Frequency Modulated Calls and Species Recognition in a Neotropical Frog

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Summary. The neotropical frog *Physalaemus pustulosus* (Leptodactylidae) has a complex advertisement call and different call components perform different functions. The whine is a necessary and sufficient stimulus for species recognition. The chuck provides information about male body size that is used by females in mate choice (Ryan 1980, 1983), but the chuck must be combined with the species-identifying whine to elicit maximum behavioral responses from males and females. One of the important features of the whine in eliciting behavioral responses from both sexes is the direction of frequency modulation. This suggests that current models of species recognition in anurans based on a frequency filtering mechanism of the peripheral auditory system and selective responses to combinations of frequencies in the central nervous system are not sufficient to explain species recognition in *P. pustulosus*. Recent neurophysiological studies of the anuran torus semicircularis are discussed in terms of a mechanism for decoding frequency sweeps.

Introduction

Anurans produce a variety of calls, most of which function in social interactions among individuals at the breeding site (Bogert 1960). With few exceptions males produce a call that contains species-specific information, and this call usually functions in both male-male interactions and attracting females. This call has been termed the advertisement call (Wells 1977). Although the temporal properties of the call often are important for species recognition (e.g. Littlejohn et al. 1960; Ger-

hardt 1974), many frogs seem to rely primarily on spectral properties of the call (Capranica 1976). This probably is accomplished, at least in part, by selective tuning of the peripheral auditory system and selective responses to combinations of frequencies in the central nervous system (Capranica 1976). Neurophysiological studies of the anuran peripheral auditory system (e.g. Frishkopf et al. 1968; Loftus Hills 1971) and central auditory pathways (e.g. Mudry et al. 1977) show that the auditory system is most sensitive to those frequencies with peak energy concentrations in the species' advertisement call.

I investigated properties of the advertisement call of *Physalaemus pustulosus* (Leptodactylidae) that are important in eliciting vocal responses from males and phonotaxis from females. This frog is unusual in having a call of varying complexity, consisting of a whine followed by 0–6 chucks (Ryan 1980, 1983; Rand and Ryan 1981; Fig. 1). Females are attracted to speakers broadcasting calls of any complexity, but when given a choice they prefer complex calls (i.e. calls with chucks; Rand and Ryan 1981). Females can use the information in the chuck to select larger males as mates (Ryan 1980, 1983). Isolated males produce the simple call (whine-only). Although females are attracted preferentially to complex calls, males only increase call complexity in response to vocal competition from other males, or when approached by another frog of either sex. This seems to be due to the counter-selective force of predation. Complex calls are more likely to attract females but they also increase the male's probability of being located by the frog-eating bat *Trachops cirrhosus* (Ryan et al. 1982).

The above studies suggest that the chuck functions in providing individual-specific information about the male, that is, information about his body

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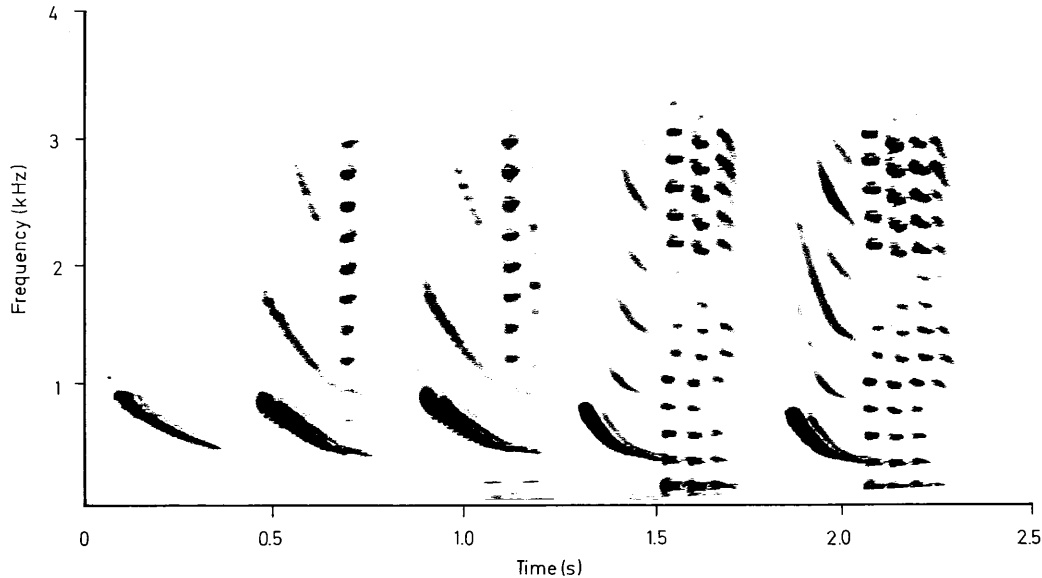
Fig. 1. A sonographic representation of the complexity series of the *Physalaemus pustulosus* advertisement call. The call contains a whine and 0, 1, 2, 3, or 4 chucks (*from left to right*). All calls are not from the same individual. Calls were recorded on Barro Colorado Island, Panama, with a Nagra IV-D tape recorder and a Sennheiser MKH 104 microphone. Air temperatures were between 24 and 26 °C. Calls were analyzed with a Kay Sonograph model 7019 A using narrow band filter

size. Since the whine alone elicits a response from females, this call component probably contains information necessary for species recognition. The whine is typical of the calls of many species of the subfamily Leptodactylinae in that it is highly frequency modulated (FM). These calls typically sweep over a frequency range of >400 Hz in >100 ms; they sweep in either direction depending on the species (Barrio 1965; Martin 1972; Straughn and Heyer 1976). Although there is a large number of FM frogs (see sonograms in Barrio 1965; Straughn and Heyer 1976; Passmore and Caruthers 1979), the importance of FM signals in species recognition has not been investigated. The purpose of this study is first to investigate the role of each call component in species recognition, and then to explore the significance of the whine's sweep in this context.

Materials and Methods

The responses of male and female *Physalaemus pustulosus* to various stimuli were determined. Both natural *P. pustulosus* calls and synthesized tones of constant frequencies were used as stimuli. These stimuli were 400 ms in duration, as is the natural whine. The chuck-only consisted of a chuck dissected from a natural call. To produce a chuck-whine, a chuck was spliced immediately in front of a whine with which it had occurred naturally. Reversed calls were natural calls played backwards. They contained the same frequencies at the same relative amplitudes as the natural calls but showed FM sweeps of reversed order (Fig. 1). All stimuli were broadcast with a Nagra IV-D tape recorder and a small extension speaker at an intensity of 75 dB SPL at the position of the frog (which was 75 cm

from the speaker), approximately the normal intensity of a call produced by a male at this distance.

I compared the ability of different stimuli to elicit a vocal response from a male. Stimuli were tested in pairs, and the responses of the male to each of the stimuli comprising the pair were compared. Table 1 shows the stimulus pairs that were tested.

A male was presented with one of the stimuli from a pair for 30 s, at a rate of 1 stimulus/2.3 s (about the normal calling rate). The male's response was recorded simultaneously with a Nagra IV-D tape recorder and a Sennheiser MKH 815 T microphone. After several minutes, the male was then tested with the second stimulus of the pair, and his response was recorded in the same manner. Each male was tested only once with each stimulus pair, and the stimulus of each pair that was presented first was alternated among males.

Several parameters of the male's response were analyzed: time to first call, time to first chuck, total number of calls, total number of chucks. For each stimulus pair, the responses to each of the stimuli were compared with a Wilcoxon matched-pairs signed-ranks test (Siegel 1956).

The response of a female was determined in a paired-choice test. A female was placed under a small cage in the center of an octagonal arena. The arena had burlap walls around its perimeter. The speakers were outside the burlap wall and faced toward the center; they were opposite each other and 1.5 m apart. Stimuli were presented alternately at a rate of 1 stimulus/2.3 s. The cage was then lifted from the female and her response was recorded. A response was noted if a female contacted the wall within 12 cm of a speaker. Female preference was tested with a two-tailed exact binomial probability test (Sokal and Rohlf 1969). See Ryan (1980) for further details of the female choice tests.

Results

In only one of the four response categories tested (i.e. time to first call, time to first chuck, total

Table 1. Evoked vocal responses of male *Physalaemus pustulosus*. Male discrimination among stimuli was tested by comparing 4 parameters of a male's response between each stimulus of a stimulus pair with a Wilcoxon matched-pairs signed-ranks test. ( ) indicates the number of ties in each analysis. # indicates that the sample size was too small for statistical analysis due to the number of ties. * indicates the stimulus that more effectively elicited a vocal response

Stimulus vs	Stimulus	Frogs tested	Probabilities of no discrimination based on			
			Time to		Number	
			first call	first chuck	of calls	of chucks
Whine-chuck*	Chuck-only	20	0.0324 (3)	0.0784 (2)	0.0070 (2)	0.0226 (3)
Whine-chuck	Chuck-whine	10	0.6744 (4)	0.4654 (4)	0.4066 (1)	0.3174 (4)
Whine*	Reversed whine	10	0.0128 (1)	0.0128 (1)	0.0070 (0)	0.0150 (1)
Whine-chuck*	Reversed whine-chuck	10	# (6)	0.1416 (4)	0.0286 (0)	0.0324 (0)

Table 2. Responses of female *Physalaemus pustulosus* in paired-choice tests. The probability of a random response was tested with a two-tailed exact binomial probability test

Stimulus vs (no. of responses)	Stimulus (no. of responses)	P
Whine-chuck (5)	Chuck-whine (5)	0.6230
Whine (9)	Reversed whine (1)	0.0214
Whine (9)	500 Hz (1)	0.0214
Whine (9)	700 Hz (1)	0.0214
Whine (12)	900 Hz (3)	0.0768
Whine-chuck (8) (70 dB SPL)	Whine (1) (75 dB SPL)	0.0390

number of calls, total number of chucks), did a chuck-only evoke a response from males as well as the whine-chuck (Table 1). The order of the call components was not important for eliciting vocal responses from males; males responded as well to a whine-chuck as they did to a chuck-whine. The direction of the frequency sweep did affect a male's response. Males responded sooner and more frequently to a whine than to a reversed whine. Similar results were obtained when a whine-chuck was paired with a reversed whine-chuck (Table 1).

The results of the female choice test paralleled those of the male test (Table 2). Again, the order of the components was not important in eliciting a response. Females did not discriminate between a whine-chuck and a chuck-whine. As with the males, females also preferred a whine to a reversed whine, and furthermore, preferred a whine to tones of constant frequency (500 Hz, 700 Hz) that were within the frequency range of, and the same duration as, the whine. Females did not significantly prefer the whine to the 900 Hz tone, but the bias was in the same direction as the response to the 500 Hz and 700 Hz tones.

Rand and Ryan (1981) previously showed that females were not attracted by a chuck-only. They also showed that females preferred a whine-chuck

to a whine when the calls were played at the same intensity. I duplicated this latter test, but the whine was played at a greater intensity: 75 dB SPL vs 70 dB SPL at the test animal (75 cm from the speaker). The females still preferred the whine-chuck (Table 2).

Discussion

Function of Advertisement Call Components

The above results demonstrate that different components of the *P. pustulosus* advertisement call perform different functions. (Here, 'function' refers to current effect and makes no assumptions about the historic roles of natural and sexual selection on the evolution of the call.) The chuck alone, which does not occur in nature, did not elicit a significant vocal response from males (when compared to a natural call). Rand and Ryan (1981) previously showed that this call component by itself does not attract females. The whine-only, however, is a necessary and sufficient stimulus to elicit responses from males and females. I also have further substantiated Rand and Ryan's (1981) results that females prefer a whine-chuck to a whine-only, and I have shown this is true even if the whine is more intense.

This study indicates that the whine is more effective in eliciting vocal responses from males and phonotaxis from females than is a chuck-only. The whine contains information necessary for species recognition. The chuck alone probably does not identify the species. The chuck does contain information about male body size that may be utilized by the female in conspecific mate choice (Ryan 1980, 1983), but the chuck must be combined with the whine to be biologically meaningful. A chuck-whine and a whine-chuck are equally effective in eliciting responses from both sexes. Thus the order of the components is not important for species rec-

ognition. Morphological constraints of the vocal apparatus of *P. pustulosus* might dictate the order of the call components, at least to the extent that the chuck must overlap the lower frequency end of the whine (Drewry et al. 1982).

Narins and Capranica (1978) showed that different notes also perform different functions in the Puerto Rican treefrog, *Eleutherodactylus coqui*. In this species the 'co' functions in male-male interactions while the 'qui' attracts females. They also showed that note order was not important. Narins and Capranica suggest that different call notes performing different functions might be a general phenomenon. I suggest that this will become even more apparent with increased studies of tropical anurans, which tend to have more complex vocal repertoires than their temperate zone counterparts.

Behavioral Sensitivity
to Frequency Sweep Direction

An obvious characteristic of the whine is the extreme frequency sweep, beginning at about 1,000 Hz and sweeping to 400 Hz in 400 ms. The female choice tests showed that the FM signal more effectively elicited phonotaxis than some single frequencies that are in the whine's frequency range. The evoked vocal responses of males, and the female choice tests both indicate that the frequency-amplitude profile of the call is not the only important cue for species recognition. The frequency sweep of the whine must be in the proper direction to best elicit a response.

My study was not designed to investigate the precise characteristics of the sweep, other than sweep direction, used in species recognition. There probably are several; certainly the frequency tuning of the peripheral auditory system places strict limitations on the frequency range of the whine. This study does show that the direction of the sweep is important, and suggests that a simple frequency filtering mechanism is not a sufficient model to account for species recognition in *P. pustulosus*, as it appears to be for some species. This does not exclude the possibility of FM signals being decoded in the periphery. For example, two-tone suppression potentially could code frequency sweeps, although this would be more likely for signals sweeping from lower to higher frequencies since, when there is a suppression tone, it is on the high frequency side of the fiber's best frequency (Capranica and Moffat 1980). However, two-tone suppression only has been investigated with simultaneous presentation of tones. The response of units to inhibitory and excitatory tones presented

sequentially has not been documented. Also, it should be noted that only the fibers from the amphibian papilla which are tuned to low frequencies (usually < 500 Hz) seem to exhibit two-tone suppression. Given the spectral properties of the advertisement calls of most frogs, which often do not contain frequencies below 500 Hz, these fibers might not function in advertisement call recognition.

Recent studies of the torus semicircularis by Narins (1976; in press), Walkowiak (1980), and Fuzessery and Feng (1982) are interesting in regards to decoding of frequency sweep direction. The 'qui' portion of the *E. coqui* call is a frequency sweep (Narins and Capranica 1978). Although Narins (1976) did not investigate the behavioral sensitivity of the frog to sweep direction, he does show that in a small number (6) of cells in the torus semicircularis the response to upward and downward sweeps can be differentiated by changes in the firing pattern, latency, and the number of spikes (Narins, in press). Walkowiak (1980), and Fuzessery and Feng (1982) characterized the response properties of single units in the torus of fire-bellied toads, grass frogs and leopard frogs. When certain combinations of tones were presented simultaneously, a large proportion of the neurons exhibited two-tone suppression. Interestingly, some neurons were inhibited by tones of lower frequency than their best excitatory frequency. In auditory nerve fibers of the frog, inhibitory tones have higher frequencies than the best excitatory frequencies of the units that they inhibit. This suggests that low-frequency inhibition in the torus semicircularis is the result of neuronal interactions. The results of Walkowiak, and Fuzessery and Feng suggest a mechanism for the decoding of frequency sweep direction that in some ways is similar to the mechanism in the inferior colliculus that has been proposed for bats (Suga and Schlegel 1973; Suga 1978). However, the responses of units to combinations of tones presented sequentially, as opposed to simultaneously, in the auditory nerve fiber and in areas of acoustic processing in the central nervous system must be investigated. Only then can a neural mechanism for decoding frequency sweep direction seriously be proposed.

Frishkopf et al. (1968) suggested that an anuran 'mating call detector' in the central nervous system would show a selective response to a combination of those frequencies which best excited the amphibian and basilar papillae. Studies of anuran acoustic processing have emphasized such combination tone selectivity (e.g. Mudry et al.

1977). However, a review of the calls of the sympatric species of *Physalaemus* in Argentina (Barrio 1965) suggests that recognition of subtle differences in the form of the FM sweep also might be necessary for species discrimination among sympatric congenerics. Sensitivity to a variety of characteristics of FM calls probably will prove an important aspect of decoding of acoustic signals for many species of anurans.

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