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Acoustic Competition in *Physalaemus pustulosus*, a Differential Response to Calls of Relative Frequency

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Abstract

The response of the male túngara frog (*Physalaemus pustulosus*) to conspecific whines of different frequencies was examined. In the first series of playback experiments (fixed frequency), three types of synthetic stimuli were used, corresponding to calls of high frequency (HFF; $\bar{x} + 2\,\mathrm{SD}$), mid-frequency (MFF; $\bar{x}$), and low frequency (LFF; $\bar{x} - 2\,\mathrm{SD}$) for the study population. In the second series of interactive playback experiments (relative frequency), whines of frequency relative to that of the male subject were used: male frequency + 2 SD (HRF), male frequency (MRF), and male frequency −2 SD (LRF). In the fixed frequency experiments, male vocal response did not vary among treatments. However, in the relative frequency experiments, males responded with more whines, and above all, with more chucks, to stimuli of similar or higher frequency than to stimuli of lower frequency than their own. In other words, male vocal competition escalates when competitors have whines with similar or relatively higher frequencies to their own, but does not increase when competitors have call frequencies that are at the mean or higher for the population. This differential response might result from competition between males of different sizes, since the frequency of the whine and male size is significantly correlated.

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Introduction

Sexual selection and communication in anurans has been studied in terms of female choice (e.g. Ryan 1980, 1983, 1985; Ryan & Rand 1990; Gerhardt 1991) and male–male competition (e.g. Schwartz 1989; Wagner 1989a, b; Wells & Taigen 1989). There are many studies of male–female interaction in sexual communication

in anurans; however, an understanding of the equally important interactions in male–male communication is less complete. Several effects of male–male acoustic competition in anurans have been described (see Gerhardt & Schwartz 1995). The most common response of males to other competing males is an increase in their call rate (e.g. Wagner 1989a; Bosch & Márquez 1996). This increase can be pronounced by up to 43% in *Alytes cisternasii*, for example (Bosch & Márquez 1996), and incurs an increase in the energetic cost of calling (see Pough et al. 1992). In addition, in some species a higher call rate increases the risk of being located by any potential acoustically oriented predator (e.g. Tuttle & Ryan 1981). In several species of anurans, a graded increase in call rate has been reported (e.g. Wagner 1989a; Bosch & Márquez 1996). Males respond with a greater increase in call repetition rate to competing males with a high repetition rate, than to competitors with a low repetition rate. In other species, male–male competition produces an increase in call complexity (e.g. Rand & Ryan 1981; Ramer et al. 1983) sometimes associated with escalating aggressive behaviour. In these species, females are usually preferentially attracted to complex calls (e.g. Rand & Ryan 1981). On the other hand, in a few species of anurans, a change in the dominant frequency of calls has been reported in the presence of competing males (Lopez et al. 1988; Wagner 1989a, b; Grafe 1995). In some species of anurans, males respond differently to calls with different frequencies (e.g. Ramer et al. 1983; Wagner 1989b; Bosch & Márquez 1996). Males may perceive the attractiveness to females of competing males because in a number of anuran species, male size is significantly correlated with the dominant frequency of the advertisement call, and females are attracted to larger males. In addition, in some species, males of different sizes respond differently to competing males (e.g. Ramer et al. 1983; Green 1990).

The túngara frog, *Physalemus pustulosus* is a small member of the family Leptodactylidae. It has a prolonged breeding period, and males often advertise from large choruses, but they are not territorial and do not defend resources. The advertisement call of *P. pustulosus* has two components: the whine and the chuck. The whine is always present and is followed by zero to six chucks. Male–male competition produces an increase in call complexity through the addition of chucks, and females prefer more complex calls (Rand & Ryan 1981). Larger males have greater mating success (Ryan 1983). There is a significant negative correlation between male size and the fundamental frequency of the chuck (Ryan 1980), although the predictability of a male's size based on the frequency of his chuck is low. The frequency structure of the chuck influences female phonotactic preferences, and females select larger males for mates, as determined by the frequency of the chuck (Ryan 1983, 1985; Wilczynski et al. 1995). However, the whine does not appear to contain any information about male body size (Ryan 1985). Ryan (1985) found that there were no significant differences in male calling behaviour in response to stimuli with different chuck frequencies. Ryan (1985) concluded that although in this species the frequency of the chuck influences female behaviour, it does not appear to influence male behaviour.

The main purposes of this study are to determine whether whine frequency is dynamically involved in male–male acoustic competition. Additionally we will

analyse whether male response depends on the absolute or relative whine frequency.

Methods

We collected male túngara frogs near the facilities of the Smithsonian Tropical Research Institute in Gamboa, Republic of Panama, during July and Aug. 1997. We maintained males under a natural light–dark cycle and temperature regime in plastic bags with water. When a male concluded all tests, or after 3–4 d, he was given a unique toe-clip to allow future identification and was returned to his capture site. Prior to testing each night, between 19.00 h and 05.00 h, each male was placed in a plastic bag inside a testing chamber. These chambers (4.7 cm × 7.1 cm × 4.7 cm) are acoustically isolated, and each chamber contains a small, wide-frequency range speaker, and a Radio Shack miniature microphone. When a male called, his call was digitized at a sampling frequency of 44.1 kHz and 16 bit resolution, using Canary 1.2 software and an Apple PowerBook 1400 cs computer. Signalyze 3.12 software was used to obtain the initial dominant frequency of the whine, through fast Fourier transform (FFT) (width 1024 points, frequency resolution 22 Hz).

The characteristics of the distribution of male advertisement calls in this population are known (Ryan & Rand, unpubl. data). The stimuli were whines synthesized using a program supplied by J. Schwartz (University of Missouri; sample rate 20000 Hz and 8 bit) of average duration (323 ms, n = 51) and average call rate (inter-call interval 1992 ms, n = 32) for this population. Rise time (42 ms) and fall time (281 ms) were linear. Only the fundamental frequency sweep of the whine was used. As phonotaxis experiments have shown, upper harmonics did not influence female behaviour (Wilczynski et al. 1995). The control used was a sequence of white noise with an average duration, call rate and amplitude envelope similar to the synthetic whine. In each experiment the control was presented twice, once before and once after presentation of the experimental stimuli. Each stimulus presentation was separated by 1 min of silence. That is, each experiment consisted of control 1 (1 min), silence 1 (1 min), experiment (1 min), silence 2 (1 min) and control 2 (1 min) (see also Ryan & Rand 1998). Stimuli were broadcast directly from the audio output of an Apple PowerBook 1400 cs computer. Stimuli were broadcast in a random order at an amplitude of 80 dB SPL at 0.5 m, measured by a GenRad sound pressure level meter (model 1982). The response of the male and the stimuli were recorded with a Sony WM D6 cassette recorder.

In the first series of playback experiments (fixed frequency), three types of synthetic stimuli were used. These stimuli corresponded to calls of high initial frequency (HFF, $\bar{x} + 2\,SD$: 1116 Hz), mid-initial frequency (MFF, $\bar{x}$: 1000 Hz), and low initial frequency (LFF, $\bar{x} - 2\,SD$: 884 Hz) for the study population (initial dominant frequency: n = 51, $\bar{x}$ = 999.7, SD = 57.8). In the second series of play-back experiments (relative frequency), whines with initial frequencies relative to that of the male subject were used: male initial frequency + 2 SD (HRF), male initial frequency (MRF), and male initial frequency − 2 SD (LRF). The final frequency was calculated in every case in the same way as the initial frequency

$- 529\,Hz$ (the average change in dominant frequency for the study population). The sample size in each experiment was 20. In each period of each experiment, the number of whines and chucks produced by the male was counted. Call complexity was calculated as the number of chucks per call and the most complex call of each period was noted. The responses to the two controls were averaged, and the ratio of experiment/(average control + 1) was calculated. The differences between experiments, after testing for normality, were analysed with an analysis of variance test with Fisher's post hoc tests. We did not use a repeated measures ANOVA because different males were used in each treatment.

Results

Male calling behaviour is completely usual in plastic bags. Acoustical distortion is minimal through the bags.

The results of playback experiments are shown in Fig. 1. In the fixed frequency experiments, no modification of male vocal response was observed. The number of whines and chucks (control-corrected) were not statistically different across the three levels of frequencies used (Whines: $F_{2,57} = 0.711$, p = 0.4953; Chucks: $F_{2,57} = 1.666$, p = 0.1980). Additionally, the maximum levels of complexity (control-corrected) were not different between frequencies ($F_{2,57} = 0.929$, p = 0.4008).
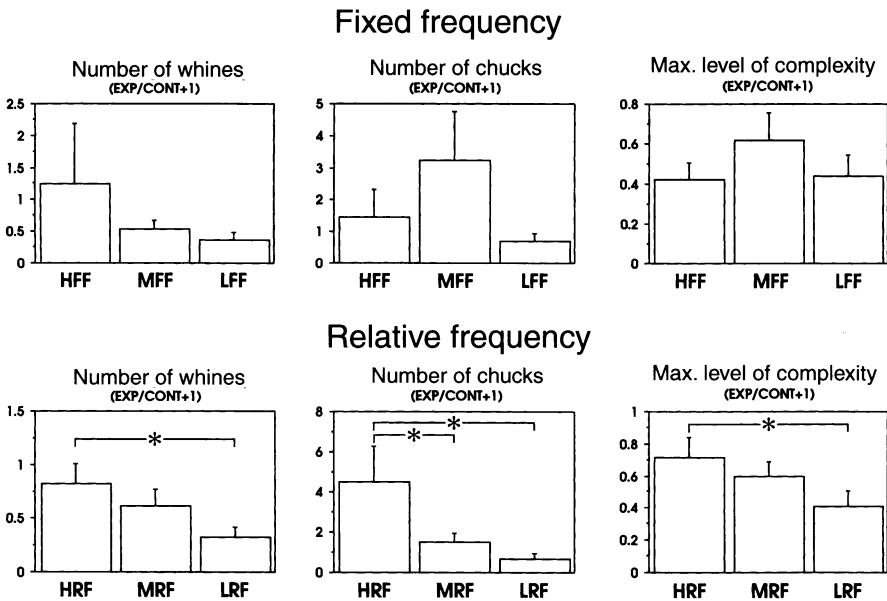

Fig. 1: Results in both fixed and relative frequency playback experiments: number of whines and chucks, and maximum levels of complexity (control-corrected). Bars show $\bar{x} \pm$ SE. HFF, high fixed frequency; MFF, middle fixed frequency; LFF, low fixed frequency; HRF, high relative frequency; MRF, middle relative frequency; LRF, low relative frequency. *P < 0.05.

In contrast, in the relative frequency experiments, males responded with more whines ($F_{2,57} = 2.911$, p = 0.0626; HRF vs. LRF, p = 0.0197) to stimuli of higher frequency than to those of lower frequency than their own. In addition, the number of chucks was different in response to stimuli of high frequency vs. stimuli of similar or lower frequency than their own ($F_{2,57} = 3.736$, p = 0.0299; HRF vs. MRF, p = 0.0460; HRF vs. LRF, p = 0.0120). The maximum level of complexity was only different between stimuli of higher frequency and stimuli of lower frequency than their own ($F_{2,57} = 2.131$, p = 0.1280; HRF vs. LRF, p = 0.0451). In other words, male vocal competition escalates when competitors have similar or relatively higher whine frequencies to their own, but does not increase when competitors have call frequencies that are at the mean or higher frequency for the population as a whole. In the fixed frequency experiments the average difference between male frequency and stimuli frequency was 131 Hz in HFF, 9 Hz in MFF, and 116 Hz in LFF. The maximum difference between male initial frequency and stimuli initial frequency was 307 Hz in HFF and 320 Hz in LFF. In the relative frequency experiments the average difference between male initial frequency and stimuli initial frequency was 116 Hz in HRF (2 SD of initial frequency of the whine), 0 Hz in MRF, and 116 Hz in LRF. Only in the LRF experiment was male size significantly and negatively correlated with male response, although the correlation coefficient was low (number of whines: $F_{1,18} = 4.605$, p = 0.0458, r = 0.451; number of chucks: $F_{1,18} = 12.494$, p = 0.0024, r = 0.64; maximum level of complexity: $F_{1,18} = 4.805$, p = 0.0418, r = 0.459). Thus there is some indication of size-mediated responses in these frogs.

Discussion

The results show that vocal competition in the male túngara frog varies among different whine frequencies when those differences are ± 2 SD relative to the focal male's call but not when they are ± 2 SD relative to the population mean. The difference in response found between the two experimental series cannot be explained by the absolute difference between the frequency of the stimulus and the frequency of the male's call, since the mean difference is very similar in both types of experiment. In the experiments with fixed frequency, the males emit a greater number of vocalizations in response to higher frequency calls, but the increment in complexity for the addition of chucks is higher for calls of mean frequency. In the relative frequency experiments, males respond with both more calls and more complex calls, to the relatively higher frequency.

In other anuran species (see Bosch & Márquez 1996), in contrast to the observations made here, males alter their vocalizations in response to lower rather than higher frequencies. This also occurs in túngara frogs when the stimulus is a complex call, i.e. whine plus chucks, rather than the simple whine used in these experiments. Males responded with more chucks to stimuli of low frequency chucks than to stimuli of high frequency chucks (Bosch, Ryan & Rand, unpubl. data).

In all of the anuran species studied, female preference for high rates of call repetition is much more marked than female preference for calls of lower frequency.

Nevertheless, the interaction between female preference for both characteristics is not well understood. In many frog species, the advertisement call does not vary in complexity. Thus calls have no secondary notes that increase their attractiveness to females, and the primary means to increase call attractiveness is by increasing the call repetition rate.

In these situations, acoustic competition is more likely to escalate when a male is confronted with competitors with more attractive calls (i.e. lower frequency calls). The only possible response a smaller male can give to increase his attractiveness would be to increase the rate of repetition. In contrast, in species with complex calls in which female preference for more complex calls is stronger than their preference for low frequency calls, one would expect a greater response to smaller competitors, if in addition, there exists a positive relationship between male size and call complexity. Green (1990) found that larger males produce more complex calls, although the same result was not found by Ryan (1985).

For the study population there is a significant relationship between body size and whine frequency (n = 46, $F_{1,44}$ = 6.057, p = 0.0178), but the predictability of body size from frequency is very low (r^2 = 0.3). In the case of the chuck, the relationship between body size and frequency is stronger (n = 47, $F_{1,45}$ = 7.273, p = 0.0098, r^2 = 0.4; Ryan & Rand, unpubl. data; see also Ryan 1980, 1985).

If males infer the size of the competitor by the frequency of the whine in the absence of the chuck, there are two reasons why it might be beneficial for the male to increase the cost of call production and the risk of predation in the face of smaller competitors who are calling without chucks. First, the calls of larger males would be preferred by females over the calls of smaller competitors because of female preference for calls of lower frequency. Even though the preference of females for low frequency calls is not very pronounced in *P. pustulosus*, females prefer both low frequency whines and low frequency chucks, although the preference is stronger for low frequency chucks (Bosch, Ryan & Rand, unpubl. data). Secondly, and perhaps more importantly, smaller competitors invest less in call production (both in rate of emission and complexity, Green 1990), thus larger males would have an advantage over them.

The negative relationship observed between the size of the male and the rate and complexity of its response to calls of a lower frequency calls than his own, is also consistent with this explanation. The test animal invested more energy in acoustic competition when the difference in frequency between the call of the test animal and the call of the larger competitor was greater.

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