

# Real estate ads in Emei music frog vocalizations: female preference for calls emanating from burrows

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**During female mate choice, both the male's phenotype and resources (e.g. his nest) contribute to the chooser's fitness. Animals other than humans are not known to advertise resource characteristics to potential mates through vocal communication; although in some species of anurans and birds, females do evaluate male qualities through vocal communication. Here, we demonstrate that calls of the male Emei music frog (*Babina dauchina*), vocalizing from male-built nests, reflect nest structure information that can be recognized by females. Inside-nest calls consisted of notes with energy concentrated at lower frequency ranges and longer note durations when compared with outside-nest calls. Centre frequencies and note durations of the inside calls positively correlate with the area of the burrow entrance and the depth of the burrow, respectively. When given a choice between outside and inside calls played back alternately, more than 70 per cent of the females (33/47) chose inside calls. These results demonstrate that males of this species faithfully advertise whether or not they possess a nest to potential mates by vocal communication, which probably facilitates optimal mate selection by females. These results revealed a novel function of advertisement calls, which is consistent with the wide variation in both call complexity and social behaviour within amphibians.**

**Keywords:** sexual selection; sound communication; mate choice; burrowing frog; phonotaxis tests

## 1. INTRODUCTION

Many studies of birds and anurans have shown that females can assess male qualities such as sexual receptivity, body physical condition and skill of vocal learning through intra-population vocal communication [1–3]. Besides male genetic qualities, resource qualities such as feeding provided, territories defended and nests constructed by the males can significantly affect the fitness of the chooser [4–6]. However, it is apparently a more serious challenge for male frogs to

acoustically advertise information about resources they occupy or nests they build than to exhibit overt secondary sexual characters to females [4]. And it is not known whether females can accurately acoustically evaluate qualities of the resource that the male occupied or the burrow that the male built. Males of the Emei music frog (*Babina dauchina*) build burrows along pond edges for mating, egg-laying and tadpole development (see electronic supplementary material, S1) and produce advertisement calls both inside the burrow (hereafter referred to as inside calls) as well as outside the burrow (hereafter referred to as outside calls) [7]. We hypothesized that males of the Emei music frogs can convey information concerning their resource qualities (i.e. the burrow) to the females by acoustic signals. To test our hypothesis, we compared the call properties between inside and outside calls, measured the structures of the burrows and tested the females' preferences between inside and outside calls with phonotaxis experiments.

## 2. MATERIAL AND METHODS

### (a) Call recordings and burrow measurements

From May to July 2009 and 2010, the vocalizations of male subjects that occupied territories in ponds (29.36° N, 103.22° E) were recorded, using a directional microphone (Sennheiser ME66 with K6 power module) connected to a digital recorder (Marantz PMD 660, 16 bit, 44.1 kHz) about 1 m from the subject. More than 150 calls produced inside burrows by 30 males (at least five calls for each male), defined as inside-nest or inside calls, and more than 100 calls produced by another 20 males in relatively free-field conditions (at least five calls for each male), defined as outside-nest or outside calls, were recorded. To investigate for possible individual effects, for some males corresponding outside calls were recorded after recording inside calls. The mouth areas and depths of 20 burrows, body mass and snout-to-vent length (SVL) were measured after the inside calls were recorded. The peak sound pressure levels (SPLs) of inside and outside calls were measured (A-weighted) with a sound level meter (AWA 6291, Hangzhou Aihua Instruments Co.) held 2 m from the subject.

### (b) Phonotaxis experiments

An open opaque plastic tank (80 × 60 × 52 cm) containing mud and water was used for phonotaxis tests. Females were placed in the centre of the tank and tested in a two-choice phonotaxis paradigm between 21.00 and 00.00 h (21.9–23.2°C). For playback of calls, two portable field speakers (SME-AFS, Saul Mineroff Electronics, Elmont, NY, USA) were placed equidistantly from the opposite points of the tank. Two speakers were set 2.8 m apart, outside of the tank with a rectangular hole (15 × 10 cm) at the central wall area of each end (see electronic supplementary material, S2).

Three types of stimulus pairs were constructed: (i) an inside call and an outside call recorded from different males, (ii) an inside call and an outside call from the same male, and (iii) an outside call paired with white noise. Five exemplars of each type, derived from five different calling males were presented to females in a randomized sequence. All calls used in the playback experiments contained five notes and were equalized for intensity (65 dB SPL; re 20 µPa, measured at the centre of the tank where the female was to be released). Stimuli were presented antiphonally with 4 s inter-stimulus intervals, approximately equal to the mean of inter-call intervals. The female under test was monitored via a closed-circuit camera. A positive response was scored if females approached the speaker within 10 cm of one of the two holes at ends of the tank. If the female failed to make a choice within 30 min, then a 'no response' was scored and the female was not tested until the next day. After one test was completed, females were returned to their temporary feeding tanks, tested with the other types of stimulus pairs on subsequent days. To control for potential side biases, we randomized the speaker assignments for each stimulus pair.

### (c) Analysis and statistics

Four acoustic properties of these calls were obtained, including note durations, inter-note intervals, fundamental frequency and centre frequency (the average frequency of the spectral components in signal). The centre frequency was extracted using PRAAT function: query centre of gravity [8]. *T*-tests were used to evaluate differences

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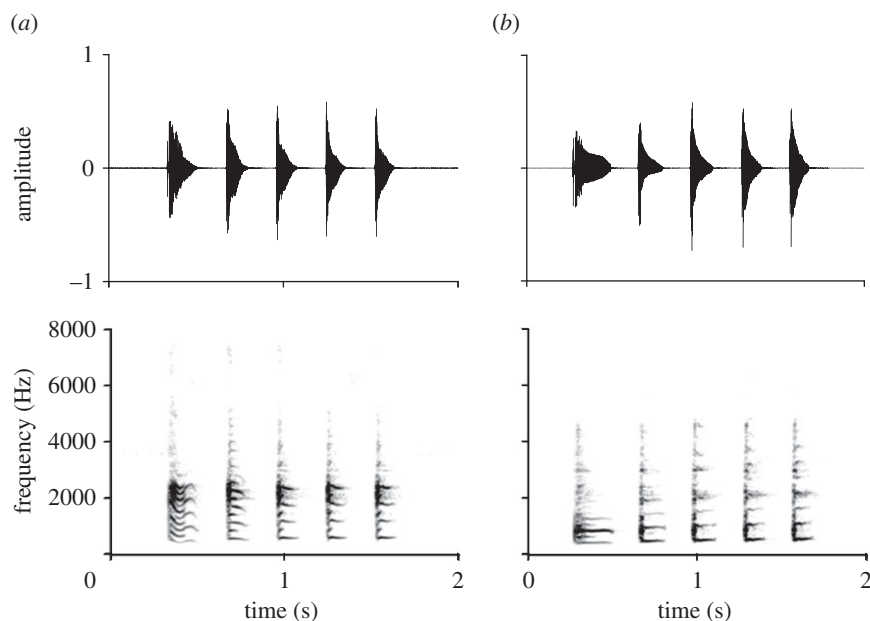


Figure 1. Amplitude-modulated waveforms and spectrograms of a typical call produced (a) outside and (b) inside the burrow.

Table 1. Note duration, centre frequency and fundamental frequency of outside and inside calls recorded from different males. N1, N2, N3, N4 and N5 represent the first, second, third, fourth and fifth note of the calls, respectively. Mean  $\pm$  s.d.,  $n = 20$  males for outside call and  $n = 30$  males for inside call.

	note duration (ms)		centre frequency (Hz)		fundamental frequency (Hz)	
	outside	inside	outside	inside	outside	inside
N1	170.0 $\pm$ 34.7	215.6 $\pm$ 29.9**	1467 $\pm$ 344	826 $\pm$ 192**	455 $\pm$ 53	427 $\pm$ 42*
N2	138.6 $\pm$ 26.1	167.6 $\pm$ 19.8**	1622 $\pm$ 347	804 $\pm$ 243**	553 $\pm$ 60	509 $\pm$ 47**
N3	129.0 $\pm$ 25.6	149.8 $\pm$ 16.8**	1563 $\pm$ 370	753 $\pm$ 175**	593 $\pm$ 87	538 $\pm$ 48**
N4	124.0 $\pm$ 21.9	139.2 $\pm$ 16.8*	1574 $\pm$ 312	749 $\pm$ 170**	594 $\pm$ 55	538 $\pm$ 44**
N5	121.2 $\pm$ 22.2	133.0 $\pm$ 14.2	1587 $\pm$ 312	785 $\pm$ 194**	587 $\pm$ 43	561 $\pm$ 47

\* $p < 0.05$ , \*\* $p < 0.01$ .

in acoustic properties between inside and outside calls. Pearson's or Spearman's correlation analysis was used to detect associations between call properties, i.e. centre frequency, fundamental frequency and duration of note with the area of the burrow mouth, depth of the burrow, body mass and SVL, respectively. The binomial test was used to evaluate the phonotaxis data. Data were expressed as mean  $\pm$  s.d.;  $p < 0.05$  was considered to be statistically significant.

### 3. RESULTS

#### (a) Differences in acoustic properties between inside and outside calls

There are clear differences in both temporal and frequency parameters between inside and outside calls (table 1 and figure 1). For the dataset consisting of inside calls from one group of individuals and outside calls from another group, the note durations of the inside calls were significantly longer than those of the outside calls (first to fourth note,  $p < 0.05$ ; table 1) whereas the inter-note intervals of inside and outside calls were not significantly different ( $p > 0.05$ , data not shown). Both the fundamental frequency (first to fourth note,  $p < 0.05$ ) and the centre frequency (first to fifth,  $p < 0.01$ ; table 1) of the notes of the inside calls were significantly lower than those of outside calls. The data comparing inside and outside calls from the same individuals showed similar patterns as

did the across-individual data. Focusing on the second of the five call notes only (for sake of brevity), the centre frequency was significantly lower for inside than for outside calls ( $776 \pm 139$  versus  $1310 \pm 331$  Hz,  $t_6 = -4.86$ ,  $p = 0.003$ , pairwise  $t$ -test,  $n = 7$  within-male measures); the mean fundamental frequency was also lower for inside calls but whereas the magnitude of the inside versus outside difference was as great as for the across frog comparison (shown in table 1), the difference was not significant ( $523 \pm 46$  versus  $572 \pm 48$  Hz,  $t_6 = -1.62$ ,  $p = 0.156$ ,  $n = 7$ ). The peak SPL (A-weighted) of the inside calls ( $59.1 \pm 4.1$  dB,  $n = 66$  males) was significantly higher than outside calls ( $54.1 \pm 3.9$  dB,  $n = 130$  males;  $t_{194} = 8.40$ ,  $p < 0.001$ ).

#### (b) Relationships between call properties and burrow structure

The centre frequency (figure 2a) and fundamental frequency (figure 2b) were positively correlated with the burrow mouth area, whereas the note durations were positively correlated with burrow depth (figure 2c;  $n = 20$  males). The average body mass of male frogs was  $10.5 \pm 1.3$  g ( $n = 21$ ) ranging from 8.5 to 14.1 g, whereas the mean SVL was  $4.4 \pm 0.2$  cm ( $n = 21$ )

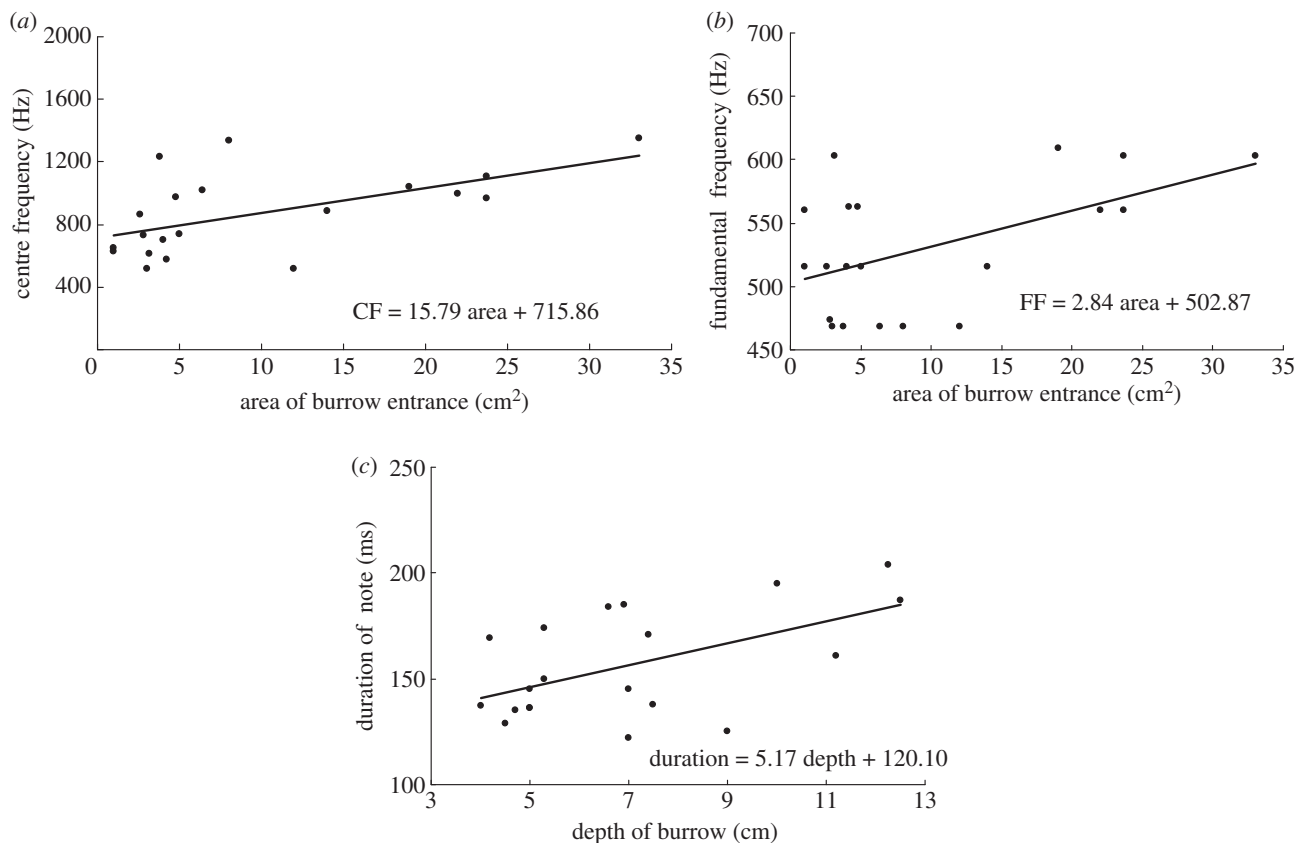


Figure 2. Correlations of area of burrow entrance with (a) centre frequency (CF;  $r = 0.570$ ,  $p = 0.009$ ). (b) Fundamental frequency (FF;  $r = 0.523$ ,  $p = 0.018$ ). (c) Depth of burrow with duration of note ( $r = 0.545$ ,  $p = 0.013$ ).

Table 2. Outside call and inside call phonotaxis data.

	stimuli		choices		preference (proportion)	$p$
	A	B	A	B		
across male	outside call	inside call	14	33	inside call (0.70)	0.008
within male	outside call	inside call	8	28	inside call (0.78)	0.001
outside call–WN	outside call	white noise	13	3	outside call (0.81)	0.021

ranging from 4.0 to 4.8 cm. No significant relationships were found between fundamental frequency, centre frequency or duration of note and body mass or body length, respectively ( $n = 21$  males, see electronic supplementary material, S3).

#### (c) Female phonotaxis experiments

When the inside and outside calls were broadcast antipodally, regardless of whether the calls were recorded from the same or different individuals, more than 70 per cent of the females tested approached the speaker broadcasting the inside calls, whereas when the outside calls and white noise were broadcast antipodally, 81 per cent of the females tested preferred the outside calls (table 2).

## 4. DISCUSSION

It has long been known that males can encode information about their physical condition in the acoustic properties of their calls, such as dominant frequency and/or fundamental frequency [1,2]. However, animals

are not known to vocally advertise nest characteristics to potential mates. The present study indicated that calls might be modified by a combination of acoustic filter properties of burrows and by the frogs actively adjusting their calls to the acoustic circumstances, and that females show a preference for calls emanating from burrows. These results suggest that females are able to evaluate the resources of their potential mates and select against males lacking such resources, mainly depending on advertisement calls.

Providing safety and security for mating, or egg-laying and offspring-raising, reproductive sites such as burrows, holes, nests, hides, dens and houses are likely to be associated with strong direct (i.e. non-genetic) benefits to females of taxa ranging from insects to humans. Females pay close attention to male-made reproductive sites; for instance, female weaver birds chose a mate based on his nest colours, profiles, materials and locations [9,10]. In the present study, two-choice experiments showed that more than 70 per cent of the female music frogs tested selected the calls produced inside the burrow and this preference

persisted when individual effects were eliminated by playback with the inside and outside calls recorded from the same individual. Consistently, the inside call with lower frequency and longer note duration is associated with a smaller burrow mouth and deeper depth, respectively, which might be advantageous to the male, female and their offspring, suggesting that the acoustic characters of the inside call convey information concerning the burrow. The inside calls differed significantly from the outside calls in the following important parameters: energy distribution, note duration and SPL. Some of this variation is caused by filter effects arising from the area of the entrance and depth of the burrow but some of it probably reflects the frogs' own physiology or calling effort [11]. Our data were supported by reports on (i) *Metaphrynella sundana* which by exploiting resonance properties of tree holes can increase the call's SPL and then, facultatively, alter their note duration [12] and on (ii) other burrowing frogs *Eupsophus emiliopugini*, *E. calcaratus* [13,14], *E. calcaratus* and *E. roseus* [15] in which some acoustic properties were affected by burrows.

Taken together, we demonstrate that males of this species faithfully advertise whether or not they possess a nest to potential mates mainly by vocal communication, facilitating mate selection by females. These results reveal a novel function of advertisement calls and provide an excellent model for investigating the role of sexual selection in mating systems in which resource quality is emphasized and in need of quantification.

Animal procedures were in agreement with the Animal Care and Use Committee of Chengdu Institute of Biology.

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- 1 Morris, M. R. & Yoon, S. L. 1989 A mechanism for female choice for large males in the treefrog *Hyla chrysocelis*. *Behav. Ecol. Sociobiol.* **25**, 65–71. (doi:10.1007/BF00299712)

- 2 Gerhardt, H. C. & Huber, F. 2002 *Acoustic communication in insects and frogs: common problems and diverse solutions*. Chicago, IL: University of Chicago Press.
- 3 Nowicki, S., Searcy, W. A. & Peters, S. 2002 Quality of song learning affects female response to male bird song. *Proc. R. Soc. Lond. B* **269**, 1949–1954. (doi:10.1098/rspb.2002.2124)
- 4 Wells, K. D. 1977 Territoriality and male mating success in the green frog (*Rana clamitans*). *Ecology* **58**, 750–762. (doi:10.2307/1936211)
- 5 Howard, R. D. 1978 The influence of male-defended oviposition sites on early embryo mortality in bullfrogs. *Ecology* **59**, 789–798. (doi:10.2307/1938783)
- 6 Kirkpatrick, M. & Ryan, M. J. 1991 The evolution of mating preferences and the paradox of the lek. *Nature* **350**, 33–38. (doi:10.1038/350033a0)
- 7 Cui, J. G., Wang, Y. S., Brauth, S. & Tang, Y. Z. 2010 A novel female call incites male–female interaction and male–male competition in the Emei music frog, *Babina daunchina*. *Anim. Behav.* **80**, 181–187. (doi:10.1016/j.anbehav.2010.05.012)
- 8 Boersma, P. & Weenink, D. 2010. See [http://www.fon.hum.uva.nl/praat/manual/Spectrum\\_Get\\_centre\\_of\\_gravity\\_.html](http://www.fon.hum.uva.nl/praat/manual/Spectrum_Get_centre_of_gravity_.html)
- 9 Collias, N. E. & Victoria, J. K. 1978 Nest and mate selection in the village weaverbird (*Ploceus cucullatus*). *Anim. Behav.* **26**, 470–479. (doi:10.1016/0003-3472(78)90064-7)
- 10 Quader, S. 2005 Elaborate nests in a weaverbird: a role for female choice? *Ethology* **111**, 1073–1088. (doi:10.1111/j.1439-0310.2005.01134.x)
- 11 Lardner, B. & bin Lakim, M. 2004 Female call preferences in tree-hole frogs: why are there so many unattractive males? *Anim. Behav.* **26**, 265–272. (doi:10.1016/j.anbehav.2004.05.003)
- 12 Lardner, B. & bin Lakim, M. 2002 Tree-hole frogs exploit resonance effects. *Nature* **420**, 475. (doi:10.1038/420475a)
- 13 Penna, M. & Solis, R. 1999 Extent and variation of sound enhancement inside burrows of the frog *Eupsophus emiliopugini* (Leptodactylidae). *Behav. Ecol. Sociobiol.* **47**, 94–103. (doi:10.1007/s002650050654)
- 14 Penna, M. 2004 Amplification and spectral shifts of vocalizations inside burrows of the frog *Eupsophus calcaratus* (Leptodactylidae). *J. Acoust. Soc. Am.* **116**, 1254–1260. (doi:10.1121/1.1768257)
- 15 Márquez, R., Penna, M., Marques, P. & Do Amaral, J. P. 2005 Diverse types of advertisement calls in the frogs *Eupsophus calcaratus* and *E. roseus* (Leptodactylidae): a quantitative comparison. *Herpetol. J.* **15**, 257–263.