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How female barking treefrogs, *Hyla gratiosa*, use multiple call characteristics to select a mate

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Female mate choice is an important mechanism of sexual selection, and in many species, females assess multiple characteristics of potential mates. Numerous studies have documented individual traits that females use to select a mate, but few have investigated how females use multiple male sex traits to choose a mate. In this study, we tested four hypotheses to determine how female barking treefrogs use multiple call characteristics concurrently to select a mate: (1) females may perceive as a single trait characteristics that researchers have defined as separate; (2) females may assess traits hierarchically, using the lower-level trait only when the higher-level trait is difficult to distinguish between males; (3) one trait may not be directly assessed by females, but may amplify another trait that is directly assessed; and (4) females may assess several traits at once, weighting them either equally or differently. We tested these hypotheses with two-speaker playback experiments that varied call rate, duration, or both. We also compared preference functions for rate and duration using a logistic regression model. Playback experiments supported simultaneous assessment and refuted the other three hypotheses. Females weighted duration more heavily than rate when percentage difference between stimuli was 29%, but not when percentage difference was 100%. Preference functions revealed that, for a given percentage difference between stimuli, percentage preferences were greater for higher call rates than for longer calls. Thus, female barking treefrogs appear to treat rate and duration as two separate traits and combine preferences for these traits nonadditively.

Keywords: Anura; barking treefrogs; *Hyla gratiosa*; mate choice; playback; sexual selection; vocal communication; vocalization

Numerous studies have documented the individual traits that females use to select mates (reviewed in Andersson 1994; Gerhardt & Huber 2002). In many species, however, males have multiple traits on which females base their choice of mates (reviewed in Møller & Pomiankowski 1993; Jennions & Petrie 1997; Candolin 2003). Females may benefit from attending to more than one cue because each trait may indicate a different aspect of a male's fitness (Møller & Pomiankowski 1993), multiple sex traits may provide redundant information about the same fitness quality (Møller & Pomiankowski 1993), or some traits that do not indicate fitness directly may instead increase the ability of females to assess other cues that do (Hasson 1991; Taylor et al. 2000). Finally, one or more of the multiple sex traits may no longer reliably signal male quality but are instead maintained by their low cost and a weak female preference for them (Møller & Pomiankowski 1993). A number of theoretical studies have investigated the evolutionary dynamics of multiple traits and preferences, and these investigations show that multiple preferences can coevolve with male traits by both Fisherian and good-genes processes (Pomiankowski & Iwasa 1993; Iwasa & Pomiankowski 1994; Johnstone 1996; Fawcett & Johnstone 2003; van Doorn & Weissing 2004). However, relatively few empirical studies have explicitly examined how females combine multiple preferences to choose mates (Jennions & Petrie 1997; Candolin 2003; Scheuber et al. 2004; Kirkpatrick et al. 2006). How females use multiple cues has important consequences for the strength of sexual selection, the maintenance of variation in sexually selected traits, the evolution of alternative signalling tactics and speciation (Candolin 2003).

There are a number of ways that females might combine preferences for multiple cues to choose a mate

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(reviewed in Jennions & Petrie 1997; Candolin 2003). Females could assess cues sequentially, screening males based on one cue before using a different cue to assess those males who pass the initial assessment. For example, female field crickets, *Gryllus lineaticeps*, use long-range calling song to determine which male to approach and then accept or reject approached males after assessing close-range courtship song (Wagner & Reiser 2000). Alternatively, females could assess multiple cues concurrently, with preferences for these cues combined in a number of ways (Jennions & Petrie 1997; Candolin 2003).

(1) *Single-trait Hypothesis*. Females may perceive multiple cues as a single trait, even though researchers may identify them as separate traits. For example, female swordtail fish, *Xiphophorus cortezi*, base their choice of mates on vertical, pigmented bars on the sides of males, but females do not separately assess the number or width of bars; instead, they appear to assess the total pigmented area (Morris et al. 2001).

(2) *Amplifier Hypothesis*. A trait may not be directly assessed by females but may amplify another trait that is assessed directly, thereby allowing females to better discern differences between males in the assessed trait (Hasson 1991; Taylor et al. 2000). For example, in male guppies, *Poecilia reticulata*, areas of orange are often surrounded by black pigment; this black pigment does not appear to be the object of a female preference per se but instead appears to improve the ability of females to assess the amount of orange on the body, which does directly influence choice (Brooks 1996).

(3) *Hierarchical Hypothesis*. Females may assess traits in a hierarchical fashion, basing their choice on the higher-level trait whenever this trait differs between males and using lower-level traits only when higher-level traits are difficult to discern between males. For example, mate choice by female field crickets, *Gryllus campestris*, is affected by variation in chirp rate, the lower-level trait, only when alternative calls do not differ in carrier frequency, the higher-level trait (Scheuber et al. 2004).

(4) *Simultaneous Hypothesis*. Females may base their choice of mates on multiple traits, combining preferences for individual traits. Preferences for traits may be combined additively. For example, the percentage preference by female sticklebacks, *Gasterosteus aculeatus*, for the more attractive of two choices increases linearly with the number of traits that differ between choices (Künzler & Bakker 2001). Alternatively, traits may be combined non-additively. For example, females may weight some traits more heavily than others, as do female grey treefrogs, *Hyla versicolor*. Females of this species weight call duration more heavily than call rate, choosing a long call with low call rate over a short call with a high call rate, even when the total amount of acoustic energy is equalized between the two alternatives (Gerhardt 2001).

To date, no published study has attempted, within one species, to distinguish among all four hypotheses for how females might concurrently combine preferences, although several studies have supported one or more hypotheses without explicitly testing all four. In the present study, we tested these four hypotheses and thereby determined how female barking treefrogs use multiple call traits in mate selection. Female barking treefrogs select their mates based on the traits of the male call (Gerhardt 1981), and two-choice playback experiments have revealed preferences based on several traits (Gerhardt 1981; Murphy & Gerhardt 1996, 2000). We tested the four hypotheses by conducting playback experiments in which we manipulated two traits, call rate and duration; females prefer higher call rates to lower call rates (Murphy & Gerhardt 1996, 2000) and longer calls over shorter calls (Poole & Murphy 2007). We also combined data from the present study and previous studies to construct preference functions for call rate and duration, and we used these preference functions to further elucidate how preferences are combined by female barking treefrogs.

METHODS

Study Site and Species

Barking treefrogs are found in the coastal plains of the southeast United States. This study was conducted from June to August 2002 and 2003, in the Apalachicola National Forest, Leon County, Florida, U.S.A. (30°22′N, 84°20′W), where barking treefrogs breed from March through August (Murphy 1994). Males and females spend the day in trees surrounding the breeding ponds (Murphy 1994), and males move to the ponds shortly after sunset and begin calling. Choruses last for 3–5 h, with individual males present for an average of 2.5 h (Murphy 1999). Males float on the surface of the water while calling, producing calls about once every second. The advertisement call consists of a short (100–200 ms) burst of sound with two or three spectral peaks, the fundamental and one or two upper harmonics (Oldham & Gerhardt 1975). Females arrive at the ponds shortly after males and approach and nudge a male to initiate amplexus. After oviposition, both the male and female return to the forest.

Playback Experiments

Methods for playback experiments follow Murphy & Gerhardt (1996, 2000). We collected females in amplexus from three small breeding ponds and brought pairs to one of two playback arenas. Both arenas were unpaved roads with sandy substrate lacking vegetation; one was approximately 0.5 km from two of the ponds and the other was 0.25 km from the third pond. At the arena, we separated the female from her mate and placed her in an acoustically transparent, hardware-cloth cage located 4.4 m from each of two speakers, which were separated by 3.5 m; the cage and two speakers formed a triangle. Playback calls were repeated five times from each speaker before the cage was opened remotely to release the female. We observed the female with an infrared video camera. We