

# Eavesdropping Micropredators as Dynamic Limiters of Sexual Signal Elaboration and Intrasexual Competition

Brian C. Leavell,<sup>1,\*</sup> Lynne E. Beaty,<sup>2</sup> Gordon G. McNickle,<sup>3</sup> and Ximena E. Bernal<sup>1,4</sup>

1. Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907; 2. School of Science, Penn State Erie, Behrend College, Erie, Pennsylvania 16563; 3. Department of Botany and Plant Pathology and Center for Plant Biology, Purdue University, West Lafayette, Indiana 47907; 4. Smithsonian Tropical Research Institute, Apartado, Balboa, Ancón, Panamá

Submitted September 17, 2020; Accepted November 10, 2021; Electronically published March 25, 2022

Online enhancements: supplemental PDF. Dryad data: <https://doi.org/10.5061/dryad.7wm37pvr5>.

**ABSTRACT:** To thoroughly understand the drivers of dynamic signal elaboration requires assessing the direct and indirect effects of naturally interacting factors. Here, we use structural equation modeling to test multivariate data from in situ observations of sexual signal production against a model of causal processes hypothesized to drive signal elaboration. We assess direct and indirect effects, and relative impacts, of male-male competition and attacks by eavesdropping frog-biting midges (Diptera: Corethrellidae) on call elaboration of male túngara frogs (*Engystomops pustulosus*). We find that the intensity of attacks by these micropredator flies drives the extent to which frogs elaborate their calls, likely due to a temporal trade-off between signaling and antimicropredator defense. Micropredator attacks appear to dynamically limit a male's call rate and complexity and consequently dampen the effects of intrasexual competition. In accounting for naturally interacting drivers of signal elaboration, this study presents a counterpoint to the mechanisms traditionally thought to drive sexual selection in this system. Moreover, the results shed light on the relatively unexamined and potentially influential role of eavesdropping micropredators in the evolution of sexual communication systems.

**Keywords:** communication, foraging, male-male competition, plasticity, predator-prey, structural equation modeling.

## Introduction

Despite a rich history of studies dating back to Darwin (1871) and Wallace (1891), evolutionary biologists continue to investigate and debate the mechanisms that shape the extravagant sexual signals observed across organisms (Kokko et al. 2006; Prum 2010). It is clear, however, that the evolution of secondary sexual traits emerges from a balance struck between benefits and costs (Wallace 1891; Kirkpatrick and

Ryan 1991; Andersson 1994). Depending on the communication context (i.e., inter- vs. intrasexual), target receivers of sexual signals often prefer or are more threatened by elaborated signal components (i.e., traits of greater quantity; Ryan and Keddy-Hector 1992) and thus drive the reproductive benefits of signal elaboration. While research on the costs of sexually selected traits has largely focused on energetic requirements for production and maintenance (e.g., Eberhardt 1994; Simmons and Emlen 2006; Somjee et al. 2018), there is a building body of empirical work on how mating communication systems are shaped by the costs imposed by eavesdropping natural enemies (i.e., predators, parasitoids, and parasites that exploit communication systems to find and attack their signaling victims; Zuk and Koluru 1998). Eavesdropping enemies are potentially strong agents of selection on sexual signal elaboration given that elaborate signal components that increase attractiveness to a potential mate or degree of threat to competitors are often also more conspicuous to eavesdropping enemies. Examples of such eavesdropping enemies that overlap with potential mates in their enhanced attraction to elaborated sexual signals include frog-eating bats (*Trachops cirrhosus*) and frog-biting midges (*Corethrella* spp.; Ryan et al. 1982; Bernal et al. 2006), as well as parasitoid flies (*Ormia ochracea* [Wagner 1996] and *Therobia leonidei* [Lehmann et al. 2001]). In these cases, the signaling sex must navigate a fundamental trade-off between the potential cost of predation risk and the reproductive benefit of signaling.

It is then unsurprising that some organisms dynamically alter components of their sexual signals to optimize fitness in response to variation in perceived costs of signal elaboration (Lindström et al. 2009), including costs imposed by predation (e.g., Candolin 1997). Risk-dependent responses of prey to perceived predation risk is indeed a well-studied phenomenon within predator-prey interactions (Miner et al.

\* Corresponding author; email: [bleavell@purdue.edu](mailto:bleavell@purdue.edu).

**ORCID:** Leavell, <https://orcid.org/0000-0003-4443-9309>; Beaty, <https://orcid.org/0000-0003-0778-1922>; McNickle, <https://orcid.org/0000-0002-7188-7265>; Bernal, <https://orcid.org/0000-0001-6155-5980>.

2005; Stevens 2016). Given that the intensity of predation risk varies in time and space (Valeix et al. 2009; Palmer et al. 2017) and that antipredatory strategies come at a cost, the threat sensitivity hypothesis predicts that antipredator behavior is scaled in proportion to the magnitude of predation risk (Sih 1986; Helfman 1989). Thus, for animals capable of dynamically elaborating conspicuous mating signals, this hypothesis predicts that the extent of elaboration is inversely related to perceived predation risk from eavesdropping enemies.

While examinations of the threat sensitivity hypothesis have focused on mechanisms that indirectly reduce predation risk, use of defensive behaviors to directly counter predation risk is also expected. Such strategies may be common, for instance, when confronting micropredators, such as herbivores and blood-sucking flies, which individually have limited impacts on their victim's fitness (Lafferty and Kuris 2002). Regarding threats from eavesdropping enemies, signaling prey may employ a range of defenses that are unrelated to signal elaboration but also scale in proportion to the threat. Calling male túngara frogs (*Engystomops pustulosus*), for instance, exhibit graded evasive responses to differing degrees of predation risk from the eavesdropping fringe-lipped bat (Tuttle et al. 1982). The armored cricket (*Acanthopplus speiseri*) also shows risk-dependent antipredator behaviors when calling, such as alarm stridulation and autohemorrhaging (Bateman and Fleming 2013). Such physical defenses, while preventing depredation, may come with immediate reproductive costs if they force the signaler to allocate time and energy away from signal production and elaboration.

The degree of risk imposed by eavesdropping enemies is only one of multiple factors that can dynamically shape sexual signal elaboration. Variation in intrasexual competition also strongly influences the extent to which a signaler (typically male) modifies a sexual signal. To counter the threat of increased competition for mates, the signaler may elaborate signal characteristics, such as intensity, size, rate, or complexity (e.g., Ryan 1985; Salazar and Stoddard 2009; Kim and Velando 2014). While ecological factors, such as food availability, can influence the behaviors of male competitors in the long-term (e.g., Marler and Ryan 1996; Koluru et al. 2007), it remains unclear how ecological modifiers of sexual signals interact with intrasexual competition in the short-term. The interaction between the risks imposed by eavesdropping enemies and threats from intrasexual competitors presents one such short-term, dynamic challenge—to suppress or enhance signal elaboration—that males must navigate to optimize fitness.

Here, our specific objectives are to (1) define and evaluate hypothesized causal processes through which intrasexual competition and eavesdropping enemies are thought to drive mating signal elaboration, (2) determine the poten-

tial direct and indirect effects of both intrasexual competition and attacks from eavesdropping enemies on mating signal elaboration, (3) test the threat sensitivity hypothesis's prediction that antipredator strategies scale with risk of attack, and (4) assess the relative impacts of intrasexual competition and attacks from eavesdropping enemies on mating signal elaboration. To accomplish these objectives, we used piecewise structural equation models (SEMs) to examine the impact of naturally varying intensities of male competitors and frog-biting midges (obligate eavesdropping micropredators) on call properties of male túngara frogs. We hypothesize that calling males assess the risk of midge attacks relative to the degree of intrasexual competition to adjust their call rate and complexity. While micropredators, because of their unique consumer strategy, have been predicted to elicit relatively weak avoidance and defensive responses in their victims (Daversa et al. 2019), on the basis of preliminary observations, we predict that anti-midge swatting defense intensifies with increasing midge attacks. We predict that, as a by-product, more frequent midge attacks suppress the impact of intrasexual competition on signal rate and complexity. This effect may result because of a temporal trade-off between swatting and calling, direct reduction in call elaboration in response to perceived risk, or a combination of the two. We expect, however, that greater numbers of neighboring rival males will result in fewer midge attacks per capita, as frog-biting midges will be spread across more frogs (i.e., the dilution effect; Foster and Treherne 1981; Alem et al. 2011). In testing these predictions, this study parses the relative impacts of two distinct and potentially antagonistic modifiers of signaling plasticity—intrasexual competition and eavesdropping micropredators.

## Methods

### *Study System*

In this study, we investigated the calling strategies of túngara frogs in Gamboa, Panamá, in and around the facilities of the Smithsonian Tropical Research Institute. Like most frogs, túngara frogs heavily invest in signaling to attract mates. The túngara frog's call is composed of a simple "whine" component, to which additional complex "chucks" can be added (up to seven chucks per call; Bernal et al. 2007a). In response to rival calls, male túngara frogs dynamically modify their call elaboration by adjusting their call rate and number of chucks per call (i.e., "complexity"), typically by adding or removing a single chuck at a time (Rand and Ryan 1981; Bernal et al. 2007a, 2009a; Goutte et al. 2010). That is, to increase his likelihood of mating, a male will increase his call elaboration in response to the elevated call elaboration of neighboring males.

Increasing call rate and adding complex chucks make calling males more attractive to females and eavesdropping enemies, such as bats and frog-biting midges (Ryan et al. 1982; Bosch et al. 2000b; Bernal et al. 2006; Akre et al. 2011; Akre and Ryan 2011; Aihara et al. 2016). Female frog-biting midges use their prey's mating calls to detect and localize the source of their blood meals (McKeever 1977; Borkent 2008; Bernal and de Silva 2015). A single motivated calling frog can attract hundreds of midges in just 30 min (Bernal et al. 2006). As a result, túngara frogs can incur a loss of ~10% of their total blood volume throughout a night (X. E. Bernal, unpublished data) and risk infection from the midge-vectored blood parasite (*Trypanosoma tungarae*; Bernal and Pinto 2016). Thus, over the course of a night, male túngara frogs are forced to balance the benefits and costs of signaling that are imposed by multiple types of receivers.

Frogs, however, are not without a defense—they swat with their arms and legs to dislodge blood-sucking midges (McKeever 1977; Bernal et al. 2006; Borkent 2017). Such a response is not unique. For instance, many mammalian victims of attacks by sanguivorous flies employ an array of behaviors akin to the túngara frog's swat—they might flick, toss, twitch, switch, swipe, or stamp to fend off the attackers (reviewed in Hart and Hart 2018). Swatting is common when túngara frogs are attacked by midges in natural conditions (up to a rate of 0.78 swats/s; de Silva et al. 2014). Yet combating midges potentially presents a temporal trade-off between signaling and defense, as males do not call while swatting (X. E. Bernal and B. C. Leavell, personal observation), likely because of a physical constraint imposed by the frog's inflated vocal sac (see the supplemental videos available via the Dryad Digital Repository [https://doi.org/10.5061/dryad.7wm37pvr5; Leavell et al. 2021]).

### Behavioral Observations

During the summers of 2010 and 2012, we observed calling male túngara frogs in situ. For each observation, which took place between 1900 and 2300 hours, we illuminated a calling male (hereafter, “focal frog”) with infrared LED arrays (Sima SL-201R) and recorded audio and video using a Sony DCR-SR220D 120-GB Handycam camcorder. Intrasexual competition was estimated by counting all of the calling males within 1 m of the focal frog. We chose a 1-m radius because túngara frogs have been shown to respond only to local competitors (~3 males maximum) within a chorus and this distance allows for comparison with past studies of male-male competition in this species (Greenfield and Rand 2000; Bernal et al. 2007a). Additionally, as a proxy for overall social activity in the chorus, each observation was assigned a number along a scale from 0

to 3 (following Heyer et al. 1994) according to a researcher's estimate of audible calling males at the time and place when recording a focal frog (0 = only focal frog heard calling; 1 = individual calling frogs could be counted; 2 = calls of frogs overlapping but individuals distinguishable; 3 = full chorus, cannot distinguish individuals). Following an observation, the focal frog was toe clipped to prevent pseudoreplication and released at point of capture in adherence to protocols established by the American Society of Ichthyologists and Herpetologists (https://asih.org/animal-care-guidelines). We conducted all research in compliance with Panamanian legal and ethical regulations (Mi Ambiente collection permits: SE/A-67-10, SC/A-20-12) and following institutional animal care and use committee protocols (Texas Tech University: 11056-08; Smithsonian Tropical Research Institute: 2011-0616-2014-11).

To analyze calling behaviors, we selected a sequence of the first 50 consecutive calls per focal frog, ensuring that the recordings maintained sufficient signal-to-noise ratios. We used the duration of the sequence, which varied per frog, to derive the call rate (i.e., the total number of calls, minus one, divided by the time from the beginning of the first call to the beginning of the last call; Cocroft and Ryan 1995). The total number of chucks produced was counted over the same time. To assess the threat of midge attacks, the total number of instances in which midges landed on the focal frog were counted from video playback during the 50-call sequence. We also counted the total number of times the focal frog swatted during the same sequence. Out of a total of 100 focal frogs that were recorded, we omitted data from 15 individuals because of high background noise levels or poor video quality that precluded behavioral analysis. The observed data set thus includes data from a total of 85 males (for matrix plot of behavioral data, see fig. S1; figs. S1–S6 are available online).

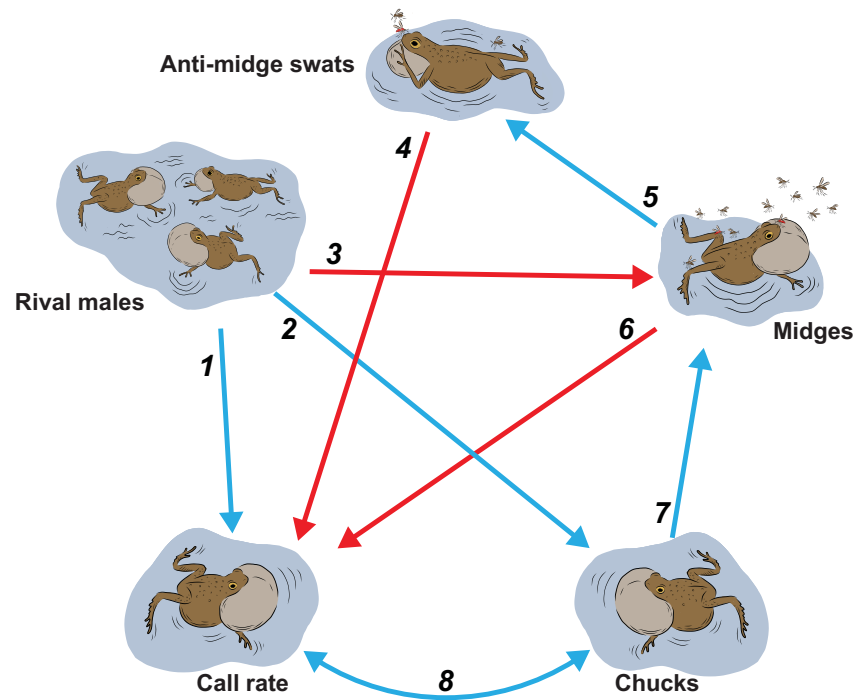
### Piecewise Structural Equation Model

To assess potential direct and indirect effects, and relative impacts, of male-male competition and frog-biting midges on male call elaboration, we analyzed the observed data set using piecewise SEM in the package piecewiseSEM in R (ver. 3.5.1; R Core Team 2018). Piecewise SEM, also referred to as confirmatory path analysis or a form of third-generation SEM, takes a graph-theoretic approach to causal modeling and relaxes assumptions of traditional path analyses (Grace et al. 2012; Lefcheck 2016). This approach integrates a network of local estimations that are modeled individually (i.e., piecewise) to disentangle complex relationships among variables. As a result, one can quantitatively test indirect and hierarchical effects within complex natural systems (e.g., Grace et al. 2007). The piecewise approach offers added flexibility over traditional SEMs in

that local-level models can accommodate random effects and nonnormal error structures. Additionally, by relying on local estimations, piecewise SEM prevents local errors from compounding throughout the network—a potential issue for SEMs that rely on global estimation (Grace et al. 2015). The network of local estimations is typically depicted by path diagrams, wherein arrows indicate the hypothesized relationships among observed variables, which can be assigned as predictors, responses, or both.

Here, following SEM conventions (Grace 2006), we established an initial hypothesized causal network based on prior knowledge of the midge–túngara frog system (fig. 1; see the supplemental PDF, available online, for extended justifications of hypothesized causal paths). We confirmed

that the paths in the network did not feed back on each other (i.e., were acyclic), which is a key assumption of piecewise SEM (Shipley 2009; Lefcheck 2016). We also confirmed that the number of samples per parameter fell within the recommended range of 5–20 (Grace et al. 2015). While SEM is amenable to both confirmatory and exploratory analytical approaches (Grace 2006), for the purpose of this study we take an exploratory approach to assess how modifying paths of the initial network affects the model's fit with the observed data. That is, we were interested in uncovering alternative, more robust explanations of our observations, which might guide future studies, rather than simply testing our initial hypothesized network. In accordance with recommended practices, modifications were



**Figure 1:** Initial hypothesized causal network. Blue and red paths indicate hypothesized positive and negative effects, respectively. Single-headed arrows represent direct effects, while double-headed arrows represent partial correlations. See the supplemental PDF for detailed path justifications. Briefly, hypothesized causal paths are based on the following: (1) male túngara frogs increase their call rate in response to increased numbers of calling rival males (Green 1990; Bernal et al. 2009a, 2009b), (2) male túngara frogs dynamically modify the number of chucks they add per call in response to rival calls (Ryan 1985; Bernal et al. 2007a, 2009a; Goutte et al. 2010), (3) increases in the number of aggregated prey reduce an individual's predation risk via the dilution effect (Foster and Treherne 1981; Alem et al. 2011). Here, we predict that more rivals will result in fewer midge attacks of the focal frog; (4) there is likely a temporal trade-off between swatting and calling, as males do not appear to be capable of performing both tasks simultaneously (X. E. Bernal and B. C. Leavell, personal observation; see the supplemental videos available via the Dryad Digital Repository [<https://doi.org/10.5061/dryad.7wm37pvr5>; Leavell et al. 2021]). Thus, swatting should reduce an individual's call rate; (5) following the threat sensitivity hypothesis, males will swat more frequently in response to greater numbers of midge attacks (Sih 1986; Helfman 1989); (6) similar to how the threat sensitivity hypothesis (Sih 1986; Helfman 1989) predicts that physical defensive behaviors scale in intensity with predation risk, if males scale their calling behaviors in response to the risk of midge attacks, then males should reduce calling rates, independent of swatting frequency, in response to greater numbers of midges; (7) midges prefer calls with chucks to calls without chucks (Bernal et al. 2006). Also, males that produce more chucks attract more midges (Aihara et al. 2016); (8) males often change their call rate and the number of chucks concomitantly (Green 1990; Bernal et al. 2009a). Illustrations by Razi Hedström.

made only if they were consistent with our knowledge of the system (Grace 2006).

The overall fit of the SEM was assessed by Shipley's test of directed separation, which tests causal independence claims among the missing paths in the model (Shipley 2009) and is common practice in evaluating piecewise SEMs (Lefcheck 2016). The fit of the data to the hypothesized causal relationships of the model was deemed adequate when the observed data underlying the causal independence claims could have occurred by chance (Lefcheck 2016). Specifically, the  $\chi^2$  distributed Fisher's *C* statistic is generated by the d-separation test, with  $P < .05$  indicating poor model fit. Thus, SEM is used to falsify a hypothesized network of causal relationships (Shipley 2016).

#### *Modeling Local Estimations and Statistical Analyses*

Before modeling local relationships between endogenous (response) and exogenous (predictor) variables in the SEM, we used variance inflation factors to determine collinearity among variables (Zuur et al. 2009). The number of neighboring rival males and level of the background calling environment exhibited high collinearity (generalized variance inflation factors  $>5$ ). We kept the former and removed the latter variable given that a main objective of the model was to assess the role of male rivals on call plasticity. Once this variable was removed, all remaining variables exhibited generalized variance inflation factor values  $<3$  and were thus included in subsequent models.

To generate local estimates for each path in the hypothesized network, we first formulated linear mixed effects models using the package *lme4* (Bates et al. 2015). All modeled relationships included date as a random effect. We compared models with varying random effect structures (slope and intercept) to determine the best fit, as we did not have a priori justifications to omit specific random effect structures. To assess whether each model in this study met assumptions of normality and homoscedasticity, we visually inspected residual plots and implemented Shapiro-Wilk tests (see the R code available in the Dryad Digital Repository [https://doi.org/10.5061/dryad.7wm37pvr5; Leavell et al. 2021]). For endogenous variables of nonnormally distributed count data, we formulated generalized linear mixed effects models with Poisson error structures and compared models with log and square root link functions. We accounted for overdispersion as indicated by tests from the packages *blme* (Korner-Nievergelt et al. 2015) and *DHARMA* (Hartig 2019) by including individual frog identity as an observational level random effect (Elston et al. 2001; Harrison 2014). We also tested for zero inflation using *DHARMA*. While one local-level model indicated zero-inflated data, we elected to still include it in the piecewiseSEM because (i) piecewiseSEM does not currently support zero-inflated

or zero-altered models and (ii) the fitted values and error from the local-level model were consistent with a separate hurdle model that accounted for zero inflation (fig. S2; see the supplemental PDF for detailed methods). All local-level models were compared and selected using conditional AIC (cAIC) values from the *cAIC4* package to control for biases when comparing models with differing random effects (Säfken et al. 2018). For each response variable, the model with the lowest cAIC value was integrated in the SEM. See tables S1–S9, available online, and R code for detailed overviews of model structures, their outputs, and subsequent analyses.

#### *Evaluating Structural Equation Models*

To assess and compare the relative magnitudes of effects across a network, we calculated the standardized regression coefficient for each path. Although  $\beta$  estimates are often standardized according to variables' standard deviations, this can lead to incorrect interpretations, especially when derived from nonnormal data, as present in this study (Grace and Bollen 2005). Standardizing across the relevant range of each variable, rather than the standard deviation, is considered to be a more robust and meaningful approach (Grace and Bollen 2005; Grace et al. 2012). Following this approach, we took the observed minimum and maximum values of a given predictor while holding all of the other predictors at their mean values to derive the change in the response variable (e.g., Grace et al. 2012; Dorresteijn et al. 2015). We divided the difference in response values from the previous step by the range of the response variable. This generated the standardized estimate for the given predictor—that is, the proportion that a response variable changes relative to its total (i.e., relevant) range as the predictor varies across its own range.

To further examine the effects of male rivals relative to attacking frog-biting midges, we evaluated and compared two additional SEMs that excluded the effects of either rivals or midges on call plasticity. SEM comparisons were based on sample size-corrected Akaike information criterion (AICc) values, following recommendations for piecewise SEMs (Lefcheck 2016). A top model was defined as having the lowest AICc value that was at least two units less than that of the next-best model.

### **Results and Discussion**

#### *Fit of Observational Data to Hypothesized Causal Network*

Our first objective was to define and evaluate a network of causal processes that we hypothesized to drive signal elaboration plasticity. The initial hypothesized causal network

was a poor fit to the observed data and therefore was falsified (fig. 1;  $C_4 = 11.041$ ,  $P = .026$ ). Although we had no a priori reason to expect male swatting effort to directly influence the number of chucks in his calls (or vice versa), the test of directed separation suggested a significant missing relationship between antimidge swats and the focal male's chucks. Still, the revealed correlation between swats and chucks, while not explicitly accounted for in the initial SEM, is not in conflict with our initial hypothesized network. The correlation can be explained by our predictions that swats drive call rate and call rate is partially correlated with chucks, a relationship likely driven by extraneous factors known to influence calling behaviors (e.g., hormones; Marler and Ryan 1996; Kime et al. 2007; Still et al. 2019). We controlled for this relationship in a revised (hereafter, "global") SEM by assuming a partial correlation between the two variables, as there was (i) no compelling biological basis to include a causal relationship between swats and chucks in the SEM, (ii) no impact on the significance and magnitude of effects of other paths in the network or the resulting conclusions of the study when this relationship was included in a subsequent SEM (see the supplemental PDF), and (iii) plausible, noncausal processes that explain their correlation. The test of directed separation for the global model indicated no significant missing pathways and an acceptable data-model fit (i.e., a lack of falsification; fig. 2;  $C_2 = 0.524$ ,  $P = .769$ ), supporting the notion that the hypothesized causal pathways align with natural processes in this system.

#### *Effects of Eavesdropping Micropredators*

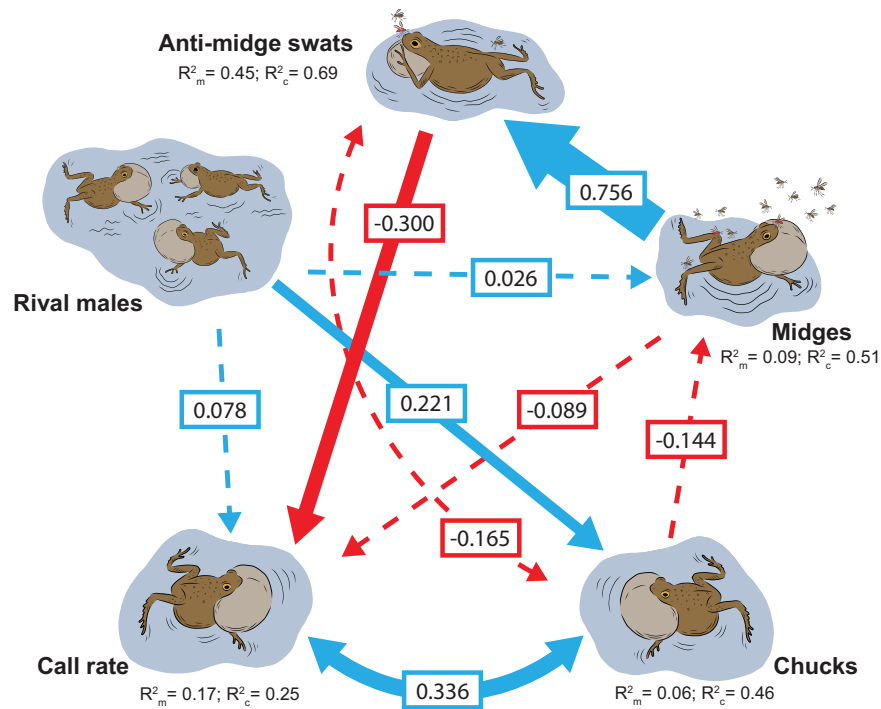
Our second objective in this study was to determine the direct and indirect effects of both intrasexual competition and eavesdropping enemies on signal elaboration plasticity. The global SEM revealed that the number of midge attacks was a strong positive predictor of a male's propensity to swat (standardized direct effect = 0.756,  $P < .001$ ; fig. 2; table 1; see fig. S3 for individual effect plots), and swatting had a negative impact on a male's call rate (standardized direct effect =  $-0.300$ ,  $P = .014$ ). These significant direct effects supported our initial predictions. Contrary to our prediction, however, the intensity of midge attacks had no direct effect on call rate ( $P = .451$ ; fig. 2). That is, even though reducing call rate can be an effective strategy to decrease the chances of midge attacks (Aihara et al. 2016) and many animals reduce signaling rates in response to elevated predation risk (e.g., Farr 1975; Jones et al. 2002; Simon 2007), males do not appear to compromise signaling rates directly in response to the intensity of micropredator attack. Yet in capitalizing on SEM's capacity to parse direct and indirect effects, our results reveal that midges impose a strong indirect, negative impact on

call rate by eliciting antimidge swats in their victims (standardized indirect effect =  $-0.227$ ; table S7).

Such a paradox, in which the intensity of midge attacks indirectly affects call rate despite having no direct effect, is likely due to unexplained among-male variation in response to midge attacks. It is unclear, however, what might underlie this variation. The physiological state of a male, such as his hormone levels or body condition, may affect his tendency to call versus swat. Factors external to males seem less likely to drive such heterogeneity. Temperature and precipitation are, for example, abiotic factors that seem to affect both frogs and midges in similar ways and would result in a positive correlation between midge attacks and call rate, rather than the observed indirect, negative effect and lack of direct effect. Overall, further investigation of this relationship reveals no conclusive ecological driver that could generate heterogeneity resulting in subgroups with different responses (see the supplemental PDF for a full description of these analyses).

Males that invest in swatting to combat midge attacks thus do so at the expense of a reduced call rate. This trade-off between swatting and calling is not surprising given that males do not and likely cannot call while swatting because of their sizeable inflated vocal sacs (X. E. Bernal and B. C. Leavell, personal observation; see the supplemental videos available via the Dryad Digital Repository [<https://doi.org/10.5061/dryad.7wm37pvr5>; Leavell et al. 2021]). Furthermore, considering the influence of swatting on call rate and that the partial correlation between a male's call rate and number of chucks was significant and positive (standardized estimate = 0.336,  $P = .001$ ; fig. 2; table 1), it appears that males indirectly scale multiple dimensions of call elaboration (i.e., rate and complexity) in response to the intensity of attacks by frog-biting midges. These findings collectively suggest that males are forced into a trade-off between benefiting from increasing signal elaboration (to increase attractiveness to potential mates) and mitigating the costs of conspicuous calling by swatting (to remove micropredators).

While frog-biting midges are shown here to dynamically influence male signal elaboration, the effects of eavesdropping micropredators may also extend to the female receiver. By limiting male signal elaboration, frog-biting midges may curtail intraspecific signal variation, directly impacting female choice (Ryan et al. 2007; Akre et al. 2011). Here, however, we find considerable variation in individual call rates that are not explained by midge attacks, swats, or male competitors ( $R_m^2 = 0.17$ ). This large unexplained variation may again reflect how the degrees to which a male invests in antimidge swats and subsequently adjusts his call rate are driven by state-dependent fitness payoffs, which can be influenced by a suite of factors, including each male's physiological condition and previous reproductive opportunities (e.g., Badyaev and Duckworth 2003; de Moraes et al. 2019).



**Figure 2:** Effects of rival male competition and frog-biting midges on call elaboration, estimated by piecewise structural equation model. Blue and red paths indicate positive and negative direct effects, respectively. Solid lines indicate significant effects. Path width reflects relative effect size for only significant effects. Dashed lines indicate nonsignificant effects. Numbers along paths are standardized estimates (single-headed arrows) or partial correlations (double-headed arrows). The term  $R_m^2$  is the marginal coefficient of determination, which represents the proportion of variance explained solely by the fixed effects. The term  $R_c^2$  is the conditional  $R^2$ , which represents the proportion of variance explained by both the fixed effects and the random effects. Illustrations by Razi Hedström.

Although the number of midge attacks explains little of the variance in call rates among males, the total effect of midges on call rates underscores the impact of antimidge swatting on within-male call plasticity in this system (total effect equals the sum of direct and indirect effects; standardized total effect =  $-0.316$ ; table S7). Evidence from frogs and crickets suggests that the degree of within-male variation in sexual signals is generally associated with particular receiver preference functions—static signal traits are often associated with unimodal female preference functions, while plastic traits are linked with open-ended female preference functions (Gerhardt 1991; Shaw and Herlihy 2000). An untested but exciting prospect is that, by suppressing within-male variation in signal elaboration, frog-biting midges may indirectly influence the evolution of female preferences.

While the results here shed light on the role of midges on a male's call plasticity, we also examined whether call properties could in turn drive the intensity of midge attacks. We had included the effect of chucks on midges based on (i) the preferences of midges in two-choice playback ex-

periments showing that midges are more attracted to calls with chucks (Bernal et al. 2006) and (ii) field collection of midges attacking naturally interacting, calling males in small choruses ( $<4$  males), which shows that frogs producing calls with more chucks attract more midges (Aihara et al. 2016). Therefore, we predicted that the more a male chucked, the more he would be attacked by midges. Yet the impact of chucks on the magnitude of midge attack was nonsignificant ( $P = .182$ ). It appears, then, that chucks do not drive midge attacks under natural conditions as we had initially predicted. A potential explanation for this finding could be that, because of the correlation between a male's call rate and complexity, the negative, indirect effect of midge attacks on a male's call rate is large enough to suppress any direct effect of the number of chucks on the intensity of midge attacks. Another possible mechanism is that swatting directly decreases a male's propensity to chuck, thus attracting fewer midges thereafter. For instance, it is possible that a male's own swat might cause a temporary reduction in his call complexity before he once again ramps up the number of chucks added to his calls. A temporary

**Table 1:** Unstandardized and standardized coefficients for the global piecewise structural equation model

| Response, predictor         | Unstandardized estimate | Standard error | Critical value | <i>P</i> | Standardized estimate (relevant range) |
|-----------------------------|-------------------------|----------------|----------------|----------|----------------------------------------|
| Swats:                      |                         |                |                |          |                                        |
| Midges                      | .037                    | .006           | 6.235          | .000***  | .756                                   |
| Midges:                     |                         |                |                |          |                                        |
| Chucks                      | −.014                   | .010           | −1.336         | .182     | −.144                                  |
| Rival males                 | .091                    | .350           | .260           | .795     | .026                                   |
| Call rate:                  |                         |                |                |          |                                        |
| Rival males                 | .010                    | .010           | .999           | .322     | .078                                   |
| Swats                       | −.002                   | .001           | 6.298          | .014*    | −.300                                  |
| Midges                      | −.0003                  | .000           | .573           | .451     | −.089                                  |
| Chucks:                     |                         |                |                |          |                                        |
| Rival males                 | 8.058                   | 3.732          | 4.435          | .038*    | .221                                   |
| Correlated error structure: |                         |                |                |          |                                        |
| Chucks ~ call rate          | .336                    | NA             | 3.232          | .001***  | NA                                     |
| Chucks ~ swats              | −.165                   | NA             | −1.517         | .067     | NA                                     |

\*  $P < .05$ .\*\*\*  $P < .001$ .

interruption owing to swatting may then temporarily reduce midge attraction. Such an effect and its underlying mechanism is purely speculative, which is why a causal relationship between swats and chucks was not included in the global SEM. Inclusion of this path in an alternative, exploratory SEM, however, indicates an acceptable model-data fit, suggesting that this hypothesized mechanism deserves future empirical work ( $C_2 = 0.524$ ,  $P = .769$ ; see the supplemental PDF for full analysis and discussion). Considering either mechanism, a male's antimidge swatting defense could effectively negate any influence of chucks on midge attraction. Indeed, midges shown to prefer males producing more complex calls were collected before they landed on their frog prey (Aihara et al. 2016), precluding any antimidge swatting response. When viewed alongside the results here, it appears that while relative call complexity guides midge foraging decisions, antimidge swatting offsets this effect by suppressing a male's call elaboration relative to the intensity of midge attacks.

Given that call complexity did not drive the number of midge attacks, we performed a post hoc test of the hypothesis that to regulate the costs imposed by these micropredators, males actively modify their call complexity in response to the number of midge attacks. We compared the global SEM with an SEM in which we reversed the directionality of the path between midges and chucks (fig. S4; table S2). Modeling midge attacks as a predictor of a male's number of chucks resulted in an SEM of equally adequate fit to the observed data compared with the global SEM ( $\Delta AIC_c = 0$ ,  $C_2 = 0.524$ ,  $P = .769$ ). The direct effect of midges on chucks was still not significant ( $P = .369$ ). This finding indicates that, as with call rate, males do not

adjust their number of chucks per se in response to the intensity of midge attacks. When attacked by these eavesdropping micropredators, males appear to alter their sexual signal elaboration as part of a cascade of indirect effects stemming from their defensive swats.

#### *Threat Sensitivity Hypothesis*

Our third aim for this study was to test a prediction of the threat sensitivity hypothesis that antipredator strategies scale with risk of attack (Sih 1986; Helfman 1989). Examinations of this hypothesis generally address how physical defensive behaviors scale in intensity with risk. Physical defenses, however, are only one of the multiple strategies used by animals to avoid attacks. As animals can actively reduce their degree of signal elaboration to avert the attraction of eavesdropping enemies (e.g., Montgomerie et al. 2001; Steinberg et al. 2014), our use of "antipredator strategies" here includes both physical and signaling behaviors that reduce the risk of attack from eavesdropping micropredators.

While we expected a male to alter his call rate and complexity directly in response to the number of attacking midges, as shown above, there were no such effects. Male túngara frogs do not appear to directly alter the attractiveness of their signals relative to their risk of attack. Conversely, calling males scale their antimidge swatting defense with the intensity of midge attacks. While attracting a predator such as a frog-eating bat carries potential high risk (i.e., death), the risk of attracting micropredators is likely density dependent (Lafferty et al. 2015) and comparatively low if the male can swat them off before they begin feeding. Considering that males also indirectly reduce their signal

elaboration when swatting, there has likely been weak selective pressure for males to directly scale call elaboration with risk of attack. Altogether, we find support for the threat sensitivity hypothesis only in the form of an antimidge physical defense.

### *Effects of Intrasexual Competition*

While midges suppress male call rate and complexity, the effects of rival males are mixed. As the number of rivals increased, focal males added more attractive, conspicuous chucks to their calls (standardized direct effect = 0.221,  $P = .038$ ; fig. 2). The direct effect of competition from neighbors on calling behavior is not surprising given that previous field observations (Ryan 1985) and playback experiments (Bosch et al. 2000a; Bernal et al. 2007b, 2009a; Goutte et al. 2010) have shown that increased male competition causes a male to increase his call rate and number of chucks. The effect of calling rivals on call complexity has been mainly examined in one-on-one interactions between a focal male and a playback of a rival male, but the findings extend to more natural interactions at a chorus (Ryan 1985; Greenfield and Rand 2000). No study of intrasexual competition, however, has explicitly accounted for the effect of frog-biting midge attacks and the antimidge swatting behaviors they induce in their victims. Here, the SEM reveals that, while the number of rival males drove a focal male's propensity to add chucks, it did not impact the focal male's call rate ( $P = .322$ ).

The social calling dynamics of a chorus may explain why the intensity of male-male competition does not impact call rate. One potential mechanism is that a male's call rate is constrained by the túngara frog's alternating signal-timing strategy. Males appear to attend to a maximum of two or three nearby males and time their own calls to avoid following these rivals—a strategy that has likely evolved in response to female túngara frogs' strong preference for "leading" males (Greenfield and Rand 2000; Legett et al. 2020). Therefore, while competition intensifies with increasing numbers of neighbor males, a focal male's call rate is limited as a by-product of avoiding lagging behind the calls of his rivals. Fine temporal signal dynamics between males may thus explain why focal males increased complexity, but not call rate, in response to increased numbers of rivals.

An alternative, but not mutually exclusive, explanation for why the males we observed did not change their call rate in response to the number of rival males is that the indirect effect of eavesdropping midges outweighs any effect of intrasexual competition on call rate. In a natural and dynamic setting, such as the in situ observations here, males might perceive their nearby competitors' reduced call rate (caused by midge-induced swatting) and the correlated re-

duction in number of chucks as reduced competition. This subdued acoustic social environment may then dampen the effects competitors would otherwise have on a male's call elaboration. Additionally, if the focal male's total chuck number and call rate are also reduced in response to midge attacks, his competitors may perceive him as reduced competition and, in turn, reduce their call elaboration. Thus, midges may cause a feedback loop among the neighboring males until all males equilibrate their call rate and complexity. This mechanism, however, is dependent on the untested assumption that all nearby males incur similar numbers of midge attacks.

Although we did not record the number of midges that attacked rival males or their calling behaviors, the SEM revealed that the number of rival males did not directly affect the intensity at which midges attacked the focal male ( $P = .795$ ); the same was true when considering the total effect of rival males on midge attack intensity, which includes the indirect effect via chucks (standardized total effect =  $-0.006$ ; table S7). This result does not support our prediction that more neighboring rivals reduces the number of midge attacks per frog. A potential explanation for this finding could be that frog and midge activity levels are driven by similar environmental factors, such as temperature, precipitation, and ambient light conditions. One hypothesis for why animals broadcast mating signals in groups, as seen in many frogs and other animals, is that aggregations dilute predation risk (Foster and Treherne 1981). Indeed, chorus size is negatively correlated with a male túngara frog's predation risk from the eavesdropping fringe-lipped bat (Ryan et al. 1981). In contrast, for many host-parasite systems, infection risk is typically assumed to positively covary with host density, though the dilution of risk is predicted to occur when a parasite's infective-stage production and transmission are spatially or temporally decoupled (Buck et al. 2017). Here, however, a male's predation risk from foraging midges was unaffected by the number of neighboring rivals (i.e., local "host" density). Future research is needed to address whether such lack of relationship extends beyond this study to micropredators and their victims in general.

### *Relative Effects of Eavesdropping Micropredators and Intrasexual Competition*

To more thoroughly examine the effects of male rivals relative to attacking frog-biting midges—the fourth and last objective of this study—we compared the global SEM with two additional independent SEMs that considered the hypothesized effects of either male-male competition or midge attacks (fig. S5). In the first additional model, we removed the effect of male rivals while preserving the effects of midges directly on calling behavior and indirectly through swatting

(fig. S5A, table S3). This model fit the data well and was a better fit than our global SEM ( $\Delta AIC_c = 15.358$ ,  $C_6 = 3.252$ ,  $P = .777$ ). Furthermore, the effect sizes were similar to those of the global SEM (table S4), confirming that frog-biting midges can be important drivers of call plasticity in túngara frogs. In the second additional model, we removed the effect of midges (and thus swatting) while preserving the effects of male rivals (fig. S5B; table S5). In contrast, this second model was a poor fit to the observed data ( $C_6 = 25.342$ ,  $P < .001$ ), confirming that intrasexual competition, by itself, is not predictive of call elaboration when frogs are under attack from frog-biting midges. When examined together, and in light of the aforementioned findings from this study, these SEMs suggest that frog-biting midges are dynamic limiters of signal elaboration in naturally occurring choruses of túngara frogs and can outweigh the effects of intrasexual competition.

### Conclusion

Elaboration of the male túngara frog's sexual signal is among the most well-supported targets of female preference (Ryan et al. 2019). Here, we show that eavesdropping frog-biting midges, by eliciting antimidge defenses in calling male túngara frogs, suppress the elaboration of their victims' signals and concomitantly dampen the effects of intrasexual competition. Furthermore, the degree to which these micropredators limit signal elaboration scales with the intensity of their attacks. Through either direct effects or indirect effects, eavesdropping micropredators offer a particularly intriguing opportunity to investigate how their signaling victims modify plastic signal traits in response to graded levels of risk. These ecological interactions have the potential to drive the evolution of sexual communication systems. Similar to herbivores that are hypothesized to have constrained the elaboration of female floral traits in pistillate plants by eavesdropping on the chemical signals sent by flowers to pollinators (Theis et al. 2007), eavesdropping micropredators have the potential to determine the evolutionary path of sexual signals. Incorporating the effects of eavesdropping micropredators as part of a more complete set of ecological costs of sexual signals may reveal more robust predictions in models of sexual selection and new insights in the fields of animal communication and predator-prey ecology.

### Acknowledgments

We are grateful for Kristyn McCoy, who helped collect the field data. We thank Erik Galindo and Cameron Smith for their help with behavioral coding, Smithsonian Tropical Research Institute for logistical support, and Jeff Lucas, Rachel Page, members of the Bernal Lab, the editors, and

two anonymous reviewers for constructive feedback that improved the manuscript. This work was supported by National Science Foundation grants to X.E.B. (IOS-1433990 and IOS-2054636). B.C.L. acknowledges the National Science Foundation for support under the Graduate Research Fellowship Program (grant DGE-1842166).

### Statement of Authorship

X.E.B. and B.C.L. were responsible for conceptualization and funding acquisition. X.E.B. and L.E.B. developed field methods and collected data. B.C.L. and L.E.B. analyzed data. B.C.L. visualized data. X.E.B. provided resources. B.C.L., G.G.M., and X.E.B. were responsible for model development and analysis. B.C.L. created the R Code and wrote the original draft of the manuscript. All authors contributed to reviewing and editing the manuscript.

### Data and Code Availability

All data, code, and other supplemental materials have been deposited in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.7wm37pvr5>; Leavell et al. 2021).

### Literature Cited

- Aihara, I., P. de Silva, and X. E. Bernal. 2016. Acoustic preference of frog-biting midges (*Corethrella* spp.) attacking túngara frogs in their natural habitat. *Ethology* 122:105–113.
- Akre, K. L., H. E. Farris, A. M. Lea, R. A. Page, and M. J. Ryan. 2011. Signal perception in frogs and bats and the evolution of mating signals. *Science* 333:751–752.
- Akre, K. L., and M. J. Ryan. 2011. Female túngara frogs elicit more complex mating signals from males. *Behavioral Ecology* 22:846–853.
- Alem, S., K. Koselj, B. M. Siemers, and M. D. Greenfield. 2011. Bat predation and the evolution of leks in acoustic moths. *Behavioral Ecology and Sociobiology* 65:2105–2116.
- Andersson, M. B. 1994. *Sexual selection*. Princeton University Press, Princeton, NJ.
- Badyaev, A. V., and R. A. Duckworth. 2003. Context-dependent sexual advertisement: plasticity in development of sexual ornamentation throughout the lifetime of a passerine bird. *Journal of Evolutionary Biology* 16:1065–1076.
- Bateman, P. W., and P. A. Fleming. 2013. Signaling or not-signaling: variation in vulnerability and defense tactics of armored ground crickets (*Acanthopplus speiseri*: Orthoptera, Tettigoniidae, Heterodinae). *Journal of Insect Behavior* 26:14–22.
- Bates, D., M. Mächler, B. M. Bolker, and S. C. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67:1–48.
- Bernal, X. E., K. L. Akre, A. T. Baugh, A. S. Rand, and M. J. Ryan. 2009a. Female and male behavioral response to advertisement calls of graded complexity in túngara frogs, *Physalaemus pustulosus*. *Behavioral Ecology and Sociobiology* 63:1269–1279.
- Bernal, X. E., and P. de Silva. 2015. Cues used in host-seeking behavior by frog-biting midges (*Corethrella* spp. Coquillett). *Journal of Vector Ecology* 40:122–128.

- Bernal, X. E., R. A. Page, A. S. Rand, and M. J. Ryan. 2007a. Cues for eavesdroppers: do frog calls indicate prey density and quality? *American Naturalist* 169:409–415.
- Bernal, X. E., and C. M. Pinto. 2016. Sexual differences in prevalence of a new species of trypanosome infecting túngara frogs. *International Journal for Parasitology: Parasites and Wildlife* 5:40–47.
- Bernal, X. E., A. S. Rand, and M. J. Ryan. 2006. Acoustic preferences and localization performance of blood-sucking flies (*Corethrella* Coquillett) to túngara frog calls. *Behavioral Ecology* 17:709–715.
- . 2007b. Sexual differences in the behavioral response of tungara frogs, *Physalaemus pustulosus*, to cues associated with increased predation risk. *Ethology* 113:755–763.
- . 2009b. Task differences confound sex differences in receiver permissiveness in túngara frogs. *Proceedings of the Royal Society B* 276:1323–1329.
- Borkent, A. 2008. The frog-biting midges of the world (Corethrellidae: Diptera). *Zootaxa* 1804:1–456.
- . 2017. Corethrellidae (frog-biting midges). Pages 665–670 in A. H. Kirk-Spriggs, B. J. Sinclair, S. A. Marshall, and South African National Biodiversity Institute, eds. *Manual of Afrotropical Diptera*. SANBI, Pretoria.
- Bosch, J., A. S. Rand, and M. J. Ryan. 2000a. Acoustic competition in *Physalaemus pustulosus*, a differential response to calls of relative frequency. *Ethology* 106:865–871.
- . 2000b. Signal variation and call preferences for whine frequency in the tungara frog, *Physalaemus pustulosus*. *Behavioral Ecology and Sociobiology* 49:62–66.
- Buck, J. C., R. F. Hechinger, A. C. Wood, T. E. Stewart, A. M. Kuris, and K. D. Lafferty. 2017. Host density increases parasite recruitment but decreases host risk in a snail-trematode system. *Ecology* 98:2029–2038.
- Candolin, U. 1997. Predation risk affects courtship and attractiveness of competing threespine stickleback males. *Behavioral Ecology and Sociobiology* 41:81–87.
- Cocroft, R. B., and M. J. Ryan. 1995. Patterns of advertisement call evolution in toads and chorus frogs. *Animal Behaviour* 49:283–303.
- Darwin, C. 1871. *The descent of man, and selection in relation to sex*. J. Murray, London.
- Daversa, D. R., R. F. Hechinger, E. Madin, and A. Fenton. 2019. Beyond the ecology of fear: non-lethal effects of predators are strong whereas those of parasites are diverse. *bioRxiv*, <https://doi.org/10.1101/766477>.
- de Moraes, P. Z., P. Diniz, E. Fernandez-Juricic, and R. H. Macedo. 2019. Flirting with danger: predation risk interacts with male condition to influence sexual display. *Behavioral Ecology* 30:1265–1272.
- de Silva, P., C. Jaramillo, and X. E. Bernal. 2014. Feeding site selection by frog-biting midges (Diptera: Corethrellidae) on anuran hosts. *Journal of Insect Behavior* 27:302–316.
- Dorresteyn, L., J. Schultner, D. G. Nimmo, J. Fischer, J. Hanspach, T. Kuemmerle, L. Kehoe, and E. G. Ritchie. 2015. Incorporating anthropogenic effects into trophic ecology: predator-prey interactions in a human-dominated landscape. *Proceedings of the Royal Society B* 282:20151602.
- Eberhardt, L. S. 1994. Oxygen consumption during singing by male Carolina wrens (*Thryothorus ludovicianus*). *Auk* 111:124–130.
- Elston, D. A., R. Moss, T. Boulinier, C. Arrowsmith, and X. Lambin. 2001. Analysis of aggregation, a worked example: numbers of ticks on red grouse chicks. *Parasitology* 122:563–569.
- Farr, J. A. 1975. The role of predation in the evolution of social behavior of natural populations of the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). *Evolution* 29:151–158.
- Foster, W. A., and J. E. Treherne. 1981. Evidence for the dilution effect in the selfish herd from fish predation on a marine insect. *Nature* 293:466–467.
- Gerhardt, H. C. 1991. Female mate choice in treefrogs: static and dynamic acoustic criteria. *Animal Behaviour* 42:615–635.
- Goutte, S., N. M. Kime, T. F. Argo IV, and M. J. Ryan. 2010. Calling strategies of male túngara frogs in response to dynamic playback. *Behaviour* 147:65–83.
- Grace, J. B. 2006. *Structural equation modeling and natural systems*. Cambridge University Press, Cambridge.
- Grace, J. B., T. M. Anderson, M. D. Smith, E. Seabloom, S. J. Andelman, G. Meche, E. Weiher, et al. 2007. Does species diversity limit productivity in natural grassland communities? *Ecology Letters* 10:680–689.
- Grace, J. B., and K. A. Bollen. 2005. Interpreting the results from multiple regression and structural equation models. *Bulletin of the Ecological Society of America* 86:283–295.
- Grace, J. B., S. M. Scheiner, and D. R. Schoolmaster. 2015. *Structural equation modeling: building and evaluating causal models*. Pages 168–199 in G. A. Fox, S. Negrete-Yankelevich, and V. J. Sosa, eds. *Ecological statistics: contemporary theory and application*. Oxford University Press, Oxford.
- Grace, J. B., D. R. Schoolmaster, G. R. Guntenspergen, A. M. Little, B. R. Mitchell, K. M. Miller, and E. W. Schweiger. 2012. Guidelines for a graph-theoretic implementation of structural equation modeling. *Ecosphere* 3:art73.
- Green, A. J. 1990. Determinants of chorus participation and the effects of size, weight and competition on advertisement calling in the tungara frog, *Physalaemus pustulosus* (Leptodactylidae). *Animal Behaviour* 39:620–638.
- Greenfield, M. D., and A. S. Rand. 2000. Frogs have rules: selective attention algorithms regulate chorusing in *Physalaemus pustulosus* (Leptodactylidae). *Ethology* 106:331–347.
- Harrison, X. A. 2014. Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ* 2:e616.
- Hart, B. L., and L. A. Hart. 2018. How mammals stay healthy in nature: the evolution of behaviours to avoid parasites and pathogens. *Philosophical Transactions of the Royal Society B* 373:20170205.
- Hartig, F. 2019. DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. <https://CRAN.R-project.org/package=DHARMa>.
- Helfman, G. S. 1989. Threat-sensitive predator avoidance in damselfish-trumpetfish interactions. *Behavioral Ecology and Sociobiology* 24:47–58.
- Heyer, W. R., M. A. Donnelly, R. W. McDiarmid, L.-A. C. Hayek, and M. S. Foster, eds. 1994. *Measuring and monitoring biological diversity: standard methods for amphibians*. Smithsonian Institution, Washington, DC.
- Jones, G., A. Barabas, W. Elliott, and S. Parsons. 2002. Female greater wax moths reduce sexual display behavior in relation to the potential risk of predation by echolocating bats. *Behavioral Ecology* 13:375–380.
- Kim, S. Y., and A. Velando. 2014. Stickleback males increase red coloration and courtship behaviours in the presence of a competitive rival. *Ethology* 120:502–510.
- Kime, N. M., T. K. Whitney, E. S. Davis, and C. A. Marler. 2007. Arginine vasotocin promotes calling behavior and call changes in male túngara frogs. *Brain, Behavior and Evolution* 69:254–265.

- Kirkpatrick, M., and M. J. Ryan. 1991. The evolution of mating preferences and the paradox of the lek. *Nature* 350:33–38.
- Kokko, H., M. D. Jennions, and R. Brooks. 2006. Unifying and testing models of sexual selection. *Annual Review of Ecology, Evolution, and Systematics* 37:43–66.
- Kolluru, G. R., G. F. Grether, and H. Contreras. 2007. Environmental and genetic influences on mating strategies along a replicated food availability gradient in guppies (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology* 61:689–701.
- Korner-Nievergelt, F., T. Roth, S. von Felten, J. Guelat, B. Almasi, and P. Korner-Nievergelt. 2015. Bayesian data analysis in ecology using linear models with R, BUGS and Stan. Elsevier, Amsterdam.
- Lafferty, K. D., G. DeLeo, C. J. Briggs, A. P. Dobson, T. Gross, and A. M. Kuris. 2015. A general consumer-resource population model. *Science* 349:854–857.
- Lafferty, K. D., and A. M. Kuris. 2002. Trophic strategies, animal diversity and body size. *Trends in Ecology and Evolution* 17:507–513.
- Leavell, B. C., L. E. Beaty, G. G. McNickle, and X. E. Bernal. 2021. Data from: Eavesdropping micropredators as dynamic limiters of sexual signal elaboration and intrasexual competition. *American Naturalist*, Dryad Digital Repository, <https://doi.org/10.5061/dryad.7wm37pvr5>.
- Lefcheck, J. S. 2016. piecewiseSEM: piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods in Ecology and Evolution* 7:573–579.
- Legett, H. D., C. T. Hemingway, and X. E. Bernal. 2020. Prey exploits the auditory illusions of eavesdropping predators. *American Naturalist* 195:927–935.
- Lehmann, G. U. C., K. G. Heller, and A. W. Lehmann. 2001. Male bushcrickets favoured by parasitoid flies when acoustically more attractive for conspecific females (Orthoptera: Phanopteridae/Diptera: Tachinidae). *Entomologia Generalis* 25:135–140.
- Lindström, J., T. W. Pike, J. D. Blount, and N. B. Metcalfe. 2009. Optimization of resource allocation can explain the temporal dynamics and honesty of sexual signals. *American Naturalist* 174:515–525.
- Marler, C., and M. J. Ryan. 1996. Energetic constraints and steroid hormone correlates of male calling behavior in the tungara frog. *Journal of Zoology* 240:397–409.
- McKeever, S. 1977. Observations of *Corethrella* feeding on tree frogs (*Hyla*). *Mosquito News* 37:522–523.
- Miner, B. G., S. E. Sultan, S. G. Morgan, D. K. Padilla, and R. A. Relyea. 2005. Ecological consequences of phenotypic plasticity. *Trends in Ecology and Evolution* 20:685–692.
- Montgomerie, R. D., B. E. Lyon, and K. Holder. 2001. Dirty ptarmigan: behavioral modification of conspicuous male plumage. *Behavioral Ecology* 12:429–438.
- Palmer, M. S., J. Fieberg, A. Swanson, M. Kosmala, and C. Packer. 2017. A “dynamic” landscape of fear: prey responses to spatiotemporal variations in predation risk across the lunar cycle. *Ecology Letters* 20:1364–1373.
- Prum, R. O. 2010. The Lande-Kirkpatrick mechanism is the null model of evolution by intersexual selection: implications for meaning, honesty, and design in intersexual signals. *Evolution* 64:3085–3100.
- Rand, A. S., and M. J. Ryan. 1981. The adaptive significance of a complex vocal repertoire in a Neotropical frog. *Zeitschrift für Tierpsychologie* 57:209–214.
- R Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
- Ryan, M. J. 1985. The túngara frog: a study in sexual selection and communication. University of Chicago Press, Chicago.
- Ryan, M. J., K. L. Akre, A. T. Baugh, X. E. Bernal, A. M. Lea, C. Leslie, M. B. Still, D. C. Wylie, and A. S. Rand. 2019. Nineteen years of consistently positive and strong female mate preferences despite individual variation. *American Naturalist* 194:125–134.
- Ryan, M. J., K. L. Akre, and M. Kirkpatrick. 2007. Mate choice. *Current Biology* 17:R313–R316.
- Ryan, M. J., and A. Keddy-Hector. 1992. Directional patterns of female mate choice and the role of sensory biases. *American Naturalist* 139(suppl.):S4–S35.
- Ryan, M. J., M. D. Tuttle, and A. S. Rand. 1982. Bat predation and sexual advertisement in a Neotropical anuran. *American Naturalist* 119:136–139.
- Ryan, M. J., M. D. Tuttle, and L. K. Taft. 1981. The costs and benefits of frog chorusing behavior. *Behavioral Ecology and Sociobiology* 8:273–278.
- Säfken, B., D. Rügamer, T. Kneib, and S. Greven. 2018. Conditional model selection in mixed-effects models with cAIC4. *Journal of Statistical Software* 99:1–31.
- Salazar, V. L., and P. K. Stoddard. 2009. Social competition affects electric signal plasticity and steroid levels in the gymnotiform fish *Brachyhyphopomus gauderio*. *Hormones and Behavior* 56:399–409.
- Shaw, K. L., and D. P. Herlihy. 2000. Acoustic preference functions and song variability in the Hawaiian cricket *Laupala cerasina*. *Proceedings of the Royal Society B* 267:577–584.
- Shipley, B. 2009. Confirmatory path analysis in a generalized multi-level context. *Ecology* 90:363–368.
- . 2016. Cause and correlation in biology: a user’s guide to path analysis, structural equations and causal inference with R. 2nd ed. Cambridge University Press, Cambridge.
- Sih, A. 1986. Antipredator responses and the perception of danger by mosquito larvae. *Ecology* 67:434–441.
- Simmons, L. W., and D. J. Emlen. 2006. Evolutionary trade-off between weapons and testes. *Proceedings of the National Academy of Sciences of the USA* 103:16346–16351.
- Simon, V. B. 2007. Not all signals are equal: male brown anole lizards (*Anolis sagrei*) selectively decrease pushup frequency following a simulated predatory attack. *Ethology* 113:793–801.
- Somjee, U., H. A. Woods, M. Duell, and C. W. Miller. 2018. The hidden cost of sexually selected traits: the metabolic expense of maintaining a sexually selected weapon. *Proceedings of the Royal Society B* 285:10–12.
- Steinberg, D. S., J. B. Losos, T. W. Schoener, D. A. Spiller, J. J. Kolbe, and M. Leal. 2014. Predation-associated modulation of movement-based signals by a Bahamian lizard. *Proceedings of the National Academy of Sciences of the USA* 111:9187–9192.
- Stevens, M. 2016. Color change, phenotypic plasticity, and camouflage. *Frontiers in Ecology and Evolution* 4:1–10.
- Still, M. B., A. M. Lea, H. A. Hofmann, and M. J. Ryan. 2019. Multimodal stimuli regulate reproductive behavior and physiology in male túngara frogs. *Hormones and Behavior* 115:104546.
- Theis, N., M. Lerdau, and R. A. Raguso. 2007. The challenge of attracting pollinators while evading floral herbivores: patterns of fragrance emission in *Cirsium arvense* and *Cirsium repandum* (Asteraceae). *International Journal of Plant Sciences* 168:587–601.
- Tuttle, M. D., L. K. Taft, and M. J. Ryan. 1982. Evasive behaviour of a frog in response to bat predation. *Animal Behaviour* 30:393–397.

- Valeix, M., A. J. Loveridge, S. Chamaillé-Jammes, Z. Davidson, F. Murindagomo, H. Fritz, and D. W. MacDonald. 2009. Behavioral adjustments of African herbivores to predation risk by lions: spatio-temporal variations influence habitat use. *Ecology* 90:23–30.
- Wagner, W. E. 1996. Convergent song preferences between female field crickets and acoustically orienting parasitoid flies. *Behavioral Ecology* 7:279–285.
- Wallace, A. R. 1891. *Natural selection and tropical nature: essays on descriptive and theoretical biology*. Macmillan, London.
- Zuk, M., and G. R. Kolluru. 1998. Exploitation of sexual signals by predators and parasitoids. *Quarterly Review of Biology* 73:3–22.
- Zuur, A. F., E. N. Ieno, N. Walker, A. A. Saveliev, and G. M. Smith. 2009. *Mixed effects models and extensions in ecology with R*. Statistics for Biology and Health. Vol. 36. Springer, New York.

#### References Cited Only in the Online Enhancement

- Bickel, P. J., E. A. Hammel, and J. W. O'Connell. 1975. Sex bias in graduate admissions: data from Berkeley. *Science* 187:398–404.
- Bolker, B. M., and R Development Core Team. 2020. *bbmle: tools for general maximum likelihood estimation*. R package version 1.0.23.1.
- Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Mächler, and B. M. Bolker. 2017. *glmmTMB* balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R Journal* 9:378–400.
- McElreath, R. 2020. *Statistical rethinking*. CRC, Boca Raton, FL.

Associate Editor: Emily H. DuVal

Editor: Erol Akçay



Frog-biting midges (*Corethrella* spp.) attacking a calling male túngara frog (*Engystomops pustulosus*). Photo by Ximena E. Bernal.