



Decoupled auditory perception from acoustic signal divergence hinders species recognition in territorial poison frogs

Marco González-Santoro^{1,2} · Pablo Palacios-Rodríguez¹ · Mabel Gonzalez^{3,4} · Jack Hernández-Restrepo¹ · Daniel Mejía-Vargas¹ · Adolfo Amézquita^{1,5}

Received: 8 July 2022 / Revised: 1 December 2022 / Accepted: 5 December 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract

Species recognition, encompassing mate and competitor recognition, is an important mechanism for establishing and maintaining species boundaries. The effective communication between a sender and a receiver is crucial for species recognition to occur. In this sense, intraspecific signals are expected to evolve coupled to recognition systems to avoid reproductive interference with other species. Empirical evidence suggests, however, that the asymmetrical evolution of signaling and recognition systems may be widespread in nature. Moreover, the study of recognition systems has classically focused on the perception by females, but the role of males has been largely overlooked. We studied the divergence of acoustic traits and their perception in two nominal species and an intermediate population that form a phenotypic cline in color and body size. We used males' recognition of and competition with other males as the predicted drivers of the communication system. We found wide and asymmetrical levels of recognition in both species and the intermediate population, which we argue reflects the prevalence of reproductive interference across the system.

Significance statement

The effective recognition of aggressive signals between males of incipient lineages is expected to prevent costly interactions, such as competing for mates. Therefore, diverging signals should evolve coupled to perceptual systems that allow their recognition. When this process is broken, reproductive interference or the competition between males of distinct lineages for the access to mates will prevail. In our study, we prove that the coupled divergence of acoustic signals and their perception is hindered in incipient lineages of territorial poison frogs (*Phylllobates*). We studied two species and an intermediate population that represent a phenotypic cline in color and body size. Territoriality in poison frogs is widely acknowledged as a predictor of reproductive success in males. Therefore, we argue that males' auditory perception remained flexible to recognize divergent acoustic traits that could put at risk males' territory ownership and by extension mate acquisition.

Keywords Species recognition · Reproductive interference · Auditory perception · Acoustic traits · Territorial males · Poison frogs

Communicated by A. Taylor Baugh

✉ Marco González-Santoro
mdg82@pitt.edu

Pablo Palacios-Rodríguez
p.palacios10@uniandes.edu.co

Mabel Gonzalez
mabelg@stanford.edu

Jack Hernández-Restrepo
jack.ph93@gmail.com

Daniel Mejía-Vargas
pyrrhura@gmail.com

Adolfo Amézquita
adolfo.amezquita@gmail.com

¹ Department of Biological Sciences, Universidad de los Andes, 4976 Bogotá, AA, Colombia

² Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, USA

³ Department of Biology, Stanford University, Stanford, USA

⁴ Department of Chemistry, Universidad de los Andes, 4976, AA, Bogotá, Colombia

⁵ Laboratory of Biodiversity and Cloud Forests Conservancy Bioconservancy, Jardín, Colombia

Introduction

The evolution of species recognition signals remains a pivotal topic in the study of adaptation and speciation (Ryan and Rand 1993a; Panhuis et al. 2001; Ritchie 2007; Rosenthal 2017). The display of species recognition signals is thought to reflect two fundamental trade-offs. On one hand, they must be conspicuous enough to be detected and recognized by conspecifics, yet conspicuousness often increases the risk of being detected by potential predators (Ryan 1988; Rosenthal 2017). On the other hand, they must be specific enough to reduce the risk of reproductive interference (i.e., mating/aggression attempts with heterospecific individuals (Gröning and Hochkirch 2008; Grether et al. 2017)), yet the lack of variation would severely constrain the potential to encode condition dependent information such as body size, sex, motivational status, and, in at least some species, individual or group identity (Ryan et al. 1990; Pryke et al. 2001; Ladich and Myrberg 2006; Tumulty and Bee 2021). Therefore, species recognition signals can diverge rapidly when two (or more) incipient lineages experience divergent selective pressures on intraspecific communication systems (Ryan and Rand 1993a; Panhuis et al. 2001; Ritchie 2007; Rosenthal 2017).

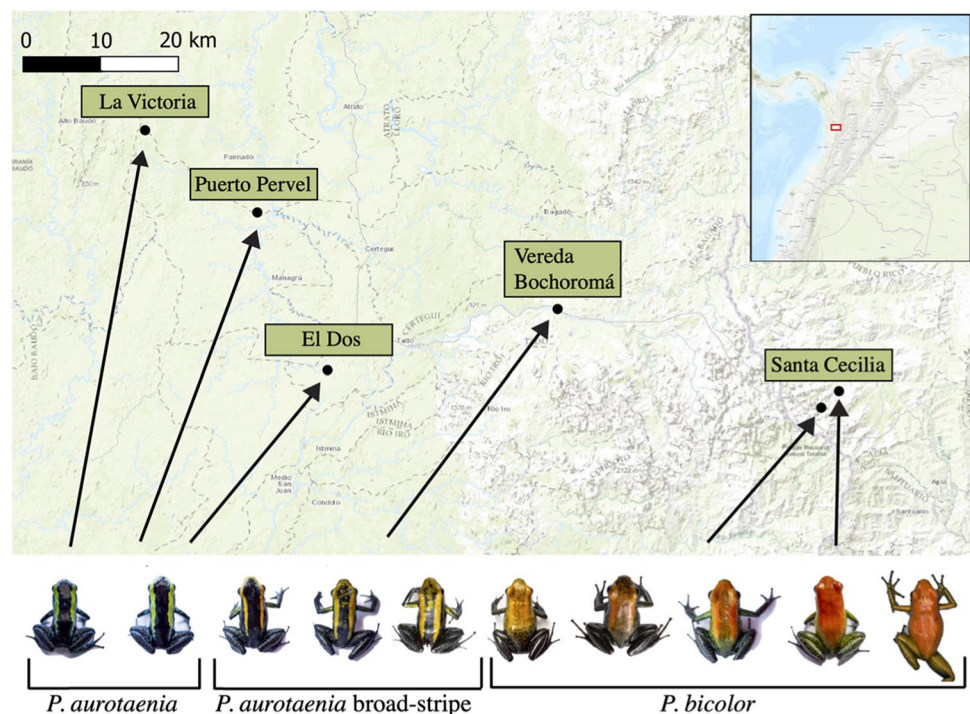
The process described above is complicated in many ways. The study of species recognition signals traditionally focuses on signal variation but less often addresses the variation in the sensory systems needed to perceive the signals (Betancourth-Cundar et al. 2016). However, variation in sensory systems can be as important as variation in signals in determining receivers' reaction to detected signals (Ryan 1988; Ryan and Rand 2003; Wilczynski et al. 1993; Dawkins and Guilford 1996; Gerhardt and Huber 2003). It is often presumed that signals evolve in tandem with recognition systems (Rosenthal 2017). However, the decoupled evolution between the sender and the receiver components of intraspecific communication signals help to explain the growing evidence of introgression, hybridization, and other evolutionary outcomes that represent incomplete reproductive isolation (Gerhardt and Schwartz 1995; den Hartog et al. 2008; Greig and Webster 2013; Twomey et al. 2016). In this sense, most research focuses on the role of females as the main receivers of species recognition signals, using the operational term “mate recognition signals” (Qvarnström et al. 2012; Servedio and Boughman 2017; Lipshutz 2018; Tinghitella et al. 2018). However, empirical evidence reveal that male-male interactions underlie among-male differences in reproductive success (Wong and Candolin 2005; Qvarnström et al. 2012; Yang et al. 2020). This is particularly true in species where territory ownership is the main determinant of access to limited females. In this scenario, reproductive

interference (i.e., competing for mates with heterospecific individuals) can be very costly for males in terms of spent energy, territory loss, and predation risk (Gröning and Hochkirch 2008; Grether et al. 2017). Therefore, male-male communication systems can be under social selection (i.e., selection arising from competition for resources, including territories/mates) and can be an important component of species recognition between divergent lineages (West-Eberhard 1983; Price 2008; Avendaño and Cadena 2021). The male signals under such scenarios have been called “competitor recognition signals” (Gröning and Hochkirch 2008; Grether et al. 2017).

For more than five decades, anuran amphibians have been one of the most thoroughly studied taxa regarding acoustic communication. Courtship in most anuran species is relatively simple and amenable to experimental manipulation: males emit species-specific advertisement calls, reproductively motivated females approach or touch some of them to signal interest, and males proceed to amplexus and external fertilization (Wells 1977; Gerhardt 1988; Ryan and Rand 1993b). However, for many anuran taxa, particularly focused on neotropical poison frogs, we now know that males are strongly territorial and detailed studies show that territory ownership determines, to a great extent, the reproductive success of males (Roithmair 1992, 1994; Pröhl 1997; Ursprung et al. 2011; Ringler et al. 2012; Meuche and Pröhl 2011; Yang and Richards-Zawacki 2021). Therefore, although territorial defense could also be directed towards heterospecifics for shared resources other than females, it is hypothesized that the main function of territoriality in frogs is associated with reproduction (Pröhl 2005). Under this scenario, the male advertisement call, widely acknowledged as the mate recognition signal in anurans, also serves as a means of communication among males. This makes territorial male anurans a suitable system to test the hypothesis of concomitant evolution of signal and recognition across diverging lineages.

We studied the sender and the receiver components of the advertisement call of three lineages of poison dart frogs (Dendrobatidae: *Phyllobates*) that form a geographic and phenotypic cline in color and body size that is most probably maintained by gene flow (Silverstone 1976; Márquez et al. 2020) (Fig. 1). At the current state of knowledge, we can rule out the possibility that the cline represents variation within a single species (Márquez et al. 2020). Instead, these poison dart frogs are currently assigned to three putative lineages: *Phyllobates aurotaenia*, *Phyllobates aurotaenia* broad-stripe morph, and *Phyllobates bicolor* (Fig. 1). *P. aurotaenia* broad-stripe morph was suggested as a putative hybrid by Silverstone (1976) given their intermediate color, body size, and geographic locality between *P. aurotaenia* and *P. bicolor*. However, recent research suggests that the cline we studied may have emerged from parapatric differentiation

Fig. 1 Color phenotypes and the geographic localities where we recorded the acoustic signals and performed the playback experiments



during a range expansion rather than by secondary contact (R. Márquez et al., unpublished data). Therefore, in our study, we recognize *P. aurotaenia* and *P. bicolor* as two nominal species at the geographic extremes of the cline and *P. aurotaenia* broad-stripe morph as an intermediate population, which likely emerged during the parapatric differentiation of the cline. Interestingly, the two nominal species interbreed in captivity suggesting the lack of postzygotic reproductive barriers (R. Márquez, personal communication).

As in most dendrobatid species, the males within the cline are strongly territorial, announce territory ownership by uttering advertisement calls, and will eagerly react to other males' calls (or to the playback of similar synthetic calls) by rapidly approaching the sound source. We address three main questions: (1) Does the advertisement call represent a continuum of variation across the phenotypic cline? (2) Does the auditory recognition space, as estimated from playback experiments with males, vary in tandem with the signal? (3) Does the degree of matching between signal and recognition spaces facilitate competitor recognition?

Methods

To characterize the frogs' acoustic signal and recognition space, we conducted call recording and playback experiments in six different localities along an ~100 km geographic cline, between 200 and 1400 m.a.s.l., in the western slope of the Colombian Western Andes (Fig. 1). The field work was conducted between March 2018 and June 2019. Both call

recordings and playback experiments were conducted between 6:00 and 17:00, during the peak calling activity in these lineages. All individual frogs were encountered in primary or slightly disturbed rainforests which agrees with the typical habitats reported for *Phyllobates* species (Kahn et al. 2016).

Characterizing the signal space

In 2018, we recorded the advertisement calls of 26 focal males distributed along the populations described in Fig. 1, with a directional microphone (ME66/K6P, Sennheiser, Wedemark, Germany) coupled to a digital recorder (H4n pro, Zoom Corporation, Tokyo, Japan) placed 1 m in front of a spontaneously calling male. We recorded the advertisement calls of twelve *P. aurotaenia* males at three localities: La Victoria, Puerto Pervel, and El Dos (Chocó); eight *P. aurotaenia* broad-stripe males in Vereda Bochoromá (Chocó); and six *P. bicolor* in Santa Cecilia, Risaralda. Immediately after recording, we measured the temperature of the frog dorsum using an infrared thermometer (Oakton model WD-35639). The temperature was measured at the frog's original calling site at the closest distance possible before the frog initiated its escape response (usually < 1 m). The frogs were thereafter captured and measured in body size (snout-vent-length, SVL) to the nearest 0.01 mm with a digital caliper. Sound recordings were deposited at the Colección de Sonidos Ambientales of the Instituto Alexander von Humboldt, Colombia (IAvH-CSA 36874 to IAvH-CSA 36885 for *P. aurotaenia*; IAvH-CSA 36886 to IAvH-CSA 36893 for *P. aurotaenia* broad-stripe; IAvH-CSA-34251 to IAvH-CSA-34256 for *P. bicolor*).

To describe each lineage's advertisement call, we measured two temporal (note and internote duration) and three spectral (low, peak and high frequency) traits of the advertisement calls (Fig. S2). We used the parameters peak frequency, frequency 5%, and frequency 95% to determine the peak, low, and high frequency, respectively, based in spectrograms created in the Raven Pro 1.3 software (K. Lisa Yang Center for Conservation Bioacoustics 2011). The acoustic parameters were chosen considering a single note as a homologous sound unit across dendrobatid frogs (Erdtmann and Amézquita 2009). The digital recordings were analyzed using a sampling rate of 44.1 kHz, and with an FFT of 512 points, allowing 50% of overlap between points, using the Blackman window.

To minimize redundancy between the measured acoustic traits, we conducted a Principal Component Analysis (PCA). To test whether the acoustic signals represented a continuum of variation along the geographic cline, we used the acoustic signal variables summarized (linear regression line \pm 95% confidence interval) by the first principal component (PC1) and plotted the values against the study localities along a West–East axis represented by longitude. Using a linear model, we tested the effect of the geographic locality on the PC1 and added temperature as a covariate given that there are altitudinal differences in each locality. Subsequently, to select the call parameters that best discriminated between lineages, we coupled the principal components with a discriminant function analysis (DFA) using the lineage as grouping factor. We then examined the variation among PCs in the discriminant function that best discriminated between lineages (DF1). Based on the PC that explained most of the variation among the other PCs, we selected the original spectral and temporal acoustic parameters with highest representation: peak frequency (PF) and note duration (ND). To understand and eventually rule out the effect of temperature and body size on these call traits, we fit linear models of PF and ND using temperature and body size as explanatory variables. Finally, to create the acoustic signal space, the main call parameters (PF and ND) were differentiated in a two-dimensional space using a scatterplot and Kernel density function. The PCA was performed using the package *psych* (Revelle and Revelle 2015); The DFA was performed using the package *MASS* (Ripley et al. 2013); and all figures were created using the package *ggplot2* (Wickham et al. 2016).

Delimiting the recognition space

In 2019, we conducted ninety-eight playback experiments per lineage to delimit the signal recognition space. We first synthesized calls using the software Audacity (Audacity Team 2021) modifying the peak frequency and note duration parameters of the calls, which were the focal temporal and spectral acoustic traits obtained by the DFA. We created every combination of 7 note durations (8, 15, 21, 31,

41, 51, and 61 ms) and 14 peak frequencies (every 200 Hz from 1800 to 4400 Hz) (type WAVE; sample rate: 44,100 Hz; 16-bit signed) (Fig S1). The values were chosen to include the natural variation of acoustic signals among lineages plus supernormal values outside the natural range of variation (Table S1). For every synthetic note, we recreated the signal envelope of a *Phylllobates* call, used a frequency modulation of 300 Hz, and added 45 ms silences between notes (internote silences), which were consistent across the different signals in the cline (Table S1; Fig. S2). Each call had a total duration of 5 min divided in series of 60 notes. Each call was separated by a silence of 5 s. Before starting an experiment, we made sure that our playback recordings had a sound pressure level of ~60 dB from approximately 3 m away of the speaker. To achieve this, we stood in front of the speaker with a sonometer which allowed us to record the sound pressure levels for every synthetic call. The sound pressure value was chosen based on levels recorded for individuals in natural conditions.

To broadcast the synthetic calls, we set up a loudspeaker (SRS-XB20, Sony, Tokyo, Japan) wirelessly connected to an iPod (Apple, Cupertino, CA) about 3 m from the selected male. Audio compression via Bluetooth from the iPod to the loudspeaker may affect the quality of the synthetic calls. However, this method allowed us to observe from a safe distance as to not interfere during the experiment. Moreover, the use of Bluetooth speakers has been used in multiple previous studies (e.g., Yang et al. 2016; Avendaño and Cadena 2021). We started a field playback experiment by carefully approaching a randomly chosen male that was emitting advertisement calls, which suggested that they were actively defending their territories. One of us sat without moving and waited until the male resumed calling vigorously, which indicated that the male was no longer disturbed by the presence of the observer. To minimize observer bias, we played a randomly chosen call for 5 min, or until the male exhibited a positive response, which was defined as his phonotactic approach to an area within 30 cm of the loudspeaker (a positive response can be seen at <https://youtu.be/fZbm3DleZvE>). To rule out lack of motivation (or disturbance) in those males that did not react to the signal during the first playback, we conducted a second playback with the average signal parameters for the male's own lineage. Only those experiments in which males failed to respond to the experimental signal (the first one) but responded positively to the control signal (the second one) were interpreted as true negatives, i.e., experiments in which the male did not recognize the signal.

We recognize that presenting different synthetic calls to different males in each experiment or including the identity of the individual male in our statistical models would have helped to ensure that individual consistent behaviors did not affect our results. However, in weighing the pros and cons, we decided against using invasive methods (e.g., toe clipping) to identify individual frogs and non-invasive

methods (e.g., using color variation or territory to identify frogs) would not have provided accurate identification either. The lack of individual identity information could increase the likelihood of pseudoreplication (i.e., the potential non-independence of the subjects tested). To minimize the possibility of testing the same individual more than once, we performed each playback experiment in a different site of the forest. Moreover, we followed steps from protocols developed to increase the quality and standardization of playback experiments (Kroodsma 1989, 1990; McGregor, 1992; Kroodsma et al. 2001) which have been extensively used in published literature (Amézquita et al. 2005, 2006, 2011; Rojas et al. 2006; Betancourth-Cundar et al. 2016): we randomized the sequence in which the synthetic calls were presented, never presented the same signal twice, and never repeated trials with the same individual in the same day. The first two steps are justified when the relevant unit of analysis is the signal presented (Amézquita et al. 2006), while the latter prevents habituation or the effect of hormonal changes that could affect aggressive responses immediately after the first playback experiment (Rodríguez et al. 2022). It was not possible to record the data blind because our experimental design involved focal animals in the field.

Lastly, to model each lineage's auditory recognition space, we used a generalized-additive model (GAM) (binomial family function, logit link and $k=5$). A different GAM was done for every lineage. We employed the variation in the two main lineages' discriminatory call parameters (PF and ND) as predictor variables and fitted binary responses from field playbacks (either approached the loudspeaker or not) as the response variable. We thus defined the operational recognition space as the area within the bivariate space (peak frequency and note duration) where the fitted GAM predicted that a male will most probably react, i.e., where the probability of male reaction was beyond 0.99. The GAM modelling was performed with the package *mgcv* in R (Wood and Wood 2015).

Results

Three principal components (PCs) explained 98% of the covariation matrix of the original five acoustic variables. The first PC (77%) was associated with all three spectral parameters and the note duration. The second (18%) and third PC (5%) were associated mainly with temporal parameters (see Table S2). Using the three PCs, we conducted a discriminant function analysis (DFA), with the lineage as the grouping factor. We found two discriminant functions (DF) explaining 91% and 9% of the variation in the PCs respectively. The first DF that explained the greatest percentage of variation was strongly represented by the PC1, hence, affected by spectral parameters and the note duration (see

Table S3). For further analysis, we used the original temporal and spectral variables that had the most weight (coefficient of the linear discrimination equations) in the DFA: peak frequency (PF) and note duration (ND).

We then analyzed the effect that temperature and body size may have on the two main variables. Larger frogs uttered significantly longer notes and at lower frequencies (linear model, beta coefficient = 0.69, $p < 0.05$ for ND; beta coefficient = -0.94 , $p < 0.05$ for PF). Temperature, however, did not have a significant effect on any of the two call parameters (beta coefficient = -0.21 , $p = 0.11$, for ND; beta coefficient = -0.005 , $p = 0.94$, for PF) (Fig. S3).

The acoustic signals summarized by the PC1 represented a continuum of variation along the geographic cline (Fig. 2), which resembled the continuum of variation in coloration and body size reported elsewhere (Silverstone 1976; Márquez et al. 2020) which conformed with our data (Fig. S4). Geographic locality but not temperature had a significant effect on the PC1 (locality (beta coefficient = 0.79, $p < 0.05$); temperature (beta coefficient = -0.02 , $p = 0.85$)). After representing call parameters in a two-dimensional space, PF matched the continuous pattern, yet ND was undistinguishable between *P. aurotaenia* broad-stripe and *P. aurotaenia* (Fig. 3). We found that lineages' recognition spaces were wider than their signal spaces, estimated by a 99% probability of positive response to a playback (Fig. 4). This means that we only consider the recognition of a conspecific when the acoustic signal falls within the narrowest ellipse of the auditory recognition space. Moreover, we found that the recognition spaces differed between groups (Fig. 4). PF and ND were significant predictors of the phonotactic response

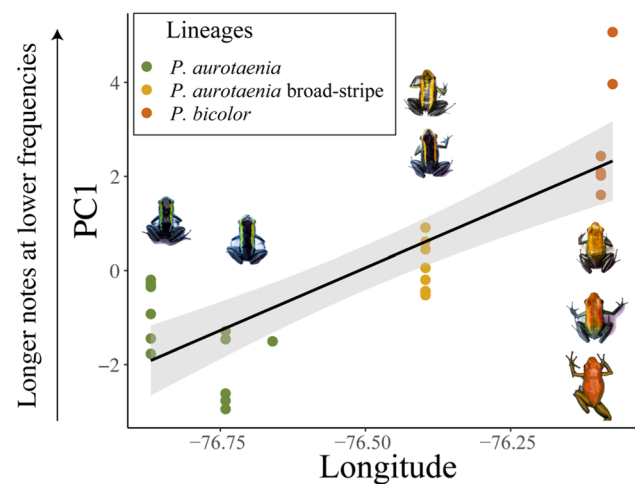


Fig. 2 Call parameters in three lineages of *Phylllobates* along a geographic cline. The PC1 is associated with spectral features and note duration of the advertisement call (longer calls and at lower frequencies at greater positive values). Dots represent each specimen in each locality. The black line represents the trending line, and the light grey area represents the confidence interval at 0.95

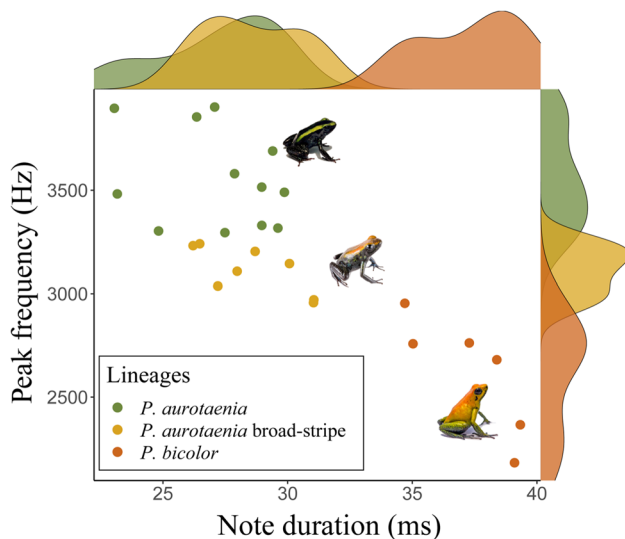


Fig. 3 Scatterplot and kernel density functions (sides) of two call parameters among three lineages of *Phylllobates*. The height of the kernel density functions represents the proportion of data points for both peak frequency and note duration. Each dot represents a specimen and dot colors denote lineage identity

of individuals to experimental playbacks for *P. aurotaenia* ($R^2=0.78$; $edf=3.864$ and $p=0.007$ for PF; $edf=3.632$ and $p=0.006$ for ND) and *P. aurotaenia* broad-stripe ($R^2=0.72$; $edf=2.892$ and $p=0.024$ for PF; $edf=2.671$ and $p=0.003$ for ND). PF but not ND was a significant predictor of male reaction for *P. bicolor* ($R^2=0.87$; $edf=2.612$ and $p=0.047$ for PF; $edf=3.957$ and $p=0.187$ for ND). An edf stands for

effective degrees of freedom. An edf of 1 would represent a linear relationship, while $edf > 2$ represents a highly non-linear relationship (Zuur et al. 2009).

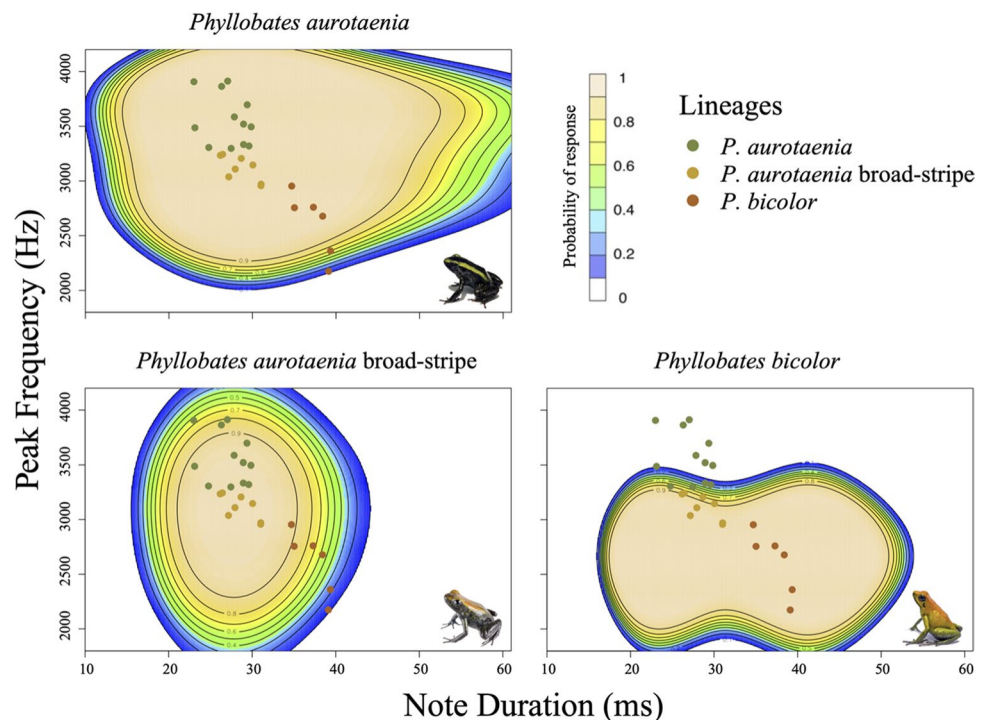
Discussion

Our results show the decoupled divergence of males' auditory perception from acoustic signals across a geographic and phenotypic cline in color and body size, which include two nominal species and an intermediate population of *Phylllobates* poison frogs. Individuals of *P. aurotaenia* responded to the calls of the other lineages reasonably well, except for the signals from the other extreme of the cline. Individuals bearing the intermediate phenotype failed to respond to *P. bicolor* calls and some individuals of *P. aurotaenia*, suggesting they do not recognize these calls. Individuals of *P. bicolor*, in turn, did not respond to the *P. aurotaenia* calls but responded to the signals of intermediate individuals. We discuss herein: (1) the pattern of acoustic signal divergence, (2) the decoupled divergence of auditory perception from acoustic signals, (3) the role of male-male communication in reproductive interference, and (4) the alternative sources of lineages' interference.

Acoustic traits divergence

We found a pattern of continuous divergence on the peak frequency but not the note duration of the advertisement call across the cline in color and body size (Fig. 3). The

Fig. 4 GAM modelling of the recognition space in three lineages of *Phylllobates* as estimated from the phonotactic response to playback experiments. Black lines and gradient fill denote increasing probability of male response to advertisement calls of variable frequency and note duration values. The acoustic signal space of the three lineages (colored dots) is overlapped to show which vocalizations are recognized by conspecific and heterospecific males



evolution of the peak frequency may have been driven by concomitant covariation in body size as the frequency of anuran calls is predominantly affected by the mass of the laryngeal apparatus, causing larger animals to call at lower frequencies (reviewed in Duellman and Trueb 1994). The evolution of the signal frequency in these poison dart frogs could thus be a pleiotropic effect of differences in body size, as *P. bicolor* is larger than *P. aurotaenia* and *P. aurotaenia* broad-stripe bears an intermediate phenotype (see Fig. S3, S4A). Divergent selective pressures in body size may indeed promote and maintain differences in incipient lineages, particularly when body size is functionally or pleiotropically linked to the species recognition signal (Gerhardt 2013).

On the other hand, the note duration values from individuals bearing the phenotypes *P. aurotaenia* broad-stripe and *P. aurotaenia* were overlapping (Fig. 3). There are two phenomena that could explain this lack of variation: First, research in insects and frogs has found that complex mating patterns among divergent lineages may be hindering the divergence in certain communication traits (Gerhardt 1991; Mavárez et al. 2006). For example, asymmetrical introgression from hybrids to one of the parental species may erode the trait divergence that first arose in hybrids (Mavárez et al. 2006). Second, different traits within the acoustic signal phenotype may be under different evolutionary pressures (Gerhardt 1991), leading to certain properties of the acoustic signals to diverge, whereas others may remain stable between species (Erdtmann and Amézquita 2009; Greig and Webster 2013; Vargas-Salinas and Amézquita 2013).

Decoupled auditory perception from acoustic signals divergence

Our playback experiments revealed wide perceptual fields and asymmetry in the mutual recognition capabilities by males of the three lineages. The recognition space of *P. aurotaenia* was not restricted either to the temporal or the spectral domain of acoustic traits; the recognition space of *P. bicolor* was restricted mainly in the spectral domain; and the recognition space of the intermediate population was restricted in both the temporal and the spectral domains. It is theorized that signals of recently diverged lineages evolve in tandem with recognition systems as to reduce the costs of competition with heterospecifics, either for mates or ecological resources (Gröning and Hochkirch 2008; Grether et al. 2017; Rosenthal 2017). Empirical evidence suggests, however, that the maintenance of partial acceptance thresholds (i.e., recognizing heterospecific individuals as competitors) may indeed be common in emergent lineages. Moreover, this process may depend on the signal being assessed, which means that different heterospecific signals could induce different behavioral responses (Bernal et al. 2007; Ritchie 2007; Shurtliff et al.

2013; Yang et al. 2016; Shizuka and Hudson 2020). Several processes have been used to explain this pattern: lineages' evolutionary history (i.e., currently allopatric populations that were once sympatric), the generalization of perceptual systems to divergent stimuli (Ryan et al. 2003), and the evolutionary flexibility of perceptual systems in comparison to signaling systems (Tumulty et al. 2022). In our study, the interaction between the mentioned processes could prevent the symmetrical evolution of communication systems among incipient lineages given the different levels of isolation and connectivity that may have occurred during the formation of the cline. Moreover, given the recent divergence of the clade we studied (< 3 MYA, Márquez et al. 2020), our results provide evidence for the prevalence of the decoupled evolution of males' signaling and recognition systems in recently diverged lineages.

The males of the two nominal species we studied, whose signals were represented at the extremes of the cline, responded reasonably well to some heterospecific signals across the cline. Our results indicate that although competitor recognition could be evident for distant signals, suggesting the incipient divergence of recognition systems, a low acceptance threshold (i.e., recognizing as a competitor a signal outside the range of variation within their own lineage) is prevalent in the two species we studied. The intermediate phenotypes, in turn, failed to respond to most of the individuals of *P. bicolor*, and some individuals of *P. aurotaenia*. A recent phylogenetic reconstruction in the genus *Phylllobates* hypothesized that *P. aurotaenia* broad-stripe (referred as *P. aurotaenia* San Juan in Márquez et al. 2020), represented a distinct lineage from *P. aurotaenia*. Our results could be evidence of the incipient divergence in both signaling and recognition systems in this population, which has remained stable for at least 50 years (first described in Silverstone 1976). Nonetheless, further work is required to untangle the relationships and more importantly, the reproductive boundaries between these lineages.

The small sample size of recordings in our study may limit us from considering potential larger variation in the signal space. Consequently, if signal variation is larger than the one we found, the mismatch between the signal space and recognition space could be less evident in the three lineages. However, our recording values fall within other published recordings of the species (Erdtmann and Amézquita 2009). Moreover, we found that body size determines the spectral and temporal parameters of the acoustic signals. We found similar values in body size as Silverstone (1976) for the three lineages, which would limit the possible variation in their acoustic signals. Therefore, although a larger sample size would give us a better representation of reality, we argue that it is unlikely to find such extreme values as to change the interpretation of our results.

The role of male-male communication in reproductive interference

We found wide and intermediately sized zones of recognition by territorial males, suggesting relaxed levels of selection on competitor recognition. Aggressive responses towards heterospecifics may be explained merely by the defense of ecological resources (Grether et al. 2017). However, complex patterns of gene flow and isolation have been found along the cline (Márquez et al. 2020). Moreover, recent research hypothesized that the cline may have emerged from parapatric differentiation during a range expansion (Márquez et al., unpublished data). This suggests that some populations were historically connected and that reproductive isolation has not completely raised between the lineages in the system. In fact, viable offspring between *P. aurotaenia* and *P. bicolor* can be obtained in captivity (Márquez, personal communication). Therefore, in this section, we interpret our results solely in the light of reproductive interference. Under this scenario, competition for potential mates can lead to heterospecific males to recognize each other as sexual competitors (Gröning and Hochkirch 2008; Grether et al. 2017). Given that territory ownership is the main determinant for reproductive success in poison frogs, competitor recognition may have remained flexible to divergent acoustic signals that could still come from potential territory/sexual competitors, leading to non-assortative male-male competition. Therefore, strong selection that would narrow the recognition space of male receivers may not occur if the potential benefits to defend a territory (i.e., reproductive success) outstand the associated costs of recognizing heterospecifics as potential sexual competitors, such as spent energy and predation risk (Gröning and Hochkirch 2008; Grether et al. 2017). Our results are supported by similar patterns of interference caused by territorial competition in the brilliant-thighed poison frog *Allobates femoralis* and in *Hetaerina* damselflies, which has hindered the coupled divergence of traits and perception (Drury et al. 2015; Betancourth-Cundar et al. 2016). Surprisingly, the role of male-male competition as a mechanism driving communication systems that are important for competitor recognition remains poorly studied (Tinghitella et al. 2018).

Although we lack data of females' species recognition, the presence of gene flow along the cline suggests low levels of assortative mating. On one hand, males' reproductive interference could prevent females from expressing their mate preferences as females would be limited to mate with territory owners of either lineage ("hampered detection of mates", Wong and Candolin 2005). Therefore, lineages divergence could be hindered if male-male competition overcomes the role of female preferences (Ritchie 2007). Alternatively, the role of males may not overcome the role of females' preferences. In that case, females could be under

stronger selection pressures to differentiate heterospecific from conspecific calls, as they would incur in higher costs of misrecognizing an individual, such as wasted eggs. Past studies exploring the species recognition patterns in both males and females of other species have encountered contrasting results, where species recognition is constrained in females (Bernal et al. 2007), or males (Nelson and Soha 2004). Elucidating the potential role of females in the evolution of the communication system and the existence of gene flow along the cline remains to be tested in future studies.

Alternative sources of lineages' interference

The relevance of territories for reproductive success in neotropical poison frogs leads us to suggest reproductive interference as the main obstacle for assortative male-male recognition. Nonetheless, two other factors could also affect heterospecific recognition between males in our system: differences in body size and competition for resources other than mates. First, some species of frogs may use the call frequency of potential competitors as a measure of their body size and therefore the likelihood to win a confrontation (Bee et al. 2016). This could lead to the larger *P. bicolor* to ignore the calls of the smallest *P. aurotaenia* as they would pose little threat. However, we would also expect a similar opposite pattern in which *P. aurotaenia* would not engage in a fight with a much larger individual. Nonetheless, we did not see such pattern in our study. Second, territories hold food resources that several species use and males could have wide recognition capabilities towards lineages that use similar resources (Grether et al. 2017). For example, resource use but not social selection predicts aggressiveness towards heterospecific morphs in two species of the neotropical passerine bird genus *Arremon* (Avendaño and Cadena 2021). In this case, we should observe escalated territorial behaviors towards other species that use similar resources. Nonetheless, we often observed other species of poison frogs including *Allobates talamancae* and *Oophaga histrionica* in close proximity to *Phyllobates* without the latter responding aggressively to them. In contrast, aggressive encounters are common between individuals of *Phyllobates aurotaenia* (González-Santoro et al. 2021). Moreover, it is unlikely that the three lineages that we studied defend ecological resources from only some individuals along the cline. Finally, males' territories are relatively small (approximately < 5 m radius), and no clear resource partitioning was evident between territories to suggest a main role of ecological resources as the source of interference. Therefore, we argue that males defend territories from mainly potential sexual competitors and suggests that reproductive interference is a main driver of the communication among males. The decoupled divergence of signaling and recognition systems in nature may be more common than previously

thought, and the role of male-male interactions in the evolution of communication systems remains to be extensively explored.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00265-022-03281-8>.

Acknowledgements We are highly thankful with Corinne Richards-Zawacki, the RZ lab and Tessa Rhinehart for helpful comments that improved the manuscript. We thank Laura Lopera-Congote for her guidance and suggestions on figure editing and the construction of the map. We thank the two reviewers and the editor that guided us to improve our manuscript. Finally, we are especially grateful with La Victoria community and Blas in Santa Cecilia for their hospitality and knowledge shared about the natural history of poison frogs.

Author contribution Conceptualization: MG-S, PP-R, AA. Methodology: all authors and coauthors contributed to the methodology. Formal analysis and investigation: MG-S, AA. Writing-original draft preparation: MG-S, AA. Writing-review and editing: MG-S, MG, AA. Funding acquisition: MG-S, AA.

Funding This work was supported by Facultad de Ciencias, Universidad de los Andes-Colombia (Seed Grant to MGS).

Data availability All data used for our study is included in the supplementary material document. This Data set can be found at https://www.researchgate.net/publication/366124036_Gonzalez-Santoro_et_al_2022_Data_Set

Declarations

Ethics approval All authors confirm that the welfare of animals was prioritized and respected in the study. We followed the recommended guidelines for fieldwork with animals set forth by the Animal Behavior Society. No ethics committee approval was required as all experiments were observational. Individuals were only temporarily caught to measure the body size and immediately released. Neither the experiments nor the handling harmed any individual.

Competing interests The authors declare no competing interests.

References

- Amézquita A, Castellanos L, Hödl W (2005) Auditory matching of male *Epipedobates femoralis* (Anura: Dendrobatidae) under field conditions. *Anim Behav* 70:1377–1386. <https://doi.org/10.1016/j.anbehav.2005.03.012>
- Amézquita A, Hödl W, Lima AP, Castellanos L, Erdtmann L, De Araujo MC (2006) Masking interference and the evolution of the acoustic communication system in the Amazonian Dendrobatid frog *Allobates Femoralis*. *Evolution* 60:1874–1887. <https://doi.org/10.1111/j.0014-3820.2006.tb00531.x>
- Amézquita A, Flechas SV, Lima AP, Gasser H, Hödl W (2011) Acoustic interference and recognition space within a complex assemblage of dendrobatid frogs. *P Natl Acad Sci USA* 108:17058–17063. <https://doi.org/10.1073/pnas.1104773108>
- Audacity Team (2021) AudacityI: Free Audio Editor and Recorder, version 3.0.0. <https://audacityteam.org/>. Accessed 01 May 2019
- Avendaño JE, Cadena CD (2021) Territorial males do not discriminate between local and novel plumage phenotypes in a tropical songbird species complex: implications for the role of social selection in trait evolution. *Behav Ecol Sociobiol* 75:37. <https://doi.org/10.1007/s00265-021-02976-8>
- Bee MA, Reichert MS, Tumulty J (2016) Assessment and recognition of rivals in anuran contests. *Adv Stud Behav* 48:161–249
- Bernal XE, Rand AS, Ryan MJ (2007) Sex differences in response to nonconspecific advertisement calls: receiver permissiveness in male and female túngara frogs. *Anim Behav* 73:955–964. <https://doi.org/10.1016/j.anbehav.2006.10.018>
- Betancourth-Cundar M, Lima AP, Hödl W, Amézquita A (2016) Decoupled evolution between senders and receivers in the neotropical *Allobates femoralis* frog complex. *PLoS ONE* 11:e0155929. <https://doi.org/10.1371/journal.pone.0155929>
- Dawkins MS, Guilford T (1996) Sensory bias and the adaptiveness of female choice. *Am Nat* 148:937–942. <https://doi.org/10.1086/285964>
- den Hartog PM, Slabbekoorn H, ten Cate C (2008) Male territorial vocalizations and responses are decoupled in an avian hybrid zone. *Phil Trans R Soc B* 363:2879–2889. <https://doi.org/10.1098/rstb.2008.0046>
- Drury JP, Okamoto KW, Anderson CN, Grether GF (2015) Reproductive interference explains persistence of aggression between species. *Proc R Soc B* 282:20142256. <https://doi.org/10.1098/rspb.2014.2256>
- Duellman WE, Trueb L (1994) *Biology of Amphibians*. John Hopkins University Press, Baltimore
- Erdtmann L, Amézquita A (2009) Differential evolution of advertisement call traits in dart-poison frogs (Anura: Dendrobatidae). *Ethology* 115:801–811. <https://doi.org/10.1111/j.1439-0310.2009.01673.x>
- Gerhardt HC (1988) Acoustic properties used in call recognition by frogs and toads. In: Frittsch B, Walkowiak W, Ryan MJ (eds) *The evolution of the amphibian auditory system*. Wiley, New York, pp 455–483
- Gerhardt HC (1991) Female mate choice in treefrogs: static and dynamic acoustic criteria. *Anim Behav* 42:615–635. [https://doi.org/10.1016/S0003-3472\(05\)80245-3](https://doi.org/10.1016/S0003-3472(05)80245-3)
- Gerhardt HC (2013) Geographic variation in acoustic communication: reproductive character displacement and speciation. *Evol Ecol Res* 15:605–632
- Gerhardt HC, Huber F (2003) *Acoustic communication in insects and anurans: common problems and diverse solutions*. University of Chicago Press, Chicago
- Gerhardt HC, Schwartz JJ (1995) Interspecific interactions in anuran courtship. *Amphib Biol* 2:603–663
- González-Santoro M, Hernández-Restrepo J, Palacios-Rodríguez P (2021) Aggressive behaviour, courtship and mating call description of the neotropical poison frog *Phylllobates aurotaenia* (Anura: Dendrobatidae). *Herpetol Notes* 14:1145–1149
- Greig EI, Webster MS (2013) Spatial decoupling of song and plumage generates novel phenotypes between two avian subspecies. *Behav Ecol* 24:1004–1013. <https://doi.org/10.1093/beheco/art005>
- Grether GF, Peiman KS, Tobias JA, Robinson BW (2017) Causes and consequences of behavioral interference between species. *Trends Ecol Evol* 32:760–772. <https://doi.org/10.1016/j.tree.2017.07.004>
- Gröning J, Hochkirch A (2008) Reproductive interference between animal species. *Q Rev Biol* 83:257–282. <https://doi.org/10.1086/590510>
- K. Lisa Yang Center for Conservation Bioacoustics (2011) Raven Pro: interactive sound analysis software, version 1.3. The Cornell Lab of Ornithology, Ithaca, NY. <https://ravensoundsoftware.com>. Accessed 30 July 2018
- Kahn TR, La Marca E, Lötters S, Brown JL, Twomey E, Amézquita A (eds) (2016) *Aposematic poison frogs (Dendrobatidae) of the Andean countries: Bolivia*. Tropical Field Guide Series,

- Conservation International, Arlington. USA, Colombia, Ecuador, Peru and Venezuela
- Kroodsma DE (1989) Suggested experimental designs for song playbacks. *Anim Behav* 37:600–609
- Kroodsma DE (1990) Using appropriate experimental designs for intended hypotheses in ‘song’ playbacks, with examples for testing effects of song repertoire sizes. *Anim Behav* 40:1138–1150
- Kroodsma DE, Byers BE, Goodale E, Johnson S, Lui WC (2001) Pseudoreplication in playback experiments, revisited a decade later. *Anim Behav* 61:1029–1033
- Ladich F, Myrberg AA Jr (2006) Agonistic behavior and acoustic communication. In: Ladich F, Collin SP, Moller P, Kapoor BG (eds) *Communication in fishes*. Science Publishers, Enfield, NH, pp 121–148
- Lipshutz SE (2018) Interspecific competition, hybridization, and reproductive isolation in secondary contact: missing perspectives on males and females. *Curr Zool* 64:75–88. <https://doi.org/10.1093/cz/zox060>
- Márquez R, Linderot TP, Mejía-Vargas D, Nielsen R, Amézquita A, Kronforst MR (2020) Divergence, gene flow, and the origin of leapfrog geographic distributions: the history of colour pattern variation in *Phylllobates* poison-dart frogs. *Mol Ecol* 29:3702–3719. <https://doi.org/10.1111/mec.15598>
- Mavárez J, Salazar CA, Bermingham E, Salcedo C, Jiggins CD, Linares M (2006) Speciation by hybridization in *Heliconius* butterflies. *Nature* 441:868–871. <https://doi.org/10.1038/nature04738>
- McGregor PK (1992) Quantifying responses to playback: one, many, or composite multivariate measures? In: McGregor PK (ed) *Playback and studies of animal communication*. Plenum, New York, pp 79–96
- Meuche I, Röhl H (2011) Alternative mating tactics in the strawberry poison frog (*Oophaga pumilio*). *Herpetol J* 21:275–277
- Nelson D, Soha J (2004) Male and female white-crowned sparrows respond differently to geographic variation in song. *Behaviour* 141:53–69. <https://doi.org/10.1163/156853904772746600>
- Panhuis TM, Butlin R, Zuk M, Tregenza T (2001) Sexual selection and speciation. *Trends Ecol Evol* 16:364–371. [https://doi.org/10.1016/S0169-5347\(01\)02160-7](https://doi.org/10.1016/S0169-5347(01)02160-7)
- Price T (2008) *Speciation in birds*. Roberts and Company, Greenwood Village CO
- Röhl H (1997) Territorial behaviour of the strawberry poison-dart frog, *Dendrobates pumilio*. *Amphibia-Reptilia* 18:437–442
- Röhl H (2005) Territorial behavior in dendrobatid frogs. *J Herpetol* 39:354–365. <https://doi.org/10.1670/162-04A.1>
- Pryke SR, Andersson S, Lawes MJ (2001) Sexual selection of multiple handicaps in the red-collared widowbird: female choice of tail length but not carotenoid display. *Evolution* 55:1452–1463. <https://doi.org/10.1111/j.0014-3820.2001.tb00665.x>
- Qvarnström A, Vallin N, Rudh A (2012) The role of male contest competition over mates in speciation. *Curr Zool* 58:493–509. <https://doi.org/10.1093/czoolo/58.3.493>
- Revelle W, Revelle MW (2015) Package ‘psych.’ *Cran r* 337:338. <https://cran.r-project.org/web/packages/psych/psych.pdf>. Accessed 30 Aug 2019
- Ringler E, Ringler M, Jehle R, Hödl W (2012) The female perspective of mating in *A. femoralis*, a territorial frog with paternal care—a spatial and genetic analysis. *PLoS ONE* 7:e40237. <https://doi.org/10.1371/journal.pone.0040237>
- Ripley B, Venables B, Bates DM, Hornik K, Gebhardt A, Firth D, Ripley MB (2013) Package ‘mass.’ *Cran r* 538:113–120. <https://cran.r-project.org/web/packages/MASS/MASS.pdf>. Accessed 30 Aug 2019
- Ritchie MG (2007) Sexual selection and speciation. *Annu Rev Ecol Evol S* 38:79–102
- Rodríguez C, Fusani L, Raboisson G, Hödl W, Ringler E, Canoine V (2022) Androgen responsiveness to simulated territorial intrusions in *Allobates femoralis* males: evidence supporting the challenge hypothesis in a territorial frog. *Gen Comp Endocrinol* 326:114046. <https://doi.org/10.1016/j.ygcen.2022.114046>
- Roithmair ME (1992) Territoriality and male mating success in the dart-poison frog, *Epipedobates femoralis* (Dendrobatidae, Anura). *Ethology* 92:331–343. <https://doi.org/10.1111/j.1439-0310.1992.tb00970.x>
- Roithmair ME (1994) Field studies on reproductive behaviour in two dart poison frog species (*Epipedobates femoralis*, *Epipedobates trivittatus*) in Amazonian Peru. *Herpetol J* 4:77–85
- Rojas B, Amézquita A, Delgadillo A (2006) Matching and symmetry in the frequency recognition curve of the poison frog *Epipedobates trivittatus*. *Ethology* 112:564–571
- Rosenthal GG (2017) *Mate choice: the evolution of sexual decision making from microbes to humans*. Princeton University Press, Princeton
- Ryan MJ (1988) Constraints and patterns in the evolution of anuran acoustic communication. The evolution of the amphibian auditory system. Wiley, New York, pp 637–677
- Ryan MJ, Rand AS (1993a) Species recognition and sexual selection as a unitary problem in animal communication. *Evolution* 47:647–657. <https://doi.org/10.1111/j.1558-5646.1993.tb02118.x>
- Ryan MJ, Rand AS (1993b) Phylogenetic patterns of behavioural mate recognition systems in the *Physalaemus pustulosus* species group (Anura: Leptodactylidae): the role of ancestral and derived characters and sensory exploitation. In: Lees DR, Edwards D (eds) *Evolutionary Patterns and Process*. Academic Press, London, pp 251–267
- Ryan MJ, Rand AS (2003) Sexual selection in female perceptual space: how female túngara frogs perceive and respond to complex population variation in acoustic mating signals. *Evolution* 57:2608–2618. <https://doi.org/10.1111/j.0014-3820.2003.tb01503.x>
- Ryan MJ, Fox JH, Wilczynski W, Rand AS (1990) Sexual selection for sensory exploitation in the frog *Physalaemus pustulosus*. *Nature* 343:66–67. <https://doi.org/10.1038/343066a0>
- Ryan MJ, Rand W, Hurd PL, Phelps SM, Rand AS (2003) Generalization in response to mate recognition signals. *Am Nat* 161:380–394. <https://doi.org/10.1086/367588>
- Servedio MR, Boughman JW (2017) The role of sexual selection in local adaptation and speciation. *Annu Rev Ecol Evol S* 48:85–109. <https://doi.org/10.1146/annurev-ecolsys-110316-022905>
- Shizuka D, Hudson EJ (2020) To accept or reject heterospecific mates: behavioural decisions underlying premating isolation. *Phil Trans R Soc B* 375:20190484. <https://doi.org/10.1098/rstb.2019.0484>
- Shurtliff QR, Murphy PJ, Yeiter JD, Matocq MD (2013) Experimental evidence for asymmetric mate preference and aggression: behavioural interactions in a woodrat (*Neotoma*) hybrid zone. *BMC Evol Biol* 13:220. <https://doi.org/10.1186/1471-2148-13-220>
- Silverstone P (1976) A revision of the poison arrow frogs of the genus *Phylllobates* Bibron in Sagra (family Dendrobatidae). Natural History Museum of Los Angeles County, Los Angeles
- Tinghitella RM, Lackey AC, Martin M, Dijkstra PD, Drury JP, Heathcote R (2018) On the role of male competition in speciation: a review and research agenda. *Behav Ecol* 29:783–797. <https://doi.org/10.1093/beheco/arx151>
- Tumulty JP, Bee MA (2021) Ecological and social drivers of neighbor recognition and the dear enemy effect in a poison frog. *Behav Ecol* 32:138–150. <https://doi.org/10.1093/beheco/araa113>
- Tumulty JP, Lange ZK, Bee MA (2022) Identity signaling, identity reception, and the evolution of social recognition in a Neotropical frog. *Evolution* 76:158–170. <https://doi.org/10.1111/evo.14400>
- Twomey E, Vestergaard JS, Venegas PJ, Summers K (2016) Mimetic divergence and the speciation continuum in the mimic poison frog

- Ranitomeya imitator*. Am Nat 187:205–224. <https://doi.org/10.1086/684439>
- Ursprung E, Ringler M, Jehle R, Hödl W (2011) Strong male/male competition allows for nonchoosy females: high levels of polygynandry in a territorial frog with paternal care. Mol Ecol 20(8):1759–1771. <https://doi.org/10.1111/j.1365-294X.2011.05056.x>
- Vargas-Salinas F, Amézquita A (2013) Stream noise, hybridization, and uncoupled evolution of call traits in two lineages of poison frogs: *Oophaga histrionica* and *Oophaga lehmanni*. PLoS ONE 8:e77545. <https://doi.org/10.1371/journal.pone.0077545>
- Wells KD (1977) The courtship of frogs. In: Taylor DH, Guttman SI (eds) The reproductive biology of amphibians. Springer, Boston, MA, pp 233–262
- West-Eberhard MJ (1983) Sexual selection, social competition, and speciation. Q Rev Biol 58:155–183. <https://doi.org/10.1086/413215>
- Wickham H, Chang W, Wickham MH (2016) Package ‘ggplot2.’ Create elegant data visualisations using the grammar of graphics. Cran r 2:1–189. <https://ggplot2.tidyverse.org>. Accessed 30 Aug 2019
- Wilczynski W, McClelland BE, Rand AS (1993) Acoustic, auditory, and morphological divergence in three species of neotropical frog. J Comp Physiol A 172:425–438. <https://doi.org/10.1007/BF00213524>
- Wong BB, Candolin U (2005) How is female mate choice affected by male competition? Biol Rev 80:559–571. <https://doi.org/10.1017/S1464793105006809>
- Wood S, Wood MS (2015) Package “mgcv.” R package version 1.29, Cran r 1:29. <https://cran.r-project.org/web/packages/mgcv/mgcv.pdf>. Accessed 30 Aug 2019
- Yang Y, Richards-Zawacki CL (2021) Male–male contest limits the expression of assortative mate preferences in a polymorphic poison frog. Behav Ecol 32:151–158. <https://doi.org/10.1093/beheco/araa114>
- Yang Y, Richards-Zawacki CL, Devar A, Dugas MB (2016) Poison frog color morphs express assortative mate preferences in allopatry but not sympatry. Evolution 70:2778–2788. <https://doi.org/10.1111/evo.13079>
- Yang Y, Prémel V, Richards-Zawacki CL (2020) Prior residence effect determines success of male–male territorial competition in a color polymorphic poison frog. Ethology 126:1131–1140. <https://doi.org/10.1111/eth.13093>
- Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer, New York

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.