Call Answering and Male Phonotaxis in the Gray Treefrog, *Hyla versicolor*

BIOS 569 - Practicum in Aquatic Biology

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Introduction

Male gray treefrogs, *Hyla versicolor*, like many anurans, rely upon calling as the basis of their courtship behavior. The call characteristics preferred by females have been well defined; females prefer values of static properties (dominant frequency and pulse rate) at or near the mean value of the natural chorus and supernormal values of dynamic properties such as call rate, pulse number and call duration (Gerhardt, 1991; Gerhardt, 1995.) In fact, preferred values of dynamic properties may exceed those observed in the natural chorus (Gerhardt, 1987.) Female selection is non-arbitrary; preferred call characteristics have been shown to be correlated with evolutionarily relevant properties such as fitness and size, but not conspecificity (Klump, 1987; Gerhardt, 1994.) Females respond to males exhibiting attractive call characteristics through phonotaxis, purposeful movement towards acoustic stimuli. This behavior ultimately results in amplexus and fertilization.

Similar phonotactic behavior by males is relatively unstudied. Although less important evolutionarily since male phonotaxis does not result in mating, male phonotaxis, if it exists, may constitute a key factor in shaping the configuration of the natural chorus. Phonotaxis is exhibited in males of other genera, although generally as a means of satellite predation on prey rather than as a technique to increase mating efficacy (Sakaluk, 1984.) Based on what is already known about female preference, there are three possible male responses to calls:

1. Males may not display phonotaxis at all; this would suggest that other stimuli, e.g. olfactory cues, may act as the impetus for chorus attendance and distribution. Alternatively, this could also suggest that a male's position or attendance in the chorus is independent of the behavior of other males.
2. Males may be attracted to the same call characteristics as females; males may recognize the same sets of stimuli as females to determine the location of a group of the same species, thereby increasing their chance of reproductive success. Male phonotactic selectivity which parallels that of females may also represent a quasi-parasitic attempt to intercept females responding to more attractive calls.
3. Males may be repulsed by the characteristics that attract females. This would imply that males attempt to increase their reproductive chances by moving to areas less dense in "attractive" males, thereby making themselves more competitive within the chorus.

It is commonly known that *Hyla versicolor* males "answer" other calling males. This phenomenon is also relatively little studied, but its prevalence suggests that there may be some significance associated with this behavior. Male call answering responses to stimuli exhibiting variations in call rate may reveal the presence or absence of a proactive response to intra-chorus competition. Although it is relatively difficult to make a prediction on the outcome of this experiment due to its novelty, I tentatively will conjecture that males will respond differently to stimuli in order to maintain their level of relative attractiveness.

This study will use call rate as a representative mate call characteristic; deductions based on male responses to changes in call rate may be extrapolated to achieve a reasonable conclusion about the nature of male phonotaxis to biologically relevant acoustical phenomena.

Materials and Methods

Hyla versicolor males were captured in vernal ponds at the University of Notre Dame Environmental Research Center in Vilas County, Wisconsin. Although all frogs were located using mate calling as a reference, sex was verified surgically after testing to insure the maleness of all participants.

Male Phonotaxis

Experiments were performed in a darkened room less than 1 mile from the collection sites, no later than 48 hr. post-capture. The room that contained the arena, 22'9" x 15'5", was large enough to minimize interference from echoes. A 10' x 10' arena was demarcated with masking tape into 1' x 1' squares on a concrete substratum. The walls of the arena, approximately 3' high, were constructed from cardboard boxes and styrofoam. Recordings used were obtained from Michelle Tito and transferred to Maxell XLII 90 min audio tapes. Recordings were played from Radio Shack desktop cassette tape recorders (CTR-69) at volume setting 4. The tape players were repositioned both within and between experiments to avoid directional bias. The duration of all experiments was 10 minutes, and the movement of the frogs was recorded over this interval. Each experiment consisted of five parts: two no-choice set-ups using natural field calls and calls from arginine vasotocin (AVT)-injected frogs as the stimuli, and three choice experiments. The choice experiments offered a choice betwen AVT calls and another stimuli. These stimuli were: natural field calls, calls from saline injected frogs, and regular frog calls at 11.6s intervals. Calls from AVT-injected frogs have an increased call rate (Tito, unpublished data); the use of these calls was preferred to the use of regular frog calls at an artificially manipulated higher rate because these calls were recorded from frogs answering in a chorus and more closely mimicked calls in a natural population. I considered a response positive when a frog moved to within 10 cm of a recorder (Diekamp, Gerhardt, 1995). The no-choice experiments exposed the frog to a 10 minute recording of either natural field calls or calls from AVT injected frogs. Again, a positive response was recorded when a frog moved to within 10 cm of a recorder.

Call Answering

Experiments were performed in a darkened room less than 1 mile from the collection sites, no later than 5 hours post-capture. In identical buckets, frogs were first exposed to a series of natural field calls to mimic the natural chorus from which they had been removed. This had been found to reduce stress and increase the likelihood of a response. After a frog began to answer, he was removed, allowed to acclimate to the absence of the natural field calls, and then exposed to a random series of 4 min. intervals of stimuli, each separated by a 2 minute pause. Seven stimuli were used: natural field calls, calls from saline-injected frogs, calls from AVT-injected frogs, regular frog calls at 2.93s intervals, regular frog calls at 11.6s intervals, regular frog calls at 5.81s intervals, and a synthesized call at 2000 and 1000 Hz at 11.6s intervals. Times at which the frog answered were recorded; call rate, average call interval, and call latency were determined through calculation.

Results

Male Phonotaxis

Males exhibited no significant phonotaxis in no-choice experiments for either stimuli, calls from AVT-injected frogs or natural field calls (Table 1). They also showed no preferences in the choice experiments (Table 1). When I include positive responses to calls from AVT-injected frogs and positive responses to the alternative stimuli (natural field calls, saline-injected frog calls, and regular frog calls at 11.6s intervals) and negative responses, extremely small p-values are obtained, seemingly disproving the null hypothesis, that there is no difference in the frog's response between the treatments (Table 1). This conclusion, however, is invalid because the apparent difference may be attributed to the negative responses. When negative responses are removed from the data set, there are no differences in the frogs' responses between the AVT-injected vs. natural field calls treatments ($P>.999$) or in the AVT-injected frogs vs. calls from saline-injected frogs treatments ($.75>P>.50$.) The choice experiment which offered calls from AVT-injected frogs vs. regular frog calls at 11.6s intervals may demonstrate some degree of selectivity ($.25>P>.10$); 6 frogs exhibited phonotaxis to AVT-injected frog calls, while only one frog displayed a preference for regular frog calls at 11.6s intervals.

Call Answering

There was a significant effect of call source on the rate at which frogs answered (Friedman's test, $S=25$; $P<0.001$), but not on the call interval ($S=5.23$; $P=0.45$) or call latency ($S=5.8$; $P=0.44$). For the call rates, frogs responded to the synthesized calls significantly less frequently than to the regular frog calls at 5.81s and 2.93s intervals, AVT-injected frog calls, and saline-injected frog calls. Moreover, frogs responded to regular frog calls at 2.93s intervals significantly more frequently than they did to regular frog calls at 11.6s intervals. No other comparisons were statistically significant.

Table 1. Male *Hyla versicolor* responses in no-choice and two-choice phonotactic playback experiments.

Stimuli	Positive Response to AVT: Positive Response to Alternative Stimuli: Negative Response	P value	Positive Response: Negative Response	P value	Positive Response to AVT: Positive Response to Alternative Stimuli	P value
AVT	N/A	N/A	2:17	.005>P>.001	N/A	N/A
Natural	N/A	N/A	1:18	P<.001	N/A	N/A
AVT vs. Natural	3:2:14	P<.001	5:14	P<.001	3:2	P>.999
AVT vs. Saline	4:2:13	.005>P>.001	6:13	.005<P<.001	4:2	.75>P>.50
AVT vs. Regular call, 11.6s btw.	6:1:12	.01>P>.005	7:12	.01>P>.005	6:1	.25>P>.10

N, number of males, equals 19. Probability in a chi-squared test shown as P. A response was scored when a male moved to within 10 cm of a speaker. α=.05.

Discussion

Sample sizes used in both experiments were relatively small (call answering, N=11, male phonotaxis, N=19) and we are therefore unable to make predictions of fine-scale preferences. Sample sizes smaller than 20 have been shown to be effective, however, in predicting female preferences (Klump and Gerhardt, 1987), thus I assume that general predictions of behavior based on these data are legitimate.

Call rate is significantly affected by exposure to different stimuli. It seems very possible that males vary their call rates based on the calling behavior of other males in the chorus. Regular frog calls at 2.93s intervals induced a higher call rate than regular frog calls at 11.6s intervals. Because females are known to prefer increased and even supernormal values of call rates, we may assume that this modification of calls by the males is in response to other males' calls, an attempt to maintain their relative attractiveness within their population. It is known that males increase their call duration in competitive situations, which is associated with a compensatory decrease in call rate (Klump, 1987). It is also known that females choose mates based on the energy investiture that a particular call requires; females choose signals with combinations of duration and call rate that are even more extreme than those found in nature, and always choose calls with a higher duty-cycle ratio (more sound energy) (Klump, 1987). In previous experiments by other investigators, when alternative stimuli, 12-pulse, 900 calls per h vs 12-pulse, 1,800 calls per h, 11 of 15 female frogs chose the faster call rate, and 0 chose the slower call rate (Klump, 1987). *H. versicolor* females, in general, prefer calls played back at twice the standard call rate (Gerhardt, 1987). Thus, because increased call rates were observed in males presented with stimuli at an increased rate, I infer that male calling behavior is not independent of the calling behavior of other males in the chorus. Calling is proactive, and males modify their response based on the attractiveness of nearby males to remain competitive. The calling responses of males to the calls of other males in this experiment parallels phonotactic response of females to male calls. At least in terms of call rate (the variable examined in this study) males answer calls which females find more attractive more rapidly than they answer calls which are less preferred by females.

Additionally, because responses to synthesized calls differed significantly from responses to almost all other stimuli, we may also conclude that males are able to recognize biologically relevant acoustical phenomena. Responses to synthesized calls did not differ significantly from responses to natural field calls, which is likely a reflection of the low statistical power of the multiple comparisons technique since mean values of the responses are very different (mean value for natural field calls treatment=.205 calls/s with a standard deviation of .050, mean value for synthesized calls treatment=.044 calls/s with a standard deviation of .066). It would be interesting to extend this research to determine whether males are able to recognize, and to vary their response to, the calls of heterospecific versus conspecific males. The lack of statistical significance between call latencies in response to different stimuli suggest that call latency may be characteristic for an individual rather than a function of the stimuli itself. Alternatively, call latency may be influenced by degree or rate of oxygen consumption; call latency may increase for a stimuli introduced directly after another stimuli which generated an energy-costly response. Because the stimuli were presented in random order, this would not be readily apparent. Similarly, call interval does not appear to be affected significantly by exposure to different stimuli.

These data support that male calling behavior may elicit a response from other males in addition to the expected, well-studied female response. The possibility of the existence of male phonotaxis introduces a new consideration; males may attempt to augment their mating competence using means other than call characteristics. The fact that the choice experiment between calls from AVT-injected frogs and regular frog calls at 11.6s intervals yielded a significant response leads me to believe that the existence of male phonotaxis is a plausible conjecture. The fact that slower call rates, exemplified by the 11.6s interval call, are less attractive to females and that AVT increases call rate, thereby likely increasing the attractiveness of the call, leads me to believe that male preference for the AVT calls is biologically relevant. Furthermore, increasing the number of participants in future trials will allow the incorporation of variables such as size and physical condition of the participants; it is entirely possible that less formidable males may exhibit phonotaxis in an attempt to increase their chance of reproductive success while larger, more fit males may elect to remain stationary.

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