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Supporting Online Material for

Signal Perception in Frogs and Bats and the Evolution of Mating Signals

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Materials and Methods

Table S1

Reference (*31*)

Materials and Methods

Túngara frogs

During 2007-2009, we collected túngara frogs (*Physalaemus pustulosus*) in amplexus approximately 1-3 hours after sunset in Gamboa, Panama. The male-female pairs were brought to a laboratory at the Smithsonian Tropical Research Institute (STRI) for testing. While waiting testing, pairs were stored in dry, dark containers to reduce stress and prevent females from dropping their eggs.

We used female phonotaxis in a binary choice test to assay relative call preference. Following previous procedures (*7-8*) with some modifications, each choice test began with a female placed under an acoustically transparent funnel in the center of a sound attenuating chamber (2.75 x 1.83 m; Acoustic Systems) lined with additional anechoic foam (Sonex, 1.5 inch; NRC 0.8). The funnel was positioned between two speakers (ADS L200C; Crown XLS 202 amplifiers; Digital buffer from Windows based PC) at 50 cm on either side of the female. Each speaker broadcast 1 call / 2 sec at 180° temporal phase (antiphonal) so that there was no temporal overlap of the two calls. Based on 14 acoustic variables, calls were synthesized to match the average call characteristics of males in the population from which these females were collected (software developed by J Schwartz). Calls were calibrated by the peak amplitude of the whine (90 dB SPL re: 20μPa) using a GenRad sound level meter (Fast, linear weighting) and 0.5 inch microphone at the female's release point. Prior to release from the funnel, females were presented with a 1.5

minute pre-broadcast consisting of 1 minute of whines followed by 30 sec of the paired complex calls (i.e., the experimental stimuli). Broadcast of these calls continued and the funnel was lifted remotely, allowing a subject to approach a speaker (i.e., phonotaxis). Females control their orientation at all times, in or out of the funnel. A choice was scored if a subject approached within 10 cm of a speaker. "No choice" was scored if she did not reach a speaker within 10 min; failed to leave the position of the funnel at the center of the chamber within 5 min of release; or if she remained immobile for 2 min at any point in the test after leaving the center. All tests were conducted under infra-red light and monitored remotely with an IR camera (Fuhrman Diversified, Inc.).

Stimuli consisted of pairs of complex (whine-chuck) calls that differed in chuck number as follows: 1:2, 1:3, 1:4, 1:5, 2:3, 2:4, 3:4. Each call pair was presented (in random order) only once to 40 different females, with one call pair tested with 41, generating 281 choices. Because each female did not complete testing with every call pair, 151 females were used to reach a minimum of 40 replicates. To these data we added previously published data collected with these same females (call pairs: 0:1 and 0:3). These tests included only 25 replicates per call pair, however, so the total N for the frog portion of our experiment is 331 choices). Counting each female's response to each different stimulus pair as statistically independent is valid because there is no significant difference among females in their strength of preference for chucks. That is, members of our group have empirically shown that each female exhibits the same consistency in preference as the population in general, meaning differences in choice are not based on behavioral polymorphism, but on a population-wide strength of preference (*31*). For this reason, each response by a female in a single choice between call pairs is a replicate.

Following testing, females were toe-clipped to avoid recapture and duplicate testing. Toe-clip procedures followed the Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research, compiled by the Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists (http://www.asih.org/files/hacc-final.pdf). Females were released the same night at their original collection site. All experiments were licensed and approved by the University of Texas at Austin Institutional Animal Care and Use Committee, the Smithsonian Tropical Research Institute, and the Autoridad Nacional del Ambiente de Panama.

Fringe-lipped bats

Trachops cirrhosus were captured in Soberania National Park, near Gamboa, Panama in 2007, 2008 and 2010 using mistnets set by small streams and ponds. Bats were tested in a large outdoor flight cage (5m x 5m x 2.5 m) in Gamboa (*12*). Each bat began the trial from a perch in one corner of the flight cage; a speaker was positioned under a screen covered in leaf litter in the two opposite corners of the cage. Calls (see above) were broadcast from Dell Inspiron 8100 and Dell Latitude E4300 laptop computers, SA-150 Realistic amplifiers, and 40-1040 Radio Shack broadband speakers at call rates and amplitudes equivalent to those described in the frog tests above. We conducted tests from approximately 1900 to 0300 when the bats were motivated to feed. To maintain high levels of motivation, we offered small, bait fish (frozen then thawed) as a food reward on each speaker. We broadcast calls for 60 s or until the bat flew from the perch to a speaker, whichever came first. For a choice to be registered, the bat had to listen to both stimuli before flight from the perch and fly within 1 m of a speaker. We pseudorandomized the speaker position underneath the screen, the order of call presentation (whether the more complex call began first or second), and the side of the flight cage (whether the more complex call began from the right or the left), and monitored the bats closely for order or side biases. Responses were recorded with Sony DCR-TRV340 and Sony DCR-SR45 camcorders equipped with Sony HVL-IRH2 and Sony HVL-IRM infrared lights. Following testing, we injected each bat with a subcutaneous passive integrative transponder (Trovan, Ltd.) to avoid duplicate testing and for long-term monitoring of population dynamics. Following testing, all bats were returned to their sites of capture and released. All experiments were licensed and approved by the University of Texas at Austin, the Smithsonian Tropical Research Institute, and the Autoridad Nacional del Ambiente de Panama.

Least Squares Fit of the Psychometric Function

Strength of preference, the proportion of responses to the call with more chucks, ranged from 0.5 to 1. Thus, we used a least squares fit of the following psychometric function to test which independent variable (total call energy difference; chuck number difference; chuck number ratio) explained the most variance in the responses:

$$response\_proportion = \frac{1}{n} + [1 - \frac{1}{1 + e^{a*(m-x)}}] * \frac{n-1}{n}$$

where n is the number of possible choices (for binary choice tests $n = 2$); m is the mean of the distribution or inflection point of the psychometric function (i.e., where the proportion response is equal to 0.75, midway between 0.5 and 1); and a is the exponential slope (Table 1). This psychometric function was specifically chosen because it is valid for binomial response functions that vary from 0.5 to 1 (*10*). Preference is based on the stimulus (i.e., the independent variable) if there is a significant fit of the function. Thus, ratio based decision-making (e.g., Weber's law) was determined when the function explained a significant proportion of the variance using stimuli ratios, as opposed to absolute differences, as the independent variable (x). Note that the independent variable, total call energy difference, was calculated by integrating the rectified voltage buffer of each stimulus. By converting this variable to dB, the analysis is valid for either total call energy difference or ratio, as the energy ratio of two stimuli in dB is equal to the energy difference in dB.

References

31. Kime, N.M., Rand, A.S., Kapfer, M. & Ryan, M.J. Repeatability of female choice in the túngara frog: A permissive preference for complex characters. *Animal Behaviour* **55**, 641-649 (1998).

Table S1. **Statistical Fit of Alternative Hypotheses:** Proportion choosing the call with more chucks was fit to three stimulus variables: total call energy difference; chuck number difference, and chuck number ratio. For both frogs and bats, only chuck number ratio explained the variance in call choice.

Stimulus Independent Variable	m	a	t	R^2	P
Frog					
Call energy difference	0.742	-1.75	1.177	0.165	0.278
Chuck number difference	3.459	-0.333	0.965	0.118	0.366
Chuck number ratio	0.208	4.298	6.156	0.844	<0.0005
Bat					
Call energy difference	2.494	-0.14	0.904	0.083	0.389
Chuck number difference	0.574	-0.863	1.099	0.118	0.300
Chuck number ratio	0.265	4.193	5.047	0.739	<0.0007

m = the mean of the distribution or inflection point of the psychometric function
a = the exponential slope
t = critical value for Student's t distribution