

Lekking Behavior and Call Quality in *Hyla versicolor*

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Abstract

The genetic character of organismal communities is determined by the genotypes of males that successfully reproduce; thus, the mechanisms of female mate selection are of critical concern to evolutionary and population ecologists. In many animal species, a communal mating behavior termed “lekking” is observed. In lek systems, males convene at a single time and place to perform their advertisement displays. At these locales, higher quality mates tend to establish territories near the center of the breeding ground, and male quality degrades as you move from the middle to the perimeter of the site. This serves to enhance the number of encounters between females – attracted by high-quality displays – and their low-quality conspecifics. This enhances the likelihood of a successful reproductive event for males of all quality rankings, thereby preventing directional evolution towards that suite of traits possessed only by alpha males, who are not necessarily the best-adapted for survival. In this study, we tested the hypothesis that the gray tree frog, *Hyla versicolor*, adheres to a lek system, which is prevalent in other tree frog species. Males at vernal pond “V,” located on the UNDERC property in Northern Michigan, were recorded and then ranked on the basis of their vocal characteristics, which females of this species use to select a partner. Contrary to our predictions, no statistically significant relationship was found between location in the pond and mate quality. We believe this to be due to

confounding factors such as weather and chorus size, and therefore recommend further studies.

Introduction

Male courtship behaviors and the mechanisms of female selection are of particular ecological importance because they determine which individuals within a species pass their genetic material on to subsequent generations, thereby shaping the character and livelihood of the entire population. Males of many species engage in a behavior termed “lekking” – the convergence upon a common geographical locale to compete for mates during the breeding season. Within each lek, males establish individual territories, called courts, as a base of operations from which to solicit females via visual displays or vocal calls. While female choice may be constrained by extrinsic factors such as population density, predation, etc., courts typically harbor no resources of use to females; instead, apparent predilections are likely due to genetic traits of prospective mates that confer an advantage in the process of sexual selection (Widemo and Saether, 1999).

In the gray tree frog (*Hyla versicolor*), an arboreal species indigenous to the Northern United States and Southeastern Canada, males seek perches in or near ponds or lakes and emit advertising calls to attract females from nearby

wooded areas. Specific phonetic properties of the call – unique for each frog – determine the overall desirability of male anuran breeding candidates (Gerhardt 2008). The call frequency, pulse rate, intercall interval, and call period are the defining features of the male call, and those properties that correspond to increased sound energy output – higher pulse rate, longer call duration, smaller intercall interval, etc. – have been shown to elevate phonotactic response in females of the same species (Fellers 1979). Additionally, females in laboratory trials have expressed preferences for males who call at a lower frequency (Gerhardt 2005). These properties are therefore linked to mate desirability and can serve as a measure of overall “quality.”

But this behavior presents a conundrum. According to the lek paradox, consistent female preference should result in directional evolution toward that suite of male traits deemed optimal, reducing the genetic variation that is so crucial to the adaptability of organismal communities. This is not observed in *H. versicolor*, as lower quality males still achieve reproductive success even when in close proximity to available alpha specimens. This is explained if location within the lek arena becomes a factor in female mate choice (Tomkins 2004). For instance, if lower-quality males position themselves between females and other, higher-quality males, they maximize the number of encounters with females. This would in turn enhance the likelihood of a successful copulatory event, as studies have shown that females prefer louder calls, and sound amplitude diminishes with

increasing distance (Beckers and Schul, 2004). It is also possible that the female frog utilizes a “minimum threshold” strategy and will mate with the first conspecific it encounters fulfilling certain baseline standards (Gibson and Langen, 1996). Thus, surrounding of isolated “ideal” males by clusters of lesser specimens promotes female selection of males with a variety of vocal characteristics (Bee and Riemersma 2008).

In this investigation, the locations and vocal properties of individual male frogs will be documented in an attempt to establish a correlation between position and quality. The specific hypothesis being tested states that lower-quality males will be located closer to the periphery of the breeding site (i.e. water’s edge, the “access point” where females enter the breeding site from the woods) than higher-quality males, who are effectively cloistered in the interior of the pond habitat.

Methods

The specimens studied were 76 males from the natural population of *H. versicolor* at the University of Notre Dame Environmental Research Center field station, located in Michigan’s Upper Peninsula. Vernal pond “V” (located at a latitude of 46.2511°N, longitude of -89.5177°W, and altitude of 513 m) has been selected as the study site on the basis of exceptional levels of choral activity in previous years. “V” resides in a stand of aspen (*Populus tremuloides*), and remnants of fallen trees strewn about the pond provide a number of favorable

perch sites for advertising males. Tufts of grass around the pond's edge and floating mats of green algae are also abundant and may serve as perches. Water depth approaches 1.25 m at the center and the total area of the pond is about 290 m². Prior to data collection, "V" was transected with a 20 m x 20 m grid using wooden stakes demarking intervals of one meter and labeled with Cartesian coordinates.

The observational period was punctuated by bouts of cold or stormy weather during which the chorus was inactive; consequently, the tree frog chorus was heard on only 11 evenings spanning the breeding season from May 21st to June 4th. On each of these evenings an excursion to "V" was made, and after making a notation of air and water temperature, I located male gray tree frogs by zeroing in on their calls, which begin around sundown and lasted up to three hours. An array of random coordinate pairs produced using Excel's number generator feature was initially consulted to determine sampling order – I would record the caller closest to each listed location – but I abandoned this method when it became evident that I could sample all frogs actively participating in the chorus each night. The advertisement call of each specimen was recorded for a total of two minutes using an M-Audio Microtrack 24/96 recorder with an Audio-Technica directional microphone. For best results, the microphone was held level to the specimen at a distance of one meter. Cloacal temperature and snout-to-vent length of each male was then measured. Males were numbered using a toe-

clipping scheme and returned to the exact place they were found. A flag bearing the same number was also be placed at each location. By daylight, the grid locations (in x, y coordinates) of flags placed the previous night were documented and represented on a diagram of the breeding site (Fig. 1), creating a distribution map of advertising males.

Using a bioacoustics program called RavenPro 1.3, acoustic features of recorded vocalizations – call rate, intercall interval, call period, call duration, pulse rate, pulse number, and frequency/pitch – were quantified (averaged out from 1 minute of audio for each frog) and compared against length and temperature data using regression analysis in SYSTAT 12. Call quality and spatial data will be analyzed with the assistance of both SYSTAT and a plug-in for Microsoft Excel called “SpPack,” which includes a test for distributive randomness. From these results, inferences can be made about the relationship between the “quality” of males (predicated on the vocal properties enumerated above) and their distribution among the breeding site.

Results

My primary null hypothesis posited no relationship between “quality” and location of male *Hyla* conspecifics at vernal pond “V.” To test this I initially performed a first-order univariate nearest neighbor analysis, which yielded a Clark-Evans’ R value of < 1 (0.617) and a negative z-score (-6.311), both of

which suggest non-uniform, non-random spatial grouping of specimens. In order to see if this was an effect of mate quality, it was necessary to design a quality index whereby males were ranked according to their compatibility with presumed female phonotactic preferences. Rank was derived from four acoustic properties: intercall interval, call duration, pulse number, and frequency. The other three tabulated variables (see methods section) were omitted because they can be calculated from the previous four, and a minimalist approach eliminates the possibility of skewing rankings in favor of one particular variable; e.g. a male with a longer call duration is more likely to produce a higher number of pulses, and inclusion of both variables in the quality index would give pulse number greater weight than, say, frequency. Moreover, it is necessary that these four variables are equally weighted because they contribute equally to net energetic output, the principal criterion according to which females select males. Rank was therefore determined as follows:

First, using data from all frogs tested, I calculated the arithmetic mean and standard deviation of intercall interval (Table 1; Fig. 2). Frogs whose average intercall interval fell within one standard deviation of the mean were assigned a value of either 2 or 3, with the higher value going to those frogs that fell on the “preferred” side of the mean (in this case, a 3 was given to those frogs whose intercalls were within one SD *below* the mean). Similarly, specimens whose intercall intervals fell between 1 and 2 SD’s were given a 1 or a 4, and those

whose values fell outside of 2 SD's were awarded a 0 or a 5, where a 5 represents utmost desirability. This ranking system was repeated for the remaining three specified variables, and the overall rank of each frog was merely the sum of his rankings for each of the four acoustic properties. Quality rankings ranged from 4 to 14, where 14 was the most appealing male; male ranks and locations are portrayed in Fig. 3. Linear regression indicates that a male tree frog's quality does not vary either with its distance from the center of the pond ($p = 0.971$) or with distance from the highest-quality male observed each night ($p = 0.420$). There was also no statistically significant relationship between any individual vocal property and either of the two just-mentioned measures of relative location (Table 2). Thus, H_0 is not invalidated. The same linear regressions were performed with the data grouped by date in order to compensate for possible temporal and meteorological effects, but once again no statistically significant results were found (Table 3).

Acoustic properties and quality ranking were then regressed in turn against both snout-to-vent length and cloacal temperature (Table 4). The only statistically significant result was an inverse relationship between snout-to-vent length and call frequency ($p = 0.017$; Fig. 4).

Next, rank was regressed against chorus size (number of frogs calling each night), and yet again, no statistically significant relationship was found ($F = 1.422$, $df = 1$, $p = 0.237$). Surprisingly, however, one-way ANOVA evinced a statistically significant variation in frog rank by night ($F = 3.571$, $df = 10$, $p <$

0.001), which the Tukey test showed existed between the evenings of June 16th and May 20th ($p = 0.007$), June 16th and May 21st ($p = 0.015$), and June 17th and May 21st ($p = 0.002$). While these differences were not explicable in terms of either temperature or choral activity taken individually, two-way ANOVA indicated a statistically significant interaction of these two variables in determining frog rank ($F = 2.545$, $df = 17$, $p = 0.005$).

To justify use of parametric statistical tests, chi-squared normality tests were performed on all continuous variables measured. All variables fit a normal distribution to a statistically significant degree (frequency was least normally distributed with $\chi^2 = 6.126$, $df = 4$, $p = 0.190$).

Discussion

My hypothesis that male vocal quality would degrade with increasing distance from the center of the pond was not supported. However, this does not necessarily rule out lekking in *H. versicolor*, especially in light of a recent study – published during the course of my own – confirming the prevalence of such behavior in two other related species, *Hyla intermedia* and *Hyla viviana* (Castellano et al. 2009).

Indeed, this study was conducted under the assumption that the center of the pond serves as the most desirable advertising perch, and that all areas of the pond that are equidistant from the center are equally attractive and accessible to

male frogs. These assumptions are contradicted both by statistical analysis and my own qualitative observations, as frogs were clustered on tufts of grass or atop physical obstacles such as logs, algae mats, and even my transect posts. No frogs whatsoever were observed calling while floating in open water, which constituted a significant proportion of the surface area of the pond. Males also seemed to avoid areas with especially dense grass cover, which would presumably dissipate sound waves and thereby reduce the clarity/amplitude of their calls as perceived by prospective mates. Thus, while all calling frogs were situated near or within the pond (copulation occurs in water), it seems probable that the pond itself was not the lek domain, but rather an aggregate of sub-leks separated by stretches of essentially unusable space. Whereas a conventional lek is modeled on the king-of-the-hill paradigm – one hill, one king – the pond instead represents a countryside marked by multiple hills, each with its own “alpha” baron around which other males in the immediate vicinity are distributed according to a quality hierarchy. Such a phenomenon is encountered in many migratory avian species (Westcott 1994), where an island serves as the communal mating site, but individual leks are limited to alcoves in the sides of cliffs. In future, it may therefore be beneficial to characterize the different areas of the pond and determine whether lekking behavior is evidenced when observations are restricted to small regions of homogenous topography. Another possibility would be to replicate this study using an artificial pond with uniform characteristics, such as evenly-spaced

floating blocks. It is also possible that diurnal perches were unevenly situated around the perimeter of the pond, and I would be interested to know whether there is a tradeoff between quality and proximity when male frogs attempt to select an advertisement perch during their crepuscular descent to water's edge. One could settle this question in a future study by tracking the movements of male frogs.

The finding that call frequency/pitch is inversely related to snout-to-vent-length makes sense because we expect larger frogs to have larger vocal sacs, and frequency is inversely proportional to the amount of air expelled (Gerhardt 2005). However, since size did not have a significant effect on any other acoustic property, its role in determining male "quality" was minimal, as is to be expected in a species wherein mates are selected on the basis of aural cues.

More significant was the observation that both temperature and chorus size affect male call properties. As ectotherms, tree frog metabolic processes are highly sensitive to external temperature, and heating a single calling male will increase that male's call rate (Fellers 1979). However, there is also likely to be a higher number of frogs participating in the chorus on warmer nights, and the presence of other males can serve to counteract the increase of call rate with temperature. That is, as other frogs enter the chorus, calling males will *decrease* call rate (though relative rank in the chorus is maintained). This is done in order to reduce the chances of call overlap/interference, which degrades the quality of the call as perceived by females. Since the energetic output per unit time and weight

of a calling frog is equivalent to that of an Olympic sprinter at top velocity, such modification of calling patterns can save precious energy (Schwartz et al. 2002). Unfortunately, my study was not designed to measure such variables, and since these vary both within and between nights, quality assignments are likely distorted. In other words, because specific call properties of individual males are subject to change, absolute qualities of frogs cannot be compared unless they are assessed simultaneously. Indeed, to say that frog A at time 1 is “better” than frog B at time 2 is a bit like saying that Timmy was taller at age 12 than Johnny was at age 4 – quite an unenlightening bit of information.

Technical errors may also have occurred. Cloacal temperature measurements are suspect because simply the act of holding the frog drastically raised its body temperature. Smaller frogs heated faster than larger ones due to their larger surface-area-to-volume ratios, and different amounts of time were required to take the temperature of each frog. Additionally, positions of transect posts may have shifted as they settled in the mud, distorting the size of the grid. Abiotic variables such as moonlight, wind, and water levels – all amenable to the weather – may also have had an uncalculated effect on the calls of males. Then, too, I wonder to what extent my presence in the pond and the light of a head lamp disrupted the normal behavioral patterns of these wild organisms. If I were to repeat this experiment, I would use remote microphones stationed at periodic intervals around the pond, significantly reducing observer effects and allowing for

cotemporaneous recording of all organisms. I would also like to corroborate my assumptions about male desirability by collecting and identifying males participating in amplexus (mating).

Acknowledgments

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Table 1. Average values for acoustic properties of 76 male gray tree frogs.

	mean	SD
intercall interval(s)	6.188	0.454
call duration(s)	1.069	0.025
pulse #	16.818	0.383
frequency(Hz)	2101.991	11.637

Table 2. Coefficients and p values for regression of vocal properties against distance from center of the pond and distance from alpha males.

	<u>Intercall interval</u>	<u>Call duration</u>	<u>Pulse number</u>	<u>Frequency</u>	<u>Rank</u>
Distance from center	-0.065 (p = 0.729)	-0.009 (p = 0.379)	-0.075 (p = 0.636)	-9.474 (p = 0.046)	-0.004 (p = 0.971)
Distance from "best" frog	0.019 (p = 0.869)	0.001 (p = 0.900)	-0.089 (p = 0.346)	0.265 (p = 0.927)	-0.052 (p = 0.420)

Table 3. Coefficients and p values for regression of vocal properties against distance from the center of the pond, grouped by date.

<u>Date</u>	<u>Intercall interval</u>	<u>Call duration</u>	<u>Pulse number</u>	<u>Frequency</u>	<u>Rank</u>
20-May	-0.806 (p = 0.432)	0.015 (p = 0.441)	-0.031 (p = 0.943)	-12.006 (p = 0.418)	0.230 (p = 0.467)
22-May	-0.222 (p = 0.331)	-0.019 (p = 0.321)	0.028 (p = 0.914)	-4.928 (p = 0.432)	-0.089 (p = 0.620)
28-May	-0.246 (p = 0.296)	-0.003 (p = 0.911)	0.150 (p = 0.644)	-8.469 (p = 0.347)	0.109 (p = 0.673)
4-Jun	1.683 (p = 0.394)	0.050 (p = 0.104)	0.394 (p = 0.442)	-20.985 (p = 0.664)	0.018 (p = 0.981)
12-Jun	0.102 (p = 0.800)	-0.005 (p = 0.888)	-0.057 (p = 0.905)	-24.181 (p = 0.168)	0.131 (p = 0.696)
14-Jun	0.032 (p = 0.927)	0.011 (p = 0.780)	-0.116 (p = 0.857)	-7.309 (p = 0.659)	0.178 (p = 0.544)
15-Jun	0.146 (p = 0.598)	-0.035 (p = 0.165)	-0.393 (p = 0.318)	0.940 (p = 0.928)	-0.343 (p = 0.201)

Table 4. P values for regression of various properties (first column) against length and temperature data. Statistically significant values are **bolded**.

	Length	Temp
Temp	0.594	
Intercall interval	0.2	0.955
Call duration	0.741	0.067
Pulse #	0.859	0.211
Frequency	0.017	0.085
Rank	0.123	0.233
Distance from center	0.323	0.676

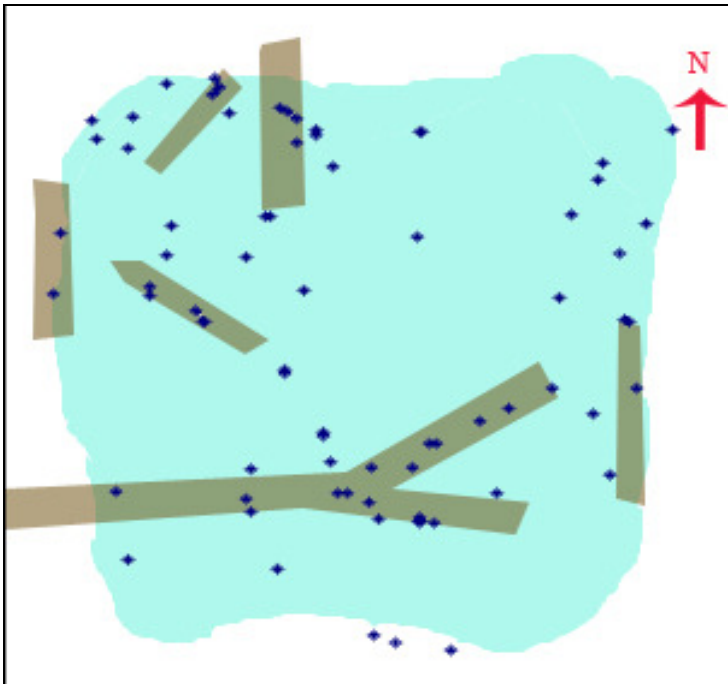


Fig. 1. Locations of surveyed males. Coordinate locations of each surveyed specimen were imposed over a rudimentary map of the study site, vernal pond “V.” Each blue diamond represents a different male specimen, light blue signifies water, and brown signifies emergent branches from fallen trees male specimen.

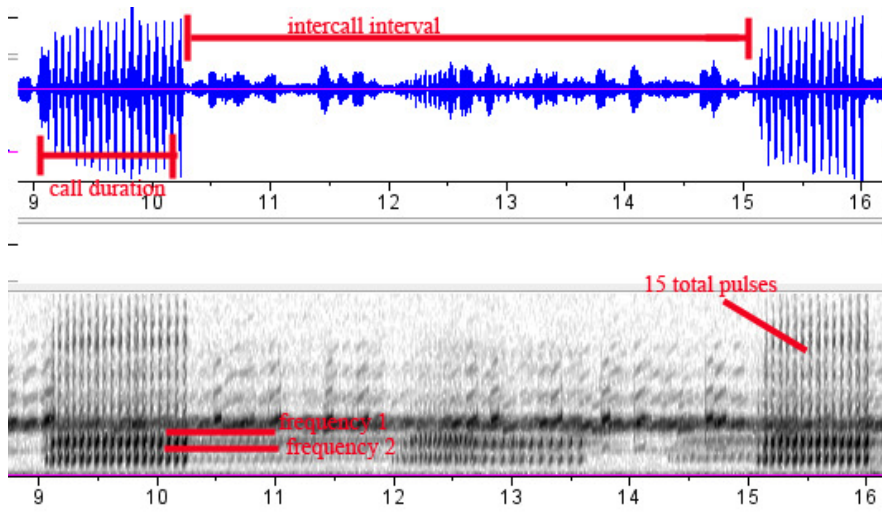


Fig. 2. Sonogram of male frog call created in RavenPro. The four portrayed variables were those used to calculate quality rankings of male frogs.

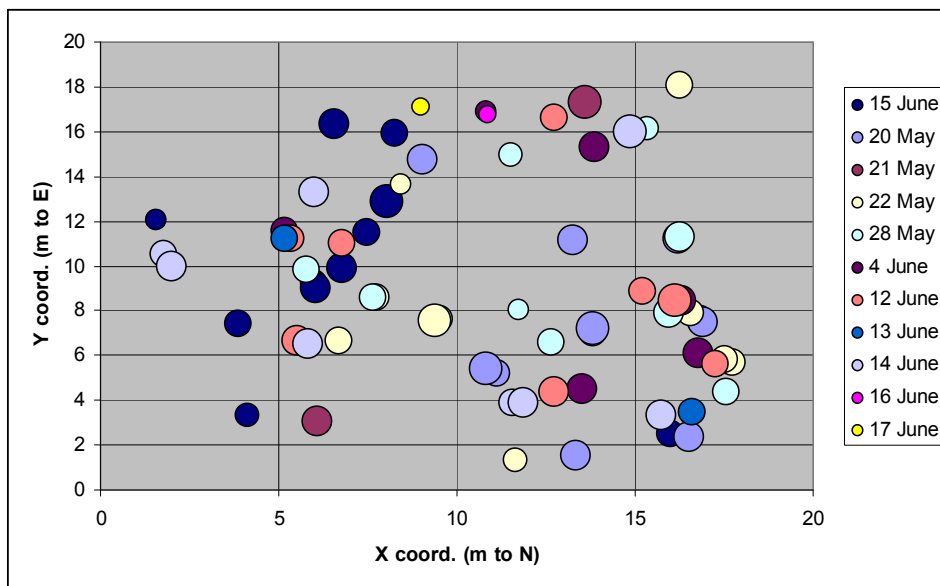


Fig. 3. Relative call quality indices of tested males. Diameter of each bubble is proportional to call “quality,” derived from four acoustic attributes: intercall interval, pulse number, call duration, and frequency/pitch. No variation in quality by coordinate position is evident.

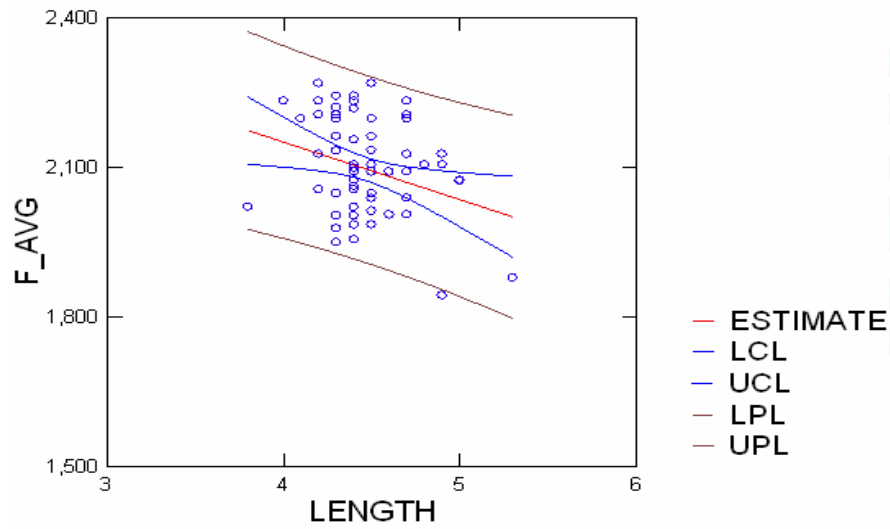


Fig. 4. Frequency vs. length. Linear regression found a statistically significant relationship between frequency (Hz) and snout-to-vent length (cm); ($p=0.017$).
Frequency = $-115.413(\text{length}) + 2612.045$.