternatively, it is possible that combining *P. shermani* and *P. teyahalee* into one category, “parental”, to compare against hybrids, may be masking population-specific variation in thermal plasticity in our all-individual models. Regardless of the mechanism underlying these contrasting results, it is clear that thermal plasticity in social behavior varies among populations in this hybrid zone.

We also observed thermal plasticity in population boldness, or the frequency of bouts initiated by an individual from a given population. Hybrid initiation frequency increased with warming from 40% initiation to 60%. In contrast, parent initiation frequency was not sensitive to temperature, remaining relatively unchanged in response to warming. Some studies on ectotherms have provided evidence that initiators win bouts more frequently (Guderley and Couture 2005; Hsu, Earley, and Wolf 2006; Stapley and Whiting 2006; Garcia et al. 2012; Umbers, Osborne, and Keogh 2012), though this trend may disappear in long-lasting bouts (Breed and Rasmussen 1980; Moretz 2003) or when comparing across taxa (Wrobell, Gergits,

and Jaeger 1980). If hybrid boldness is sensitive to temperature, then it may improve competitive ability, especially when considering that evasion behavior increases with temperature in both parental species. Future studies should continue to explore this trend in amphibians, examining the outcomes of interspecific bouts in relation to temperature and initiation frequencies.

Because population-level differences exist in the thermal plasticity of social behaviors, our findings suggest a connection between temperature and competitive ability. Recent studies of climate-induced range shifts have emphasized the importance of including species interactions in predictive models, namely competition (Urban, Tewksbury, and Sheldon 2012; Urban, Zarnetske, and Skelly 2013; HilleRisLambers et al. 2013; Legault et al. 2020; Freeman, Strimas-Mackey, and Miller 2022). Here, we provide empirical evidence that social behaviors are sensitive to thermal variation and that plasticity varies by population, raising questions about how salamander behavior might shift as the environment continues to warm. Temperature increases can lead to range shifts toward more favorable thermal conditions (Parmesan and Yohe 2003), which are often upslope in salamanders (Hairston et al. 1992; Walls 2009), although their mobility is generally low (Buckley, Tewksbury, and Deutsch 2013). Though the lower boundaries of salamander populations may continue to climb, upper boundaries are limited by the elevational extremes of their habitats. Climate-induced range shifts may therefore decrease space availability, creating greater demand for favorable microclimates (Kelly and Goulden 2008; Urban 2018). The *shermani-teyahalee* hybrid zone itself is moving upslope, a trend that may be in response, at least in part, to environmental warming (Walls 2009). Similarly, some ectotherm defensive behaviors are more sensitive to temperature than others. Many species rely on evasion for predator avoidance (Domenici, Blagburn, and Bacon 2011; Grovenburg et al. 2012; Samia et al. 2016), but some studies have argued that thermal plasticity can prompt shifts between evasive and aggressive strategies (Hertz, Huey, and Nevo 1982; Crowley and Pietruszka 1983; Ratnasabapathi, Burns, and Soucek 1992). For example, sprint speed (an evasive strategy) in *Trapelus* lizards is thermally dependent while bite force (an aggressive strategy) is largely independent of temperature (Herrel, James, and Van Damme 2007). Other studies of evasive behavior have examined flight initiation distance (FID), which is the closest distance a potential predator can approach an individual before it flees (Ydenberg and Dill 1986). Small increases in ambient temperature are sometimes linked to decreased FID, in which an individual can afford to wait a little longer before fleeing because its improved performance can increase its odds of survival (Braun, Baird, and LeBeau 2010; Boronow, Shields, and Muñoz 2018; Gutiérrez et al. 2023). Similarly, exposure to cold temperatures should increase FID because of the decrement in performance, like sprint speed (Rand 1964). However, other taxa have exhibited no thermal plasticity of FID (Smith and Lemos-Espinal 2005; Samia et al. 2016; Lemos-Espinal and Smith 2021; Krenhardt et al. 2024). Despite its importance in both competitive bouts and predator avoidance, the potential for thermal plasticity in evasive behavior remains unclear.

Our findings reveal a connection between warming and competitive ability in lungless salamanders. Our full-sample models identified temperature as a significant predictor of TEI, but not TAI, suggesting that salamander evasive behaviors (such as

fleeing or tail twitching) may be more thermally plastic than those associated with aggression (such as bites or nose taps). Thus, elevated temperatures may disproportionately increase the frequency of evasive behaviors compared to aggressive behaviors. Therefore, we can infer that temperature increases may alter both the frequency and intensity of competition; warming adjusts the rate at which bouts occur by increasing competition for preferred microclimates, as well as the performance of those involved. Biotic interactions such as ecological competition are known to affect range shifts under climate change (Pigot and Tobias 2013; HilleRisLambers et al. 2013; Tingley et al. 2014). As different taxa may have different degrees of plasticity, even minute changes in temperature may have the capacity to reshape how competition unfolds.

Notably, our dataset did not include the sex of individuals. Though some sex-based differences in behavior have been documented, they are sometimes attributed to reproductive behavior and territoriality (Tornick 2010; Lynn, Dalton, and Mathis 2019). Other studies of plethodontids have described no relationship between aggression and sex (Thurow 1976; Jaeger, Kalvarsky, and Shimizu 1982; Keen and Sharp 1984; Nishikawa 1985). Correspondingly, recent literature on salamander aggression has varied in its inclusion of sex as a behavior-determinant (Cunningham and Rissler 2013; Clay and Gifford 2016; Dallalio, Brand, and Grant 2017; Bissell and Cecala 2019). Sexing live salamanders is difficult and often species-specific. Neither *P. shermani* nor *P. teyahalee* exhibit any external recorded sexual dimorphism (Bruce 2000; Rucker et al. 2021) beyond reproductive characteristics, such as enlarged ova or mental glands, which were not present in our sample individuals (Nishikawa 1990; Palmer and Houck 2005). Our dataset also did not include the degree of hybridization of individuals. Given that our analysis focuses on differences in thermal plasticity between populations rather than individual variation, we elected to rely on categorical descriptions of home population rather than establish degree of hybridization as a continuous feature. Future examinations of hybridization should incorporate salamander population genomics as they become more accessible, but the extraordinarily large genome sizes of salamanders have posed a significant challenge to genetic analyses (Gregory 2024). We suggest that future studies combine physiology, genomics, and behavior as a framework for understanding interspecific variation.

This is one of the first investigations to explore evasive behavior in lungless salamanders. We observed that individuals devoted a considerable portion of time and energy to evasive behavior, flinging themselves away from the aggressor, or jerking back to avoid contact. Thus, examining aggression alone does not provide the full picture of behavioral diversity in lungless salamanders. Additionally, this is one of the first studies examining the relationship between hybridization and thermal plasticity. Taxon-level differences between hybrid and parent individuals have previously been taken as evidence of hybrid vigor (Trotta et al. 2010; Bernatowicz, Dawidowicz, and Pijanowska 2021; Singh et al. 2022), which supposes a fitness benefit for hybrid individuals relative to their parent taxa. Our study suggests that thermal plasticity, or lack thereof, may underlie fitness differences across populations. The exact direction of this fitness change, however, remains unclear. Increased aggression in salamanders corresponds to improved competitive ability (Wrobell, Gergits,

and Jaeger 1980), suggesting that thermally plastic taxa that can fine-tune performance with respect to temperature, such as *P. shermani*, may win interspecific bouts more often when paired with competitors that cannot. Furthermore, aggression is not particularly taxing for salamanders—energy expenditure is largely unchanged by aggressive intensity (Bennett and Houck 1983). This seems to support the strategy of extreme aggression, and thus extreme thermal plasticity. These outcomes, however, may be tempered when individuals are faced with multiple competing demands; hydroregulation, for example, may take precedence over thermoregulation (Brattstrom 1979; Anderson and Andrade 2017). Both behavior and physiology are intrinsically tied to environmental temperature (Riddell et al. 2018, 2024; Navarro et al. 2018), and these trait-environment relationships influence how ranges will shift under climate change (Riddell et al. 2023). Some populations may be more plastic to temperature than others, altering the competitive landscape and determining persistence under warming, but many of the tradeoffs associated with plasticity have yet to be identified.

Author Contributions

Emmy James: conceptualization, data curation, formal analysis, visualization, writing – original draft, methodology, investigation, writing – review and editing, project administration. **Martha M. Muñoz:** writing – review and editing, conceptualization, funding acquisition, resources, supervision, project administration.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data and code are available on GitHub at https://github.com/emmyl18/salamander_hybrid_behavior.

References

- Abhinandini, D. I., and M. G. Venkatesha. 2015. “Antagonistic Interactions Among Common Tropical Household Ant Species (Hymenoptera: Formicidae).” *Journal of Entomology and Zoology Studies* 3: 27–32.
- Allaire, J. J., Y. Xie, C. Dervieux, et al. 2024. “rmarkdown: Dynamic Documents for R.” <https://github.com/rstudio/rmarkdown>.
- AmphibiaWeb. 2024. “AmphibiaWeb.” <https://amphibiaweb.org/>.
- Anderson, R. C. O., and D. V. Andrade. 2017. “Trading Heat and Hops for Water: Dehydration Effects on Locomotor Performance, Thermal Limits, and Thermoregulatory Behavior of a Terrestrial Toad.” *Ecology and Evolution* 7: 9066–9075.
- Angilletta, M. J., Jr. 2009. *Thermal Adaptation: A Theoretical and Empirical Synthesis*. London: Oxford University Press.

- Bennett, A. F., and L. D. Houck. 1983. "The Energetic Cost of Courtship and Aggression in a Plethodontid Salamander." *Ecology* 64: 979–983.
- Bernatowicz, P., P. Dawidowicz, and J. Pijanowska. 2021. "Phenotypic Plasticity and Developmental Noise in Hybrid and Parental Clones of *Daphnia longispina* Complex." *Aquatic Ecology* 55: 1179–1188.
- Biro, P. A., and J. A. Stamps. 2010. "Do Consistent Individual Differences in Metabolic Rate Promote Consistent Individual Differences in Behavior?" *Trends in Ecology & Evolution* 25: 653–659.
- Bissell, K. E., and K. K. Cecala. 2019. "Increased Interspecific Aggression Between Appalachian Stream Salamanders at Elevated Temperatures." *Freshwater Science* 38: 834–841.
- Bogert, C. M. 1949. "Thermoregulation in Reptiles, A Factor in Evolution." *Evolution; International Journal of Organic Evolution* 3: 195–211.
- Boronow, K., I. Shields, and M. Muñoz. 2018. "Parallel Behavioral Divergence With Macrohabitat in *Anolis* (Squamata: Dactyloidae) Lizards From The Dominican Republic." *Breviora* 561: 1–17.
- Bozinovic, F., P. Calosi, and J. Spicer. 2011. "Physiological Correlates of Geographic Range in Animals." *Annual Review of Ecology, Evolution, and Systematics* 42, 155–179.
- Brattstrom, B. H. 1979. "Amphibian Temperature Regulation Studies in the Field and Laboratory." *American Zoologist* 19: 345–356.
- Braun, C. A., T. A. Baird, and J. K. LeBeau. 2010. "Influence of Substrate Temperature and Directness of Approach on the Escape Responses of Juvenile Collared Lizards." *Herpetologica* 66: 418–424.
- Breed, M. D., and C. D. Rasmussen. 1980. "Behavioural Strategies During Intermale Agonistic Interactions in a Cockroach." *Animal Behaviour* 28: 1063–1069.
- Bruce, R. C. 2000. "Sexual Size Dimorphism in the Plethodontidae." In *The Biology of Plethodontid Salamanders*, edited by R. C. Bruce, R. G. Jaeger, and L. D. Houck, 243–260. Boston, MA: Springer US.
- Buckley, L. B., J. J. Tewksbury, and C. A. Deutsch. 2013. "Can Terrestrial Ectotherms Escape the Heat of Climate Change by Moving?" *Proceedings of the Royal Society B: Biological Sciences* 280: 20131149.
- Burger, I. J., E. T. Carter, L. M. Magner, et al. 2024. "Assessing Hybrid Vigour Using the Thermal Sensitivity of Physiological Trade-Offs in Tiger Salamanders." *Functional Ecology* 38: 143–152.
- Carvalho, F., R. Sousa, F. Cássio, and C. Pascoal. 2022. "Temperature and Interspecific Competition Alter the Impacts of Two Invasive Crayfish Species on a Key Ecosystem Process." *Biological Invasions* 24: 3757–3768.
- Clay, T. A., and M. E. Gifford. 2016. "Thermal Sensitivity of Aggression in Two Terrestrial Salamanders, *Plethodon cylindraceus* and *P. montanus*." *Ethology* 122: 127–133.
- Costaz, T. P. M., P. W. De Jong, J. A. Harvey, J. J. A. Van Loon, M. Dicke, and R. Gols. 2023. "Temperature Affects the Outcome of Competition Between Two Sympatric Endoparasitoids." *Animal Behaviour* 203: 11–20.
- Crowley, S. R., and R. D. Pietruszka. 1983. "Aggressiveness and Vocalization in the Leopard Lizard (*Gambelia wislizenii*): The Influence of Temperature." *Animal Behaviour* 31: 1055–1060.
- Cunningham, H. R., and L. J. Rissler. 2013. "Investigating Behavioral Shifts in Aggression Between a Naturalized and Native Salamander Species of the Genus *Plethodon*." *Herpetological Conservation and Biology* 8: 276–287.
- Dahl, D. B., D. Scott, C. Roosen, A. Magnusson, and J. Swinton. 2019. "xtable: Export Tables to LaTeX or HTML." <https://CRAN.R-project.org/package=xtable>.
- Dallalio, E. A., A. B. Brand, and E. H. C. Grant. 2017. "Climate-Mediated Competition in a High-Elevation Salamander Community." *Journal of Herpetology* 51: 190–196.
- Davis, T. M. 2002. "An Ethogram of Intraspecific Agonistic and Display Behavior for the Wandering Salamander, *Aneides vagrans*." *Herpetologica* 58: 371–382.
- Domenici, P., J. M. Blagburn, and J. P. Bacon. 2011. "Animal Escapology I: Theoretical Issues and Emerging Trends in Escape Trajectories." *Journal of Experimental Biology* 214: 2463–2473.
- Drummond, J. P., Jr. 2015. "Aggression Affects Distributions and Hybrid Zone Expansion in Montane *Plethodon* Salamanders." ProQuest Dissertations and Theses.
- Engler, J. O., D. Rödder, O. Elle, A. Hochkirch, and J. Secondi. 2013. "Species Distribution Models Contribute to Determine the Effect of Climate and Interspecific Interactions in Moving Hybrid Zones." *Journal of Evolutionary Biology* 26: 2487–2496.
- Farallo, V. R., M. M. Muñoz, J. C. Uyeda, and D. B. Miles. 2020. "Scaling Between Macro- to Microscale Climatic Data Reveals Strong Phylogenetic Inertia in Niche Evolution in Plethodontid Salamanders." *Evolution* 74: 979–991.
- Feldmeier, S., B. R. Schmidt, N. E. Zimmermann, M. Veith, G. F. Ficetola, and S. Lötters. 2020. "Shifting Aspect or Elevation? The Climate Change Response of Ectotherms in a Complex Mountain Topography." *Diversity and Distributions* 26: 1483–1495.
- Fox, J., and S. Weisberg. 2019. *An R Companion to Applied Regression*. 3rd ed. Thousand Oaks, CA: Sage.
- Freeman, B. G., M. Strimas-Mackey, and E. T. Miller. 2022. "Interspecific Competition Limits Bird Species' Ranges in Tropical Mountains." *Science* 377: 416–420.
- Gade, M. R., Q. Zhao, and W. E. Peterman. 2022. "Spatial Variation in Demographic Processes and the Potential Role of Hybridization for the Future." *Landscape Ecology* 37: 2671–2687.
- Garcia, M. J., L. Paiva, M. Lennox, B. Sivaraman, S. C. Wong, and R. L. Earley. 2012. "Assessment Strategies and the Effects of Fighting Experience on Future Contest Performance in the Green Anole (*Anolis carolinensis*)." *Ethology* 118: 821–834.
- Gaston, K. J. 2009. "Geographic Range Limits: Achieving Synthesis." *Proceedings of the Royal Society B: Biological Sciences* 276: 1395–1406.
- Giacometti, D., and G. J. Tattersall. 2024. "Seasonal Variation of Behavioural Thermoregulation in a Fossorial Salamander (*Ambystoma maculatum*)." *Royal Society Open Science* 11: 240537.
- Gregory, T. R. 2024. "Animal Genome Size Database." <http://www.genomesize.com>.
- Grovenburg, T. W., K. L. Monteith, R. W. Klaver, and J. A. Jenks. 2012. "Predator Evasion by White-Tailed Deer Fawns." *Animal Behaviour* 84: 59–65.
- Grover, C. D., A. D. Kay, J. A. Monson, T. C. Marsh, and D. A. Holway. 2007. "Linking Nutrition and Behavioural Dominance: Carbohydrate Scarcity Limits Aggression and Activity in Argentine Ants." *Proceedings of the Royal Society B: Biological Sciences* 274: 2951–2957.
- Guderley, H., and P. Couture. 2005. "Stickleback Fights: Why Do Winners Win? Influence of Metabolic and Morphometric Parameters." *Physiological and Biochemical Zoology* 78: 173–181.
- Gutiérrez, J. S., T. Cattray, M. Espinosa-Colin, J. A. Masero, and J. P. Granadeiro. 2023. "Heat Stress Increases Risk Taking in Foraging Shorebirds." *Functional Ecology* 37: 1005–1019.
- Gvoždík, L. 2022. "Thermoregulatory Opportunity and Competition Act Independently on Life-History Traits in Aquatic Ectotherms." *Functional Ecology* 36: 2520–2530.
- Hairston, N. G., K. A. Kneidel, R. H. Wiley, and C. K. Smith. 1992. "The Dynamics of Two Hybrid Zones in Appalachian Salamanders of the Genus *Plethodon*." *Evolution; International Journal of Organic Evolution* 46: 930–938.

- Havird, J. C., J. L. Neuwald, A. A. Shah, A. Mauro, C. A. Marshall, and C. K. Ghalambor. 2020. "Distinguishing Between Active Plasticity due to Thermal Acclimation and Passive Plasticity due to Q10 Effects: Why Methodology Matters." *Functional Ecology* 34: 1015–1028.
- Hedrick, B. P., A. Estrada, C. Sutherland, and A. M. Barbosa. 2023. "Projected Northward Shifts in Eastern Red-Backed Salamanders due to Changing Climate." *Ecology and Evolution* 13: e9999.
- Herrel, A., R. S. James, and R. Van Damme. 2007. "Fight Versus Flight: Physiological Basis for Temperature-Dependent Behavioral Shifts in Lizards." *Journal of Experimental Biology* 210: 1762–1767.
- Hertz, P. E., R. B. Huey, and E. Nevo. 1982. "Fight Versus Flight: Body Temperature Influences Defensive Responses of Lizards." *Animal Behaviour* 30: 676–679.
- HilleRisLambers, J., M. A. Harsch, A. K. Ettinger, K. R. Ford, and E. J. Theobald. 2013. "How Will Biotic Interactions Influence Climate Change-Induced Range Shifts?" *Annals of the New York Academy of Sciences* 1297: 112–125.
- Hoffmaster, D. K. 1986. "Aggression in Tropical Orb-Weaving Spiders a Quest for Food?" *Ethology* 72: 265–276.
- Hsu, Y. Y., R. L. Earley, and L. L. Wolf. 2006. "Modulation of Aggressive Behaviour by Fighting Experience: Mechanisms and Contest Outcomes." *Biological Reviews* 81: 33–74.
- Hubbs, C. L. 1955. "Hybridization Between Fish Species in Nature." *Systematic Biology* 4: 1–20.
- Huey, R. B., P. E. Hertz, and B. Sinervo. 2003. "Behavioral Drive Versus Behavioral Inertia in Evolution: A Null Model Approach." *American Naturalist* 161: 357–366.
- Huey, R. B., and J. G. Kingsolver. 1989. "Evolution of Thermal Sensitivity of Ectotherm Performance." *Trends in Ecology & Evolution* 4: 131–135.
- Huey, R. B., and R. D. Stevenson. 1979. "Integrating Thermal Physiology and Ecology of Ectotherms: A Discussion of Approaches." *American Zoologist* 19: 357–366.
- Hunter, E. A., M. D. Matocq, P. J. Murphy, and K. T. Shoemaker. 2017. "Differential Effects of Climate on Survival Rates Drive Hybrid Zone Movement." *Current Biology* 27: 3898–3903.e4.
- IPCC. 2021. "Summary for Policymakers." In *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. London: Cambridge University Press.
- Jaeger, R. G. 1984. "Agonistic Behavior of the Red-Backed Salamander." *Copeia* 1984: 309.
- Jaeger, R. G., D. Kalvarsky, and N. Shimizu. 1982. "Territorial Behaviour of the Red-Backed Salamander: Expulsion of Intruders." *Animal Behaviour* 30: 490–496.
- Kassambar, A. 2023. "ggpubr: 'ggplot2' Based Publication Ready Plots." <https://CRAN.R-project.org/package=ggpubr>.
- Kearney, M., and W. Porter. 2009. "Mechanistic Niche Modelling: Combining Physiological and Spatial Data to Predict Species' Ranges." *Ecology Letters* 12: 334–350.
- Keen, W. H., and S. Sharp. 1984. "Responses of a Plethodontid Salamander to Conspecific and Congeneric Intruders." *Animal Behaviour* 32: 58–65.
- Kelly, A. E., and M. L. Goulden. 2008. "Rapid Shifts in Plant Distribution With Recent Climate Change." *Proceedings of the National Academy of Sciences* 105: 11823–11826.
- Krenhardt, K., J. Martinez-Padilla, D. Canal, et al. 2024. "The Effect of Environmental Variation on the Relationship Between Survival and Risk-Taking Behaviour in a Migratory Songbird." *Journal of Evolutionary Biology* 37: 566–576.
- Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2017. "lmerTest Package: Tests in Linear Mixed Effects Models." *Journal of Statistical Software* 82: 1–26.
- Legault, G., M. E. Bitters, A. Hastings, and B. A. Melbourne. 2020. "Interspecific Competition Slows Range Expansion and Shapes Range Boundaries." *Proceedings of the National Academy of Sciences* 117: 26854–26860.
- Lemos-Espinal, J. A., and G. R. Smith. 2021. "Flight Initiation Distance of *Urosaurus ornatus* From the Sierra de Samalayuca, Mexico." *Acta Herpetologica* 16: 53–57.
- Liles, L. A., K. K. Cecala, J. R. Ennen, and J. M. Davenport. 2017. "Elevated Temperatures Alter Competitive Outcomes and Body Condition in Southern Appalachian Salamanders." *Animal Conservation* 20: 502–510.
- Losey, G. S. 1982. "Intra- and Interspecific Aggression by the Central American Midas Cichlid Fish, *Cichlasoma citrinellum*." *Behaviour* 79: 39–80.
- Lynn, C. S., B. Dalton, and A. Mathis. 2019. "Territorial Behaviour in Southern Red-Backed and Ozark Zigzag Salamanders: Effects of Sex, Species and Ownership." *Behaviour Research and Therapy* 156: 1017–1037.
- Mazerolle, M. J. 2023. "AICcmodavg: Model Selection and Multimodel Inference Based on (Q)AIC(c)." <https://cran.r-project.org/package=AICcmodavg>.
- Messerman, A. F., M. Turrell, and M. Leal. 2022. "Divergent Physiological Acclimation Responses to Warming Between Two Co-Occurring Salamander Species and Implications for Terrestrial Survival." *Journal of Thermal Biology* 106: 103228.
- Metcalfe, N. B., T. E. Van Leeuwen, and S. S. Killen. 2016. "Does Individual Variation in Metabolic Phenotype Predict Fish Behaviour and Performance?" *Journal of Fish Biology* 88: 298–321.
- Meza-Parral, Y., C. Garcia-Robledo, E. Pineda, F. Escobar, and M. A. Donnelly. 2020. "Standardized Ethograms and a Device for Assessing Amphibian Thermal Responses in a Warming World." *Journal of Thermal Biology* 89: 102565.
- Moldowan, P. D., G. J. Tattersall, and N. Rollinson. 2022. "Climate-Associated Decline of Body Condition in a Fossorial Salamander." *Global Change Biology* 28: 1725–1739.
- Montanucci, R. R. 1983. "Natural Hybridization Between Two Species of Collared Lizards (*Crotaphytus*)." *Copeia* 1983: 1.
- Moretz, J. A. 2003. "Aggression and RHP in the Northern Swordtail Fish, *Xiphophorus cortezi*: The Relationship Between Size and Contest Dynamics in Male–Male Competition." *Ethology* 109: 995–1008.
- Muñoz, D., D. Miller, R. Schilder, and E. H. Campbell Grant. 2022. "Geographic Variation and Thermal Plasticity Shape Salamander Metabolic Rates Under Current and Future Climates." *Ecology and Evolution* 12: e8433.
- Muñoz, M. M. 2022. "The Bogert Effect, a Factor in Evolution." *Evolution* 76: 49–66.
- Muñoz, M. M., and J. B. Losos. 2018. "Thermoregulatory Behavior Simultaneously Promotes and Forestalls Evolution in a Tropical Lizard." *American Naturalist* 191: E15–E26.
- Nishikawa, K. C. 1985. "Competition and the Evolution of Aggressive Behavior in Two Species of Terrestrial Salamanders." *Evolution* 39: 1282–1294.
- Nishikawa, K. C. 1990. "Intraspecific Spatial Relationships of Two Species of Terrestrial Salamanders." *Copeia* 1990: 418.
- Novarro, A. J., C. R. Gabor, C. B. Goff, T. D. Mezebish, L. M. Thompson, and K. L. Grayson. 2018. "Physiological Responses to Elevated Temperature Across the Geographic Range of a Terrestrial Salamander." *Journal of Experimental Biology* 221: jeb178236.

- Palmer, C. A., and L. D. Houck. 2005. "Responses to Sex- and Species-Specific Chemical Signals in Allopathic and Sympatric Salamander Species." In *In Chemical Signals in Vertebrates*. New York, NY: Springer.
- Parmesan, C., and G. Yohe. 2003. "A Globally Coherent Fingerprint of Climate Change Impacts Across Natural Systems." *Nature* 421: 37–42.
- Parris, M. J. 1999. "Hybridization in Leopard Frogs (*Rana pipiens* Complex): Larval Fitness Components in Single-Genotype Populations and Mixtures." *Evolution* 53: 1872–1883.
- Pedersen, T. L., J. Ooms, and D. Govett. 2024. "Systemfonts: System Native Font Finding." <https://CRAN.R-project.org/package=systemfonts>.
- Pigot, A. L., and J. A. Tobias. 2013. "Species Interactions Constrain Geographic Range Expansion Over Evolutionary Time." *Ecology Letters* 16: 330–338.
- R Core Team. 2022. "R: A Language and Environment for Statistical Computing." <https://www.R-project.org/>.
- Rand, A. S. 1964. "Inverse Relationship Between Temperature and Shyness in the Lizard *Anolis lineatopus*." *Ecology* 45: 863–864.
- Ratnasabapathi, D., J. Burns, and R. Soucek. 1992. "Effects of Temperature and Prior Residence on Territorial Aggression in the Convict Cichlid *Cichlasoma nigrofasciatum*." *Aggressive Behavior* 18: 365–372.
- Riddell, E. A., I. J. Burger, M. M. Muñoz, S. J. Weaver, and M. C. Womack. 2024. "Amphibians Exhibit Extremely High Hydric Costs of Respiration." *Integrative and Comparative Biology* 64, no. 2: icae053. <https://doi.org/10.1093/icb/icae053>.
- Riddell, E. A., I. J. Burger, T. L. Tyner-Swanson, et al. 2023. "Parameterizing Mechanistic Niche Models in Biophysical Ecology: A Review of Empirical Approaches." *Journal of Experimental Biology* 226: jeb245543.
- Riddell, E. A., J. P. Odom, J. D. Damm, and M. W. Sears. 2018. "Plasticity Reveals Hidden Resistance to Extinction Under Climate Change in the Global Hotspot of Salamander Diversity." *Science Advances* 4, no. 7: eaar5471. <https://doi.org/10.1126/sciadv.aar5471>.
- Rillich, J., and P. A. Stevenson. 2019. "Fight or Flee? Lessons From Insects on Aggression." *e-Neuroforum* 25: 3–13.
- Rodriguez-Sanchez, F., and C. P. Jackson. 2023. "Grateful: Facilitate Citation of R Packages." <https://pakillo.github.io/grateful/>.
- Rucker, L. E., D. J. Brown, C. D. Jacobsen, K. R. Messenger, E. R. Wild, and T. K. Pauley. 2021. "A Guide to Sexing Salamanders in Central Appalachia, United States." *Journal of Fish and Wildlife Management* 12: 585–603.
- Samia, D. S. M., D. T. Blumstein, T. Stankowich, and W. E. Cooper Jr. 2016. "Fifty Years of Chasing Lizards: New Insights Advance Optimal Escape Theory." *Biological Reviews* 91: 349–366.
- Scott, P. A., T. C. Glenn, and L. J. Rissler. 2019. "Formation of a Recent Hybrid Zone Offers Insight Into the Geographic Puzzle and Maintenance of Species Boundaries in Musk Turtles." *Molecular Ecology* 28: 761–771.
- Sears, M. W., E. Raskin, and M. J. Angilletta Jr. 2011. "The World Is Not Flat: Defining Relevant Thermal Landscapes in the Context of Climate Change." *Integrative and Comparative Biology* 51: 666–675.
- Sears, M. W., E. A. Riddell, T. W. Rusch, and M. J. Angilletta. 2019. "The World Still Is Not Flat: Lessons Learned From Organismal Interactions With Environmental Heterogeneity in Terrestrial Environments." *Integrative and Comparative Biology* 59: 1049–1058.
- Sexton, J., P. McIntyre, A. Angert, and K. Rice. 2009. "Evolution and Ecology of Species Range Limits." *Annual Review of Ecology, Evolution, and Systematics* 40: 415–436.
- Singh, D., S. Ramniwas, P. K. Tyagi, G. Kumar, and D. Gola. 2022. "Melanization Plasticity of *Drosophila kikkawai*, *Drosophila leontia* and Reciprocal Hybrids Under Different Temperatures." *Journal of Asia-Pacific Entomology* 25: 101865.
- Smith, G. R., and J. A. Lemos-Espinal. 2005. "Comparative Escape Behavior of Four Species of Mexican Phrynosomatid Lizards." *Herpetologica* 61: 225–232.
- Spotila, J. R. 1972. "Role of Temperature and Water in the Ecology of Lungless Salamanders." *Ecological Monographs* 42: 95–125.
- Stapley, J., and M. J. Whiting. 2006. "Ultraviolet Signals Fighting Ability in a Lizard." *Biology Letters* 2: 169–172.
- Tanner, C. J. 2006. "Numerical Assessment Affects Aggression and Competitive Ability: A Team-Fighting Strategy for the Ant *Formica xerophila*." *Proceedings of the Royal Society B: Biological Sciences* 273: 2737–2742.
- Tattersall, G. J. 2007. "Skin Breathing in Amphibians." In *Endothelial Biomedicine*, edited by W. C. Aird, 85–91. Cambridge: Cambridge University Press.
- Taylor, S. A., T. A. White, W. M. Hochachka, V. Ferretti, R. L. Curry, and I. Lovette. 2014. "Climate-Mediated Movement of an Avian Hybrid Zone." *Current Biology* 24: 671–676.
- Thurrow, G. 1976. "Aggression and Competition in Eastern *Plethodon* (Amphibia, Urodela, Plethodontidae)." *Journal of Herpetology* 10: 277.
- Tingley, R., M. Vallinoto, F. Sequeira, and M. R. Kearney. 2014. "Realized Niche Shift During a Global Biological Invasion." *Proceedings of the National Academy of Sciences* 111: 10233–10238.
- Tornick, J. K. 2010. "Factors Affecting Aggression During Nest Guarding in the Eastern Red-Backed Salamander (*Plethodon cinereus*)." *Herpetologica* 66: 385–392.
- Trotta, V., C. Pertoldi, A. Rudoy, T. Manenti, S. Cavicchi, and D. Guerra. 2010. "Thermal Plasticity of Wing Size and Shape in *Drosophila melanogaster*, *D. Simulans* and Their Hybrids." *Climate Research* 43: 71–79.
- Umbers, K. D. L., L. Osborne, and J. S. Keogh. 2012. "The Effects of Residency and Body Size on Contest Initiation and Outcome in the Territorial Dragon, *Ctenophorus decresii*." *PLoS One* 7: e47143.
- Urban, M. C. 2018. "Escalator to Extinction." *Proceedings of the National Academy of Sciences* 115: 11871–11873.
- Urban, M. C., J. J. Tewksbury, and K. S. Sheldon. 2012. "On a Collision Course: Competition and Dispersal Differences Create No-Analogue Communities and Cause Extinctions During Climate Change." *Proceedings of the Royal Society B: Biological Sciences* 279: 2072–2080.
- Urban, M. C., P. L. Zarnetske, and D. K. Skelly. 2013. "Moving Forward: Dispersal and Species Interactions Determine Biotic Responses to Climate Change." *Annals of the New York Academy of Sciences* 1297: 44–60.
- Walls, S. C. 2009. "The Role of Climate in the Dynamics of a Hybrid Zone in Appalachian Salamanders." *Global Change Biology* 15: 1903–1910.
- Wickham, H. 2016. *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer.
- Wilke, C. O., and B. M. Wiernik. 2022. "ggtext: Improved Text Rendering Support for ggplot2." <https://CRAN.R-project.org/package=ggtext>.
- Wrobell, D. J., W. F. Gergits, and R. G. Jaeger. 1980. "An Experimental Study of Interference Competition Among Terrestrial Salamanders." *Ecology* 61: 1034–1039.
- Xie, Y. 2014. "In Implementing Reproducible Computational Research." In *Knitr: A Comprehensive Tool for Reproducible Research in R*, edited by V. Stodden, F. Leisch, and R. D. Peng. Boca Raton, Florida: Chapman and Hall/CRC.
- Xie, Y. 2015. *Dynamic Documents With R and Knitr*. 2nd ed. Boca Raton, FL: Chapman and Hall/CRC.
- Xie, Y. 2024. "knitr: A General-Purpose Package for Dynamic Report Generation in R." <https://yihui.org/knitr/>.

Xie, Y., J. J. Allaire, and G. Golemund. 2018. *R Markdown: The Definitive Guide*. Boca Raton, Florida: Chapman and Hall/CRC.

Xie, Y., C. Dervieux, and E. Riederer. 2020. *R Markdown Cookbook*. Boca Raton, Florida: Chapman and Hall/CRC.

Ydenberg, R., and L. Dill. 1986. "The Economics of Fleeing From Predators." *Advances in the Study of Behavior* 16: 229–249. [https://doi.org/10.1016/s0065-3454\(08\)60192-8](https://doi.org/10.1016/s0065-3454(08)60192-8).

Zhu, H. 2017. "kableExtra: Construct Complex Table With "kable" and Pipe Syntax." <https://CRAN.R-project.org/package=kableExtra>.

Supporting Information

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