



Modality interactions alter the shape of acoustic mate preference functions in gray treefrogs

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Sexual selection takes place in complex environments where females evaluating male mating signals are confronted with stimuli from multiple sources and modalities. The pattern of expression of female preferences may be influenced by interactions between modalities, changing the shape of female preference functions, and thus ultimately altering the selective landscape acting on male signal evolution. We tested the hypothesis that the responses of female gray treefrogs, *Hyla versicolor*, to acoustic male advertisement calls are affected by interactions with visual stimuli. We measured preference functions for several call traits under two experimental conditions: unimodal (only acoustic signals presented), and multimodal (acoustic signals presented along with a video-animated calling male). We found that females were more responsive to multimodal stimulus presentations and, compared to unimodal playbacks, had weaker preferences for temporal call characteristics. We compared the preference functions obtained in these two treatments to the distribution of male call characteristics to make inferences on the strength and direction of selection expected to act on male calls. Modality interactions have the potential to influence the course of signal evolution and thus are an important consideration in sexual selection studies.

KEY WORDS: Anuran, multimodal integration, preference function, sexual selection.

In sexual selection, female mate choice leads to nonrandom mating success among males, and is thus a potent evolutionary force in generating selection on male traits (Andersson 1994). Thus, a major focus in evolutionary biology has been to understand what influences a female's decision to mate with a particular male (Jennions and Petrie 1997). The mate evaluation process is potentially complex, and preference expression is likely to be influenced by multiple, possibly interacting, factors (Hohenlohe and Arnold 2010). However, most studies have taken a reductionist, univariate, approach in which the effects of a single male characteristic or environmental factor on the expression of female preferences is examined (Chenoweth and Blows 2006). For example, in species that signal to attract mates, female preference functions, which describe the pattern of female response across variation in male signal characteristics (Wagner 1998), have traditionally been estimated for a single signal characteristic at a time. However, recent studies of multivariate preference functions demon-

strate that females can evaluate multiple signal characteristics, and this has important, and sometimes unexpected, implications for the patterns of selection expected to act upon male traits (Blows et al. 2003; Brooks et al. 2005; Gerhardt and Brooks 2009). Nonetheless, even these measurements of female preference functions have generally been limited to stimuli in a single sensory modality (but see, e.g., Bailey, 2011). Animals possess multiple sensory systems, and their behaviors are potentially influenced by stimuli in multiple sensory modalities (Bradbury and Vehrencamp 2011; Stevens 2013). In this study, we thus ask if female preference functions for male signal traits in one modality are influenced by a cue in a different sensory modality.

Cross-modal interactions occur whenever the response to a signal in one modality is influenced by stimuli in other modalities (Shimojo and Shams 2001; Calvert et al. 2004; Munoz and Blumstein 2012). These stimuli can be part of a multimodal signal (Partan and Marler 1999; Candolin 2003; Hebets and Papaj

2005) or may involve other characteristics of the signaler or its environment. The shape of preference functions influences the strength and direction of selection on male signal characteristics (Ritchie 1996; Shaw and Herlihy 2000), and is thus an important metric of the potential for evolution by sexual selection. If preference expression is influenced by stimuli in multiple modalities, the resultant effects on the shape of female preference functions have important implications for understanding potential drivers of signal evolution. Nonetheless, little is known about multimodal effects on preference function shape.

At first glance, the nocturnal breeding choruses of anuran amphibians seem like an unlikely case for the importance of multiple sensory modalities in mate selection. Males in these choruses produce loud advertisement vocalizations to attract females, and the combined effect of many males vocalizing in the densest choruses is a complex and overwhelming acoustic display (Gerhardt and Huber 2002). Nevertheless, there is increasing evidence that even in nocturnal anurans other sensory modalities influence mate selection. Male calling and female mate searching behavior is affected by general visual stimuli in the environment such as ambient light levels (Rand et al. 1997; Baugh and Ryan 2010; Bonachea and Ryan 2011b,c) and the movement of other animals, for example, that of bats flying overhead (Tuttle and Ryan 1982; Tuttle et al. 1982). Anurans also attend to cues in multiple sensory modalities that are associated with calling males, including seismic vibrations produced by males calling on the ground (Lewis et al. 2001), waves produced by males calling on the water surface (Höbel and Kolodziej 2013; Halfwerk et al. 2014), and visual cues associated with the movement of the male vocal sac (Rosenthal et al. 2004; Taylor et al. 2008, 2011aa; Gomez et al. 2009). In some cases, there is evidence that males actually produce multimodal signals, that is, that both the acoustic component and components in other modalities evolved as signals and influence the response of females (reviewed by Starnberger et al. 2014). Thus, females potentially attend to a wide range of sensory stimuli in the choruses of nocturnal anurans, providing an ideal opportunity to assess the effects of stimuli in multiple modalities on the shape of female preference functions.

We selected our study species, the gray treefrog *Hyla versicolor*, for two reasons. First, previous measurements of female preference functions for acoustic characteristics of male advertisement calls indicate the potential for selection on multiple call characteristics. Females have directional preferences for long call durations (Gerhardt et al. 2000; Bush et al. 2002) and short call periods (= high call rates; Klump & Gerhardt, 1987; Gerhardt et al., 1996). In contrast, female preference functions for advertisement call frequency are generally closed and centered at or near the population mean (Gerhardt 1991, 2005). Second, there is evidence that visual stimuli are relevant in *H. versicolor*. Males of this species produce courtship calls in response to the visual

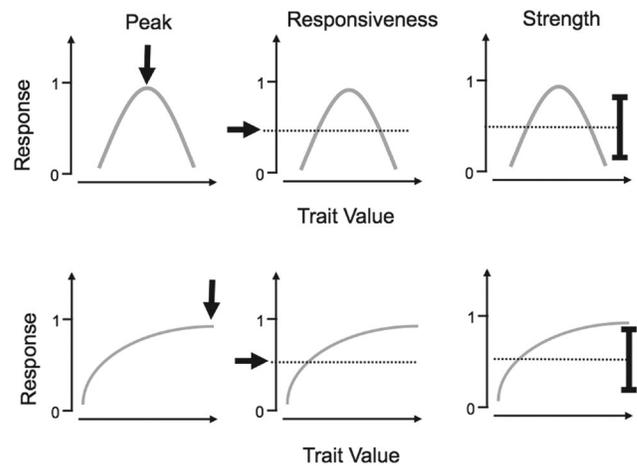


Figure 1. Traits used to describe variation in female preference functions. **Peak:** the trait value eliciting the fastest response from a female, indicated by the vertical arrow; **Responsiveness:** mean response across the range of stimuli, indicated by the horizontal arrow; **Strength:** the square of the coefficient of variation in responses across the range of presented stimuli (Schluter 1988), represented by the length of the bracketed bar. Top row shows examples of closed preference functions, bottom row shows examples of open preference functions.

stimulus of an approaching female (Reichert 2013). In addition, in a video playback experiment, females showed phototaxis to a variety of visual stimuli presented on a computer monitor (Reichert et al. 2014).

We tested whether the shape of female preference functions for acoustic characteristics of male advertisement calls in *H. versicolor* was affected by cues in the visual modality. Female preferences were measured for three call characteristics under both unimodal (acoustic signal only) and multimodal (acoustic signal and visual cue) conditions. We used a function-valued approach (Stinchcombe and Kirkpatrick 2012) in which we compared the effects of these two treatments on the shape of the whole preference curve, as well as on specific features of the preference curve that indicate the center and strength of the preference function. The function-valued approach allowed us to interpret the effects of stimuli in multiple sensory modalities in the context of the selection pressures acting on male advertisement signaling. We compared preference function shapes to the distribution of natural male call characteristics to test the following hypotheses of the potential selective consequences of modality interactions in female preferences. (1) Compared to acoustic stimuli alone, multimodal stimuli affect the peak (most preferred signal trait; Fig. 1) of the preference function. The location of the peak preference relative to the center of the distribution of male call characteristics determines whether there is directional selection on male calls. Peak preferences may be affected by multimodal interactions if, for example, these directly affect a female's perception of the acoustic

signal (McGurk and Macdonald 1976), or if females favor different signal characteristics under different environmental conditions (Gray et al. 2008). (2) In comparison with acoustic stimuli, multimodal stimuli affect the responsiveness of females to male calls. If female responsiveness is increased by visual cues, then males that signal in conditions that allow for visual stimuli to be perceived by females will have a selective advantage. (3) Multimodal stimuli affect the strength of female preferences for male call characteristics. Females with stronger preferences (sometimes referred to as “choosier” females: Reinhold and Schielzeth 2015) are less responsive to calls that deviate from the peak preference and thus will exert stronger selection than females with weaker preferences. Variation in both responsiveness and strength of female response to acoustic cues in multimodal conditions could be due to different patterns of searching behavior under different visual conditions (Rand et al. 1997; Baugh and Ryan 2010; Bonachea and Ryan 2011b,c).

Methods

We captured gravid female *H. versicolor* from two local ponds in Ozaukee Co., WI (University of Wisconsin-Milwaukee Field Station, $n = 57$ females; Riveredge Nature Center, $n = 3$ females). All females were captured in amplexus and were brought to our laboratory at the University of Wisconsin-Milwaukee on the night of capture where they were held in containers placed in melting ice to prevent oviposition. Females were acclimated prior to testing by placing them in a light-safe box maintained at the testing temperature of 20°C for 30 min. All tests were performed during the natural breeding season of *H. versicolor* (May 2012 and 2013).

STIMULUS PREPARATION

Acoustic stimuli

Male *H. versicolor* have pulsed advertisement calls consisting of a series of short pulses that, at our test temperature of 20°C, have a duration of approximately 25 msec and that are repeated after a pause of 25 msec (thus, at a given temperature the duration of the call can be expressed in number of pulses or in milliseconds; for full description of the call, see Gerhardt 2001). Two frequency peaks are emphasized in the call. The second frequency peak is the dominant frequency (i.e., the frequency peak with the most energy), and is approximately 7–10 dB higher in amplitude than the first frequency peak (G. Höbel pers. obs., Gerhardt 2005). To describe the average call characteristics of male *H. versicolor*, we recorded 54 calling males at our main study population (UWM Field Station pond) over the course of the 2011 field season. We sampled the pond two to three times a week, thus capturing the range of variation in the social environment that can affect male call traits. Recordings were obtained in the field using a Marantz

PMD670 digital recorder (Mahwah, NJ, USA) with a Sennheiser K3-ME88 microphone (Sennheiser, Wedemark, Germany). We calculated average values of call traits (call duration [in pulses per call]; call period [time between consecutive calls]; first and second frequency peaks) for each male, based on analysis of 10 calls per male. Recordings were analyzed using Raven Pro 1.3 (1024-point fast Fourier transform, Hann window, 50% overlap, 43.1 Hz resolution; Cornell Lab of Ornithology, Ithaca, NY). Average values (\pm SE) in our study population are as follows: call duration = 17 ± 0.5 pulses/call, call period = 7750 ± 3027 msec, first frequency peak = 1071 ± 99 Hz, second (dominant) frequency peak = 2142 ± 192 Hz.

Acoustic stimuli (16-bit WAV file, 20 kHz sampling rate) were generated with software provided by J. Schwartz, and the characteristics of the stimuli were based on measurements of males of our local population (see above). Each of the three call traits (call duration, call period, or call frequency) was varied in a separate experiment, with the other traits set to the average values. For each call trait, the range of tested trait values was the population mean \pm 2 SD, thus spanning and exceeding the range of natural variation found in the population. We varied call duration between 5 and 33 pulses per call (increasing in increments of four pulses in eight steps; i.e., 5, 9, 13, 17, 21, 25, 29, and 33 pulses). Call period varied between 1361 and 15802 msec (increasing in increments of 2063 msec in eight steps; i.e., 1361, 3424, 5487, 7550, 9513, 11,676, 13,739, and 15,802 msec). Call frequency (low frequency peak) varied between 820 and 1405 Hz (increasing in increments of 84 Hz in eight steps; i.e., 820, 904, 987, 1071, 1154, 1238, 1321, and 1405 Hz). As is typical for natural calls (Gerhardt 2005), the second frequency peak of our playback stimuli was always twice as high as the low peak, that is, if the low frequency peak was 820 Hz, the high frequency peak was 1640 Hz.

Visual stimuli

The visual stimulus was a video playback of a computer-animated model of a calling male *H. versicolor*. Details of video stimulus preparation can be found in Reichert et al. (2014). Briefly, the animated frog stimuli consisted of a still image of a male *H. versicolor* with an appended image of a vocal sac that was animated to mimic the inflation and deflation that takes place during the production of a natural advertisement call (see Supporting Information Movie S1 in Reichert et al. 2014).

To create the animated stimuli, we obtained still images of naturally calling male *H. versicolor* from video recordings ($n = 14$). We paused the videos at a frame at which the vocal sac was fully extended and measured both the area of each male's body and the vocal sac at that point. We then selected five images each of male bodies and vocal sacs (each image was from a different male, but four males that contributed body images also contributed

vocal sac images from a different video frame) that most closely approximated the average value for each of these traits. From these we randomly selected three of the five body exemplars and paired these with three of the five randomly selected vocal sac exemplars, resulting in a total of nine exemplars consisting of different combinations of body and vocal sac images. These composite exemplars ensured that our conclusions are not based on a response to the specific characteristics (e.g., color pattern, body shape) of any given frog image but rather are generalizable across multiple visual stimuli. Vocal sac color in *H. versicolor* can range from white to almost black (G. Höbel, pers. obs.); we colored the vocal sac of each stimulus to a uniform medium gray (RGB = 128, 128, 128), but left the male body color of the stimuli unaltered.

Body and vocal sac images were manipulated in animation software (Motion 2.1; Apple Computer Inc., Cupertino, CA) to create stimuli that mimicked the natural movements of the vocal sac during calling. Images were scaled on the screen so that they would appear life-sized on the playback monitor. The background of all videos was a uniform black. Prior to the animated calling event, only the image of the frog body with no vocal sac was presented. The stimulus was animated so that the vocal sac inflated at the onset and deflated at the offset of the acoustic stimulus (rise and fall times of the vocal sac inflation were kept constant at 0.167 sec each), as would be the case in a naturally calling male frog. All animations were saved as lossless plus alpha MOV files. Videos were created with a monitor resolution of 1920 × 1200, a bit depth of 8 bits, and a frame rate of 30 frames per second.

Playback and monitor adjustment

We played back videos using the VLC media player (VideoLAN) onto a Dell U2412M LCD computer monitor. Videos were presented from a PC laptop computer located outside of the testing chamber connected by a video cable fed through the chamber's walls to the monitor that was placed inside the testing chamber. We adjusted the brightness of the computer monitor to its lowest setting so that the stimuli presented mimicked those potentially available to females in the dim conditions of a nocturnal chorus (average stimulus brightness measured for one of the presented exemplars at 1 m from the monitor: 0.301 nW/cm²; Reichert et al. 2014). We also adjusted the color output of the monitor so that the color of the animated male vocal sac would simulate a medium gray as perceived by the female's visual system (Fleishman et al. 1998; Fleishman and Endler 2000). We followed the methods of Gomez et al. (2009) to characterize and adjust our monitor's pixel output so that the vocal sac stimulus would equally stimulate the two photoreceptors, based on the spectral sensitivity function for the closely related congener *H. cinerea* presented in King et al. (1993). Detailed parameters of the monitor adjustment procedure are given by Reichert et al. (2014).

We covered the computer monitor with an opaque black cloth that was cut so that only the portion of the monitor displaying the frog image was visible (the visible portion of the monitor was approximately 7.5 cm high and 10 cm wide). This step was performed to minimize light leakage from the monitor unrelated to the stimulus itself. Between tests, we entered the chamber to reposition the female in the release cage. We ensured that females' eyes remained as dark-adapted as possible during this process by performing the tests in a darkened laboratory and using a headlamp with a red filter to search for the female in the testing chamber.

GENERAL PREFERENCE TESTING PROCEDURES

Females were tested in two experimental conditions: (1) unimodal (audio only), and (2) multimodal (audio + video combined). All tests took place in a darkened semi-anechoic chamber (3.3 × 3.3 × 2 m). Within the chamber, we placed the female underneath a visually and acoustically transparent release cage in the center of a circular arena (2 m diameter) that was surrounded by hardware cloth covered with an opaque black sheet. A computer monitor was placed facing the female along the border of the arena such that it was 1 m away from the female release point. A small speaker (Vifa C11WG-09) was positioned immediately beneath the computer monitor. The acoustic stimuli were broadcast from a PC laptop computer through a Radio Shack 32–2054 amplifier (Fort Worth, TX, USA) to the speaker, which was adjusted to broadcast at 85 dB SPL (measured at the female release point with an Extech 407764 sound-level meter set to measure fast RMS with “C” weighting Extech Instruments Corporation, Nashua, NH, USA). The computer monitor was switched on during multimodal trials, but powered off during the unimodal (audio only) trials to eliminate the faint glow of the black monitor screen, to which females are known to respond (Reichert et al. 2014).

We broadcast a stimulus for 1 min while the female was confined in the arena's center and then released her by pulling on a rope that was attached to the top of the release cage. We monitored the female's movements remotely using an infrared camera system placed directly above the female release point. In order for the female's behavior to be scored as a “response,” we required her to enter an area within a 10 cm radius of the stimulus after making deliberate movements toward the stimulus. If the female failed to leave the release cage or failed to meet our response criteria, we gave her a rest of 5 min and retested her once with the same stimulus. Females that remained unresponsive following this rest period were excluded from analyses (call duration tests: $n = 2$; call rate tests: $n = 4$; call frequency tests: $n = 3$).

A total of 60 females contributed data in the experiments described below. Different sets of females ($n = 20$ each) were tested in each of the three call trait experiments (call duration, call period, call frequency). Within each of the three call trait experiments each female was tested with 16 different stimuli

(eight call trait values each in both the unimodal and multimodal treatments). We randomly assigned females to either start with the unimodal or multimodal treatment, and each individual was tested with all eight stimuli within a treatment before testing them with the stimuli in the other treatment. Within each treatment, each female was presented with the stimuli in a random order. For the multimodal stimuli, each acoustic stimulus was coupled with a randomly selected video stimulus from among the nine video exemplars described above. We also randomly placed the stimuli at one of four locations within the arena after every four trials to control for any localization biases of the females. For each female, we measured latency to respond, that is, the amount of time from the start of the trial to the moment the female first met our response criteria. In addition, we analyzed the length and directionality of the approach path each female took to reach the stimulus (see Bonachea and Ryan 2011a). Briefly, videos of the phonotaxis trials were broken into JPEG single-frame image stacks using Avidemux (<http://fixounet.free.fr/avidemux/>), which were then combined to produce a single composite image of the approach path taken by females using the Extended Depth of Field plugin for ImageJ (Schneider et al. 2012). Based on these composite images, we then used ImageJ to measure the approach path length (by tracing the path the female took from the release cage to the monitor/speaker stimulus source), and the maximum deviation angle (by measuring the greatest angular distance from the straight line between release point and stimulus source; measured relative to the release point). After testing was completed, we measured each female's mass and snout-vent length, and marked them with a subcutaneous fluorescent tag (VI Alpha tags, Northwest Marine Technology, Shaw Island, WA) to ensure individual identification and to prevent females from being tested twice with the same stimuli.

TESTING VARIATION IN PREFERENCE FUNCTIONS

We examine variation in call trait preferences using preference functions, which are curves that describe female responses as a function of variation in call traits (i.e., Ritchie 1996; Meyer & Kirkpatrick, 2005; Rodríguez et al. 2006; Fowler-Finn and Rodríguez 2012a). Our assay of female preferences is based on the phonotaxis behavior typical for anuran amphibians, where mate-searching females approach stationary displaying males (Wells 2007). Here, a strong preference for a given signal is indicated by a fast approach toward the stimulus target (i.e., a short response latency). Because interpretation of results is more intuitive if a female's most preferred value is shown as the highest point in a curve, not the lowest one, we converted raw female response latencies (in seconds) obtained during preference trials to preference scores (in fractions of 1). To do this, we first subtracted the female's approach latency value from 300 sec (the slowest latency possible, as our observation period was capped at 5 min). Then we

determined each female's highest ($300 - x$) value (i.e., the fastest approach latency during her 16 trials). We set each female's highest value to 1, and calculated all other responses as a fraction of this highest (fastest) value. We used these preference scores for (1) statistical significance testing examining whether unimodal and multimodal preference functions differed from each other (mixed models, see below), and (2) to generate individual preference functions that allowed us to explore in more detail how unimodal and multimodal preference functions differed from each other (cubic splines and preference function trait analysis, see below).

We were interested in the effects of visual stimuli on the shape of the whole female preference function for male acoustic signal characteristics, and therefore we used a function-valued approach for the analysis of the female preference data (Meyer and Kirkpatrick 2005; McGuigan et al. 2008; Stinchcombe and Kirkpatrick 2012), using the methods described by Fowler-Finn and Rodríguez (2012a,b). We entered the preference scores as response variables in a mixed model (standard least squares) implementing expected mean squares (EMS) to test for differences in preference function shape between treatments. Female identity was entered as a random effect because we estimated two preference functions for each female (i.e., individual females contributed multiple datapoints, both in response to the individual stimuli used to construct a single preference function and to estimate the preference functions in the two different experimental treatments). We also included a trial sequence term in the model, to account for potential fatigue effects of repeated tests of the same female. Additional terms were included in the model for treatment, the linear and quadratic call trait terms, the treatment \times linear call trait interaction, and the treatment \times quadratic call trait interaction. Effects of these latter terms indicate how the preference function is shaped by variation in male call traits (the linear call trait term indicates whether the female response increases or decreases with increases in the value of the male call trait; the quadratic call trait term addresses the curvature of this response), and by the experimental treatment (the linear interaction term indicates how the treatment affects the slope of the preference function and the quadratic interaction term indicates whether the function's curvature differed between treatment groups).

Preference functions vary in multiple dimensions, and we were interested in which of these were most affected by the effects of treatment (unimodal/multimodal). We visualized preference functions with cubic spline regressions (on preference scores) (Schluter 1988). We generated the splines in R version 2.14.1 (R Development Core Team, 2011) with the *mgcv* package and the *gam* function, using a custom written R script (courtesy of J. Kilmer, available in online Supporting Information of Rebar and Rodríguez 2015). This script generates individual female preference functions, and extracts three traits that describe

the shape of the preference function (Fig. 1): (1) Peak describes the female's most preferred signal value (i.e., signal eliciting the fastest response from a female), expressed as the highest point in the preference function. (2) Responsiveness quantifies the average height of the curve; it is calculated as the mean height of the preference function. (3) Strength describes the extent to which a female's response is reduced to signals that deviate from the peak preferred signal; it is calculated as the square of the coefficient of variation of points in the preference function (Schluter 1988). Responsiveness and strength are components of female mate choice selectivity; more selective females have lower responsiveness and greater preference strength (Fowler-Finn and Rodríguez 2012a). We analyzed each preference function trait using a mixed model (implementing EMS) with the preference function trait (i.e., peak, responsiveness, or strength) as the response variable, and the treatment (unimodal/multimodal) as the independent variable. We also included female identity as a random term in the model because each female contributed two datapoints per preference function trait (one from the unimodal and one from the multimodal preference function). All statistical analyses were performed in JMP Version 8.0 (SAS Institute, Inc., Cary, NC). Raw data from this study are available from the Dryad data repository (doi: 10.5061/dryad.pm37b).

TESTING EFFECTS OF STIMULUS MODALITY ON APPROACH PATH LENGTH AND APPROACH ANGLE

We tested for differences in approach behavior (path length, approach angle) between treatments using a mixed model (standard least squares) implementing EMS. We entered approach path length or approach angle, respectively, as response variables, and treatment, the linear and quadratic call trait terms, the treatment \times linear call trait interaction, and the treatment \times quadratic call trait interaction as predictor variables. We also included female identity (random term) and trial sequence in the model, because each female had responded in multiple trials.

Results

VARIATION IN PREFERENCES FOR CALL TRAITS

Call duration preferences

Females had significant preferences for call duration (significant linear and quadratic call duration stimuli terms; Table 1), and their response was affected by the modality treatment (significant treatment term, Table 1). Moreover, the significant treatment \times call interaction term shows that the preference functions for call duration under unimodal and multimodal presentations differed in slope (Table 1, Fig. 2A). Females in the multimodal treatment showed overall higher responsiveness, but lower preference strength, toward calls varying in duration (Table 2, Fig. 2A). Visual inspection of the preference functions clearly shows the

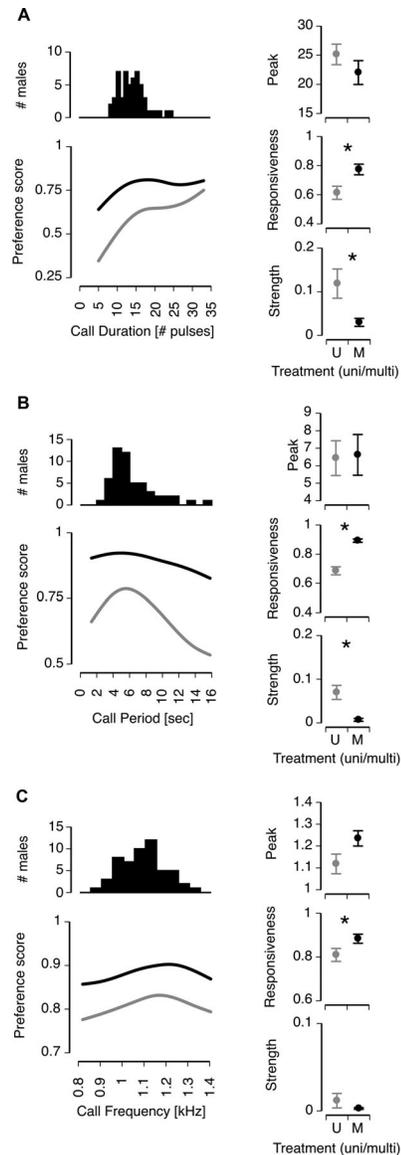


Figure 2. Test of variation in female preference functions for (A) call duration, (B) call period, and (C) call frequency according to variation in stimulus modality (unimodal shown in gray, multimodal shown in black). The top left panel in (A)–(C) is a histogram showing the distribution of average values of call traits in males of the study population. The bottom left panels show variation in the overall shape of the preference functions for call traits; note that the multimodal preference function (black line) is always above the unimodal one, indicating a greater response to these stimuli. Male call histograms are aligned with female preference functions to enable comparisons of the shape of female preference functions to the distribution of male call characteristics. The panels on the right show variation in preference function traits (peak, responsiveness and strength); note that responsiveness was always higher during multimodal presentation. Multimodal presentation decreased preference strength for call duration and call period, but not call frequency. Significant differences are indicated by an asterisk; U indicates unimodal stimulus presentation, M indicates multimodal presentation. Shown are least square means \pm SE.

Table 1. Mixed model analysis of the variation in *H. versicolor* female call trait preference scores, according to variation in experimental treatment (unimodal/multimodal) and call characteristics (call).

Variable	Parameter estimate (\pm SE)	df	F-ratio	P
Call duration preferences				
Treatment	-0.082 \pm 0.025	1,294	10.49	0.0013
Call	0.008 \pm 0.002	1,294	20.72	<0.0001
Call \times call	-0.0005 \pm 0.0002	1,294	5.09	0.025
Treatment \times call	0.004 \pm 0.002	1,294	4.98	0.026
Treatment \times call \times call	-2.9 $\times 10^{-5}$ \pm 0.0002	1,294	0.02	0.90
Sequence	-0.0004 \pm 0.004	1,294	0.01	0.92
Female ID and random		19,294	3.07	<0.0001
Call period preferences				
Treatment	-0.082 \pm 0.017	1,294	22.14	<0.0001
Call	-9.3 $\times 10^{-6}$ \pm 2.4 $\times 10^{-6}$	1,294	14.73	0.0002
Call \times call	-1.76 $\times 10^{-9}$ \pm 5.9 $\times 10^{-10}$	1,294	9.03	0.003
Treatment \times call	-3.59 $\times 10^{-6}$ \pm 2.4 $\times 10^{-6}$	1,294	2.21	0.14
Treatment \times call \times call	-9.3 $\times 10^{-10}$ \pm 5.8 $\times 10^{-10}$	1,294	2.91	0.11
Sequence	0.003 \pm 0.002	1,294	1.31	0.25
Female ID and random		19,294	2.17	0.004
Call frequency preferences				
Treatment	-0.036 \pm 0.01	1,293	13.37	0.0003
Call	-3.9 $\times 10^{-5}$ \pm 3.4 $\times 10^{-5}$	1,293	1.39	0.24
Call \times call	-4.6 $\times 10^{-7}$ \pm 2.0 $\times 10^{-7}$	1,293	5.17	0.02
Treatment \times call	-4.4 $\times 10^{-6}$ \pm 3.4 $\times 10^{-5}$	1,293	0.02	0.90
Treatment \times call \times call	-4.2 $\times 10^{-8}$ \pm 2.0 $\times 10^{-7}$	1,293	0.04	0.83
Sequence	-6.2 $\times 10^{-5}$ \pm 0.001	1,293	0.002	0.97
Female ID and random		19,293	17.23	<0.0001

Significant *P*-values are highlighted in bold.

more elevated, yet somewhat flatter shape of the functions under multimodal presentation. Peak preference did not vary between treatments and generally favored call durations that were longer than those given by most males in the population (Table 2, Fig. 2A).

Call period preferences

Females had significant preferences for call period (significant linear and quadratic call period stimuli terms; Table 1), and their response was affected by the modality treatment (significant treatment term, Table 1). The treatment \times call interaction terms were not statistically significant. The analyses of spline-based preference function traits showed that females in the multimodal treatment had generally higher responsiveness, as well as lower preference strength, toward calls varying in period than females in the unimodal treatment (Table 2, Fig. 2B). Peak preference did not vary between treatments and generally matched the peak of the distribution of male call periods (Table 2, Fig. 2B).

Call frequency preferences

Females had significant preferences for call frequency (quadratic call frequency stimulus term; Table 1), and their response was affected by the modality treatment (significant treatment term, Table 1). Preference function shape for call frequency did not differ significantly between unimodal and multimodal presentation (nonsignificant treatment \times call interaction terms). Females showed higher responsiveness under multimodal presentation, but in both treatments had a similar, and overall low, preference strength for calls varying in frequency (Table 2, Fig. 2C). This is corroborated by visual inspection of the rather flat and almost perfectly parallel preference functions (Table 1, Fig. 2C). Peak preference was marginally higher in the multimodal compared to the unimodal treatment (Table 2, Fig. 2C). The unimodal peak preference matched reasonably well the peak of the distribution of male call frequencies, whereas the multimodal peak preference favored call frequencies somewhat higher than those given by most males in the population.

Table 2. Mixed model analysis of the effect of treatment on variation in preference function traits for different call characteristics.

Trait	Variable	Parameter estimate (\pm SE)	df	F-ratio	P
Call duration preferences					
Peak	Treatment	1.56 \pm 1.61	1,19	0.93	0.35
	female ID and random		19,19	0.39	0.98
Responsiveness	Treatment	-0.08 \pm 0.03	1,19	7.36	0.01
	female ID and random		19,19	0.93	0.56
Strength	Treatment	0.04 \pm 0.02	1,19	6.04	0.02
	female ID and random		19,19	0.85	0.64
Call period preferences					
Peak	Treatment	-93.7 \pm 814.3	1,19	0.01	0.91
	female ID and random		19,19	30.77	0.71
Responsiveness	Treatment	-0.10 \pm 0.02	1,19	46.8	<0.0001
	female ID and random		19,19	60.96	0.54
Strength	Treatment	0.03 \pm 0.009	1,19	13.26	0.002
	female ID and random		19,19	0.82	0.67
Call frequency preferences					
Peak	Treatment	-59.1 \pm 30.1	1,19	3.85	0.06
	female ID and random		19,19	0.77	0.71
Responsiveness	Treatment	-0.04 \pm 0.01	1,19	6.58	0.02
	female ID and random		19,19	2.14	0.05
Strength	Treatment	0.004 \pm 0.004	1,19	1.39	0.25
	female ID and random		19,19	1.47	0.20

Multimodal presentation increased responsiveness across the range of tested call trait preferences, and decreased the strength of call duration and call period preferences (see Fig. 2). The only trait for which multimodal presentation resulted in a marginally significant shift in peak preference was call frequency (Fig. 2). Significant *P*-values are highlighted in bold.

EFFECT OF STIMULUS MODALITY ON APPROACH PATH LENGTH AND APPROACH ANGLE

During multimodal trials, females usually walked an almost straight path toward the stimulus source. Consequently, multimodal presentation resulted in average approach path lengths only slightly longer than the distance between the release box and stimulus source, and a more directed approach (lower deviation angles) (Fig. 3A). For all three tested call traits, treatment affected both approach path length (Table 3, Fig. 3B) and maximum deviation angles (Table 4, Fig. 3C). There was an effect of variation in call period, and the interaction between treatment and call period, on both path length and deviation angle, and a quadratic effect of call duration on path length (Tables 3, 4). Otherwise, there were no effects of call trait variation on these variables.

Discussion

IMPLICATIONS FOR SIGNAL EVOLUTION

The expression of female preferences is variable, and a major source of this variability is likely to be that the evaluation of unimodal signal characteristics involved in mate choice is not an isolated process but rather is affected by simultaneous reception of stimuli in other modalities (Munoz and Blumstein 2012; Ronald et al. 2012). There were two general effects of the addition of visual cues to playbacks of acoustic signals on the shape of female preference functions in *H. versicolor*. First, female responsiveness was greater in the multimodal treatment. Second, the strength of female preferences was weaker in the multimodal treatment.

The most important finding of our study was that preference functions, which estimate the selective landscape acting on

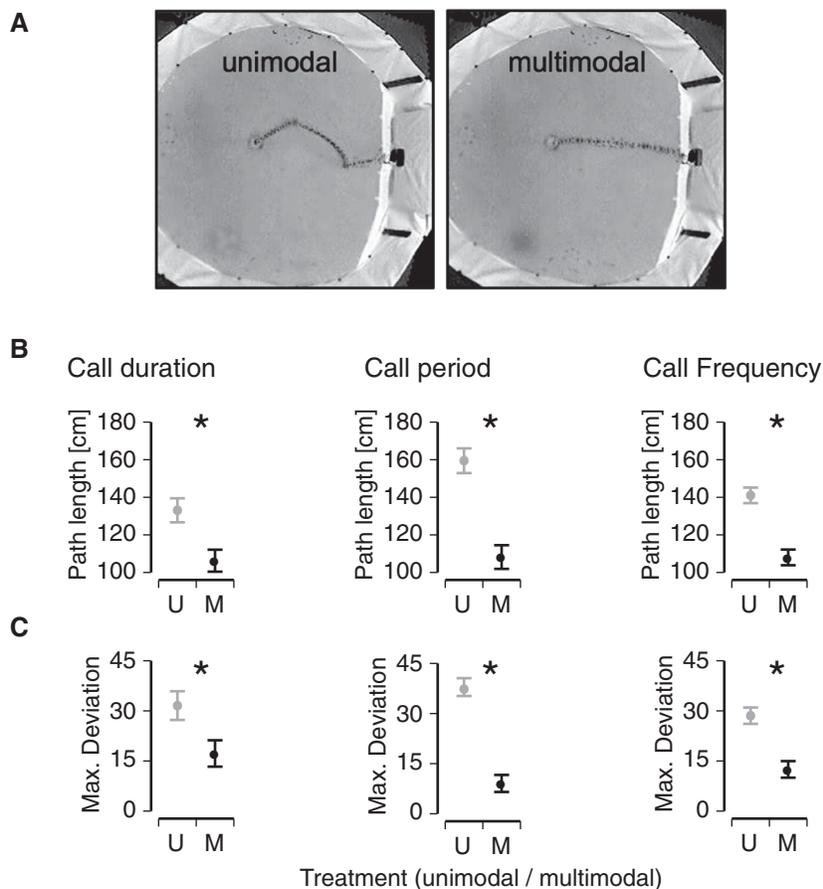


Figure 3. (A) Representative example of a female approaching the 7.55 sec call period stimulus (in the call period experiment) when presented alone (unimodal) or combined with a visual stimulus (multimodal). (B) Approach path length was shorter and (C) maximum deviation from straight path was smaller when the auditory stimulus was paired with a visual stimulus (multimodal condition). Significant differences are indicated by an asterisk; U indicates unimodal stimulus presentation, M indicates multimodal presentation. Shown are least square means \pm SE.

acoustic signal evolution, had a different shape in the unimodal and multimodal treatments. The interaction between the characteristics of acoustic signals and stimuli in other modalities, including those arising from environmental conditions, is predicted to determine the pattern of variation in male mating success, which will determine the strength and potentially the direction of the selective pressures acting on male signal evolution (Endler 1992; Boughman 2002). If there is consistent variation between populations in environmental conditions that affect multimodal stimulus processing, the effects on female preference shape could ultimately lead to reproductive isolation by divergence in the sexual signaling system (Wilkins et al. 2013).

Much debate in sexual selection studies centers around the issue of what maintains genetic variation in sexually selected traits (Pomiankowski and Moller 1995; Rowe and Houle 1996; Kotiaho et al. 2001, 2008). One explanation that is likely to apply to many species is that patterns of sexual selection are influenced by environmental characteristics, and if these characteristics are

variable in space or time then there will be spatial or temporal variation in patterns of male mating success, which will help to maintain variation in male traits (Bussière et al. 2008; Bro-Jørgensen 2010; Ingleby et al. 2010). Our finding that the shape of female preference functions for acoustic signals differed in the presence of a visual cue implies that, in *H. versicolor*, sexual selection for acoustic signals will operate differently in environments that vary in visual characteristics. Such variation is likely to be present in anuran breeding choruses at both temporal and spatial scales due to variation in vegetation cover, patterns of cloud cover and the lunar cycle (Cummings et al. 2008; Grant et al. 2013), and the presence of anthropogenic light sources (Gaston et al. 2013). Similar processes are likely to take place in other taxa and signaling modalities (Bro-Jørgensen 2010). Our findings thus emphasize that environmental context and receiver sensory processing capabilities are key considerations in the understanding of sexual selection and the evolution of reproductive isolation.

Table 3. Mixed model analysis of the variation in the approach path length of *H. versicolor* females, according to variation in experimental treatment (unimodal/multimodal) and call characteristics (call).

Variable	Parameter estimate (\pm SE)	df	F-ratio	P
Call duration preferences				
Treatment	13.6 \pm 4.3	1,244	9.89	0.002
Call	-0.56 \pm 0.31	1,244	3.15	0.08
Call \times call	0.10 \pm 0.03	1,244	6.66	0.01
Treatment \times call	0.29 \pm 0.31	1,244	0.84	0.36
Treatment \times call \times call	0.02 \pm 0.03	1,244	0.29	0.59
Sequence	0.51 \pm 0.63	1,244	0.67	0.41
Female ID and random		19,244	1.65	0.05
Call period preferences				
Treatment	25.5 \pm 4.6	1,280	31.36	<0.0001
Call	0.002 \pm 0.001	1,280	8.34	0.004
Call \times call	-9.4 $\times 10^{-8}$ \pm 1.5 $\times 10^{-7}$	1,280	0.37	0.55
Treatment \times call	0.002 \pm 0.001	1,280	10.56	0.001
Treatment \times call \times call	-1.4 $\times 10^{-7}$ \pm 1.5 $\times 10^{-7}$	1,280	0.79	0.38
Sequence	-0.55 \pm 0.65	1,280	0.79	0.40
Female ID and random		19,280	3.0	<0.0001
Call frequency preferences				
Treatment	16.7 \pm 2.9	1,277	32.02	<0.0001
Call	-0.003 \pm 0.01	1,277	0.11	0.73
Call \times call	3.1 $\times 10^{-4}$ \pm 6.0 $\times 10^{-5}$	1,277	0.27	0.60
Treatment \times call	0.001 \pm 0.01	1,277	0.01	0.92
Treatment \times call \times call	2.8 $\times 10^{-5}$ \pm 6.0 $\times 10^{-5}$	1,277	0.22	0.64
Sequence	0.34 \pm 0.43	1,277	0.63	0.43
Female ID and random		19,277	2.60	0.0004

Approach path length was shorter when auditory stimuli were combined with a visual cue (i.e., multimodal condition; see Fig. 3B). Significant *P*-values are highlighted in bold.

Our stimuli presented a visual cue from the playback monitor, but we do not necessarily consider this to have been a simulation of a multimodal signal (in the sense that the video stimulus was perceived as a visual signal produced by the calling male; Higham & Hebets, 2013). Instead, we interpret female responses to the visual playback as an example of a general effect of the visual environment on response to acoustic signals (see below). Nevertheless, it is interesting to compare our findings to previous studies of multimodal signaling to gain general insights into the potential evolutionary consequences of multimodal stimulus processing. Several studies have shown that female responses to signals in one modality can be modulated by the presence, and in some cases by the magnitude, of stimuli in other sensory modalities. For instance, in the cricket *Gryllus integer*, females responded to close-range chemical courtship cues more rapidly if these were preceded by an attractive long-range calling song than if these were preceded by less attractive songs (Leonard and Hedrick 2010). In another cricket, *Teleogryllus oceanicus*, females responded more quickly to playbacks of male songs when their preferences were measured on a substrate containing chem-

ical cues from male cuticular hydrocarbons (Bailey 2011). However, female choosiness for acoustic signal characteristics was not affected by the chemical signal treatment (Bailey 2011). As we found in *H. versicolor*, both cricket examples demonstrated that exposure to stimuli in one modality influences the overall responsiveness of females to stimuli in another modality. In the wolf spider *Rabidosa rabida*, females showed no preferences for variation in a visual signal when it was presented in isolation, but preferred visually ornamented males over unornamented males when these were accompanied by a seismic signal (Wilgers and Hebets 2012). In this case, the presence of the seismic signal influenced the strength of female preferences for the visual signal. In general, these intersignal interactions may be a powerful explanation for the evolution of multimodal signals (Hebets and Papaj 2005). By analogy, in our study the cross-modal interaction between male acoustic signals and visual cues also represents a potentially important consideration in the evolution of the sexual signaling system in *H. versicolor*, and our measurement of whole female preference functions allows for direct estimates of the evolutionary consequences.

Table 4. Mixed model analysis of the variation in maximum angular deviation from a straight path of *H. versicolor* females, according to variation in experimental treatment (unimodal/multimodal) and call characteristics (call).

Variable	Parameter estimate (\pm SE)	df	F-ratio	P
Call duration preferences				
Treatment	7.49 \pm 2.9	1,244	6.65	0.01
Call	-0.39 \pm 0.21	1,244	3.42	0.07
Call \times call	0.05 \pm 0.03	1,244	3.49	0.06
Treatment \times call	0.29 \pm 0.21	1,244	1.85	0.17
Treatment \times call \times call	0.05 \pm 0.03	1,244	3.22	0.07
Sequence	0.84 \pm 0.42	1,244	3.97	0.05
Female ID and random		19,244	2.11	0.005
Call period preferences				
Treatment	14.4 \pm 1.8	1,280	60.88	<0.0001
Call	0.0006 \pm 0.0003	1,280	4.66	0.03
Call \times call	$-3.5 \times 10^{-8} \pm 6.2 \times 10^{-8}$	1,280	0.31	0.58
Treatment \times call	0.0006 \pm 0.0003	1,280	5.19	0.02
Treatment \times call \times call	$-1.1 \times 10^{-7} \pm 6.2 \times 10^{-8}$	1,280	3.16	0.08
Sequence	0.36 \pm 0.26	1,280	1.86	0.17
Female ID and random		19,280	1.38	0.15
Call frequency preferences				
Treatment	8.35 \pm 1.7	1,277	23.2	<0.0001
Call	-0.001 \pm 0.006	1,277	0.04	0.84
Call \times call	$5.1 \times 10^{-5} \pm 3.5 \times 10^{-5}$	1,277	2.08	0.15
Treatment \times call	0.007 \pm 0.006	1,277	1.33	0.25
Treatment \times call \times call	$4.4 \times 10^{-5} \pm 3.5 \times 10^{-5}$	1,277	1.51	0.22
Sequence	0.58 \pm 0.25	1,277	5.4	0.02
Female ID and random		19,277	2.56	0.0004

Maximum deviation from the straight path was smaller when auditory stimuli were combined with a visual cue (i.e., multimodal condition; see Fig. 3C). Significant *P*-values are highlighted in bold.

MULTIMODAL INTERACTIONS AND ANURAN COMMUNICATION

A potential limitation of our study is that the video playback stimulus, which was designed to simulate a calling male, was potentially not perceived as such by the female. In a previous study we demonstrated that females responded similarly to not only animated males with different vocal sac characteristics but also to various abstract shapes (Reichert et al. 2014). Most trials in the previous study were performed without an accompanying acoustic stimulus, and the inference was that females were responsive to the visual stimulus of the glowing monitor. Thus, in this study, we describe the video playback as providing visual cues rather than signals (Higham and Hebets 2013) and do not suggest that female *H. versicolor* are necessarily attending to a multimodal signal. Because females are so responsive to visual stimuli, it could be that their increased responsiveness in multimodal compared to unimodal trials was entirely caused by the presence of a visual stimulus in the multimodal trial, i.e. that the acoustic playback was irrelevant. To the contrary, we argue that acoustic stimuli are the most important determinant of female

attraction; visual stimuli simply augment the attractiveness of advertisement calls. Although females were responsive to unimodal presentations of both visual (video animation) and acoustic (male advertisement call) stimuli, they responded more often and more rapidly to the acoustic stimulus than to the video stimulus (Fig. 4; Reichert et al. 2014). Thus, our finding in this study that females responded even more rapidly to the multimodal treatment (Fig. 4) cannot be explained by the isolated effect of female attraction to the monitor. Instead, it is the interaction between the visual and acoustic stimuli that led to the change in female responsiveness and the resultant change in preference function shape. This interaction is of significance for the understanding of sexual selection pressures in *H. versicolor* because there is natural variation in both acoustic signal traits and the light environment from which males signal.

Even if females did not recognize the video animation as a calling male, there are intriguing potential explanations for their improved responsiveness to multimodal stimuli. One possibility is that the light cue from the video playback aided in the localization of the acoustic signal. The localization of acoustic signals in

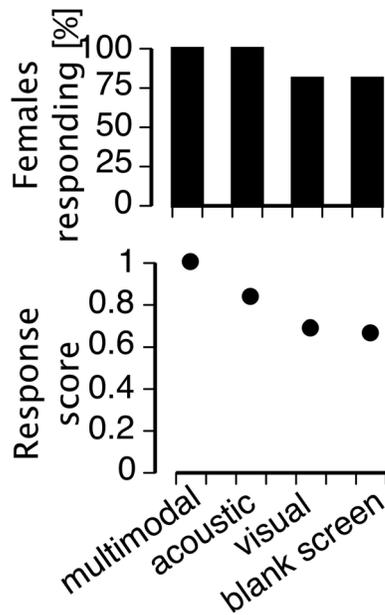


Figure 4. Responses of female *H. versicolor* to different unimodal (acoustic = call playback with average call characteristics, or visual = video playback of a male with an average sized, gray vocal sac) and multimodal (call + video animation of a male with average call and vocal sac characteristics) stimuli, along with a blank screen control (unimodal data and control from Reichert et al. 2014). Top graph shows the proportion of females approaching the stimulus, bottom graph shows mean approach latency.

anurans is characterized by a zigzag approach path in which there is some error between the actual location of the sound and the turn angle taken by females on each successive movement towards the sound's source (reviewed by Gerhardt and Bee 2007). Corrections to these angles take place with each successive reception of an acoustic signal and allow females to eventually locate the calling male (Rheinlaender et al. 1979; Klump and Gerhardt 1989). We found that female approaches to the multimodal playbacks had lower maximum angular deviations and were thus shorter than the paths taken by females in the unimodal playback. The addition of a visual stimulus that was co-localized with the acoustic signal may have allowed females to make more accurate, and hence more rapid, movements towards the speaker. In essence, the visual stimulus may have served as a beacon, allowing females to maintain orientation in a forward direction and reducing the errors from each successive movement. Effects of visual stimuli on acoustic signal localization are known from many other animals, including humans (Knudsen and Knudsen 1985; Heffner and Heffner 1992; von Helversen 1997; Bishop et al. 2011). This finding has important consequences for the evolution of male signaling strategies: if males can select favorable visual environments for signaling, they will benefit from an increased speed and directness of female ap-

proach, reducing the chances that she first encounters other males (Halliday and Tejedro 1995).

The strength of female preferences for temporal call characteristics was reduced in the multimodal treatment. Both our unimodal playbacks and previous studies of female preferences for call duration and call period showed that female *H. versicolor* generally have directional preferences that favor longer call durations and shorter call periods (= faster call rates; Klump and Gerhardt 1987; Gerhardt et al. 1996; Gerhardt et al. 2000; Gerhardt and Brooks 2009). In the multimodal treatment, although the shape of the preference function indicated that females still discriminated somewhat against males with short duration calls, their overall responsiveness to such calls was much higher and not substantially different from their response to longer-duration calls. Thus, males with less attractive temporal call characteristics may especially benefit from the multimodal stimulus interaction. At the same time, females obtain genetic benefits from mating with males with longer calls (Welch et al. 1998). Apparently such benefits would not be realized under certain visual conditions because females would mate much more indiscriminately with respect to call duration. Nocturnal choruses of anurans are characterized by highly variable light levels (Cummings et al. 2008; Grant et al. 2013), but most laboratory phonotaxis tests are performed in nearly complete darkness. Laboratory phonotaxis tests often reveal strong female preferences for male call characteristics, but variation in male calls has been shown to be only weakly, if at all, related to male mating success in the field (Gerhardt et al. 1987; Sullivan and Hinshaw 1992; Schwartz et al. 2001). Many factors probably contribute to this finding, but our study raises the possibility that the visual conditions of the environment play an important role in reducing the strength of sexual selection from female choice of male call characteristics, which may help maintain genetic variation in call traits.

Conclusions

We show that the addition of visual cues to acoustic playbacks changes the shape of female preference functions and thus the strength of selection acting on male call characteristics. How important is this effect likely to be in nature? Our multimodal stimuli consisted of an acoustic playback with a co-localized visual stimulus. Although the directionality of light sources in nocturnal chorus environments has rarely been quantified (Cummings et al. 2008), presumably most light arrives at receivers either from the moon and stars located overhead, or from the reflection of these light sources off of water surfaces. Thus, except in the special case where females recognize the conspicuous visual stimulus of a calling male (Taylor et al. 2007, 2008), such co-localization of acoustic and visual stimuli may not take place in natural choruses.

However, it is unknown whether the co-localization of multimodal stimuli was necessary to generate the observed effects on female preference functions (Taylor et al. 2011b). Higher light levels may increase female responsiveness and reduce female preference strength for male call characteristics irrespective of location. Although the specific mechanisms remain to be worked out in the field, our results demonstrate clearly that the expression of female preferences can be strongly modified by multimodal stimulus interactions. The direction and strength of sexual selection on acoustic call characteristics is therefore likely to vary across environments. Understanding the causes and consequences of this variable preference function expression is an important challenge for the study of sexual selection in natural populations.

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Data Archiving

The doi for our data is doi:10.5061/dryad.pm37b.

LITERATURE CITED

- Andersson, M. 1994. Sexual selection. Princeton Univ. Press, Princeton, NJ.
- Bailey, N. 2011. Mate choice plasticity in the field cricket *Teleogryllus oceanicus*: effects of social experience in multiple modalities. *Behav. Ecol. Sociobiol.* 65:2269–2278.
- Baugh, A. T., and M. J. Ryan. 2010. Ambient light alters temporal-updating behaviour during mate choice in a Neotropical frog. *Can. J. Zool.* 88:448–453.
- Bishop, C. W., S. London, and L. M. Miller. 2011. Visual influences on echo suppression. *Curr. Biol.* 21:221–225.
- Blows, M. W., R. Brooks, and P. G. Kraft. 2003. Exploring complex fitness surfaces: multiple ornamentation and polymorphism in male guppies. *Evolution* 57:1622–1630.
- Bonachea, L. A., and M. J. Ryan. 2011a. Localization error and search costs during mate choice in túngara frogs, *Physalaemus pustulosus*. *Ethology* 117:56–62.
- . 2011b. Predation risk increases permissiveness for heterospecific advertisement calls in túngara frogs, *Physalaemus pustulosus*. *Anim. Behav.* 82:347–352.
- . 2011c. Simulated predation risk influences female choice in túngara frogs, *Physalaemus pustulosus*. *Ethology* 117:400–407.
- Boughman, J. W. 2002. How sensory drive can promote speciation. *Trends Ecol. Evol.* 17:571–577.
- Bradbury, J. W., and S. L. Vehrencamp. 2011. Principles of animal communication. 2nd ed. Sinauer Associates, Sunderland, MA.
- Bro-Jørgensen, J. 2010. Dynamics of multiple signalling systems: animal communication in a world in flux. *Trends Ecol. Evol.* 25:292–300.
- Brooks, R., J. Hunt, M. W. Blows, M. J. Smith, L. F. Bussière, and M. D. Jennions. 2005. Experimental evidence for multivariate stabilizing sexual selection. *Evolution* 59:871–880.
- Bush, S. L., H. C. Gerhardt, and J. Schul. 2002. Pattern recognition and call preferences in treefrogs (Anura: Hylidae): a quantitative analysis using a no-choice paradigm. *Anim. Behav.* 63:7–14.
- Bussière, L., J. Hunt, K. Stölting, M. Jennions, and R. Brooks. 2008. Mate choice for genetic quality when environments vary: suggestions for empirical progress. *Genetica* 134:69–78.
- Calvert, G. A., C. Spence, and B. E. Stein, eds. 2004. The handbook of multisensory processes. The MIT Press, Cambridge, MA.
- Candolin, U. 2003. The use of multiple cues in mate choice. *Biol. Rev.* 78:575–595.
- Chenoweth, S. F., and M. W. Blows. 2006. Dissecting the complex genetic basis of mate choice. *Nat. Rev. Genet.* 7:681–692.
- Cummings, M. E., X. E. Bernal, R. Reynaga, A. S. Rand, and M. J. Ryan. 2008. Visual sensitivity to a conspicuous male cue varies by reproductive state in *Physalaemus pustulosus* females. *J. Exp. Biol.* 211:1203–1210.
- Endler, J. A. 1992. Signals, signal conditions, and the direction of evolution. *Am. Nat.* 139:S125–S153.
- Fleishman, L. J., and J. A. Endler. 2000. Some comments on visual perception and the use of video playback in animal behavior studies. *Acta Ethol.* 3:15–27.
- Fleishman, L. J., W. J. McClintock, R. B. D'Eath, D. H. Brainard, and J. A. Endler. 1998. Colour perception and the use of video playback experiments in animal behaviour. *Anim. Behav.* 56:1035–1040.
- Fowler-Finn, K. D., and R. L. Rodríguez. 2012a. Experience-mediated plasticity in mate preferences: mating assurance in a variable environment. *Evolution* 66:459–468.
- . 2012b. The evolution of experience-mediated plasticity in mate preferences. *J. Evol. Biol.* 25:1855–1863.
- Gaston, K. J., J. Bennie, T. W. Davies, and J. Hopkins. 2013. The ecological impacts of nighttime light pollution: a mechanistic appraisal. *Biol. Rev.* 88:912–927.
- Gerhardt, H. C. 1991. Female mate choice in treefrogs: static and dynamic acoustic criteria. *Anim. Behav.* 42:615–635.
- Gerhardt, H. C. 2001. Acoustic communication in two groups of closely related treefrogs. *Adv. Study Behav.* 30:99–167.
- . 2005. Acoustic spectral preferences in two cryptic species of grey treefrogs: implications for mate choice and sensory mechanisms. *Anim. Behav.* 70:39–48.
- Gerhardt, H. C., and M. A. Bee. 2007. Recognition and localization of acoustic signals. Pp. 113–146 in A. S. Feng, P. M. Narins, R. R. Fay, and A. N. Popper, eds. *Hearing and sound communication in amphibians*. Springer, New York.
- Gerhardt, H. C., and R. Brooks. 2009. Experimental analysis of multivariate female choice in gray treefrogs (*Hyla versicolor*): evidence for directional and stabilizing selection. *Evolution* 63:2504–2512.
- Gerhardt, H. C., and F. Huber. 2002. Acoustic communication in insects and anurans. The University of Chicago Press, Chicago.
- Gerhardt, H. C., R. E. Daniel, S. A. Perrill, and S. Schramm. 1987. Mating behaviour and male mating success in the green treefrog. *Anim. Behav.* 35:1490–1503.
- Gerhardt, H. C., M. L. Dyson, and S. D. Tanner. 1996. Dynamic properties of the advertisement calls of gray tree frogs: patterns of variability and female choice. *Behav. Ecol.* 7:7–18.
- Gerhardt, H. C., S. D. Tanner, C. M. Corrigan, and H. C. Walton. 2000. Female preference functions based on call duration in the gray tree frog (*Hyla versicolor*). *Behav. Ecol.* 11:663–669.
- Gomez, D., C. Richardson, T. Lengagne, S. Plenet, P. Joly, J.-P. Léna, and M. Théry. 2009. The role of nocturnal vision in mate choice: females prefer

- conspicuous males in the European tree frog (*Hyla arborea*). *Proc. R. Soc. B Biol. Sci.* 276:2351–2358.
- Grant, R., T. Halliday, and E. Chadwick. 2013. Amphibians' response to the lunar synodic cycle—a review of current knowledge, recommendations, and implications for conservation. *Behav. Ecol.* 24:53–62.
- Gray, S. M., L. M. Dill, F. Y. Tantu, E. R. Loew, F. Herder, and J. S. McKinnon. 2008. Environment-contingent sexual selection in a colour polymorphic fish. *Proc. R. Soc. B Biol. Sci.* 275:1785–1791.
- Halfwerk, W., P. L. Jones, R. C. Taylor, M. J. Ryan, and R. A. Page. 2014. Risky ripples allow bats and frogs to eavesdrop on a multisensory sexual display. *Science* 343:413–416.
- Halliday, T., and M. Tejedo. 1995. Intrasexual selection and alternative mating behavior. Pp. 419–468 in H. Heatwole and B. Sullivan, eds. *Amphibian biology volume 2: social behavior*. Surrey Beatty, Chipping Norton, Australia.
- Hebets, E. A., and D. R. Papaj. 2005. Complex signal function: developing a framework of testable hypotheses. *Behav. Ecol. Sociobiol.* 57:197–214.
- Heffner, R., and H. Heffner. 1992. Visual factors in sound localization in mammals. *J. Comp. Neurol.* 317:219–232.
- Higham, J. P., and E. A. Hebets. 2013. An introduction to multimodal communication. *Behav. Ecol. Sociobiol.* 67:1381–1388.
- Höbel, G., and R. C. Kolodziej. 2013. Wood frogs (*Lithobates sylvaticus*) use water surface waves in their reproductive behaviour. *Behaviour* 150:471–483.
- Hohenlohe, P. A., and S. J. Arnold. 2010. Dimensionality of mate choice, sexual isolation, and speciation. *Proc. Natl. Acad. Sci. USA* 107:16583–16588.
- Ingleby, F. C., J. Hunt, and D. J. Hosken. 2010. The role of genotype-by-environment interactions in sexual selection. *J. Evol. Biol.* 23:2031–2045.
- Jennions, M. D., and M. Petrie. 1997. Variation in mate choice and mating preferences: a review of causes and consequences. *Biol. Rev. Camb. Philos. Soc.* 72:283–327.
- King, R. B., J. K. Douglass, J. B. Phillips, and C. L. Baube. 1993. Scotopic spectral sensitivity of the optomotor response in the green treefrog *Hyla cinerea*. *J. Exp. Zool.* 267:40–46.
- Klump, G. M., and H. C. Gerhardt. 1987. Use of non-arbitrary acoustic criteria in mate choice by female gray tree frogs. *Nature* 326:286–288.
- . 1989. Sound localization in the barking treefrog. *Naturwissenschaften* 76:35–37.
- Knudsen, E., and P. Knudsen. 1985. Vision guides the adjustment of auditory localization in young barn owls. *Science* 230:545–548.
- Kotiaho, J. S., L. W. Simmons, and J. L. Tomkins. 2001. Towards a resolution of the lek paradox. *Nature* 410:684–686.
- Kotiaho, J. S., N. R. Lebas, M. Puurtinen, and J. L. Tomkins. 2008. On the resolution of the lek paradox. *Trends Ecol. Evol.* 23:1–3.
- Leonard, A. S., and A. V. Hedrick. 2010. Long-distance signals influence assessment of close range mating displays in the field cricket, *Gryllus integer*. *Biol. J. Linn. Soc.* 100:856–865.
- Lewis, E. R., P. M. Narins, K. A. Cortopassi, W. M. Yamada, E. H. Poinar, S. W. Moore, and X. L. Yu. 2001. Do male white-lipped frogs use seismic signals for intraspecific communication?. *Am. Zool.* 41:1185–1199.
- McGuigan, K., A. VanHomrigh, and M. W. Blows. 2008. Genetic analysis of female preference functions as function-valued traits. *Am. Nat.* 172:194–202.
- McGurk, H., and J. Macdonald. 1976. Hearing lips and seeing voices. *Nature* 264:746–748.
- Meyer, K., and M. Kirkpatrick. 2005. Up hill, down dale: quantitative genetics of curvaceous traits. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 360:1443–1455.
- Munoz, N. E., and D. T. Blumstein. 2012. Multisensory perception in uncertain environments. *Behav. Ecol.* 23:457–462.
- Partan, S. R., and P. Marler. 1999. Communication goes multimodal. *Science* 283:1272–1273.
- Pomiankowski, A., and A. P. Moller. 1995. A resolution of the lek paradox. *Proc. R. Soc. B Biol. Sci.* 260:21–29.
- Rand, A. S., M. E. Bridarolli, L. Dries, and M. J. Ryan. 1997. Light levels influence female choice in túngara frogs: predation risk assessment?. *Copeia* 1997:447–450.
- Rebar, D., and R. L. Rodríguez. 2015. Insect mating signal and mate preference phenotypes covary among host plant genotypes. *Evolution* 69:602–610.
- Reichert, M. S. 2013. Visual cues elicit courtship signals in a nocturnal anuran. *Behav. Ecol. Sociobiol.* 67:265–271.
- Reichert, M. S., H. Galante, and G. Höbel. 2014. Female gray treefrogs, *Hyla versicolor*, are responsive to visual stimuli but unselective of stimulus characteristics. *J. Exp. Biol.* 217:3254–3262.
- Reinhold, K., and H. Schielzeth. 2015. Choosiness, a neglected aspect of preference functions: a review of methods, challenges and statistical approaches. *J. Comp. Physiol. A* 201:171–182.
- Rheinlaender, J., H. C. Gerhardt, D. D. Yager, and R. R. Capranica. 1979. Accuracy of phonotaxis by the green treefrog *Hyla cinerea*. *J. Comp. Physiol. A* 133:247–255.
- Ritchie, M. G. 1996. The shape of female mating preferences. *Proc. Natl. Acad. Sci.* 93:14628–14631.
- Rodríguez, R. L., K. Ramaswamy, and R. B. Cocroft. 2006. Evidence that female preferences have shaped male signal evolution in a clade of specialized plant-feeding insects. *Proc. R. Soc. B Biol. Sci.* 273:2585–2593.
- Ronald, K. L., E. Fernández-Juricic, and J. R. Lucas. 2012. Taking the sensory approach: how individual differences in sensory perception can influence mate choice. *Anim. Behav.* 84:1283–1294.
- Rosenthal, G. G., A. S. Rand, and M. J. Ryan. 2004. The vocal sac as a visual cue in anuran communication: an experimental analysis using video playback. *Anim. Behav.* 68:55–58.
- Rowe, L., and D. Houle. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. B Biol. Sci.* 263:1415–1421.
- Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. *Evolution* 42:849–861.
- Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9:671–675.
- Schwartz, J. J., B. W. Buchanan, and H. C. Gerhardt. 2001. Female mate choice in the gray treefrog (*Hyla versicolor*) in three experimental environments. *Behav. Ecol. Sociobiol.* 49:443–455.
- Shaw, K. L., and D. P. Herlihy. 2000. Acoustic preference functions and song variability in the Hawaiian cricket *Laupala cerasina*. *Proc. R. Soc. B Biol. Sci.* 267:577–584.
- Shimojo, S., and L. Shams. 2001. Sensory modalities are not separate modalities: plasticity and interactions. *Curr. Opin. Neurobiol.* 11:505–509.
- Starnberger, I., D. Preininger, and W. Hödl. 2014. The anuran vocal sac: a tool for multimodal signalling. *Anim. Behav.* 97:281–288.
- Stevens, M. 2013. *Sensory ecology, behaviour, and evolution*. Oxford Univ. Press, Oxford, U.K.
- Stinchcombe, J. R., and M. Kirkpatrick. 2012. Genetics and evolution of function-valued traits: understanding environmentally responsive phenotypes. *Trends Ecol. Evol.* 27:637–647.

- Sullivan, B. K., and S. H. Hinshaw. 1992. Female choice and selection on male calling behaviour in the grey treefrog *Hyla versicolor*. *Anim. Behav.* 44:733–744.
- Taylor, R. C., B. W. Buchanan, and J. L. Doherty. 2007. Sexual selection in the squirrel treefrog *Hyla squirella*: the role of multimodal cue assessment in female choice. *Anim. Behav.* 74:1753–1763.
- Taylor, R. C., B. A. Klein, J. Stein, and M. J. Ryan. 2008. Faux frogs: multimodal signalling and the value of robotics in animal behaviour. *Anim. Behav.* 76:1089–1097.
- Taylor, R. C., B. A. Klein, and M. J. Ryan. 2011a. Inter-signal interaction and uncertain information in anuran multimodal signals. *Curr. Zool.* 57:153–161.
- Taylor, R. C., B. A. Klein, J. Stein, and M. J. Ryan. 2011b. Multimodal signal variation in space and time: how important is matching a signal with its signaler?. *J. Exp. Biol.* 214:815–820.
- Tuttle, M. D., and M. J. Ryan. 1982. The role of synchronized calling, ambient light, and ambient noise in anti-bat-predator behavior of a treefrog. *Behav. Ecol. Sociobiol.* 11:125–131.
- Tuttle, M. D., L. K. Taft, and M. J. Ryan. 1982. Evasive behavior of a frog in response to bat predation. *Anim. Behav.* 30:393–397.
- VonHelversen, D. 1997. Acoustic communication and orientation in grasshoppers. Pp. 301–341 in M. Lehrer, ed. *Orientation and communication in arthropods*. Birkhäuser, Basel.
- Wagner, W. E. 1998. Measuring female mating preferences. *Anim. Behav.* 55:1029–1042.
- Welch, A. M., R. D. Semlitsch, and H. C. Gerhardt. 1998. Call duration as an indicator of genetic quality in male gray tree frogs. *Science* 280:1928–1930.
- Wells, K. D. 2007. *The ecology and behavior of amphibians*. The University of Chicago Press, Chicago.
- Wiglers, D. J., and E. A. Hebets. 2012. Seismic signaling is crucial for female mate choice in a multimodal signaling wolf spider. *Ethology* 118:387–397.
- Wilkins, M. R., N. Seddon, and R. J. Safran. 2013. Evolutionary divergence in acoustic signals: causes and consequences. *Trends Ecol. Evol.* 28:156–166.

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