

Do frog-eating bats perceptually bind the complex components of frog calls?

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Abstract The mating calls of male túngara frogs, *Physalaemus pustulosus*, attract intended (conspecific females) and unintended (eavesdropping predators and parasites) receivers. The calls are complex, having two components: a frequency-modulated “whine” followed by 0–7 harmonic bursts or “chucks”. The whine is necessary and sufficient to elicit phonotaxis from females and the chuck enhances call attractiveness when it follows a whine. Although chucks are never made alone, females perceptually bind the whine and chuck when they are spatially separated. We tested whether an unintended receiver with independent evolution of phonotaxis, the frog-eating bat, *Trachops cirrhosus*, has converged with frogs in its auditory grouping of the call components. In contrast to frogs, bats approached chucks broadcast alone; when the chuck was spatially separated from the whine the bats preferentially approached the whine, and bats were sensitive to whine–chuck temporal sequence. This contrast suggests that although disparate taxa may be selected to respond to the same signals, different evolutionary histories, selective regimes, and neural and cognitive architectures may result in different weighting and grouping of signal components between generalist predators and conspecific mates.

Keywords Phonotaxis · *Physalaemus pustulosus* · Sexual advertisement signal · Túngara · *Trachops cirrhosus*

Introduction

Conspicuous sexual advertisement signals attract potential mates (Darwin 1871; Andersson 1994), but may also attract eavesdropping predators or parasitoids (Zuk and Kolluru 1998; Peake 2005; Jones et al. 2011; Page et al. 2013). Indeed, conspecific mates and heterospecific eavesdroppers use the same signals to identify, locate, and assess the quality of the signaler (Walker 1993; Wagner 1996; Haynes and Yeargan 1999; Bernal et al. 2006). The extent to which signal processing converges in these intended and unintended receivers may depend on how specialized their phonotaxis is to a particular signal. For example, specialized dipteran parasitoids of a single singing insect species exhibit similar auditory tuning and phonotactic preferences to those of mate-searching females (Fowler 1987; Robert et al. 1992; Walker 1993; Wagner 1996, 2011; Lakes-Harlan et al. 1999; Gray et al. 2007; Farris et al. 2008). In contrast, generalist parasitoids may exhibit less convergence with females of the host species (Stumpner et al. 2007; Sakaguchi and Gray 2011). Our study investigates whether a generalist acoustic predator, the frog-eating bat (*Trachops cirrhosus*), groups the complex call components of male túngara frogs (*Physalaemus*(=*Engystomops*) *pustulosus*) as female túngara frogs do.

Calls of túngara frogs have two acoustically distinct components: a frequency-modulated ~350 ms sweep (“whine”) and a broadband ~40–80 ms harmonic burst (“chuck”) (Ryan 1980). Males can produce simple calls consisting of a whine alone, or complex calls composed of a whine followed by 1–7 chucks. Both female frogs and

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frog-eating bats are more attracted to complex calls than simple calls (Ryan 1980; Ryan et al. 1982, Akre et al. 2011). Male frogs call in multi-male choruses creating a problem for female frogs and frog-eating bats that is acoustically analogous to the ‘cocktail party problem’ in humans (Cherry 1953). Female frogs and bats must determine which whine goes with which chuck, so that calls can be assigned to the correct source and thereby accurately compared. In female frogs, the whine is necessary and sufficient to elicit phonotaxis, but frogs exhibit a conditional response to the chuck: a chuck that elicits no response when presented alone is attractive and elicits phonotaxis when broadcast with the spatially separated whine (Ryan 1985; Farris et al. 2002). This conditional phonotactic response reveals auditory grouping and source assignment of the two components that are based on relative whine–chuck spatial separation and temporal sequence (Farris et al. 2002, 2005; Farris and Ryan 2011). We tested whether this grouping response found in female frogs is also exhibited by frog-eating bats that are generalist acoustic predators of several frog species (Tuttle and Ryan 1981). The results allow us to compare the weighting and grouping of complex call components by two receivers that have different evolutionary histories and are under different selective regimes in their response to the same signal.

Methods

We captured bats with mist-nets in Soberanía National Park, Panamá between February and July of 2012 ($N = 10$, 7 adult males and 3 adult non-reproductive females). Bats were released into a 5 m \times 5 m \times 2.5 m flight cage with ambient temperature and humidity, illuminated by one 25 W red light bulb. Only one bat was tested at a time. We placed Fostex FE103En speakers underneath 1.5 m \times 1.5 m screens covered in leaf-litter in two diagonally opposite corners of the cage. In the third corner, we positioned a shelter with a perch to which the bats were trained to return between stimulus presentations. The experimenters sat in the fourth corner with the playback equipment (see Page and Ryan 2005, 2006). The experimental stimuli were constructed in Adobe Audition 3 from the modal túngara frog call selected from a sample of 300 calls from 50 males (Ryan and Rand 2003). Stimulus period was 2 s and stimuli were broadcast at 75 dB SPL (re. 20 μ Pa) at 1 m from the speaker, reflecting natural call rate and amplitude (Rand and Ryan 1981; Ryan 1985). We broadcast stimuli using a Pyle Pro PTA2 amplifier and a Lenovo T500 Thinkpad laptop.

Each bat received six different stimuli (Fig. 1), four times each, presented in random order (24 presentations total). The whine (W) alone is sufficient to elicit

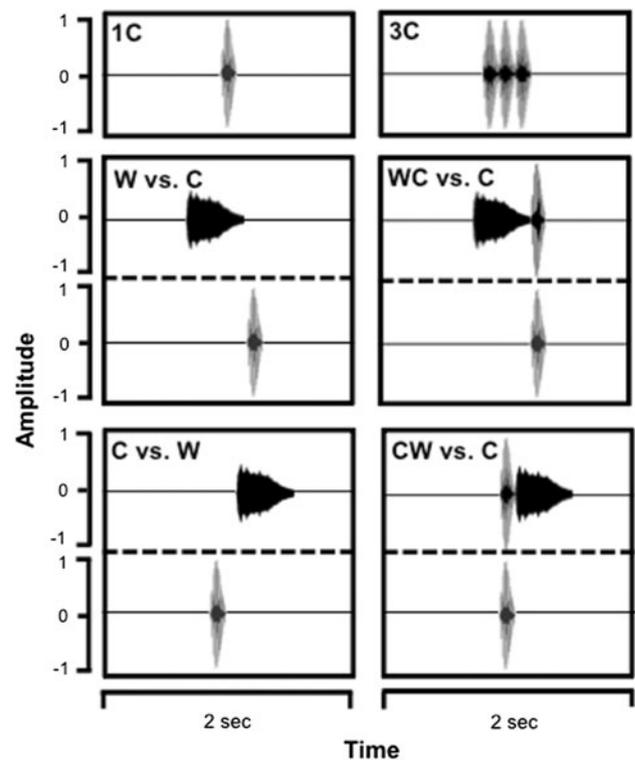


Fig. 1 Waveforms of experimental stimuli. Dotted lines separate stimuli from two-speaker experiments. 1C, a single chuck from a single speaker; 3C, three chucks from a single speaker; W versus C, whine from one speaker, the chuck from the other in natural temporal sequence; WC versus C, whine-chuck from one speaker and the identical chuck from the other speaker in natural temporal sequence; C versus W and CW versus C, as with the stimuli above except in reversed temporal sequence

phonotaxis in *T. cirrhosus* (Ryan et al. 1982). To assess whether the chuck (C) alone also elicits phonotaxis, we examined bat response in single speaker tests of either a single chuck (1C) or three consecutive chucks (3C). The 3C stimulus had a similar duration to the whine and was included in the design a priori in case bats were not responsive to a single chuck due to its short duration. The other four stimuli were broadcast with two speaker tests to determine how bats weight and group the two call components. For two of the stimuli, spatially separated whines and chucks were broadcast from the separate speakers in the two corners of the cage either in the natural (W vs. C) or reversed temporal order (C vs. W). These stimuli tested the relative weighting of the two components during phonotactic decisions and the extent that the natural temporal sequence affected such weighting. Previous research demonstrates that bats and frogs preferentially approach complex calls with higher ratios of chucks (Akre et al. 2011), indicating the importance of chucks in phonotactic decisions. We therefore also examined whether the chuck’s influence on the whine’s attractiveness was maintained even when presented without a co-localized whine. Thus,

for the fifth stimulus, a whine was broadcast from one speaker followed by the chuck from both speakers (WC vs. C). The sixth stimulus reversed the temporal sequence to assess whether such a comparison is order-dependent (CW vs. C).

To control for arena bias, the speaker side associated with the chuck component was randomly assigned for each stimulus. To maintain the bats' motivation, baitfish rewards were placed on the screens over both speakers. Tests lasted 20 s or until the bat removed the baitfish from the speaker. Observers recorded which of the two speakers the bat approached for each stimulus. Analysis was conducted in R v. 2.15 (R Development Core Team 2012) and evaluated whether the number of times bats approached each of the stimuli (each bat had a value between 0 and 4 for each stimulus) differed from 0 (0 %) in one-speaker tests (did not approach the chuck) and 2 (50 %) in two-speaker tests (no preference). Significance was determined using one-sample *t* tests for each of the stimuli.

Results

Bats showed consistency in their responses across the four presentations. All ten individuals approached both the single chuck (1C) and the three chuck (3C) stimuli in at least two of the four presentations. The number of times that the bats approached both the 1C (one-sample *t* test: $t = 19$, $df = 9$, $p < 0.0001$; Fig. 2a) and the 3C significantly differed from zero ($t = 24$, $df = 9$, $p < 0.0001$; Fig. 2b). Bats therefore showed no conditional response to the chuck; they were attracted to chucks in the absence of whines even though this stimulus does not occur in the wild. We therefore used the two-speaker tests to determine if bats weight whines and chucks equally during phonotaxis.

When presented broadcasts of a spatially separated whine and chuck that maintained natural temporal sequence (W vs. C), bats approached the whine significantly more often than would be expected if they had no preference ($t = 3.5$, $df = 9$, $p = 0.007$; Fig. 2c). These results contrast the conditional response to the chuck found in frogs. Bats are known to discriminate calls that have identical whines, but differ in their chuck number (Akre et al. 2011). When chuck number was controlled in the whine–chuck versus chuck tests (WC vs. C), however, all of the bats preferentially approached the whine–chuck for all of the presentations, showing that decisions were not based on chuck number alone and confirming that the whine plays an important role in phonotactic response (one-sample *t* test: $t = 199$, $df = 9$, $p < 0.0001$; Fig. 2d).

Bats' phonotactic preference for the whine was, in part, based on component sequence. When the call component

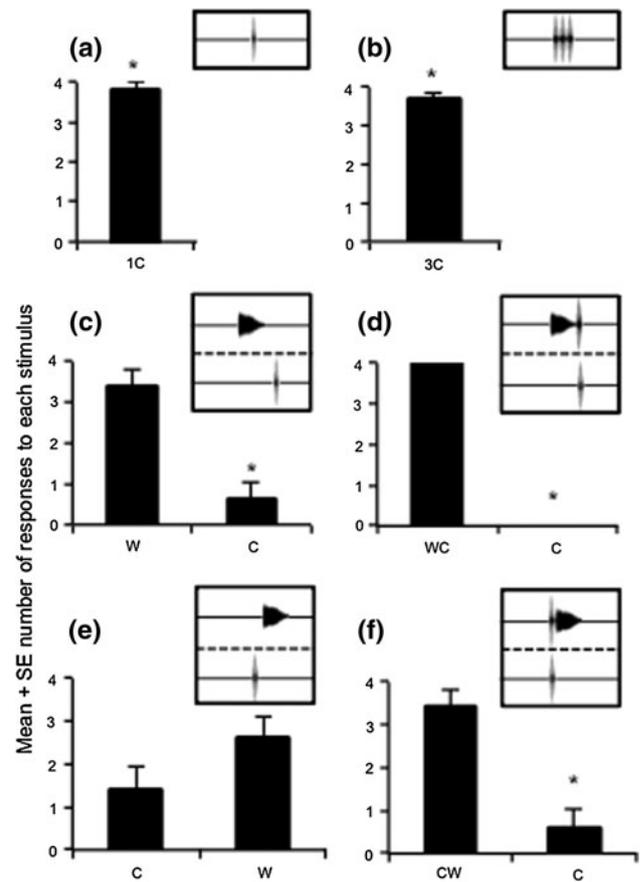


Fig. 2 Mean (+SE) number of presentations for which bats approached each of the stimuli. Each bat was tested four times for each stimulus. Stimuli are noted on the x axis *under the bars* and *insets* are waveforms. *Asterisks* indicate when the number of times bats approached the chuck significantly ($p < 0.01$) differed from 0 (0 %; one speaker tests) or 2 (50 %; two-speaker tests)

order was reversed (C vs. W), bats did not approach the whine significantly more often than would be expected if they had no preference ($t = 1.2$, $df = 9$, $p = 0.26$; Fig. 2e). The reverse order did not switch the preference to the chuck, however, suggesting that the following whine still influenced phonotaxis. The whine's influence was further confirmed when bats were given the complex calls but with the natural order reversed: chuck–whine versus chuck (CW vs. C). If only the leading calls were considered, there should have been equal attraction. The bats, however, approached the chuck–whine significantly more often than expected if they had no preference ($t = 3.5$, $df = 9$, $p = 0.007$; Fig. 2f).

Discussion

Frog-eating bats forage in a complex acoustic environment where they are faced with the perceptual problem of locating a single prey item in a chorus. Female frogs are

faced with a similar problem as they also use these signals to locate and assess potential mates amidst overlapping calls and heterospecific noise. Given that both of these receivers are localizing the same signal in the same environment, we might expect convergence in signal processing. There is evidence for convergence despite substantial differences in peripheral (e.g., cochlea vs. amphibian and basilar papillae) and central (e.g., mammalian cortex in bats) auditory processing. In particular, the auditory system of *Trachops cirrhosus* has neuroanatomical adaptations that extend its frequency sensitivity into the sonic range (Bruns et al. 1989), enabling the detection of frog calls. Túngara frogs also have adaptations in both the peripheral and central auditory systems that enable them to respond preferentially to conspecific calls (Wilczynski et al. 2001; Hoke et al. 2004). In addition, both taxa can process the two call components separately, allowing for components to be compared between calls. For example, when comparing complex calls with different numbers of chucks, both frogs and bats use the relative difference in call complexity (Akre et al. 2011). Such convergence has influenced the same phonotactic task with different functional outcomes (i.e., a mate vs. a prey item, with the latter being a more general or multi-species category). We further tested the extent of convergence by measuring bat responses to call components known to elicit specialized grouping or sorting responses in female frogs.

Our data show that the influence of the two call components on phonotaxis differs between the taxa. When female frogs group call components, the whine functions in both recognition and location (so called “what” and “where”) decisions, whereas the chuck only functions in location decisions (Farris et al. 2002; 2005; Farris and Ryan 2011). For bats, however, we found that both components can be used for recognition and location, but components are not equally weighted. First, bats prefer whines over single chucks. With the exception of temporal order, we did not test which acoustic parameter is responsible for this decision. Furthermore, although bats make phonotactic decisions by comparing the relative number of chucks between calls (Akre et al. 2011), our data (e.g., whine-chuck is more attractive than the identical chuck alone) show that this comparison requires whines in both calls. Bats’ greater perceptual weighting of the whine, however, is affected by temporal sequence, as the whine is less attractive when following a chuck. This is in contrast to female frogs, which group single whines and chucks even when presented in non-natural sequences (Farris et al. 2005; Farris and Ryan 2011). A potential reason for bat preferential weighting of the first call component may result from the need to approach a calling frog quickly without alerting the frog to predator presence, as frogs that detect approaching bats cease calling (Tuttle et al. 1982).

Bat response is not entirely dictated by component order, however, because the relative preference for the chuck and the whine does not reverse when the order is reversed: some preference for the whine remains. Given the importance of duration to signal detection (e.g. Campbell 1963), the longer duration of the whine compared to the chuck may be a factor affecting bat preference for the whine component.

Our results lead to the conclusion that bats exhibit more flexibility than frogs in call processing, as the chuck alone is sufficient to elicit phonotaxis in bats but not in frogs. The lack of flexibility is not surprising for the frogs, since females are under intense selection to mate preferentially with conspecifics due to the reproductive costs of heterospecific matings. The bat response matches the flexibility that these bats show in other assessments of prey-generated acoustic cues (Page and Ryan 2005), making it apparent that bats, unlike frogs, do not use the same simple grouping rule as a solution to the problem of assigning call components to a single male in a chorus that is analogous to the acoustic ‘cocktail party problem’ (Cherry 1953). Indeed, as a generalist predator, assigning call components to a particular source, although beneficial in comparing sources, may limit the ability to locate a source quickly. Speed notwithstanding, bats should not respond randomly to any leading acoustic stimulus. Bats must discriminate palatable from poisonous and smaller from larger anurans by their calls (Tuttle and Ryan 1981). Differences in whine and chuck weighting could therefore be based on strategies best suited for recognizing palatable prey, a selective pressure not shared by female frogs. The response of these generalist bats is likely to be a balance between speed and accuracy, and exhibits little convergence with more specialized female frogs in these complex stimulus binding tasks.

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