

LETTERS

Ultrasonic frogs show hyperacute phonotaxis to female courtship calls

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Sound communication plays a vital role in frog reproduction^{1,2}, in which vocal advertisement is generally the domain of males. Females are typically silent, but in a few anuran species they can produce a feeble reciprocal call³ or rapping sounds⁴ during courtship. Males of concave-eared torrent frogs (*Odorrana tormota*) have demonstrated ultrasonic communication capacity⁵. Although females of *O. tormota* have an unusually well-developed vocal production system⁶, it is unclear whether or not they produce calls or are only passive partners in a communication system dominated by males. Here we show that before ovulation, gravid females of *O. tormota* emit calls that are distinct from males' advertisement calls, having higher fundamental frequencies and harmonics and shorter call duration. In the field and in a quiet, darkened indoor arena, these female calls evoke vocalizations and extraordinarily precise positive phonotaxis (a localization error of $<1^\circ$), rivalling that of vertebrates with the highest localization acuity (barn owls^{7,8}, dolphins, elephants and humans⁹). The localization accuracy of *O. tormota* is remarkable in light of their small head size (interaural distance of <1 cm), and suggests an additional selective advantage of high-frequency hearing beyond the ability to avoid masking by low-frequency background noise⁵.

Odorrana tormota (formerly *Amolops tormotus*)¹⁰ is an arboreal, nocturnal frog living near noisy streams in Huangshan Hot Springs, China. On rainy nights when ovulation is imminent, gravid females can be found in numbers in their natural habitat. We recorded the vocalizations of captive females kept in a quiet darkened room using an ultrasonic microphone and PCTape, a computer-based data acquisition system¹¹. In captivity, gravid females vocalize rarely (<1 call per hour on average), but they stop calling altogether after ovulation. This suggests that these vocalizations probably serve to advertise their gravid reproductive state.

A representative female call consists of an upward and downward frequency modulated (FM) tone pip having a fundamental frequency from 7.2 to 9.8 kHz with multiple harmonics that extend into the ultrasonic range (Fig. 1a). The calls are moderately intense, with a peak intensity of ~ 80 dB sound pressure level (SPL), measured 1 m away from a calling female frog. The call duration is generally short (<150 ms), and the fundamental frequency of female calls is higher than that of the males' advertisement calls^{11,12} (see below) despite females having an unusually larger body size and more robust vocal cords⁶. Also, unlike males' advertisement calls, female calls do not display nonlinear characteristics (for example, chaos, subharmonics or frequency jumps).

To determine the functional significance of female calls, we carried out acoustic playback experiments in the field and in a quiet room indoors. In the field, broadcasting female calls at the rate of 1 call per 15 s at ~ 90 dB SPL (measured at 50 cm from the playback loudspeaker) consistently induced nearby males to call antiphonally or

to increase their calling activities. Moreover, most of these males displayed positive phonotaxis—they rapidly approached the loudspeaker with high-precision. Results of the playback experiments in the field suggest that a female call functions as a courtship call; emission of such calls not only signals a female's receptivity to males but also her location.

To validate the above hypothesis and to assess the acuity of sound localization quantitatively, we repeated the acoustic playback experiments in a quiet arena in a darkened room, this time to individual captive males. In response to female calls (intensity ~ 80 dB SPL measured at 1 m from the loudspeaker, presented at a rate of 1 call per 15 s), males produced four types of vocal responses during phonotaxis (see Supplementary Videos). The most prominent was the antiphonal response, which was time-locked (within 60.4 ± 31.7 ms from 459 antiphonal responses from 48 males) to the stimulus offset. The majority of antiphonal responses comprised a short note (duration ≤ 40 ms), with prominent upward and downward FM sweeps and multiple harmonics (Fig. 1b). The fundamental frequency (F_0) of the antiphonal responses varied with responding individuals, from 6.2 to 7.8 kHz (Fig. 1b). This variation was a function of the ambient noise level; in the field where it is noisy, males increased the F_0 of their antiphonal responses presumably in order to decrease acoustic masking by low-frequency background interference¹¹. The population mean of F_0 is 7.24 ± 0.96 kHz ($n = 43$) in the field—this is significantly higher than the value observed indoors, 5.96 ± 1.01 kHz ($n = 48$; $t = 6.21$, $P < 0.0001$).

Whereas the antiphonal response typically occurred before males jumped towards the loudspeaker broadcasting the female call, responding males switched to another type of vocalization midway during the phonotaxis—namely, the staccato call, which consisted of up to 60 triple pulses repeated at the rate of 14 Hz, lasting 3–4 s, each pulse with a duration of ~ 3 ms, and a dominant frequency of ~ 4.0 kHz (Fig. 1c). Following production of a staccato call, the male moved rapidly or hopped towards the loudspeaker. After an antiphonal response and before the next female call stimulus, males often produced two or three short (~ 50 ms) shallow FM pips. When close to the loudspeaker (20 cm), males sometimes produced a low-level long (250–300 ms) call having a fundamental frequency of ~ 4 –5 kHz and many subharmonics.

In addition to evoked vocal responses, the female call also consistently triggered positive phonotaxis. Analysis of video recordings of males' phonotactic paths in the indoor arena showed that males' localization acuity was extraordinary. On hearing a female call, a male usually oriented his body towards the loudspeaker — this was followed by a long-distance hop towards the loudspeaker (Fig. 2; see Supplementary Video). The precision of the long-distance (range: 30–75 cm) hops was remarkable, with an average azimuthal error of just $0.7^\circ \pm 3.3^\circ$ ($n = 41$). Males' localization acuity rivals that of

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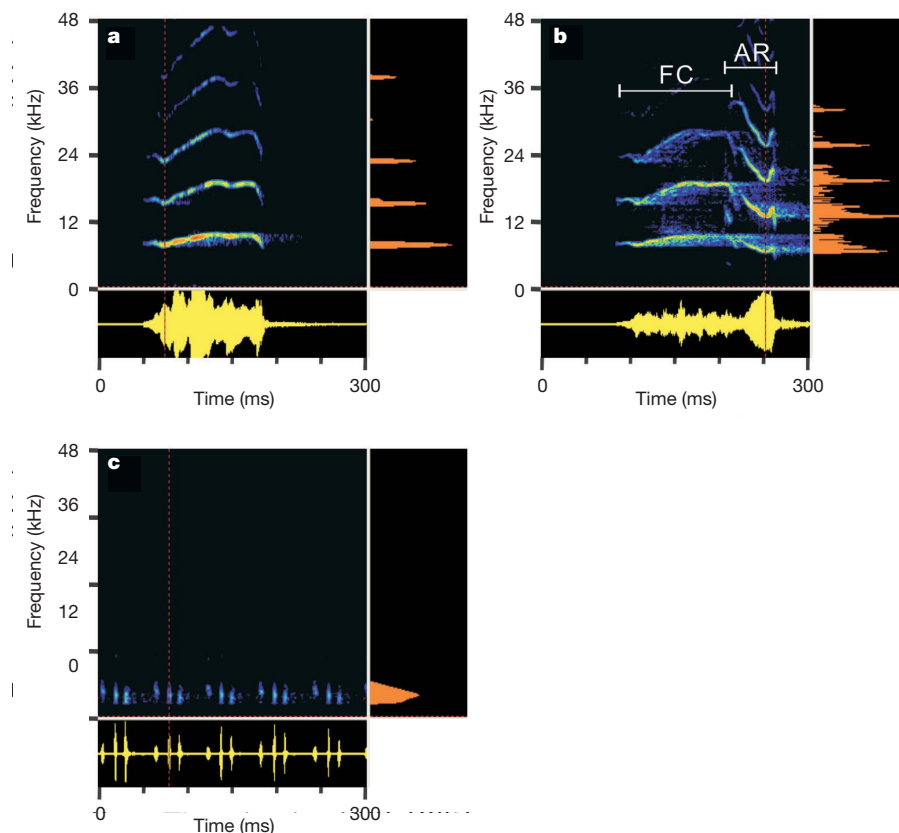


Figure 1 | Female's courtship call and male's evoked vocal responses. **a**, A female's courtship call (FC) (duration 134 ms), showing its high-fundamental frequency and rich harmonics. **b**, A male's antiphonal response (AR) emitted 8 ms before the offset of the FC. **c**, A staccato call, comprising a

string of soft clicks at low frequencies. In each panel of this figure, the sound spectrogram is shown at top left, the signal waveform at bottom left, and the instantaneous amplitude spectrum at the right.

the best vertebrate performers (barn owls^{7,8}, elephants, dolphins and humans⁹), despite their very short interaural distance (<1 cm).

O. tormota inhabits the vegetation along noisy streams, rendering communication problematic. Males have been shown to produce calls with spectral energy extending into the ultrasonic range to avoid masking from the broadband ambient noise; the fundamental frequency of male calls is around 5–7 kHz on average^{5,6,11,12}. That females of *O. tormota* produce moderate-level calls having a significantly higher F_0 (7.2–9.8 kHz) than male calls is surprising. First, females of *O. tormota* not only possess distinct vocal cords, but their vocal cords are bigger and their medial vocal ligaments are thicker compared to their male counterparts⁶. Generally, the mass of the vocal cords tends to be correlated to body size, so the larger female frogs would be expected to produce calls with a lower dominant frequency^{13–17}. Further studies are therefore necessary to determine the mechanisms underlying high-frequency sound production in

females. Second, for most anuran species, females are silent, and those that vocalize generally possess a rudimentary larynx and thus can emit low-level calls or rapping, or simple release calls^{3,4,18–22}. The high-frequency and moderately intense mating calls of female *O. tormota* are thus exceptional. Our results show that they promote reproduction by unambiguously conveying receptive state and location in the presence of a noisy background. This finding further supports the hypothesis that the frogs produce high-frequency components to overcome the masking effects of ambient noise, to increase the salience of the communication signal, to facilitate amplexus and to ensure successful reproduction in the field. Little is known, however, of the mechanisms that underlie production of the higher F_0 in the females.

Another surprising finding is that the female call is a universal signal used to both stimulate vocal responses from and attract conspecific individual males distributed at different sectors along the Tau

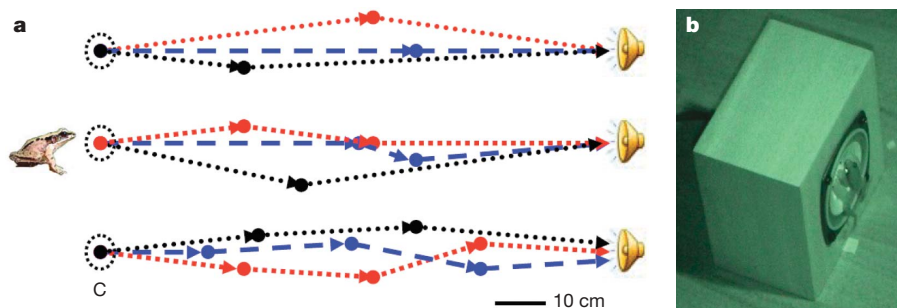


Figure 2 | Males' phonotactic responses to a female's courtship call. **a**, Representative phonotactic orienting responses from six males. Circles represent the landing site of a hop; the arrow indicates the hop's direction

towards the loudspeaker broadcasting the female's courtship call. **c**, Glass cover. **b**, Image showing a male frog reaching the centre of the diaphragm of the loudspeaker (taken from a video recording of the frog's phonotactic responses).

Hua Creek in Huangshan Hot Springs, China. This implies that males of *O. tormota* have conservative characters for female call pattern recognition and perception, although the neural mechanisms and genetic bases of these characters are unknown. Furthermore, how the female call of *O. tormota* has evolved as a sex-specific and potent signal model, how the auditory system of receivers generates perceptual biases that direct female call evolution, and how the high-frequency mating calls are produced in the female frog, are but three of the unanswered questions about this remarkable animal^{15,17,23}.

The third surprising finding is that the male *O. tormota* can localize female calls with an extraordinary acuity of just 1° despite their small head size (interaural distance ~1 cm). It is known that barn owls that prey upon small mammals can locate sound with an accuracy of 1–2° (refs 7, 8); they and dolphins, elephants and humans have the highest localization acuity among the vertebrates⁹. Amphibians are less well endowed, generally showing an acuity of ~16–23° (ref. 24), as they locate a sound source based on low-frequency perception. In contrast, ultrasonic males of *O. tormota* have the capacity to perceive higher-frequency sounds as an adaptation to their noisy habitats⁵, which may underlie their hyperacute sound localization. Additional mechanisms that underlie localization hyperacuity in these animals remain to be studied.

METHODS SUMMARY

Females of *O. tormota* were caught on rainy nights in Huangshan Hot Springs, China. In a quiet, darkened room at midnight, female frog vocalizations were recorded using an ultrasonic microphone and PCTape, a computer-based data-acquisition system. Data were saved as WAV files and analysed, and displayed using SELINA software. Acoustic playback experiments were carried out at night in the field with a peak ambient noise level of 70–88 dB SPL or in a quiet and darkened indoor room under dim infrared illumination. The WAV file of a recorded female call of *O. tormota* was stored either on an MP3 player for field tests, or on the Play unit for the indoor study. Calls were broadcast through a loudspeaker at ~80–90 dB SPL and a rate of one call per 15 s. The vocal responses of 48 males indoors and 43 males in the field, as well as their spontaneous calling (no stimulus, as control) were recorded using PCTape indoors and a digital audio recorder (frequency range: 10 Hz–96 kHz) in the field with an ultrasonically-sensitive microphone placed 10 cm from the loudspeaker to monitor the frogs' vocalizations as well as the acoustic playback stimulus for later analysis. During the phonotaxis experiments in the indoor arena, each male frog was placed on the floor under a removable glass cover, 1 m in front of the loudspeaker. Video recordings of males' phonotactic behaviours were made under infrared illumination. The trajectories for each male frog were obtained from the video recordings. The hop distance (*D*, in cm) and azimuthal jump angle (α , in degrees) were measured.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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1. Capranica, R. R. *The Evoked Vocal Response of the Bullfrog: A Study of Communication by Sound* (Research Monograph 33, MIT, Cambridge, Massachusetts, 1965).
2. Glaw, F. & Vences, M. *A Fieldguide to the Amphibians and Reptiles of Madagascar* 2nd edn (Vences & Glaw, Köln, Germany, 1994).

3. Roy, D. Communication signals and sexual selection in amphibians. *Curr. Sci.* **72**, 923–927 (1997).
4. Tobias, M. L., Viswanathan, S. & Kelley, D. B. Rapping, a female receptive call, initiates male/female duets in the South African clawed frog. *Proc. Natl Acad. Sci. USA* **95**, 1870–1875 (1998).
5. Feng, A. S. *et al.* Ultrasonic communication in frogs. *Nature* **440**, 333–336 (2006).
6. Suthers, R. A. *et al.* Voices of the dead: Complex nonlinear vocal signals from the larynx of an ultrasonic frog. *J. Exp. Biol.* **209**, 4984–4993 (2006).
7. Knudsen, E. I., Blasdel, G. G. & Konishi, M. Sound localization by the barn owl (*Tyto alba*) measured with search coil technique. *J. Comp. Physiol. A* **133**, 1–11 (1979).
8. Bala, A. D. S., Spitzer, M. W. & Takahashi, T. T. Prediction of auditory spatial acuity from neural images on the owl's auditory space map. *Nature* **424**, 771–774 (2003).
9. Heffner, R. S. & Masterton, R. B. in *Comparative Perception* Vol. I, *Basic Mechanisms* (eds Berkley, M. A. & Stebbins, W. C.) 285–314 (John Wiley & Sons, New York, 1990).
10. Cai, H.-X., Che, J., Pang, J.-F., Zhao, E.-M. & Zhang, Y.-P. Paraphyly of Chinese *Amolops* (Anura, Ranidae) and phylogenetic position of the rare Chinese frog, *Amolops tormotus*. *Zootaxa* **1531**, 49–55 (2007).
11. Narins, P. M. *et al.* Old world frog and bird vocalizations contain prominent ultrasonic harmonics. *J. Acoust. Soc. Am.* **115**, 910–913 (2004).
12. Feng, A. S., Narins, P. M. & Xu, C. H. Vocal acrobatics in a Chinese frog, *Amolops tormotus*. *Naturwissenschaften* **89**, 352–356 (2002).
13. Narins, P. M. & Smith, S. L. Clinal variation in anuran advertisement calls: Basis for acoustic isolation? *Behav. Ecol. Sociobiol.* **19**, 135–141 (1986).
14. Marquez, R. Female choice in the midwife toads (*Alytes obstetricans* and *A. cisternasii*). *Behaviour* **132**, 151–161 (1995).
15. McClelland, B. E., Wilczynski, W. & Ryan, M. J. Correlations between call characteristics and morphology in male cricket frogs (*Acris crepitans*). *J. Exp. Biol.* **199**, 1907–1919 (1996).
16. Gerhardt, H. C. & Huber, F. *Acoustic Communication in Insects and Anurans* (Univ. Chicago Press, Chicago, 2002).
17. Narins, P. M., Feng, A. S., Fay, R. R. & Popper, A. N. *Hearing and Sound Communication in Amphibians* (Springer, New York, 2007).
18. Duellman, W. E. & Trueb, L. *Biology of Amphibians* (McGraw-Hill, New York, 1986).
19. Roy, D., Borah, B. & Sarma, A. Analysis and significance of female reciprocal call in frogs. *Curr. Sci.* **69**, 265–270 (1995).
20. Kelley, D. B. & Tobias, M. L. in *The Design of Animal Communication* (eds Konishi, M. & Hauser, M.) 9–35 (MIT Press, Cambridge, Massachusetts, 1999).
21. Emerson, S. B. & Boyd, S. K. Mating vocalizations of female frogs: Control and evolutionary mechanisms. *Brain Behav. Evol.* **53**, 187–197 (1999).
22. Kelley, D. B. Vocal communication in frogs. *Curr. Opin. Neurobiol.* **14**, 751–757 (2004).
23. Rand, A. S. Is the canary singing? *Froglog* **48**, 3 (2001).
24. Christensen-Dalsgaard, J. in *Sound Source Localization* (eds Popper, A. N. & Fay, R. R.) 67–123 (Springer, New York, 2005).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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METHODS

Animal preparation. Females of *O. tormota* ($n = 4$) were caught on rainy nights along Tau Hua Creek in Huangshan Hot Springs, China, between 22 and 28 April, 2007. In a quiet, darkened room at midnight to early morning hours, female frog vocalizations were recorded using an ultrasonic microphone and PCTape, a computer-based data-acquisition system. Data were saved as WAV files and analysed (fast Fourier transform, 1,024 points), and displayed using SELENA software^{5,11,25}. Three to five males were captured each night and kept individually in small plastic cages for acoustic playback experiments.

Acoustic playback experiments. Acoustic playback experiments were carried out between 13 and 29 May 2007 at night along Tau Hua Creek with a peak ambient noise level of 70–88 dB SPL¹² or in a quiet and darkened indoor room, ~1 km from the frog's natural habitat. The WAV file of a recorded female call of *O. tormota* was stored either on an MP3 player for field tests, or on the Play unit, a computer-based playback system^{5,25}, for indoor study. The MP3 player broadcasts the calls through a power amplifier and a loudspeaker (FE87E). Both playback systems have the means to adjust the level of acoustic playback to ~80–90 dB SPL and the rate of stimulus presentation to one call per 15 s. The playback stimulus was presented over a 5-min period. The loudspeaker was positioned 1 m away from a calling frog in the field, as well as in an indoor arena. Playback experiments in the indoor arena were made under dim infrared illumination.

Audio and video recording, and data analysis. We observed the vocal responses of 48 males indoors and 43 males in the field, as well as their spontaneous calling (no stimulus, as control); for this we used a PCTape system indoors and a digital audio recorder (Sound Devices, model 702; frequency range: 10 Hz–96 kHz) in the field. During the phonotaxis experiments in the indoor arena (3.5 × 4.5 m), each male frog was placed on the floor (that is, the release site) under a removable glass cover (inside diameter 8.5 cm), 1 m in front of the loudspeaker. The peak sound pressure level of the playback stimulus was measured with a ¼-inch wide-band condenser microphone (G.R.A.S. 40BE) placed above the cage or at the release site; the sound was recorded on the digital audio recorder (Sound Devices 702) and this was compared with the output of a calibrator (Bruel and Kjaer 4231) that produces a 1-kHz tone at 94 dB SPL. Audio recordings were made with either a PCTape system^{5,6} or the digital audio recorder, with a microphone mounted on a tripod and placed 10 cm from the loudspeaker to monitor the frog's vocalizations as well as the acoustic playback stimulus for later analysis. Video recordings of males' phonotactic behaviours were made under infrared illumination, using an infrared video camera (Sony DCR-TRV30E). The trajectories for each male frog were obtained from the video recordings. We measured the hop distance (D , in cm) and azimuthal jump angle (α , in degrees); the latter was calculated using the formula $\alpha = \arcsin d/D$, where d is the shortest distance from the animal's present position to the straight line between the animal's initial position and the centre of the loudspeaker.

25. Siemers, B. M. & Schnitzler, H.-U. Echolocation signals reflect niche differentiation in five sympatric congeneric bat species. *Nature* **429**, 657–661 (2004).