

Report

Complexity Increases Working Memory for Mating Signals

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Summary

Females often prefer to mate with males who produce complex signals [1–3]. It is not clear why they do so. Females might prefer complexity if it predicts mate quality [4–6], or signals might evolve complexity to exploit females' sensory or cognitive biases [6–9]. We tested whether complexity increases active time, the period over which a signal influences a receiver's response to that signal. Mating signals are often ephemeral, yet their active time has largely been ignored. Here we demonstrate that signal complexity influences active time in túngara frogs. Male advertisement calls consist of frequency sweeps (whines) followed by 0–7 high-frequency bursts (chucks). Females preferentially approach complex (whines with chucks) over simple (whines alone) calls but do not consistently prefer greater complexity [10], so the function of multiple chucks has been uncertain. We found that females remember which speaker previously broadcast complex calls when choosing between simple calls broadcast after a delay. This effect occurred for calls with multiple chucks, but not with single chucks. Neither motivation nor orientation behavior differed with chuck number, suggesting that results are due to differential memory. Thus, female memory could favor the evolution of increasing signal complexity through sexual selection.

Results

Natural Interchorus Intervals

We measured the duration of túngara frog (*Physalaemus pustulosus*) interchorus intervals to determine the time period over which females might benefit from retaining attraction to a male's calls (Figure 1). Females choose a mate from males calling in a chorus, and assuming that they integrate calls over a substantial time period to assess males, as shown in other anurans [11], females could reduce assessment time by retaining attraction to a male through the silent intervals between chorus bouts. Reduced assessment time could ameliorate some costs of mate choice, such as increased predation risk characteristic of túngara frog choruses [12, 13]. From recordings of chorus calling activity, we determined that chorus bouts ($n = 4$ chorus groups, 109 bouts) are separated by variable silent intervals with a mean of 25 s (mean \pm standard error [SE] = 25.2 ± 1.9 s; see also Figure S1 and Supplemental Experimental Procedures available online).

Phonotaxis Experiments

Active Time

We conducted phonotaxis experiments to determine whether complexity, defined here as the number of components in a call [6], influenced active time for advertisement signals. Active time is the period over which an ephemeral signal influences a receiver's immediate response to that signal, analogous to a signal's active space, i.e., the area over which it is effective. We restrained a female under an acoustically transparent cone between two speakers. For the first 60 s, two speakers broadcast a series of alternating and identical simple whines. For the next 30 s, one speaker broadcast simple whines while a series of whines with 1 or 3 chucks broadcast from a second speaker. This presentation was followed by a silent period, after which the female was released as both speakers began to broadcast identical simple whines. If females approached the speaker that originally broadcast complex calls, this was evidence that the silent period was within the complex signal's active time. We predicted that active time would be longer for 3-chuck calls than for 1-chuck calls.

One-chuck calls did not influence choices made after a silent period, but 3-chuck calls did so significantly (Figure 2). A repeated-measures logistic regression with the general estimating equation (GEE) model showed a significant effect of chuck number on speaker choice for each of the silent periods ($p < 0.001$; Table S1). Pairwise comparisons showed that the results at each delay period differed significantly between 1-chuck and 3-chuck calls (0 s: $p < 0.009$; 15 s: $p < 0.009$; 30 s: $p < 0.009$).

We further investigated the active time for 3-chuck calls by testing the temporal limit of this signal's influence on females. Preference for the speaker that broadcast 3-chuck calls was retained after 45 s and reached the null expectation of 50% after 120 s (Figure 3). The mean chorus interval in nature is 25.2 s, thus the females retain assessment of 3-chuck but not 1-chuck calls over most interbout intervals. Because males call in leks and defend calling territories [14], if a female approaches calls that come from the same location as earlier attractive calls, she will reach the original signaling male.

Memory, Behavioral Mnemonic, and Motivation

Working memory of an ephemeral signal would extend its active time, but other factors may also influence this measure. For example, maintaining postural orientation toward a sound after it ceases could be a behavioral mechanism to retain information about an attractive signal's location. We propose the term "behavioral mnemonic" to describe this phenomenon of an adaptive strategy to retain information behaviorally without increasing memory load, as in the case of using a bookmark rather than remembering a page number. If females maintain postural orientation toward complex calls through the silent period, then orientation toward the speaker might explain the retention of attraction to complex calls. We recorded female orientation at the moment of release from the cone after the silent period. The GEE logistic model showed no influence of orientation on choice ($n = 231$; $p = 0.118$), thus females do not use a behavioral mnemonic as an alternative to memory, and this is not a strategy that interacts with memory as the mechanism for retaining attraction to calls.

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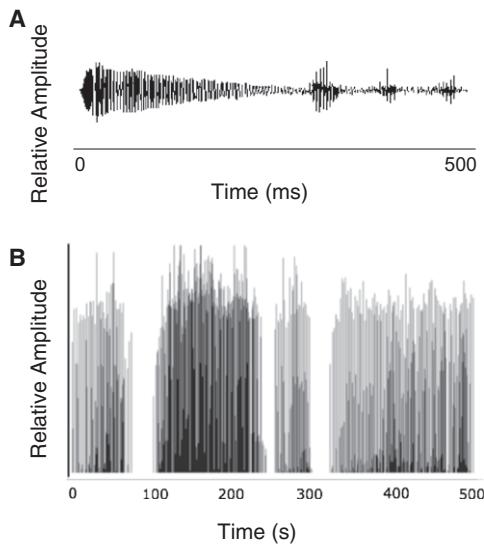


Figure 1. Túngara Frog Individual and Chorus Waveforms

Túngara frogs call in choruses that are active in bouts, separated by silent periods. Each male can produce simple and complex calls.

(A) A complex call with 3 chucks.

(B) A chorus calling in bouts. See also Figure S1.

Increased active time for calls of greater complexity could result from enhanced motivation to approach 3-chuck calls, differential memory of these calls, or an interaction between these factors. We considered several measures of motivation to test the hypothesis that females show differential motivation to approach calls with 1 or 3 chucks.

First, we conducted preference tests between a simple call and either a 1-chuck or 3-chuck call to determine the strength of preference for each type of complex call. In these tests, the female was released as calls were broadcast, and the broadcast continued until a speaker was chosen or the female failed the test by remaining still or not entering the choice zone. The strength of preference for complex calls over simple calls did not differ between 1-chuck and 3-chuck calls ($n = 25$ females; Fisher's exact test: $p = 0.668$).

We also compared latency to make a choice in the two conditions and found no significant difference ($n = 25$; 1-chuck calls: mean \pm SE = 61.0 ± 14.9 s; 3-chuck calls: mean \pm SE = 74.3 ± 15.2 s; paired 2-tailed t test: $p = 0.544$). Similarly, chuck number did not have a significant effect on latency in the active time trials ($n = 239$; 1-chuck calls: mean \pm SE = 92.3 ± 8.7 s; 3-chuck calls: mean \pm SE = 99.6 ± 8.2 s; GEE linear model; degree of freedom = 1; $p = 0.524$).

We also tested whether females differentially maintain orientation toward complex calls after 3-chuck and 1-chuck calls. The GEE logistic model showed no interaction effect for chuck number and orientation ($n = 231$; $p = 0.810$).

Our data reject the hypothesis that differential motivation influences the difference in active time for 1-chuck and 3-chuck calls. Thus, we conclude that increased active time for 3-chuck calls depends upon working memory.

Discussion

Our results show that females retain attraction to ephemeral mating signals over silent periods; when female túngara frogs chose between sources broadcasting identical whines, they

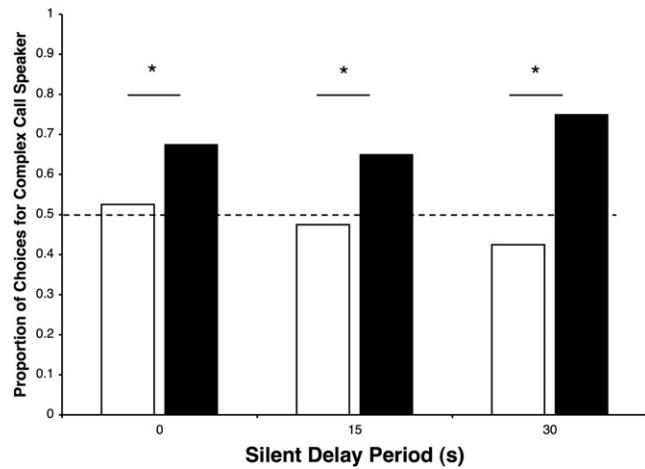


Figure 2. Proportion of Choices for Complex Call Speakers

Bars show proportion of choices for the speaker that originally broadcast complex calls before a 0 s, 15 s, or 30 s silent period. Dashed line shows the null hypothesis of 50% choice for each speaker. Females do not discriminate based on prior calls when the initial call pair was whines versus 1-chuck calls (white bars) but do discriminate when the initial call pair was whines versus 3-chuck calls (black bars), preferentially approaching the speaker that broadcast 3-chuck calls (general estimating equation: $n = 240$ choices, $p < 0.001$; pairwise comparisons with sequential Bonferroni adjustment: * = $p < 0.009$; see also Table S1).

preferred the source that previously had been broadcasting 3-chuck calls. Other studies in a variety of taxa have shown that exposure to males during a critical period as a juvenile can influence later preferences as an adult [15] and that recent exposure to males of different attractiveness can influence later female receptivity [16–19]. Studies of mate choice copying have also shown that recent experience can influence later preference [20, 21]. Our study, however, is the first to show that recent exposure to advertisement signals of certain males influences later female choice between those same males. Our study is also the first to consider the active time of mating signals and to propose its potential role in the evolution of complex mating signals.

We show that active time is enhanced by signal complexity: exposure to 3-chuck calls but not 1-chuck calls is effective after a delay. Thus, female retention of attraction to a caller over silent periods could favor the evolution of call complexity. Complexity in mating signals has been studied extensively, and many hypotheses for its evolution have been considered [3–9]. In general, hypotheses tend to fall into one of two classes: (1) complexity is an honest signal of male quality or (2) complexity is due to signal efficacy derived from the way signals interact with sensory biases or receiver psychology. Although both factors can interact, our data suggest support for the sensory bias explanation because there are no data to suggest that chuck number indicates better mates [22, 23]. All male túngara frogs can produce complex calls, and males in a chorus tend to increase chuck number together, in response to other males increasing call complexity [10, 24]. Nonetheless, it is still possible that females remember complex calls for longer because these calls indicate superior mates.

Memory is the mechanism by which females retain their attraction to complex calls. Female preference strength, latency to choice, and orientation upon release showed no difference between trials with 1-chuck and 3-chuck complex

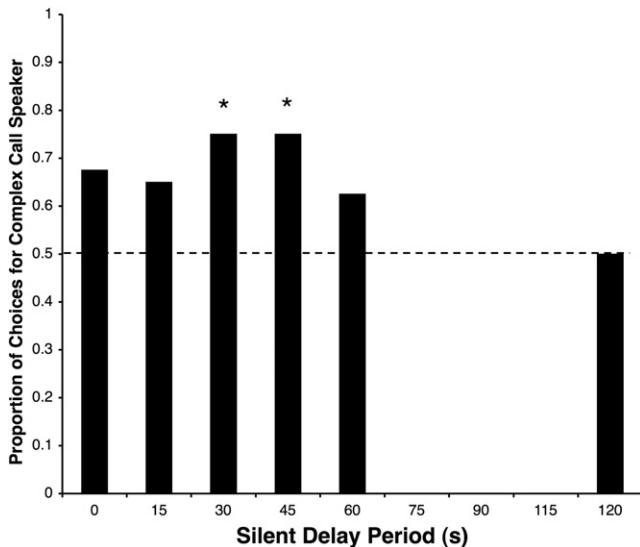


Figure 3. Proportion of Choices for 3-Chuck Calls Decreases over Time
Bars show proportion of choices for the speaker that originally broadcast 3-chuck calls before a variable silent period. The preference for complex calls fades after silent periods greater than 45 s ($n = 40$ each condition, exact binomial with Bonferroni adjustment: * = $p < 0.008$).

calls. Thus, the difference in active time is not influenced by differential motivation. We have not documented the precise parameters of complexity relevant to differential memory (i.e., duration, total energy), but as more chucks are added, the quantity of sensory stimulation from the advertisement signal increases, as is the case for many instances of complex signaling [3]. This difference in stimulation could influence the neural processing of sensory information.

Active time is a metric relevant to a variety of research related to temporal integration, such as mate assessment. For example, one area of research that would have obvious applications for active time is call rate variation [25, 26]. Faster call rates could ensure that signals with a short active time maintain a continuous influence on female behavior. A related process might explain the similarity between the duration of female túngara frog memory for 3-chuck calls and the duration of silent periods between chorus bouts.

Active time may also help us understand multimodal signaling in animal communication. Additional sensory modalities may increase the active time of a signal, as do additional components within one modality. In túngara frogs, for example, an inflating vocal sac is a visual cue that makes the mating call more attractive [27], and it might also enhance the call's active time. We predict that this active time enhancement might be a general feature of multimodal signals, which may help us understand the occurrence of multimodal signals that have been assumed to be redundant [28].

In summary, this study shows that females retain attraction to ephemeral calls over silent periods and that this effect depends upon the degree of call complexity. Females retain their attraction via working memory. This study highlights the need to consider not only how signal structure influences its attractiveness to the receiver, but also how this influence is retained once the signal ceases and, in general, how cognitive processes interact with preference in decision making. Sexual selection on signals to increase their active time by enhancing receiver memory might explain the evolution of many complex

courtship signals. Similar results have documented the influence of long-term memory on warning-signal evolution [6, 29, 30]. Because working memory also influences human language learning and comprehension [31], its influence on nonlanguage signal evolution might provide comparative perspectives on how the brain processes complex signals.

Experimental Procedures

Experiments were conducted in Gamboa, Panama at the Smithsonian Tropical Research Institute (STRI) between June and November of 2007 and 2008. We captured male-female pairs and tested females in a 2.7×1.8 m sound attenuation chamber, then released pairs after testing. Chamber temperature was controlled to be 26°C (range 25°C - 27°C). Each female performed a block of binary choice phonotaxis tests, and we followed standard toe clipping procedures to mark individuals before release. Toe clip procedures followed the Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research, compiled by the Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists, available at <http://www.asih.org/files/hacc-final.pdf>. All experiments were licensed and approved by STRI, The University of Texas at Austin Institutional Animal Care and Use Committee, and La Autoridad Nacional del Ambiente.

Stimulus Presentation

For each phonotaxis test, we placed a female under an acoustically transparent mesh funnel in the center of the chamber and between two speakers (ADS L200C) placed opposite each other at a distance of 50 cm on either side of the female. The speakers broadcast synthetic calls antiphonally, each at a call rate of one call per 2 s at a peak intensity of 90 dB sound pressure level (re: $20 \mu\text{Pa}$) at the initial location of the female. Synthetic calls were constructed (software developed by J. Schwartz) to be similar to the population average of male call variation based on seven call parameters [32].

Phonotaxis Trials

In each trial, we scored female response as a "choice" when she approached to within 10 cm of a speaker within 10 min of being released from the funnel. If she did not reach a speaker within 10 min, failed to leave the center of the chamber within 5 min, or remained immobile for 2 min at any point in the test after leaving the circle, she was scored as "no choice."

We varied which speaker played the complex call, leading call, last call before silence, and leading call after the silent delay. We used both 1- and 3-chuck complex calls and tested females with three silent delay periods, 0 s, 15 s, and 30 s, to compare these two call types. We then titrated the active time of 3-chuck calls by testing females with delay periods of 45 s, 60 s, and 120 s. Females completed a series of tests in random order. Each test had a sample size of 40 females. Most females were tested with several different stimulus combinations on the night of capture, but females were tested only once with any specific stimulus combination. For tests of preference strength, 25 females completed two tests as a repeated-measures series.

Statistical Analysis

To determine the influence of chuck number on speaker choice in these tests, we used the GEE procedure in SPSS 16 to create a binary logistic model of speaker choice predicted by the number of chucks in the complex call and the length of silent delay period. Our statistical analysis controlled for the fact that most females completed multiple tests but that few completed all conditions by using the repeated subject function. Pairwise comparisons were completed with the EMMeans contrasts with sequential Bonferroni adjustments for multiple comparisons.

We also completed GEE models to determine whether chuck number and orientation interacted to predict speaker choice and whether chuck number influenced latency to choice. The sample sizes for these models differ because orientation was not recorded for 9 of 240 trials and latency was not recorded for 1 of 240 trials.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, one figure, and one table and can be found with this article online at <doi:10.1016/j.cub.2010.01.021>.

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