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Signal variation and call preferences for whine frequency in the túngara frog, *Physalaemus pustulosus*

Abstract Properties of sexual signals can differ in the amount of within-male variability. In several species of anurans, females exert stabilizing or weakly directional preferences on less variable call properties, and highly directional preferences on more variable ones. Preferences of female túngara frogs, *Physalaemus pustulosus*, were examined for two call characteristics: a less variable spectral character, dominant frequency of the whine, and a more variable temporal character, inter-call interval. Eight phonotaxis experiments using synthetic calls were conducted with gravid females. Stimuli presented for both characters were based on the mean and standard deviation (SD) of those characters in the study population. For each character, we used four intervals of variation (1, 2, 3, and 4 SD) between stimuli in four different experiments. As has been found in some other anuran species, preference was stronger for the more variable temporal character, increasing in proportion to the difference between stimuli. Preference for the less variable spectral character was not significant until the difference between stimuli was substantial. The strength of female preference, estimated as latency to choose, the number of speakers visited, and the number of females showing phonotaxis, increased in proportion to the increase in the difference between stimuli. All these measures of strength of preference were greater in response to the more variable temporal character compared to responses to the less variable spectral character.

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Introduction

Female preferences can generate selection on signals used by males in sexual displays. Many signals are composites of a variety of properties or characterswhich can exhibit different patterns of variation within and among individuals. For example, an animal's mating or advertisement call might exhibit relatively narrow variation in dominant frequency but substantially greater variation in calling rate. Gerhardt (1994) suggested a general pattern in anuran call preferences related to within-individual signal variation. Females prefer calls with frequencies near or below the population mean over calls of higher frequency, while they tend to prefer calls played back at high rates over low-call-rate alternatives (Gerhardt 1994). In some species, the preference for high call rate can reverse a preference for low frequency when these two parameters are paired against one another (Morris and Yoon 1989).

Gerhardt (1991; see Tables 1–3) classified the acoustic properties of anuran advertisement calls into two groups: static and dynamic based on within-male variability during calling bouts. Static properties changed relatively little between calls, whereas dynamic properties showed a high degree of intra-individual variation. For the static properties, the most preferred values in phonotaxis tests are at or near the mean values for natural populations. In contrast, the most preferred values of dynamic properties equal or exceed the highest values observed in natural populations (Gerhard 1991). In our study, we use the túngara frog, *Physalaemus pustulosus* to explore some of those relationships uncovered by Gerhardt (1994). We do not use the terms static and dynamic to characterize these calls since they vary along other biologically meaningful axes as well. For example, the less variable character we test is a spectral character while the most variable is a temporal one.

The túngara frog has proven to be a useful subject for studies of the mechanisms and evolution of communication (e.g., Ryan 1985; Ryan et al. 1990). The advertisement call of *P. pustulosus* has two components: the whine (always present) and the chuck (from zero to six per call). The number of chucks incorporated into the call is influenced by male-male competition, and females prefer a more complex call (Rand and Ryan 1981). Larger males have higher mating success in the wild (Ryan 1983), and there is a significant negative correlation between male size and fundamental frequency of the chuck (Ryan 1980). Females prefer lower-frequency chucks, and this preference might account in part for the greater mating success of larger males (Ryan 1980, 1983; Wilczynski et al. 1995). The whine frequency is not correlated with body size (Ryan 1985).

Males vocalizing alone produce the simple call, perhaps because complex calls might be more attractive to acoustically orienting predators (Tuttle and Ryan 1981). Phonotaxis experiments have shown that a whine is both necessary and sufficient to elicit female phonotaxis, and the upper harmonics of the whine have no influence on female preference (Ryan and Rand 1990; Rand et al. 1992; Wilczynski et al. 1995). The main goal of this paper is to compare preferences for the less variable dominant frequency of the whine to that for the more variable inter-call interval.
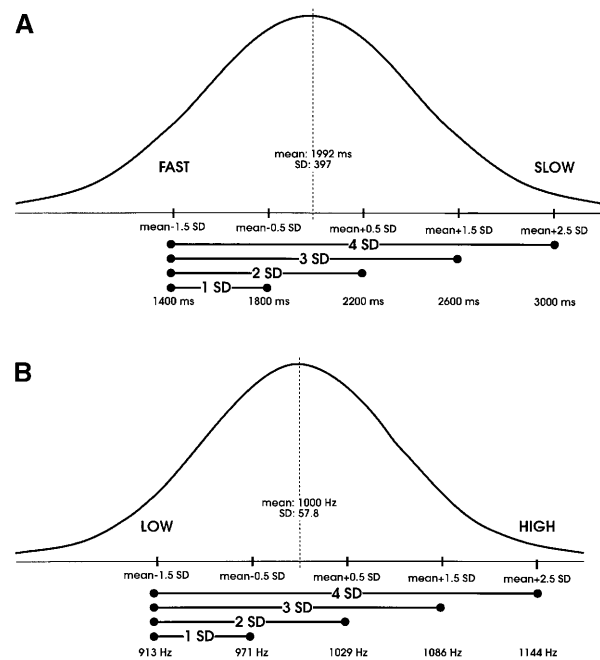
Methods

Fifty-seven amplectant females were collected near the facilities of the Smithsonian Tropical Research Institute in Gamboa, Republic of Panama, during August 1997 and June 1998. When a female had completed all tests, we gave her a unique toe-clip and returned her to the capture site.

The general methodology for the phonotaxis tests followed Wilczynski et al. (1995). In the phonotaxis experiments we used stimuli synthesized with a program written by J. Schwartz (University of Missouri; sample rate: 20,00 Hz and 8 its). We used only the fundamental frequency sweep of the whine because its upper harmonics appear to have no influence on female phonotaxis (Rand et al. 1992; Wilczynski et al. 1995).

The characteristics of the distribution of male advertisement calls in this population are known (M.J. Ryan, A.S Rand, J. Bosch, unpublished data). Stimuli presented for both characters are based on the mean and standard deviation (SD) of those characters in the study population.

The calls were emitted directly from the stereo audio output of an Apple PowerBook 1400cs computer, and we amplified them through a stereo amplifier. We broadcast synthetic whines antiphonally from two speakers (ADS L2000) in an indoor square arena (3×3 m) under red light in 1997. In 1998, we used a dark acoustic chamber (Acoustic Systems, Austin, Tex.) (182×274 cm) with a Fuhrman video monitor system illuminated with infrared light. Temperature in the arena was maintained between 24.5° and 27.6°C, and testing occurred between 2000 and 0600 hours. We adjusted the amplitude of the calls at the release point (center of the arena) to 82 dB sound pressure level (SPL; re: 20 μPascals) with a GenRad SPL meter (model 1982; flat weighting, peak response). We tested females within hours after capture. Testing began by placing a female under a restraining cone at the release site while the stimuli were broadcast. Following a 3-min. acclimation period, we raised the cone allowing the female to move about the arena. If the female approached within 10 cm of a speaker and

Fig. 1. Inter-call interval (**A**) and initial frequency (**B**) distributions in the study population, and stimuli values used in the phonotaxis experiments

ceased movement, a preference was scored. No preference was scored if a female did not move from the release site within 5 min, if she ceased moving at any time, if she remained against the near or far wall for 2 min, if the she climbed the wall, if she passed both speakers without stopping or turning back and then passed either of them again without stopping or turning back, or when no edge was reached within 15 min after initiating the trial.

In the first set of experiments we used the less variable call character, dominant frequency. The synthetic calls were sequences of whines with an average duration (323 ms) and an average inter-call interval (1,992 ms) for this population; pairs of stimuli differed only in spectral frequency. For this character, four distinct intervals of variation were used (1, 2, 3 and 4 SD), which in four different experiments translates into: mean frequency–1.5 SD vs mean frequency–0.5 SD, mean frequency–1.5 SD vs mean frequency+0.5 SD, mean frequency–1.5 SD vs mean frequency+1.5 SD, and mean frequency–1.5 SD vs mean frequency+2.5 SD (Table 1, Fig. 1B).

In the second set of experiments, the synthetic stimuli were sequences of whines of average duration (323 ms) and frequency (1,000 Hz) for this population; pairs of stimuli differed only in whine rate. Again, for this character, four distinct intervals of variation were used (1, 2, 3, and 4 SD), which translates into in four different experiments: mean interval–1.5 SD vs mean interval–0.5 SD, mean interval–1.5 SD vs mean interval+0.5 SD, mean interval–1.5 SD vs mean interval+1.5 SD, and mean interval–1.5 SD vs mean interval+2.5 SD (Table 1, Fig. 1A). In all cases, the whine frequency and whine rates used in the synthetic stimuli are within the range found in the natural population. The whine frequency referenced is the dominant frequency in the beginning of the whine. The final frequency was calculated in every case like the initial frequency–529 Hz (the average change in dominant frequency for the study population).

The sample size in each experiment was 20. Each female was tested once or twice in each of the eight experiments in random order. The females that did not provide a valid response in one experiment were tested again after several minutes in the same experiment. If the female did not respond after the second trial, she did not score for that experiment. Each experiment was concluded when we had obtained 20 valid responses.

Table 1 Population values for initial frequency and inter-call intervals of the whine, and stimuli values used in the phonotaxis experiments (*CV* coefficient of variation)

Call property	Mean population values (SD, range, CV, sample size)	Experiment	Difference between stimuli (SD)	Stimuli
Initial dominant frequency (Hz)	999.7 (57.8, 871–1,157, 0.033, 51)	Mean–1.5 SD/mean–0.5 SD	1	913/971
		Mean–1.5 SD/mean+0.5 SD	2	913/1,029
		Mean–1.5 SD/mean+1.5 SD	3	913/1,086
		Mean–1.5 SD/mean+2.5 SD	4	913/1,144
Inter-call interval (s)	1.992 (0.397, 1.426–2.921, 0.222, 32)	Mean–1.5 SD/mean–0.5 SD	1	1.4/1.8
		Mean–1.5 SD/mean+0.5 SD	2	1.4/2.2
		Mean–1.5 SD/mean+1.5 SD	3	1.4/2.6
		Mean–1.5 SD/mean+2.5 SD	4	1.4/3.0

Several measures were used to assess the strength of female preference. We measured the latency to choice, as well as the number of times the females crossed the mid-line through the chamber perpendicular to the two speakers (that is, the number of times each half of the arena was visited).

The binomial test was used to test whether the number of females attracted to each speaker was nonrandom. We use a one-tailed exact binomial probability test because there was an a priori expectation of the directionality of response: usually female frogs prefer low-frequency calls and a high call rate. A two-way ANOVA for repeated measures was used to analyze the latency to choose between experiments.

Results

The females' preference for dominant frequency remained constant in the first three experiments, and increased strongly in the fourth experiment (Table 2). Females preferred low- over high-frequency whines, but the difference was only statistically significant for the 4 SD interval of variation (16:4, binomial test, one-tailed, P=0.006). Preference was strong for variation in temporal characters (Table 2). The difference was statistically significant for the 3 and 4 SD intervals of variation (3 SD, 16:4, P=0.006; 4 SD, 17:3, P=0.001).

The three measures of strength of preference are shown in Table 3. In all experiments, the number of females that visited only one half of the arena was statistically higher than the number of females that visited both halves of the arena.

Latency to choose was highly variable between individuals (Table 3, Fig. 2B), and there were no statistically significant differences among individuals within each set of experiments (dominant frequency and inter-call interval) or between the two sets of experiments (log-transformed data; two-way ANOVA for repeated measures, for only the 1 SD to 3 SD intervals: effect "character kind" $F_{1,9}$=1.195, P=0.303; effect "interval between stimuli" $F_{2,18}$=1.462, P=0.258; effect interaction $F_{2,18}$=0.1935, P=0.826; ANOVA for repeated measures, dominant frequency vs inter-call interval for the 4 SD interval: $F_{1,14}$=0.1921, P=0.668).

The differences in females choices and number of females that only visited one half of the arena in all experiments appear in Table 4. Figure 2 shows the results of the two-speaker playback tests for both the dominant frequency and the inter-call interval experiments in increasing units of variation between stimuli. In response to variation in inter-call interval, the proportion of females that chose the faster stimulus increased in proportion to the increase in the interval between stimuli. In response to variation in dominant frequency, the trend was flat in the first three experiments (Fig. 2A). For each interval of variation between stimuli, the difference in female choices between dominant frequency and inter-call interval was not significant (Fisher's exact test, P>0.15 in all cases). The latency to choose was also not statistically different for preferences for dominant frequency versus inter-call interval between the first three experiments in each larger set of experiments. However, Fig. 2B shows a gradation in the average choice duration in both characters (that is short when the interval of variation between stimuli is high), and the slopes are very similar. In addition, we found a
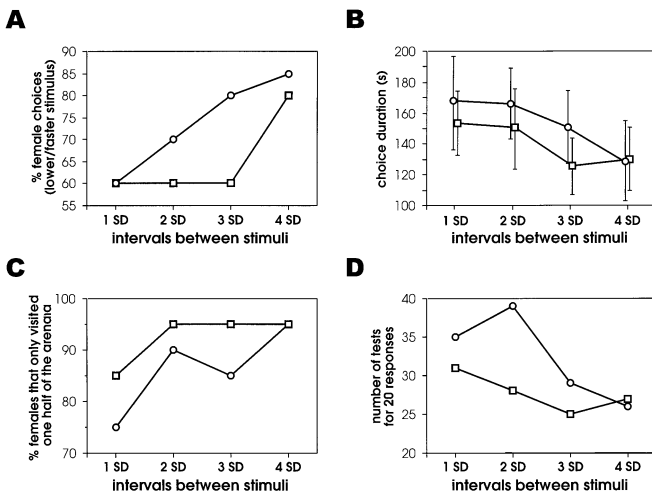
Table 2 Results of phonotaxis experiments for both call properties studied

Difference between stimuli	Number female choices	P (binomial, one-tailed)
Dominant frequency	Low:high	
1 SD	12:8	0.252
2 SD	12:8	0.252
3 SD	12:8	0.252
4 SD	16:4	0.006
Inter-call interval	Fast:slow	
1 SD	12:8	0.252
2 SD	14:6	0.058
3 SD	16:4	0.006
4 SD	17:3	0.001

Table 3 Time taken to make the choice, proportion of females that visit one/two halves of the arena, and number of tests necessary to obtain 20 valid responses, for both call properties studied

Difference between stimuli	Mean (SD, range) choice duration (s)	Number of females that visited one/two halves of the arena[a]	Number of tests for 20 responses
Dominant frequency			
1 SD	168.2 (135.2, 43–598)	15/5, $P=0.021$	35
2 SD	165.9 (102.7, 40–373)	18/2, $P<0.001$	39
3 SD	150.6 (106.6, 37–516)	17/3, $P=0.001$	29
4 SD	128.7 (115.9, 27–436)	19/1, $P<0.001$	26
Inter-call interval			
1 SD	153.5 (94.5, 49–357)	17/3, $P=0.001$	31
2 SD	150.4 (116.9, 41–466)	19/1, $P<0.001$	28
3 SD	125.5 (81.4, 16–314)	19/1, $P<0.001$	25
4 SD	130.2 (92.8, 20–347)	19/1, $P<0.001$	27

[a] P-values from two-tailed binomial tests

Fig. 2A–D Results of the phonotaxis experiments for both call properties studied in increasing units of variation between stimuli (*squares* initial frequency, *circles* inter-call interval). **A** Proportion of female choices for the lower or faster stimulus. **B** Time taken to make the choice (mean±SE). **C** Proportion of females that only visited one half of the arena. **D** Number of tests necessary to get 20 valid responses

Table 4 Differences between both characters for number of female choices and number of females that visited only one half of the arena, for every difference between stimuli

Difference between stimuli	Female choices dominant frequency inter-call interval	Number of females that visited only one half of the arena
1 SD	12:8/12:8 $P=0.626$	15:5/17:3 $P=0.347$
2 SD	12:8/14:6 $P=0.371$	18:2/19:1 $P=0.5$
3 SD	12:8/16:4 $P=0.150$	17:3/19:1 $P=0.303$
4 SD	16:4/17:3 $P=0.5$	19:1/19:1 $P=0.756$

gradation in the average choice duration in both characters [Jonckheere test for ordered alternatives (Siegel and Castellan 1988, p. 216): dominant frequency, $J^*=3.388$, $P<0.001$; inter-call interval, $J^*=2.149$, $P<0.05$]

In general, the proportion of unresponsive females, as estimated by the number of tests necessary to accumulate 20 valid responses, tended to be higher in experiments that varied dominant frequency compared to those that varied inter-call interval, but the differences were not statistically significant ($\chi^2=6.16$, $P=0.104$). The number of females that visited only one half of the arena during the tests was higher in response to variation in inter-call interval than in response to variation in dominant frequency, although not significantly so (Fisher's exact test, $P>0.303$ in all cases).

Discussion

In general, female túngara frogs tend to exhibit weak preferences for dominant frequency, a relatively less variable call character, and stronger directional preferences for inter-call interval, a relatively more variable call character. For dominant frequency, however, this is true only for smaller intervals of variation between stimuli: when the difference between stimuli is greater (4 SD in this study), females exert directional selection on dominant frequency. Similar results have been found in other studies. There was a strong preference for lower-frequency calls in *Hyla ebraccata* when the difference between stimuli was less than 2 SD of the study population (Wollerman 1998); comparable preference patterns were found in *Alytes* (Márquez 1995). Castellano and Giacoma (1998) found weak preference for lower-frequency calls in *Bufo viridis* when the alternatives were a medium-versus a higher-frequency call, but there was a strong preference for medium-frequency calls when the alternative stimulus was lower than the minimum value observed in the population. Their results together with those of Márquez and Bosch (1997) indicate that selection for low frequencies in the species they tested is not open ended.

As expected, the female response is stronger when the difference among broadcast stimuli is greater. We did not determine if the amount of the difference between stimuli that results in a preference is dependent on the pair of stimuli being tested or if it can be extrapolated to any pair of stimuli; in the absence of data show-

ing otherwise, we would assume the former. We caution, however, that static versus dynamic is a categorical classification of continuous variation. Furthermore, one cannot assume signal function from signal variation; for example, dynamic properties are important in evaluating some aspects of male quality. For example, variation between the properties of the whine and chuck does not show large differences across the range of the túngara frog (Ryan et al. 1996). The whine is both necessary and sufficient to elicit phonotaxis responses from females, while the chuck does not elicit responses if not accompanied by the whine.

Although Ryan (1985) found no relationship between body size and initial frequency of the whine, in our study population, the relationship is statistically significant ($n=46$, $F_{1,44}=6.057$, $P=0.018$; M.J. Ryan and A.S. Rand, unpublished data), but the coefficient of determination ($r^2=0.30$) indicates very low predictability for the size-frequency correlation. This female preference might result in a preference for larger males when, and only when, size differences are very large or beyond some threshold. Ryan (1985) showed that female preference for larger males in the wild might be favored by direct selection. Females are larger than males and the smaller the size difference in a pair, the more eggs are fertilized; choosing larger males usually reduces this size difference. Ryan et al. (1990) argued that the preference for lower-frequency chucks, which contributes to female preference for larger males, might be adaptive but did not evolve as an adaptation in túngara frogs. The properties of the auditory system that guide females to lower-frequency chucks is present in species in which chucks are not produced (see also Ryan 1999).The preference for lower-frequency whines could possibly also contribute to the female's bias of mating with larger males. If preference for lower-frequency whines is widespread within these frogs or is unique to the túngara frog is not yet known.

The relationship between signal variation and signal preference is an intriguing one. Here we have shown that patterns of acoustic preference in túngara frogs are similar to the findings by Gerhardt (1991, 1994) and others in different species of frog. We resist a functional or adaptive interpretation here, however. To fully understand these patterns of preference versus signal variation, one must address not only how mating preferences might generate selection on more or less variable traits, but also how the nature of the traits (e.g., spectral versus temporal) interact with the sensory system of the receiver, the biomechanical and physiological constraints on signal evolution, and something of the underlying genetic and environmental factors that might influence signal variation.

References

Castellano S, Giacoma C (1998) Stabilizing and directional female choice for male calls in the European green toad. Anim Behav 56:275–287

Gerhardt HC (1991) Female mate choice in treefrogs: static and dynamic acoustic criteria. Anim Behav 42:615–636

Gerhardt HC (1994) The evolution of vocalization in frogs and toads. Annu Rev Ecol Syst 25:293–324

Márquez R (1995) Female choice in the midwife toads (*Alytes obstetricans* and *A. cisternasii*). Behaviour 132:151–161

Márquez R, Bosch J (1997) Female preference in complex acoustical environments in the midwife toads *Alytes obstetricans* and *Alytes cisternasii*. Behav Ecol 8:588–594

Morris MR, Yoon SL (1989) A mechanism for female choice of large males in the treefrog *Hyla chrysoscelis*. Behav Ecol Sociobiol 25:65–71

Rand AS, Ryan MJ (1981) The adaptive significance of a complex vocal repertoire in a neotropical frog. Z Tierpsychol 57:209–214

Rand AS, Ryan MJ, Wilczynski W (1992) Signal redundancy and receiver permissiveness in acoustic mate recognition by the túngara frog, *Physalaemus pustulosus*. Am Zool 32:81–90

Ryan MJ (1980) Female mate choice in a neotropical frog. Science 209:523–525

Ryan MJ (1983) Sexual selection and communication in a neotropical frog, *Physalaemus pustulosus*. Evolution 37:261–272

Ryan MJ (1985) The túngara frog. University of Chicago Press, Chicago

Ryan MJ (1999) Sexual selection and sensory exploitation. Science 283:1083a

Ryan MJ, Rand AS (1990) The sensory basis of sexual selection for complex calls in the Túngara frog, *Physalaemus pustulosus* (sexual selection for sensory exploitation). Evolution 44:305–314

Ryan MJ, Fox JH, Wilczynski W, Rand AS (1990) Sexual selection for sensory exploitation in the frog *Physalaemus pustulosus*. Nature 343:66–67

Ryan MJ, Rand AS, Weight LA (1996) Allozyme and advertisement call variation in the túngara frog, *Physalaemus pustulosus*. Evolution 50:2435–2453

Siegel S, Castellan Jr NJ (1988) Nonparametric statistics for the behavioral sciences. McGraw-Hill, New York

Tuttle MD, Ryan MJ (1981) Bat predation and the evolution of frog vocalizations in the Neotropics. Science 214:677–678

Wilczynski W, Rand AS, Ryan MJ (1995) The processing of spectral cues by the call analysis system of the túngara frog, *Physalaemus pustulosus*. Anim Behav 49:911–929

Wollerman L (1998) Stabilizing and directional preferences of female *Hyla ebraccata* for calls differing in static properties. Anim Behav 55:1619–1630