



Receiver discriminability drives the evolution of complex sexual signals by sexual selection

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A hallmark of sexual selection by mate choice is the evolution of exaggerated traits, such as longer tails in birds and more acoustic components in the calls of birds and frogs. Trait elaboration can be opposed by costs such as increased metabolism and greater predation risk, but cognitive processes of the receiver can also put a brake on trait elaboration. For example, according to Weber's Law traits of a fixed absolute difference will be more difficult to discriminate as the absolute magnitude increases. Here, we show that in the Emei music frog (*Babina daunchina*) increases in the fundamental frequency between successive notes in the male advertisement call, which increases the spectral complexity of the call, facilitates the female's ability to compare the number of notes between calls. These results suggest that female's discriminability provides the impetus to switch from enhancement of signaling magnitude (i.e., adding more notes into calls) to employing a new signal feature (i.e., increasing frequency among notes) to increase complexity. We suggest that increasing the spectral complexity of notes ameliorates some of the effects of Weber's Law, and highlights how perceptual and cognitive biases of choosers can have important influences on the evolution of courtship signals.

KEY WORDS: Acoustic communication, sexual selection, signal evolution, Weber's Law.

Sexual selection has resulted in some of the most elaborate morphologies and behaviors in nature (Darwin 1872; Andersson 1994). Furthermore, signals used in sexual displays are among the most elaborate sexually selected traits, and choosers often prefer courtiers with more elaborate traits (Ryan and Keddy-Hector 1992; Andersson 1994). There is considerable interest in how this signal elaboration evolves. In some cases signal elaboration involves an increase in signal magnitude, such as longer tails and louder songs (Andersson 1994; Andersson and Simmons 2006). In other cases, novel signals such as decorations on tails or new notes in calls results in evolution of signal complexity such as the appearance of multidimensional signals. As choosers are the ones who drive evolution of courtship traits, it is critical to understand how choosers perceive and analyze such signals during mate choice (Akre and Johnsen 2014).

A recent study found that female cognitive processes can limit the evolution of sexual signal elaboration in accordance with Weber's Law, which holds that the just-noticeable-difference (JND) between two stimuli is based upon differences in ratio rather than absolute differences (Akre et al. 2011). When this is the case, then the absolute differences between traits sufficient for a JND increases with over trait magnitude, which in turn might drive the evolution of novel signal components along a separate new perceptual axis. Our previous study showed that male Emei music frogs produce very long calls (about 0.75–2.25 s) composed of 3–8 notes (up to 11 notes in response to playback stimulation) (Cui et al. 2010, 2012; Chen et al. 2011). It seems reasonable to hypothesize that a “law of diminishing returns” governs the benefits of increasing note numbers due to the difficulty for females to discriminate note numbers as predicted

Table 1. Stimulus pairs set.

Ratio	0	0	0.375	0.5	0.6	0.625	0.714	0.833	0.875
Group E	0 vs. 5	0 vs. 8	3 vs. 8	4 vs. 8	3 vs. 5	5 vs. 8	5 vs. 7	5 vs. 6	7 vs. 8
N	0 vs. 5	0 vs. 8	3 vs. 8	4 vs. 8	3 vs. 5	5 vs. 8	5 vs. 7	5 vs. 6	7 vs. 8
F	0 vs. 5	0 vs. 8	3 vs. 8	4 vs. 8	3 vs. 5	5 vs. 8	5 vs. 7	5 vs. 6	7 vs. 8

E, N, and F represent extra frequency increase group, natural group, and flat group, respectively.

by Weber's Law (Akre and Johnsen 2014). Most pertinent is the fact the fundamental frequency (F0) of male Emei music frog calls increases between successive notes. We therefore hypothesize that this pattern of successive frequency increments enhances female discrimination of note number thereby somewhat countering the effects of Weber's Law.

Materials and Methods

STIMULI SYNTHESIS

The male Emei music frog produces advertisement calls from both inside and outside burrows with the fundamental frequency of successive notes increasing from the first to the fifth notes (Chen et al. 2011; Cui et al. 2012). The calls produced inside burrows are more sexually attractive than those produced outside burrows, which possess harmonics not present for the inside call (Cui et al. 2012). Thus inside calls were selected to construct the acoustic stimuli in the present experiment. Three groups of stimuli were constructed based on calls with eight notes recorded inside the frog's burrow (Fig. 1): (1) Natural calls (N) in which the fundamental frequency (F0) increases in the species-typical manner (i.e., naturally) from note to note: 441 Hz, 551 Hz, 589 Hz, 606 Hz, 618 Hz, 628 Hz, 628 Hz, and 628 Hz, respectively; (2) Flat calls (F) in which the frequency among notes did not vary and (3) exaggerated frequency increase calls (E) in which the increase in frequency among notes was exaggerated artificially. Both flat calls and exaggerated frequency increase calls were constructed based on the natural calls. For the flat call, all frequencies of the second to eighth notes were lowered to make each note F0 equal to 441 Hz using Avisoft SAS-Lab Pro (Avisoft Bioacoustics, Berlin) while the temporal characters remained unchanged. For the exaggerated frequency increase call, the frequencies of the fourth to eighth notes were increased by 38 Hz, which was the difference between the second and third notes in the natural call while the temporal characters remained unchanged. Therefore, the F0 of the eight notes in the extra frequency increase call were set to 441 Hz, 551 Hz, 589 Hz, 627 Hz, 665 Hz, 703 Hz, 741 Hz, 779 Hz, respectively (Fig. 1). For each group, two stimuli were paired with different note numbers as follows: 0:5, 0:8, 3:8, 4:8, 3:5, 5:8, 5:7, 5:6, 7:8 (Table 1). To test the difference in attractiveness among the three call groups, nine stimulus pairs with the same note number

but differences in frequency step increases were constructed as follows: N4:F4, E4:F4, E4:N4, N5:F5, E5:F5, E5:N5, N6:F6, E6:F6, E6:N6.

PHONOTAXIS EXPERIMENTS

During the breeding seasons of 2012–2013, female Emei music frogs were collected after 1900 h for phonotaxis tests. An open tank [270 cm (l) x 95 cm (w) x 100 cm (h)] containing humid mud located outdoors was used for phonotaxis tests. Females were placed in the center of the tank and tested in a two-choice phonotaxis paradigm between 2000 and 2400 h. To broadcast the stimulus pairs, two speakers (SME-AFS, Saul Mineroff Electronics, USA) were placed equidistant from the opposite sides of the tank.

The amplitude of all stimulus pairs used in the playback experiments were equalized for 75 dB (SPL re: 20 μ Pa), which approximates the amplitude of the calls in nature, measured at the center of the tank where the subject was situated using an SPL meter (AWA 6291, Hangzhou Aihua Instruments Co., China). Stimuli were presented antiphonally with 4-s interstimulus intervals, approximately equal to the mean of intercall intervals in the chorus ponds (Cui et al. 2012). The female subject was monitored via an infrared camera (BL-3720I, WAPA, Inc., China). A positive response was scored if females approached within 10 cm of a speaker. If the female failed to make a choice within 10 min a "no response" was scored. Females who did not complete all the trials were marked with passive integrated transponder (PIT) tags (Hongteng, Inc., China), and returned to the original pond. These females were tested with the remaining stimulus pairs when next recaptured. After all tests were completed, females were marked with PIT tags and returned to their ponds. To control for possible side biases, we varied the speaker broadcasting each stimulus of a pair. Twenty-seven stimulus pairs (three call types \times nine different note number ratio = 27 pairs) were presented (in random order) only once to each individual. Fifty-nine females completed all tests with 27 stimulus pairs, generating a total of 1593 choices. To test the relative attractiveness of the different types of stimuli, stimulus pairs with the same number of notes but different frequency step increases (N4:F4, E4:F4, E4:N4, N5:F5, E5:F5, E5:N5, N6:F6, E6:F6, E6:N6) were presented (in random order) to another 58 females following the previously described procedures, generating 522 choices. Animal procedures were approved

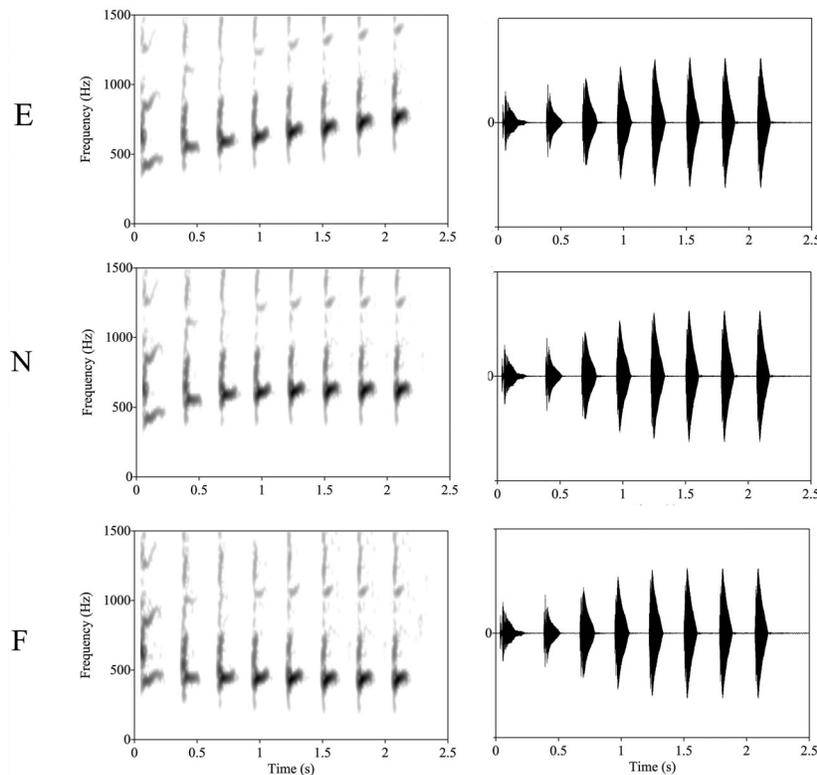


Figure 1. Spectrograms and amplitude-modulated waveforms of the stimuli. E, N, and F represent the extra frequency increase call, natural call, and flat call, respectively. The sound spectrogram is shown at left, the signal waveform at right.

by the Animal Care and Use Committee of Chengdu Institute of Biology, CAS.

ANALYSIS AND STATISTICS

The ratio of approaches to calls with more notes for each stimulus type was computed and plotted against the ratio for the stimulus pairs. The curves were fit and analyzed with GraphPad Prism Software (Version 5.01, GraphPad software Inc., California) and SigmaPlot 11 software (Systat Software Inc., San Jose). Three curves for the responses in the extra frequency increase group, natural group, and flat group treatments were separately fit using the least squares fit of the psychometric function as described by Akre et al. (Akre et al. 2011):

$$\text{response_proportion} = \frac{1}{n} + \left[1 - \frac{1}{1 + e^{a*(m-x)}} \right] \times \frac{n-1}{n}.$$

Where n is the number of possible choices (for binary choice tests $n = 2$); m is the mean of the distribution or inflection point of the psychometric function (i.e., where the response proportion is equal to 0.75, midway between 0.5 and 1); and a is the exponential slope. Three curves were statistically tested using the compare tab of the nonlinear regression dialog in GraphPad Prism to determine if these curves were significantly different from one another (Motulsky 2004). We compared the independent fits with the

global fit that shared two parameters: a and m . The null hypothesis is that one curve fits all data points. The alternative hypothesis is that the curves are distinct. The F test was used to examine the hypothesis. To further test the effect of the note frequency increases on female discriminability of note numbers, the ratios of note numbers in the pairs were divided into two categories: those for which the ratio was less than or equal to 0.5 (relatively easy ratio range for discrimination) and those for which the ratio was more than 0.5 (relatively difficult ratio range). Repeated measures one-way analysis of variance (ANOVA) was used to evaluate the female discriminability among the three groups within each ratio category. Fisher's exact test was used to compare the relative attractiveness of calls with equal note numbers but different frequency increases.

Results and Discussion

We compared the females' ability to discriminate among pairs of calls in three groups in which the magnitude of the dominant frequency of successive notes was the independent variable. Three regression curves of female preferences versus the ratios of note numbers in the stimulus pairs were fit and statistically tested. The psychometric function fit the data well for the exaggerated frequency increase (E) group ($R^2 = 0.901$, $P = 0.0003$),

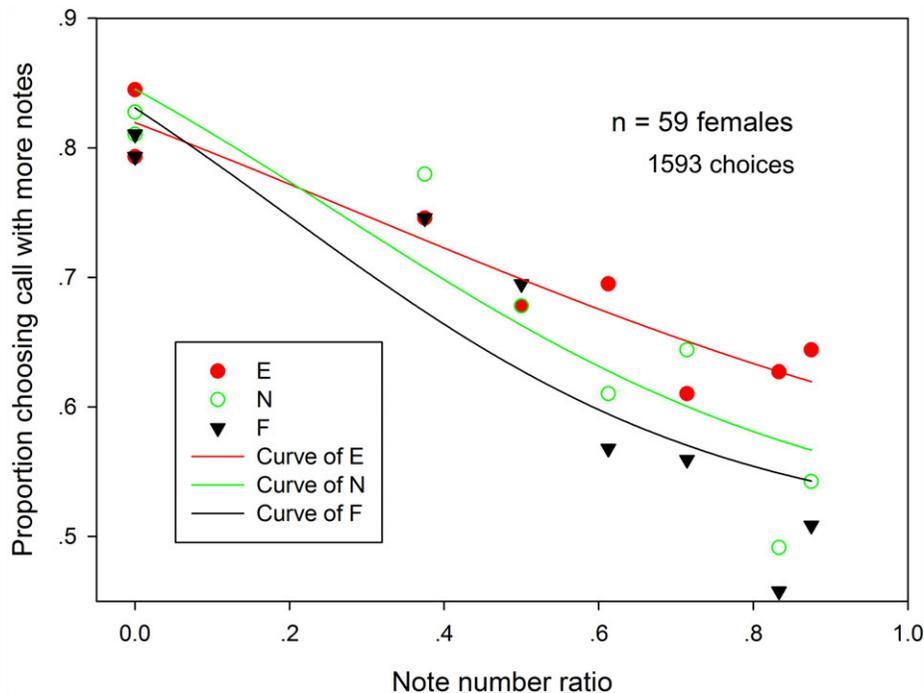


Figure 2. Effect of frequency step increases on signal discriminability by females. Proportions of females choosing the call with more notes in the exaggerated-frequency increase group (E, red), natural group (N, green), and flat group (F, black) at different ratios of note numbers. Curves are the least-squares fit of the psychometric function for data bound by 0.5 and 1.0.

natural (N) group ($R^2 = 0.847$, $P = 0.0012$), and flat (F) group ($R^2 = 0.831$, $P = 0.0016$), respectively (Fig. 2, since note ratios of 0.6 and 0.625 are very close, the corresponding data were pooled). There were significant differences in the curves between the exaggerated-frequency increase (E) group and the flat (F) group ($F_{2,12} = 4.096$, $P = 0.044$) and no significant differences between the extra frequency increase group and the natural group ($F_{2,12} = 1.954$, $P = 0.184$) and between the natural group and the flat group ($F_{2,12} = 0.522$, $P = 0.606$) (Fig. 2).

To further test the effect of the note frequency step increases on female discriminability of note number, the ratios of note number in the pairs were divided into two categories: those for which the ratio was less than or equal to 0.5 (a relatively easy ratio range for discrimination) and those for which the ratio was more than 0.5 (a relatively difficult ratio range). Repeated measures one-way ANOVA was used to evaluate female discriminability among the three groups within each ratio category. Results showed that for the ratios of note number in stimulus pairs less than or equal to 0.5, the proportions of females choosing calls with more notes were not significantly different among the three groups ($F_{2,11} = 0.466$, $P = 0.649$, Fig. 2), while for ratios more than 0.5, the proportions of females choosing calls with more notes were significantly higher in the extra frequency increase (E) group compared to the flat (F) group ($F_{2,11} = 10.380$, $P = 0.011$, Fig. 2). These results showed that the existence of frequency

increases in successive notes can facilitate note number discrimination by females for the call pairs used in this study.

Furthermore, in order to determine if internote frequency increases enhance the attractiveness of male calls, nine stimulus pairs with the same note number but differing in the magnitude of internote frequency step increases (N4:F4, E4:F4, E4:N4, N5:F5, E5:F5, E5:N5, N6:F6, E6:F6, E6:N6) were presented to 58 females, generating a total of 522 choices. Fisher's exact test showed no significant difference in the female preference between two stimuli in the pairs ($P > 0.1$, $n = 58$, Fig. 3). In addition, for combined E–F group data of playbacks with 4, 5, and 6 notes, $P = 0.237$; for combined N–F group data, $P = 0.668$; for combined E–N group data, $P = 0.582$, $n = 174$. These results show that there is no preference for change in note frequency independent of note number. Thus we suggest that changes in note frequency evolved to enhance female discriminability of note number, rather than note frequency is a separate signal component favored by female choice.

We can only speculate is exhibited by closely related species. We have recorded the calls of the other three closely related and geographically isolated *Babina* species in China. We found that both *B. adenopleura* and *B. hainanensis* have similar frequency increase among successive notes while for *B. lini* there is no frequency increase among successive notes (their calls seem to have fewer notes). However, the population of the other three species

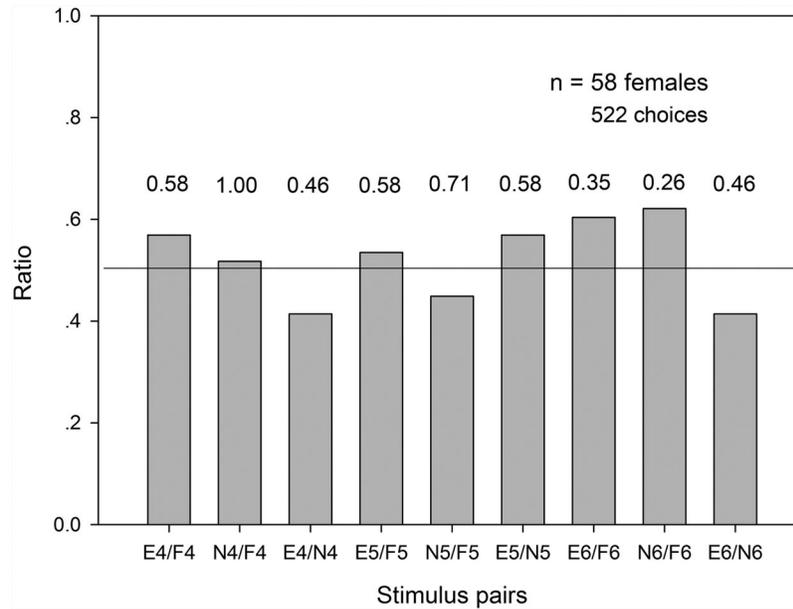


Figure 3. Relative attractiveness of calls with the same note number but differences in frequency step increases. Bars show the proportion of choices for the speaker that broadcast the exaggerated frequency increase or natural calls (the left letter of the stimulus pairs). Female preference strengths were not significantly different in all tested stimulus pairs. The number above each bar was the *P* value of the stimulus pairs. The E, N, and F represent the exaggerated frequency increase, natural, and flat stimulus type, respectively. The number such as the 4, 5, 6 represents the note number of the stimulus.

is too small to generate sufficient sample sizes for phonotaxis experiments.

Sexual selection by mate choice often favors the evolution of the more elaborate trait, but continued trait elaboration can be opposed by costs such as increased metabolic expenditure and heightened predation risk (Andersson 1994; Bradbury and Vehrencamp 2011). Perceptual and cognitive processes, such as those related to Weber's Law, can also put a brake on trait elaboration. One evolutionary response to these cognitive constraints could be the evolution of trait variation in a new dimension (Holland and Rice 1998; Ligon et al. 1998; Endler et al. 2005). In song birds, it has been suggested that decreased habituation of the listener is one of the advantages of complex song repertoires (Hartshorne 1956); behavioral (Krebs 1976) and neurobiological (Clayton 1997) evidence supports this hypothesis.

The increases in frequency of notes between calls, therefore, we could consider the frequency changes as an amplifier. For example, an amplifier can be a pattern that outlines feathers with a color that is different from the feather's background color, making each feather color appear more distinct (Hasson 1989, 1991). In the present study, we found that the female frog's ability to discriminate note number between two calls is better when there is an increase in frequency between successive notes than when the notes do not change in frequency. This effect is most pronounced when the difference in note number between stimuli is more challenging to discriminate (i.e., the note number ratio of the two calls

is close to 1.0). These results thus support the idea that the note frequency increase facilitates the female's ability to discriminate the longer call pairs by exploiting the increased discriminability of stimuli differing in frequency consistent with Weber's Law (Akre et al. 2011). Therefore, the ability of females to discriminate successive call notes may have driven the evolution of the increasing note frequency pattern in this species reflecting signal innovation rather than straightforward elaboration.

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DATA ARCHIVING

The doi of our data is 10.5061/dryad.q2764.

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