

The relative value of call embellishment in túngara frogs

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Abstract Facultative traits that have evolved under sexual selection, such as the acoustic ornaments present in the advertisement signals of male túngara frogs (*Physalaemus pustulosus*), offer a unique opportunity to examine selection for trait exaggeration with a focus on individual differences amongst signalers. By contrast, many studies of mate choice use experimental designs that obscure the inter-individual variation amongst signalers available for selection to act on—through the use of “typical” or average signals from the population. Here, we use dichotomous female phonotaxis choice tests to determine how the value of male call embellishment varies across 20 individual males frogs recorded from the wild—a sample which captures the acoustic diversity present in the population. We tested 20 females for each male call pair (i.e., 400 females). The results show widespread preference amongst females for ornamented calls (“whine–chucks”) over simple

calls (“whines”), yet also demonstrate substantial variation in the relative benefits for individual male frogs—some males enjoy appreciable benefits by using ornaments while others (30% of males in this study) do not. We also show that the relative amplitude of the chuck to the whine correlates positively with the value of call elaborations across these 20 males. Finally, by manipulating the relative amplitude of whines and chucks using both natural and synthetic calls, we demonstrate directly that this single call parameter is key to determining the relative value of call elaborations across males.

Keywords Advertisement calls · Individual differences · Mate choice · Phonotaxis · *Physalaemus* · Sexual selection

Introduction

A chief objective for the study of mate choice is to examine how females favor certain males and how such preferences are related to the male phenotype (Darwin 1871; Andersson 1994). In addition to examining natural correlations between male phenotype and female preferences (e.g., Møller 1994; Ryan and Rand 2003), studies of sexual selection have examined how males manipulated to possess one or more exaggerated or diminished traits (natural or artificial) are selected for or against by females when compared to a set of unmanipulated males (Hill 1991; Zuk et al. 1992; Møller 1998). Together, such correlational and experimental studies have yielded important insights into the nature of sexual selection, including the function of elaborated traits, such as indicators of male quality (Hill 1991; Sheldon et al. 1999) or biases in female sensory systems (Ryan et al. 1990; McClintock and Uetz 1996). For many species, however, male advertisements vary along

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multiple dimensions and investigations often fail to assess the multivariate universe of male traits—how such variables interact (including intrinsic correlations between the trait under study and other traits, measured or not) and how this complex variation impinges on the behavioral responses of females.

One weakness of the approach that attempts to attribute a particular feature of naturally occurring variation amongst males with female choice is the problem of correlated variation; traits not under study might be correlated with those that are, thus diminishing an investigator's ability to control potentially important attributes differing between individual males. Thus, experimental designs that attempt to “match” different males as closely as is practical for traits not under study but thought to be important, such as body length, assume that other relevant traits do not vary appreciably or that traits measured and “matched for” between signalers are the same ones that animal receivers attend to (Cummings et al. 2003; Cummings and Mollaghan 2006). This particular limitation could be ameliorated by testing female preferences before and after the manipulation of the same individual male. When using live signalers, however, this approach is limited to choice/no-choice testing conditions, which are often unrealistic given the natural history of many organisms (e.g., lek-breeding taxa), and are also complicated by test order effects (Dochtermann 2010). What has not been examined are female preferences under testing conditions that use the simultaneous presentation of a single natural male in two states (e.g., with and without an exaggerated trait), thus eliminating potential confounds of correlated traits between males and circumventing the limitation of one-choice testing conditions.

At the other end of the spectrum are studies that have addressed the issue of selection on multivariate signals by systematically synthesizing artificial advertisement signals in order to control equally each dimension of variation (Gerhardt 1978; Schwartz and Gerhardt 1998; Ryan and Rand 2001; Gerhardt and Brooks 2009). This strategy has also been fruitful, helping elucidate the interactions between signal parameters that lead to optimally attractive signals, and how such combinations may drive the tempo and mode of evolution by sexual selection (Gerhardt and Schwartz 2001). This approach is not without its complications as well, most importantly because the range of signals synthesized by experimenters does not necessarily reflect the variation that occurs naturally. Ideally, this type of approach is combined with studies that investigate female preferences for natural variants under controlled conditions. What is lacking are experimental designs that permit the use of naturally varying signals but avoid the problems associated with drawing inferences about female choice when the trait under study correlates with other traits, thus obscuring or confounding the role of the feature under study.

When the trait in question is dynamically expressed and facultative, there is an opportunity to do just that. Here, we have tested female frogs for their preferences in dichotomous choice tests in which they are presented with a simple natural male advertisement call and the same male call in a naturally exaggerated (complex) state. We repeated this testing condition across calls from 20 different males, examining the preferences of 400 females, 20 different females for each of the 20 pairs of calls tested. Additionally, we manipulated these same natural signals (as well as artificial stimuli) for a single particular acoustic parameter (the relative peak amplitudes of the two components of complex calls) that we predicted would significantly influence female preferences. In doing so, our study uses natural within- and between-male variation in calls, and direct tests of call manipulation on female preference, to ask how the value of embellishing a call may depend on the identity of the signaler.

The system

Túngara frogs are small anurans (ca. 30 mm snout–vent length) distributed throughout much of Middle America (Weigt et al. 2005). During the breeding season (May through December), males vocally advertise to females using a species-typical call, known as the “whine” or simple call (Ryan 1985). Males can produce a complex advertisement call by ornamenting the whine with one to seven suffixes known as “chucks” producing what are known as “whine–chuck” calls. Females use advertisement calls to localize and select a male amongst a chorus; by making physical contact with a male, a female selects a mate, after which the male mounts and clasps the female in a posture known as amplexus. In laboratory two-choice phonotaxis tests using synthetic calls, the whine–chuck calls are strongly preferred to whine calls (85% preference strength in the study population; Ryan 1985; Ryan and Rand 2003).

Individual male túngara frogs differ in the attractiveness of their calls. Ryan and Rand (2003) studied female mate choice in response to recorded natural calls from males in the present study's population (Gamboa, Panamá) and showed that some males are consistently more attractive than others. Here, we examine a different, but related question: do acoustical differences amongst male calls result in differential value of call elaboration? Male túngara frogs can produce either simple or complex calls in order to attract females to mate, and males alternate between simple and complex calls depending on the competitive vocal environment (Bernal et al. 2009; Ryan 1985) and when they detect the presence of a female (Akre, personal communication). But how does natural variation in call parameters

influence the value of such facultative elaboration? In other words, do some males benefit significantly more than others by adding a chuck to their whine, and is it possible that a proportion of males do not benefit at all from such embellishment? This latter finding would be especially interesting, given the high cost of complex call use (attracting acoustic predators and parasites). Finally, we predicted that a significant proportion of the variation across males in the value of their chucks would be predicted by one particular call variable: the relative amplitude of the chuck to the whine—an acoustic feature that varies between individual males and has been shown to be salient to females when evaluating multiple males (Ryan and Rand 2003).

Materials and methods

Stimuli

Forty natural stimuli were used in experiments 1 and 2: whine–chuck from 20 males were recorded and the chuck was removed to obtain the whine-only versions. These 20 whine–chuck calls were originally recorded in July 1996 with a Marantz PMD 420 recorder and a Sennheiser ME 80 microphone with K3U power module on magnetic cassette tape (Ryan and Rand 2003). In this previous study, the authors recorded 300 calls from 50 males in the same population used in the present study. Calls from nine of the males in this study (b, e, f, g, h, i, j, o, t) were selected because they represent the extent of acoustic variation amongst the sample of 50 males (median, \pm SD, outliers; see Ryan and Rand 2003 for an explanation of the sampling and selection procedure) and the calls of the remaining 11 males (a, c, d, k, l, m, n, p, q, r, s) were selected randomly from the remaining sample. In experiment 1, all 20 whine–chuck calls were matched for the peak amplitude of the whine (82 dB SPL) before chucks were removed to obtain the whine-only calls. Therefore, the natural whine-to-chuck relative amplitudes were preserved. In experiment 2, we also matched the whine peak amplitudes (82 dB SPL) but manipulated digitally the chuck peak amplitudes, thus generating five different treatments: for each call, chuck peak amplitudes of 62, 70, 76, 82, and 88 dB were generated. In both experiments 1 and 2, each call was low-pass filtered (5,000 Hz) and background noise was removed as much as possible from calls using a procedure in Signal RTS (Engineering Design, Belmont, MA) that defines the spectral and amplitude characteristics of each harmonic of the call and then resynthesizes the call.

Digitized calls (sampling rate of 20 kHz) were analyzed for a set of spectral and temporal variables that have been

used previously to quantify signal variation in this species (Ryan and Rand 2001, 2003). Spectral characters for the whine were derived from the fundamental frequency component. Using programs written in Signal RTS, we analyzed calls for 15 acoustic variables using spectrograms, waveforms, and fast Fourier transforms (see Fig. 1 for examples of parameters used). Values for the acoustic variables for each call and their definitions are provided as Electronic supplementary materials (S1).

Using these same 300 calls, Ryan and Rand (2003) calculated the repeatability of acoustic attributes in male túngara frog calls between repetitions and across nights using intraclass correlations (ICC, a measure of the relative within- and between-subject variance, in which an ICC value of 1.0 indicates high within-male repeatability compared to between-male, and a value of 0.0 indicates that within-subject variance is equivalent to between-subject variance). The results demonstrated that male túngara frogs have reasonably repeatable call characteristics; for each of the call variables measured (see Electronic supplementary materials S1), only the initial frequency of the whine and the dominant frequency of the chuck had ICC values less than 0.5.

In experiment 3, we used a synthetic whine and whine–chuck. The whines in these two signals were identical and consisted of only the fundamental frequency; it has been shown previously that the upper harmonics of the whine do not influence mate choice in the laboratory (Ryan and Rand 1990; Rand et al. 1992) and that these synthetic stimuli are as attractive, on average, as natural signals (Ryan, unpublished data). Information on the synthesis procedure can be found in Ryan et al. (2003). In each of three treatments, the whine stimulus had a peak amplitude of 82 dB and the chuck component varied between treatments (76, 82, and 88 dB). Therefore, in every phonotaxis trial performed in this study (experiments 1–3), the whine-only stimulus and the whine component of the whine–chuck stimulus was

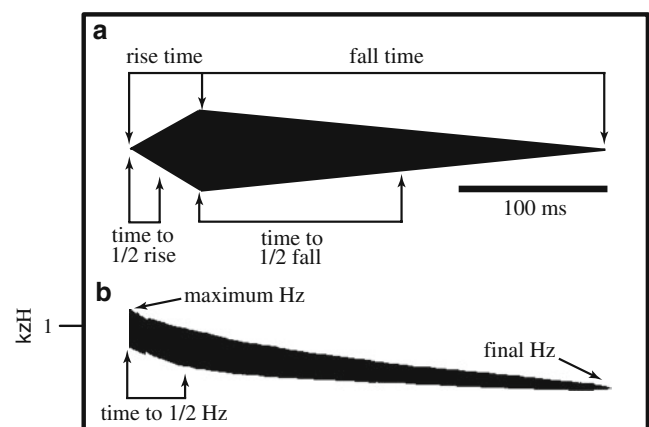


Fig. 1 **a** Stylized oscillogram and **b** spectrogram depicting the acoustic variables analyzed

calibrated to a peak amplitude of 82 dB at the center of the phonotaxis arena.

Female choice tests

We conducted phonotaxis experiments between the months of June and September in 2003 and 2005–2008 at facilities for the Smithsonian Tropical Research Institute in Gamboa, Panamá. We collected frogs as amplexant mated pairs from breeding sites between 1900 and 2200 hours and performed behavioral testing between 2000 and 0500 hours. We held animals in small plastic bags in dark, quiet conditions prior to testing. We marked individuals with a unique toe-clip combination to prevent resampling and measured the mass and snout-to-vent length and returned them to their original site of collection within 12 h. In marking frogs, we followed the Guidelines for the Use of Live Amphibians and Reptiles in Field Research, compiled by the American Society of Ichthyologists and Herpetologists, The Herpetologists' League, and the Society for the Study of Amphibians and Reptiles, and our methods were approved by the Institutional Animal Care and Use Committee at The University of Texas at Austin and La Autoridad Nacional del Ambiente in Panamá. Experiments 1–3 each used a sample size of 20 females per treatment. Experiment 1 had 20 treatments and involved testing 465 females across 481 trials, which resulted in 400 successful choices with one unique female per choice (i.e., 83.2% trials resulted in choices, and 86.0% of females were responsive). Experiment 2 (five treatments) and Experiment 3 (three treatments) resulted in 100 and 60 successful choice tests, respectively, and also used a unique set of females for each treatment.

Frogs were tested under infrared light in a sound-attenuating chamber (Acoustic Systems, Austin, TX) measuring ($L \times W \times H$, m) $2.7 \times 1.8 \times 1.78$. Before each subject was tested, we calibrated both speakers to a sound pressure level (SPL) of 82 dB (re. 20 μ Pa) at the center of the arena (1.35 m from each speaker) using a synthetic whine stimulus (peak amplitude and flat weighting settings on a GenRad 1982 SPL meter, General Radio Corporation, West Concord, MA). In each trial, we broadcast the whine–chuck of a given male versus the whine from the same male and repeated that using 20 unique females for each male's call pair. In 2007, we collected 12 choices for each of the 20 male call pairs, and in 2008, we collected the remaining eight female choices for each pair of calls.

Each trial began with the subject placed under a cone at the center of the acoustic chamber for 3 min while the two stimuli were broadcast antiphonally at a rate of one call per 2 s from each of two ADS L210 speakers opposite one another. The cone was then lifted remotely and phonotaxis was monitored via a ceiling-mounted infrared camera and television monitor outside the chamber. We scored a phonotactic choice when a

frog approached one of the speakers within a 10-cm radius without simply following the wall perimeter. A frog failed to exhibit a phonotactic choice (i.e., the trial was “disqualified”) if it was motionless for the initial 5 min after the cone was raised or during any 2-min interval thereafter, or if the animal failed to make a choice within 15 min after the cone was raised. We also measured the time lapsed from lifting the cone to choice (latency) and report the mean latency (and standard error) for each of the 20 male call conditions (Electronic supplementary materials S2). Simple and complex calls were alternated between left and right speakers between each trial to minimize any potential side bias; we also tested directly for a side bias using both simple and complex stimuli and found none (Baugh and Ryan 2010). The lack of a side bias using both simple and complex stimuli supports our assumption that the frequency responses of the two speakers are equivalent at the level of female perception.

In experiment 1, we performed an exact binomial probability test (two-tailed) on each of the 20 whine versus whine–chuck treatments ($\alpha=0.05$). We tested the null hypothesis of no among-male variation for adorned versus unadorned calls using a one-sample Kolmogorov–Smirnov test. We examined how preference strength relates to the relative peak amplitudes of chuck and whine within each call using a linear regression with the difference in the peak amplitude (dB SPL) of the chuck compared to the whine as the independent variable and the number of females choosing the whine–chuck stimulus over the whine as the dependent variable. Finally, we examined latency to choice for trials in which females chose the whine–chuck compared to trials involving a choice for whine using a t test. In Experiments 2 and 3, we performed exact binomial probability tests (two-tailed) for each treatment ($\alpha=0.05$).

Results

Experiment 1

Female preferences for the complex version of a call varied from very strong (90%) to absent (40%; see Fig. 2). This distribution differed significantly from a uniform distribution (Kolmogorov–Smirnov $Z=2.012$, $P=0.001$). In 14 of the calls, there was a significant preference for the complex version and in the remaining six there was no detectable preference (binomial probabilities, two-tailed; Fig. 2). There were no calls for which the whine was significantly preferred compared to the whine–chuck.

Sample sizes for each of the 20 treatments were not sufficiently large to permit post hoc analyses to parse out the role that each of the multiple call variables had on female preference scores. The relative amplitude of the chuck to the whine for each call, however, has been suggested as an

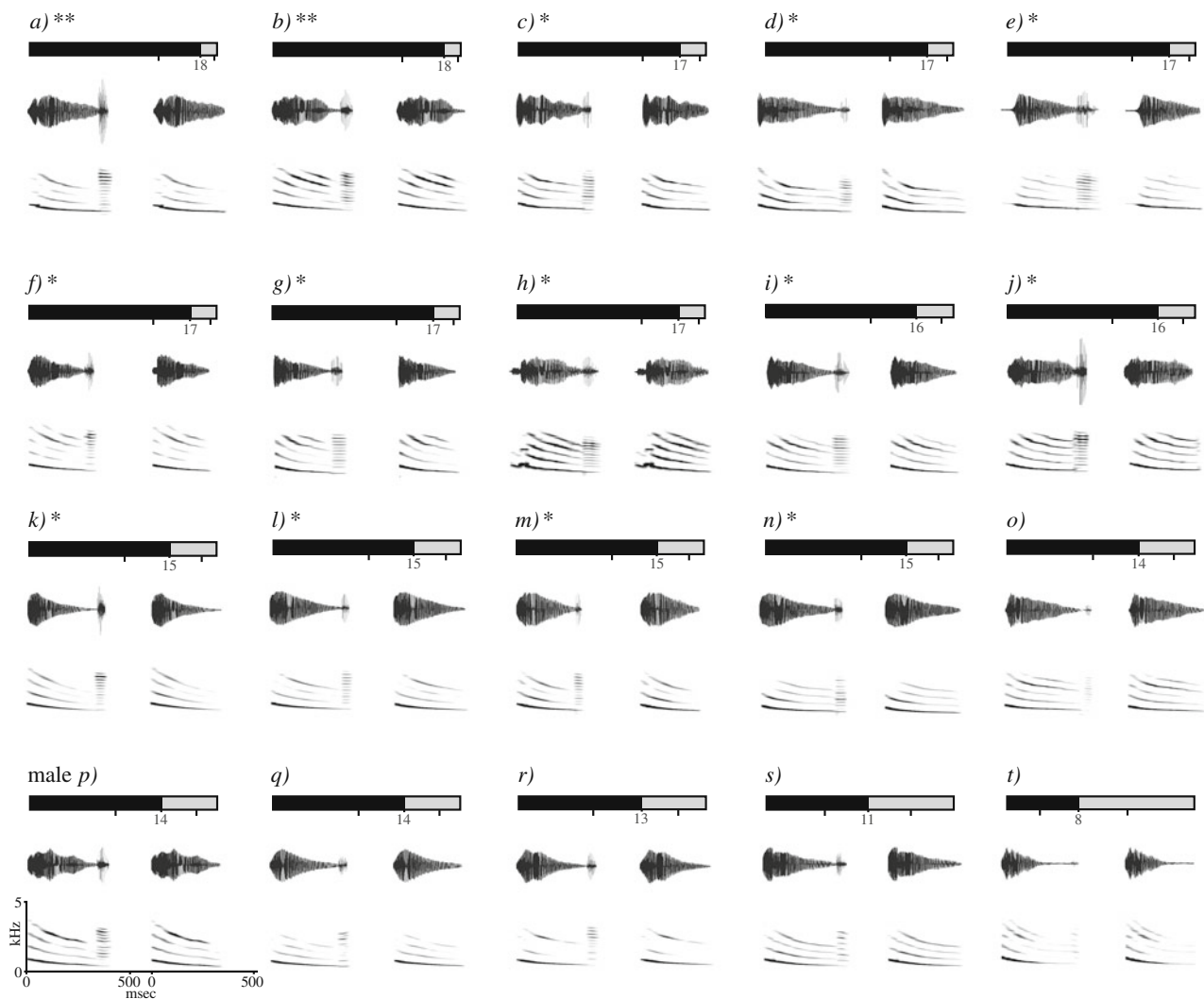


Fig. 2 An oscillogram (*top*) and a spectrogram (*bottom*) for both the white and white-chuck calls are shown from each of 20 males (all depictions are plotted at the same scale). Female choice results are depicted on the *horizontal bar* with choices for the white-chuck shown in *black* and choices for white in *gray*. *Black hash marks* indicate the boundaries for 95% confidence intervals. *Asterisks* next to the male number indicate a significant preference for the white-

chuck using a two-tailed binomial probability (** $P < 0.001$, * $P < 0.01$). Male identifiers correspond to the following male ditch call numbers: *a* d10, *b* d8, *c* d15, *d* d12, *e* d9, *f* d6, *g* d7, *h* d2, *i* d3, *j* d1, *k* d20, *l* d18, *m* d14, *n* d16, *o* d4, *p* d13, *q* d17, *r* d19, *s* d11, and *t* d5. The calls of nine males were used in Ryan and Rand (2003) with the following naming conversions: *b* Sd, *e* Od, *f* Sc, *g* Ob, *h* Oa, *i* M, *j* Oc, *o* Sa, and *t* Sb

important factor related to inter-male call attractiveness in a previous study—higher chuck relative to white amplitudes are predicted to be preferred (Ryan and Rand 2003). Our results confirm this prediction and extend this finding to intra-male variation. Four of the six treatments in which there were no preferences had some of the lowest chuck-to-white relative amplitudes (less than 1.0, see Electronic supplementary materials S1). Moreover, a linear regression revealed a significant positive relationship between the number of choices for the white-chuck in each call pair and the difference in peak amplitude (dB SPL) of the chuck compared to the white ($r^2 = 0.567$, $F_{1,18} = 23.55$, $P = 0.0001$; Fig. 3).

Whereas latencies did not differ significantly when females selected white compared to white-chuck stimuli

(experiment 1), there appeared to be a trend towards shorter latencies for white-chuck choices (latency (mean \pm SEM, s): white-chuck, 133.5 ± 6.9 , $N = 304$; white, 160.2 ± 11.9 , $N = 96$; *t* test (two-tailed): $t_{398} = 1.9$, $P = 0.058$). Likewise, the single most attractive individual male from Ryan and Rand (2003; male *i* in the present study) elicited the quickest response in the current study and the lowest frequency of disqualified trials (Electronic supplementary materials S2).

Experiment 2

In experiment 2, we used the identical natural signals used in experiment 1 but disrupted the natural ratio of white-to-chuck peak amplitudes in the complex stimulus. Here, we

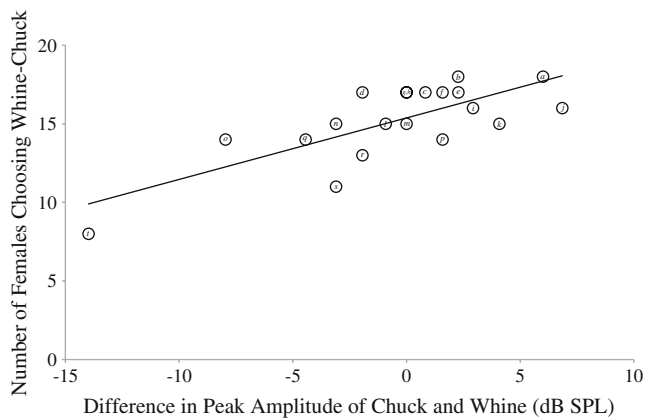


Fig. 3 Linear regression of relative amplitude of the chuck to the whine (peak amplitude, in units dB SPL, of chuck to whine) versus the number of choices for the whine–chuck stimulus in each treatment condition. Positive values therefore indicate that the chuck had a higher peak amplitude than the whine and vice versa. Letters inside the circular symbols indicate the individual male (see Fig. 2)

found that females exhibited preferences (complex–simple choices, P values) when chuck peak amplitudes were 76 (16:4, $P=0.011$), 82 (17:3, $P=0.0025$), and 88 dB (17:3, $P=0.0025$), whereas no preferences were evident when chuck peak amplitudes were 62 (12:8, $P=0.503$) or 70 dB (8:12, $P=0.503$; see Fig. 4).

Experiment 3

Experiment 3 used synthetic signals with modified chuck peak amplitudes and provided similar results as experiments 1 and 2: female preferences for complex calls were evident when the chuck was 82 (18:2, $P=0.0004$) and

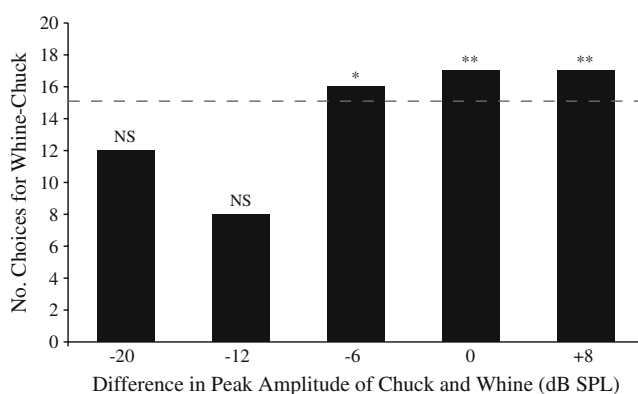


Fig. 4 Results from two-choice phonotaxis experiments in which females were presented with a natural whine stimulus (from males $a-t$; peak amplitude, 82 dB) and the identical whine stimulus with a single chuck appended. The five treatments ($N=20$ per treatment) differ in the peak amplitude (dB SPL) of the chuck in the whine–chuck stimulus, and thus in the relative amplitude of whine and chuck components (x -axis). Dashed line indicates significance criterion for complex call preference

88 dB (15:5, $P=0.041$) but not when the chuck had a peak amplitude of 76 dB (14:6, $P=0.115$; see Fig. 5).

Discussion

One area of focus in sexual selection research is to identify the modes by which trait adornments evolve and doing so requires an examination of male mating success with and without facultative adornments (i.e., at the level of the individual). Most studies focusing at the population level have demonstrated, among other things, that intra-population variation between males in sexual advertisement signals is present (Espmark 1995; Dale 2006), and in many instances, such variation has important consequences for female choice (Jang and Greenfield 1998; Zuk et al. 1990; Ryan and Rand 2003), including a female's commitment to an initial choice (Baugh and Ryan 2010). In the present study, we have taken this basic question one step further by illustrating that not only does a given male's attractiveness vary when an advertisement signal is facultatively embellished, but that the degree to which such embellishments enhance the signaler's attractiveness varies widely as well—for certain males increasing attractiveness markedly and in other males having little or no effect. This observation is particularly relevant in species such as the túngara frog, for which embellished sexual signals are costly. Because whine–chuck calls are more successful at attracting frog-eating bats (Ryan et al. 1982; Page and Ryan 2008) and acoustic parasitic flies (Bernal et al. 2006), male túngara frogs that use such calls but do not enjoy an elevated mating success might incur considerable disadvantage.

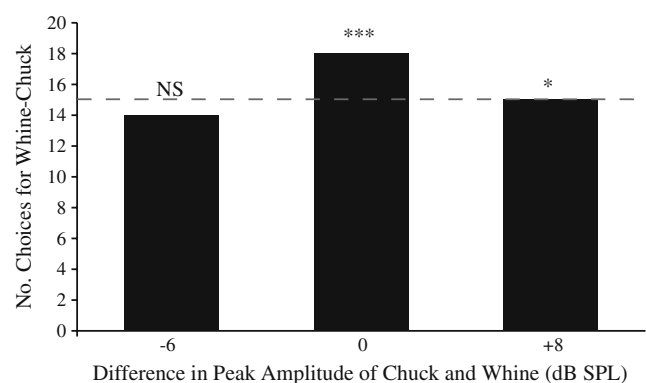


Fig. 5 Results from two-choice phonotaxis experiments in which females were presented with a synthetic whine stimulus (peak amplitude, 82 dB) and the identical whine with a single synthetic chuck appended. The three treatments ($N=20$ per treatment) differ in the peak amplitude (dB SPL) of the chuck in the whine–chuck stimulus and thus in the relative amplitude of whine and chuck components (x -axis). Dashed line indicates significance criterion for preference

This possibility begs the question as to how such variation is maintained in the population—how population-level variance in the value of chucks might be moderated or traded off against other traits under selection. There are several possible explanations, none of which are mutually exclusive: (1) males might employ individually specific calling strategies that reduce these individual differences, such as varying the use (i.e., frequency) of complex calls according to the costs and benefits of call embellishment—males that gain little or no benefit from complex call production might at minimum enjoy reduced predation/parasitism by minimizing their use of complex calls. Males with low value chucks might also simply forego calling altogether and adopt a satellite male strategy, although satellite behavior has not been reported in this species. (2) Alternatively, the acoustic parameters that endow certain males with relatively little or no benefit in female choice through the use of complex calls might be correlated with acoustic traits that attract fewer parasites and predators, thus not demanding individually specific differences in the proportions of simple and complex calls used by males. (3) Male–male competition might also explain some of this variation—although unlikely it is possible that features of the advertisement call that are ineffective in attracting females might be compensated for by an elevated value in vocal competition between males. (4) Lastly, male mating success may vary widely—as has been suggested in túngara frogs (Ryan 1985) and other species with skewed operational sex ratios (e.g., Kodric-Brown 1988; Pröhl and Hödl 1999)—but be explained by other biotic and abiotic selective pressures which dilute or counteract the consequences of variation in the relative value of sexually selected, facultative traits.

Consistent with our prediction, we showed that the relative amplitude of the chuck to the whine explains a significant portion of the variation in the value of call embellishments. The whines used in this study were equalized for peak amplitude, and the relative amplitudes of the whines and chucks were maintained at their natural ratios (experiment 1) and manipulated digitally to test directly the effects of specific whine-to-chuck relative amplitudes on natural (experiment 2) and synthetic calls (experiment 3). The range of manipulated whine-to-chuck relative amplitudes used in experiments 2 and 3 (−20 to +8 dB) encompass approximately the range of variation found in the natural whine-to-chuck relative amplitudes (experiment 1: −14 to +7 dB). Ryan and Rand (2003) showed that as an acoustic trait, the relative amplitude of whines and chucks is relatively stable within individual males ($ICC=0.63$). The evidence from their study and the present work points to a key role for relative amplitude in determining call attractiveness. Playback studies in the field in which relative amplitude is manipulated during female choice would be make it possible to test

the importance of this trait under more complex acoustic and social conditions.

These signals are multivariate, however, and while relative amplitude clearly plays a role, it is not the sole factor underlying female preferences (see Ryan and Rand 2003 for a statistical evaluation of female preferences and multivariate male traits). We conducted experiments in which we directly manipulated the relative amplitudes of whines and chucks using both natural (experiment 2) and synthetic (experiment 3) stimuli. These experiments confirmed the importance of whine-to-chuck relative amplitudes for female preferences. Experiment 2 supported the results of experiment 1 by demonstrating directly that preferences increase as chuck peak amplitudes increase across a range of naturally occurring calls. Experiment 3 showed that increasing chuck amplitudes alone (i.e., all other signal parameters held constant) significantly increases call attractiveness. The magnitude of effects were similar, but not identical, across experiments 1–3: females preferred the complex call significantly if the chuck was equal (or higher) in peak amplitude when synthetic signals were used, but significant preferences were manifest at lower chuck amplitudes (−6 dB) when natural signals were used. Upon closer inspection, however, female preferences at the relative amplitude of −6 dB do not differ significantly between natural (16:4) versus synthetic (14:6) experiments (Fisher's exact test: $P=0.71$).

The current study does not allow us to determine which additional call features, besides relative amplitude, explain the remaining variance in female preferences. Addressing that question has been approached traditionally by studies that systematically vary multiple call features simultaneously through the use of synthetic signals. Such an approach has been useful at modeling the parameter space of interest for advertisement signals and generating insights about the mode and direction of sexual selection through female choice (Ryan and Rand 2001; Gerhardt and Brooks 2009). An additional approach for future studies using natural stimuli would involve examining whether the remaining variation in enhanced relative attractiveness results from spectral properties of the chuck per se or the interaction between whine and chuck. One method for decomposing this problem would involve systematically combining the whines and chucks from different males to examine interactions that act in additive or non-additive ways to determine attractiveness.

A next step is to examine how variation and co-variation in male call parameters relates to underlying vocal mechanics and how morphological, physiological, and genetic mechanisms give rise or constrain the diversity of combinations of signal traits available for selection to act on. A study by Ryan and Rand (2003) suggested that call traits in this species appear relatively uncorrelated, thus making possible the independent evolution of multiple call

parameters. This evidence combined with the present demonstration of salient variation in relative whine-to-chuck amplitudes raises the likely possibility that other factors are acting to stabilize selection for this particular acoustic trait, thus maintaining the extant variation in the population. Addressing the nature of these factors will require an understanding of the heritability of vocal traits, auditory processing, and behavioral responses of both females (mate choice) and males (male–male competition), the possibility that vocal traits are plastic and condition-dependent, as well as the greater ecology of signaling in this system. For instance, how might unintended receivers such as eavesdropping predators respond to this call variation, and might such natural selection pressures serve to maintain variation in suites of call traits? Alternatively, complex social and acoustic conditions in the wild might greatly attenuate the preferences shown in the present phonotaxis study. We must also emphasize, however, that this study examined the value of adding a single facultative call component. Therefore, males with less preferred whine-to-chuck relative amplitudes might simply employ complex calls less often thus minimizing the costs of complex call production (e.g., increased predation) for what would be a marginally effective elaboration. Alternatively, the addition of more than one chuck might alter (e.g., attenuate or even reverse) the patterns of female preferences observed here.

Our understanding of the complex relationship between male signal variation, female preferences, and the mode and direction of sexual selection may be best approached by integrating across various levels of analysis. We believe that it is particularly useful to combine, as we have done here, the use of both natural and manipulated intra-male variation as well as synthetic stimuli because such an approach enables the experimenter to control the properties of opposing signals in all aspects except the one under study while simultaneously using signals representing real-world individual differences amongst signalers.

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