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The Affect of Male Calling Dynamics on
Mate Choice in *Hyla versicolor*

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Abstract

While females are known to exhibit preference for a number of call characteristics in acoustic advertisements by calling males, the means by which females make their mate decisions are still unclear. Chorus sampling and the selection of the best relative male in *Hyla versicolor* supply a basic model for female mate selection, but a more detailed understanding of the female's methods is still lacking. I investigated the affects of multiple male presence at equidistance to female sampling. My results showed few trends in female preference, with a preliminary finding in support of the hypothesis that females can be affected by total male sound output. Further testing is required to fully support or reject this hypothesis.

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Introduction

Female *Hyla versicolor* use temperature (Gerhardt, 1978), call amplitude (Beckers and Schul, 2004), and call length (Schwartz et al., 2001) to assess their mate choice. The method females use to find and select their optimal male involves a combination of tactics: the nearest "best" male (best of "n") (Janetos, 1980) and a series of movements which sample a number of males (no greater than 3) in a select area (relative comparison) (Murphy and Gerhardt, 2002).

While females are known to choose longer calls over shorter calls and to sample multiple males before selecting a mate, it is unclear which stimulus a female is initially attracted to: the burst of sound overall or an individual call. Murphy and Gerhardt (2002) found that in a closely related species to *H. versicolor*, females were able to detect male calls that were 3dB greater than the chorus background. While this finding can help explain female mate choice behavior when males are less numerous or more isolated from the chorus, what happens when males are clustered, when a female is able to distinguish more than one option from the chorus? Male treefrogs will often position themselves in close proximity to one another when calling, frequently overlapping one another's calls and making acoustic discrimination difficult. This behavior suggests there may be some reproductive advantage to male clustering in choruses; male grouping within choruses may be a means to increase the total sound output.

This study will examine the possible advantage in male *H. versicolor* to cluster when performing advertisement calls. My hypothesis is that the presence of additional males along a female's path to multiple males will affect her ultimate mate choice and that there is an advantage to males advertising near other males. To test this hypothesis, two and three-choice call playback experiments will be conducted with phonotactic females where amplitude and distance have been equalized. This equalization will account for the other variables in male calls that could be responsible for affecting a female's mate choice. My hypothesis in part predicts that females move up a pulse-number gradient in making their mate choices; measuring relative male call quality as

opposed to call amplitude as a means to discriminate. This would explain male behavior of clumping: by calling near one another, males are able to take advantage of a female's bias for more pulses in a call by attracting her to their proximity initially, then until causing her to choose one of them based on their relative call quality.

Methods

Female *H. versicolor* were collected from amplexing pairs on University of Notre Dame Research Center property, near Land O' Lakes, Wisconsin. Collection and test dates occurred between June and July, 2009. Following collection, females were kept in a refrigerator at approximately 1 degree Celsius until approximately one hour before testing, where they were then kept in a dark, quiet but temperature unregulated room until testing.

Testing took place in a 6m x 4.6m x 2.3m room padded with mattress foam. Four Mineroff SME-AFS speakers were placed 2 meters from the release cage in a half circle around the female at 30 degree increments (Figure 1). The female was brought into the darkened room with the use of a headlamp, placed in a petri dish, and covered with a wire or plastic mesh cage. She was allowed to acclimate to the room for approximately 2-3 minutes before any stimulus was played. Calls were played through an ESI Gigaport HD 8 channel output from Cubase LE software at 80dB 1m from the speaker. Tests were run (with the exception of 8 tests, as noted) with the observer outside the room, female choice was determined by watching on a computer outside the room using a CCD

KT6215IR security camera run through a USB-DVR to Max-DVR software; all control of the speaker play was conducted outside the room.

For each test, the stimulus was played for at least two cycles before the female was released. Speaker choice was made if the female moved within 10cm of the speaker. If the female moved beyond the speakers and did not return or began moving in an indiscriminate path for longer than 5 minutes the trial was considered an escape. Additionally, a female was considered unresponsive if she did not move from the petri dish for 2 minutes after the cage release. Latency time moving from the dish, speaker choice and time to speaker choice were recorded. A full test run included treatments in the following order: 12 pulse v. 12 pulse; 12 pulse v. 12 pulse v. 6 pulse; 12 pulse v. 15 pulse; 12 pulse v. 15 pulse v. 6 pulse; etc. with the treatments extending to 18 and 21 pulse calls with two separate treatments with the 6 pulse call speaker on either side of the 12 pulse call speaker and the respective alternate.

Calls were computer simulated with a 47ms pulse period and 25ms pulse length with 3.4 seconds between calls. Females were allowed to rest for at least 2-3 minutes between treatments. If a female was either nonresponsive or escaped for more than one treatment she was considered to not be phonotaxic and removed from further testing. Given the low number of data points, however, the results of females that did not complete the full round of treatments were still included.

Results

Females followed our expected choice model with one of the four treatment cycles. With the treatments involving 15 pulse calls, females chose the highest call rate when the lower call rates were isolated as opposed to paired. In other words, the coupling of the 12 pulse call with the 6 pulse call was enough to cause the female to choose the 12 pulse speaker over the 15 pulse speaker. This was statistically significant at the 0.05 level with a p-value of 0.044 (Figure 3). All other treatments (12 v 6, 12 v 18, 12 v 21) had statistically non-significant p-values of 0.814, 0.140, and 0.082, respectively. The control treatment of 12 v 12, 12 v 12 v 6, and 6 v 12 v 12 was the least significant of all treatments; in fact females had a greater tendency to choose the left 12 pulse speaker in both the first (6 of 10) and third (4 of 6) treatments and only chose the 12 pulse right speaker 50 % of the time in the second treatment (Figure 2). The treatments involving 18 pulse and 12 pulse calls had so few replicates that any trend in behavior was not evident. All analysis was conducted using SYSTAT and Fisher's exact test.

Discussion

The results of this experiment give little evidence to refute or support the hypothesis that male clustering can affect female mate choice, at most they give some small support. The results of the treatment cycle involving 15 pulse calls show the behavior as proposed; the female was attracted to and ended up choosing the highest pulse call when it was played in the settings where it had the highest number of pulses (in comparison to the 12 pulse call or coupled with the 6 pulse call) and had the opposite behavior when the two lower pulsed calls were paired (6 and 12). This shows a possible trend in support of the hypothesis;

suggesting that females move up a pulse-number gradient when sampling multiple males. However, the results of the control cycle (12 v 6 pulse calls) showed no or even the opposite trend to support the findings of the 15 pulse cycle. When the 6 pulse call sides were reversed on either side of the 12 pulse speakers, the female preference only followed their movement in one of the two layouts (Figure 2). Looking at the preference of the control 12 v 12 treatment in regards to this, it appears there may have been a difference in quality or amplitude between the middle two speakers as the females consistently showed a preference for the left speaker over the right. While amplitude was set equal between all speakers before testing and all speakers were of the same make and model, there is the possibility either amplitude was not equal due to operator error or the speakers were not equal due to production defects.

The results of the other cycles show no complete trends in support or otherwise of the hypothesis. The cycle involving 18 pulse calls show as expected preference for the 18 pulse call when played only with the 12 pulse call. Once the 6 pulse call was added in, the females consistently chose the 12 pulse call, regardless of the location of the 6 pulse call (Figure 2). While the results of this cycle were non-significant, looking at the numbers alone can possibly suggest that the addition of the 6 pulse call to the choices may have confounded the field enough to bring the female to choose the 12 pulse call instead of the 18 pulse call (Figure 4). On the other hand, this behavior was not the case with the 15 pulse call cycle, and there is no plausible reason why a call of higher number of pulses would make the female's choice turn to the 12 pulse call, given that we know and

have seen from the initial 12 v 18 pulse test that there is a preference for the 18 pulse call normally. As with the 21 pulse cycle (Figure 5), the low number of data points failed to provide sufficient evidence for any trend in female choice along a pulse-number gradient that may exist. This year's breeding season was unusually cold and made the collection of females difficult, leading to such low amounts of data. Retesting of this hypothesis during a different breeding season may yield more compelling results.

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Figures

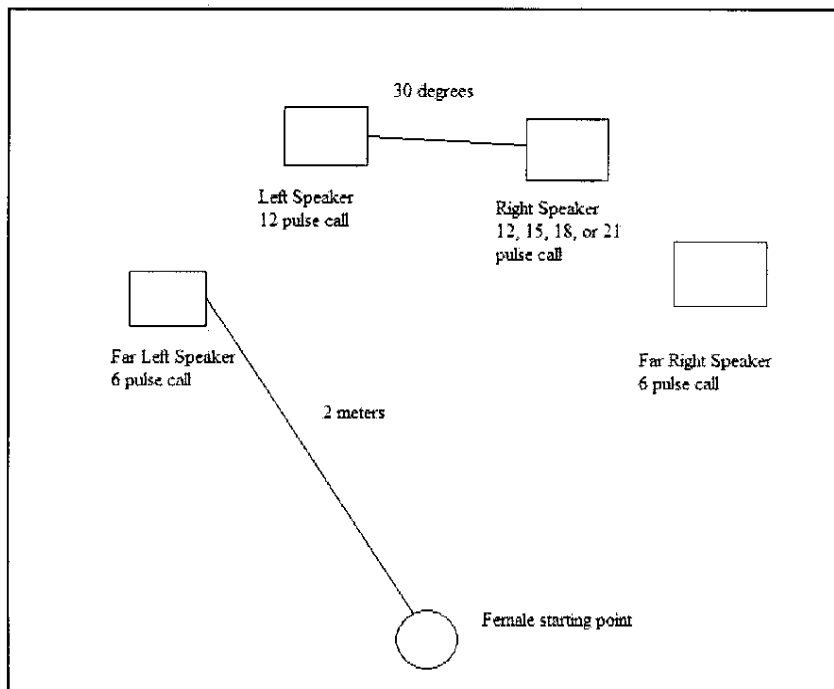


Figure 1: Diagram of room layout used for testing

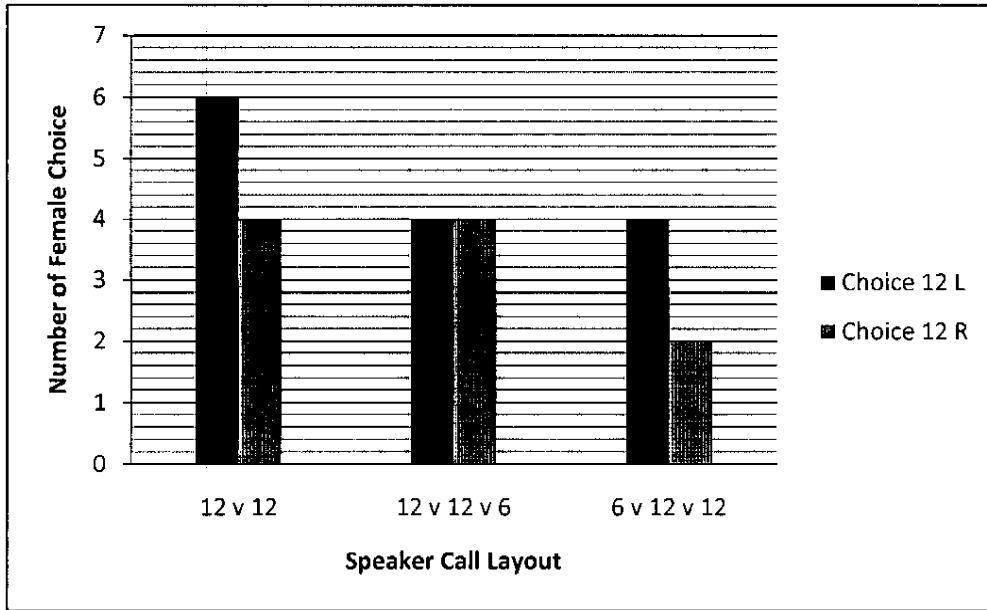


Figure 2: Results of treatment cycle 1; p-value of 0.814

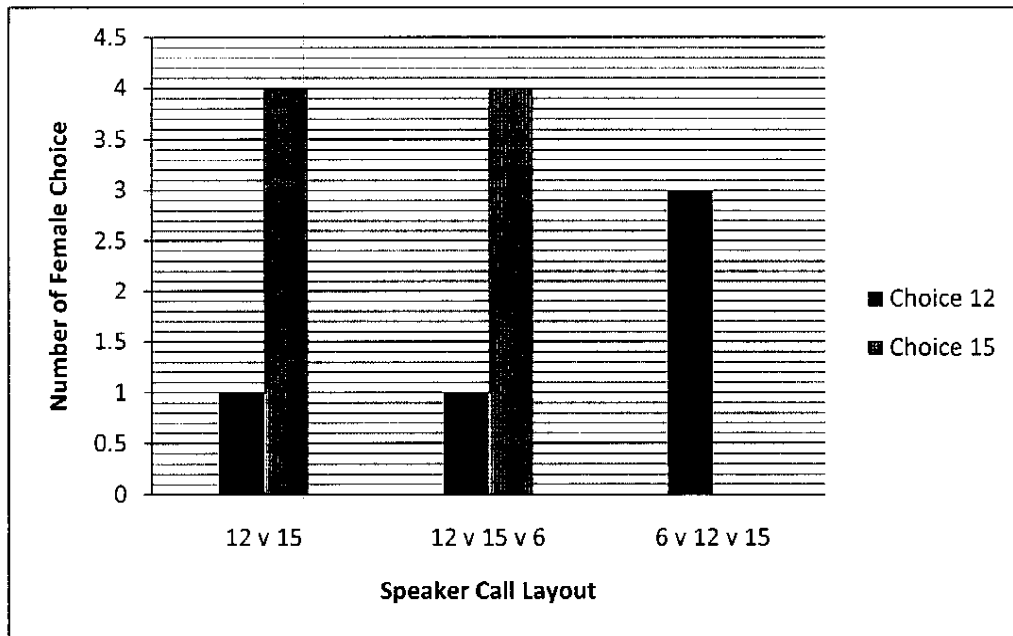


Figure 3: Results of treatment cycle 2; p-value of 0.044

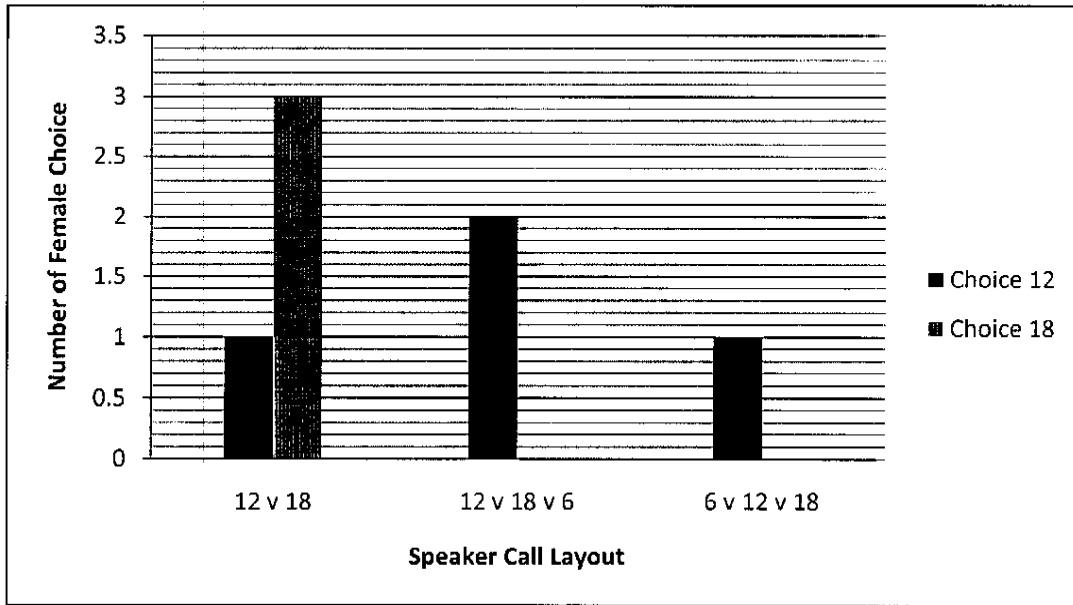


Figure 4: Results of treatment cycle 3; p-value of 0.140

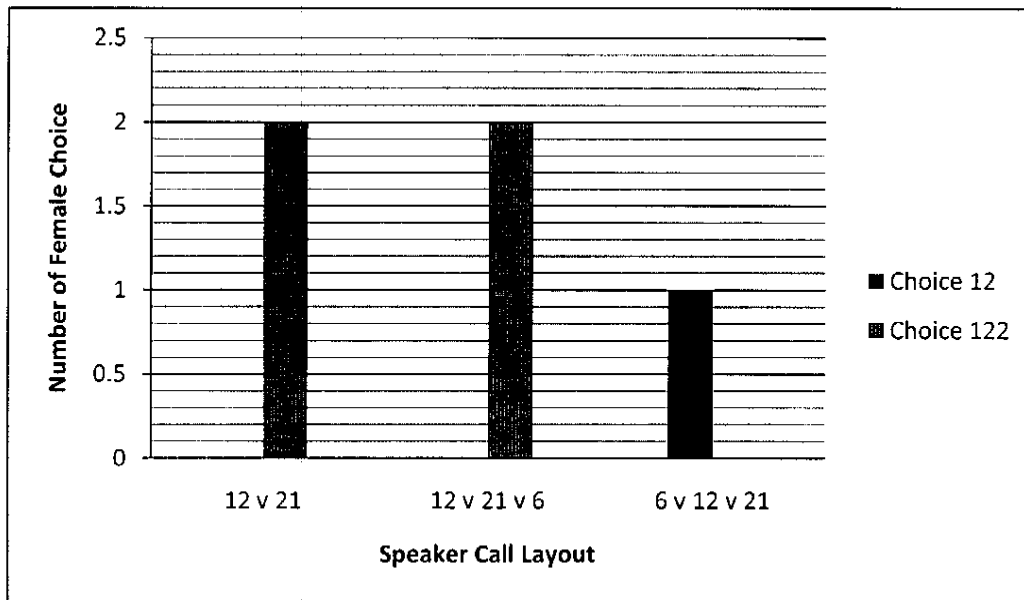


Figure 5: Results of treatment cycle 4; p-value of 0.082