

# Bimodal signal requisite for agonistic behavior in a dart-poison frog, *Epipedobates femoralis*

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**Animal acoustic signals play seminal roles in mate attraction and regulation of male spacing, maintenance of pairbonds, localization of hosts by parasites, and feeding behavior. Among vertebrate signals, it is becoming clear that no single stereotyped signal feature reliably elicits species-specific behavior, but rather, that a suite of characters is involved. Within the largely nocturnal clade of anuran amphibians, the dart-poison frog, *Epipedobates femoralis*, is a diurnal species that physically and vigorously defends its calling territory against conspecific intruders. Here we report that physical attacks by a territorial male are provoked only in response to dynamic bimodal stimuli in which the acoustic playback of vocalizations is coupled with vocal sac pulsations, but not by either unimodal cues presented in isolation or static bimodal stimuli. These results suggest that integration of dynamic bimodal cues is necessary to elicit aggression in this species.**

animal communication | territorial defense | anuran | amphibian | Dendrobatidae

Anuran amphibians (frogs and toads) provide excellent model systems for the investigation of signal function because their calls are frequently discrete, periodic, and stereotyped in both the frequency and time domains (1), although extreme variability in advertisement-call complexity has been documented (2, 3). Territories provide their residents with space for mate attraction and reproduction, and thus form the basis of resource-defense mating systems of many animal species (4, 5). One form of agonistic interaction between animals, aggressive behavior, occurs when one individual attempts to acquire a contested resource, such as a territory, at the expense of another (6). Although intraspecific fighting has been observed under natural conditions during territorial interactions between male frogs (7–9), it has heretofore not been possible to evoke this behavior under controlled experimental conditions in any amphibian. This inability is in contrast to the situation in birds, for which a skin mounted in a life-like position (10), or just a simple tuft of breast feathers attached by wires to a perch (11), is sufficient to evoke clear aggressive behavior in some species. Electromechanically sophisticated, life-like models have recently been used to quantify courtship behavior in satin bowerbirds (12).

To minimize the risk of physical damage from combat, territorial male frogs display a graded series of prefighting behaviors, many of which have been elicited solely by acoustic cues in loudspeaker playback experiments. Thus, in response to either an intruding male's vocalizations or the broadcasting of conspecific calls, territorial frogs have been reported to adjust their calling pattern (*i*) by alternating their call with the perceived acoustic stimulus (13, 14) or shifting their call dominant frequency (15) to avoid acoustic interference; (*ii*) by vocalizing concurrently with the intruder to "jam" his advertisement call (16); (*iii*) by increasing vocalization complexity by adding new call notes to signal an escalating state of aggression (17); or, in response to extremely high playback levels, (*iv*) by ceasing calling completely (18). In some species, males orient toward the sound source and then physically approach the loudspeaker (phonotactic response; refs. 19 and 20).

We studied the territorial-defense behavior of males of the dart-poison frog *Epipedobates femoralis* (Dendrobatidae), which produce frequency-modulated advertisement calls. At Arataï, French Guiana, the call consists of four notes, each sweeping up in frequency within a range from  $\approx 3.0$  to 3.9 kHz. Calls are repeated by individual males every 458 ms on average, forming bouts containing up to 40 calls. Although some frog species have evolved rather elaborate intraspecific visual displays, the vocal sac (VS) in *E. femoralis* is not brightly colored or highly reflective as it is in *Colostethus inguinalis*, or in various other species (see ref. 21 for review). Nevertheless, the most salient dynamic visual cues produced by *E. femoralis* males are the conspicuous VS pulsations, being inevitable consequences of call generation. Using a model, we now dissociate the natural inextricable linkage between sound production and VS movement in dart-poison frogs to test the efficacy of each cue individually in its ability to evoke aggressive behavior.

Acoustic playback of *E. femoralis* advertisement calls at intensities between 56 and 68 dB sound pressure level (SPL) measured at the conspecific focal male generally evokes complete body orientation toward the broadcasting speaker and subsequent antiphonal calling (22). Species-specific advertisement calls played back to males at intensities above  $\approx 68$  dB SPL reliably result in a sudden cessation of calling activity, head and body reorientation toward the sound source, and a rapid zigzag approach toward the broadcasting speaker (22). Whereas prior workers have sometimes interpreted positive phonotaxis as the "behavioral indicator of a male's propensity to fight" (19) or as an "aggressive approach" (23), evoking actual fighting behavior by using acoustic playback alone has heretofore not been possible. We took advantage of the stereotyped, species-specific approach behavior of *E. femoralis* and used an electromechanical model frog (EMF) mounted on an artificial log designed to mimic simultaneously the morphology, calling posture, VS motion, and species-specific vocal behavior of the calling male. By providing controlled unimodal and bimodal (acoustic and visual) static and dynamic stimuli, we were able to identify those signal cues necessary to elicit fighting behavior in an amphibian.

## Methods

**Study Animals and Field Site.** Dart-poison frogs (*E. femoralis*) are distributed throughout the Amazonian lowlands and the Guiana Shelf (24). Males are diurnal and inhabit the leaf litter in which they produce advertisement calls from exposed sites on fallen branches or leaves (25). Territorial individuals were located by their continuous vocal activity and exposed calling positions. Territories in this species are sites for mate attraction and egg deposition; in one population they range in area between 0.25 and 26.2 m<sup>2</sup> (5). Typical interindividual distances are 4–30 m and calling positions within these territories may be occupied continuously for as long as 103 days (5). Conspecific incursions into their territory are readily

Abbreviations: EMF, electromechanical model frog; VS, vocal sac.

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investigated by male residents temporarily leaving their calling site and approaching a vocalizing intruder (22).

All experiments were undertaken with individual *E. femoralis* males (mean snout-urostyle length: 25.5 mm,  $n = 27$ ) calling in the primary forest near the field station at Arataï, French Guiana (3° 59' N, 52° 35' W) at the onset of the rainy seasons during December 2000 and January 2001. The study site is in a lowland wet tropical forest (elevation 23 m), in which the mean annual rainfall and temperature are 3,000–3,250 mm and 26°C, respectively.

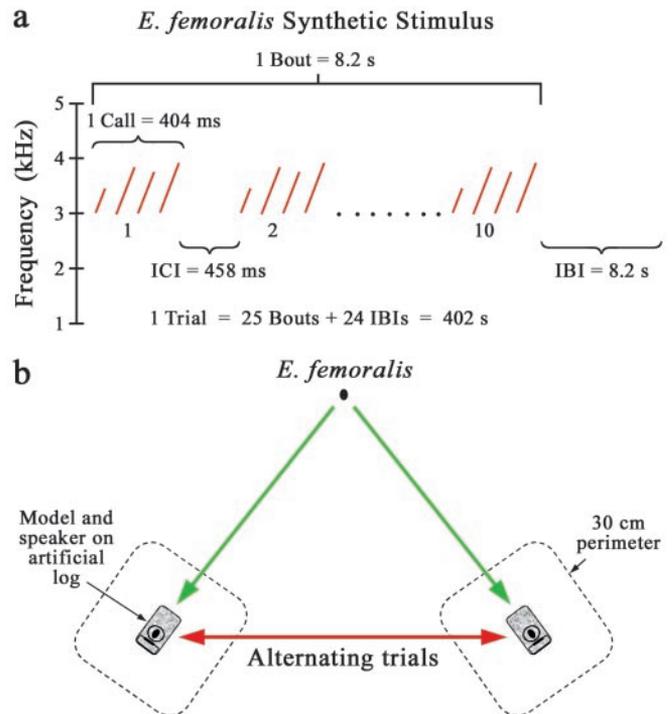
**Playback Experiments.** Unimodal and bimodal stimuli were delivered by an EMF of *E. femoralis* made from silicon rubber and painted to mimic an adult male. The frog model, in an upright (forelimbs extended) posture, was positioned such that the fully inflated VS cleared the substrate by 5 mm, thus minimizing the seismic correlates of vocalization. The model was fixed to a rotatable turntable (Graupner, Modellbau, Kirchem, Germany; maximum angular velocity: 1.9 rad·s<sup>-1</sup>) placed on top of an artificial log made from epoxy resin and colored to mimic tree bark. A loudspeaker (FNX140X tweeter, Rockford Fosgate, Tempe, AZ) was installed in an artificial branch emerging from the upper surface of the log just behind the frog model. The loudspeaker was directed toward the frog to simulate the natural directivity pattern of a calling male (26). The log cavity housed the loudspeaker power amplifier (XVR 10075, Rockford Fosgate) and a servo-activated (HS-300, Hitec, Seoul, South Korea) air pump to inflate and deflate the vocal pouch. The model was connected by means of a 7-m cable to a control box containing the stimulus preamplifier (no. M32, Kemo Electronic, Langen, Germany), batteries, and control switches for activating the sound, VS pulsations, and turntable rotation.

**Playback Stimuli and Presentation.** Synthetic advertisement calls were prerecorded on cassette tape and could be broadcast through the loudspeaker behind the model during playback trials. The stimulus parameters represent the mean values for 15 males from the Arataï population and are as follows: number of notes per call, 4; note duration and frequency sweep range of note 1, 32.4 ms, 3,011–3,450 Hz; note 2, 66.1 ms, 2,985–3,846 Hz; note 3, 50.8 ms, 3,004–3,767 Hz; note 4, 64.0 ms, 3,026–3,932 Hz; internote intervals: notes 1 and 2, 50.2 ms; notes 2 and 3, 96.2 ms; notes 3 and 4, 43.9 ms; number of calls per bout, 10; intercall interval (ICI), 458 ms; and interbout interval (IBI), 8.2 s. Each experimental trial consisted of broadcasting 25 calling bouts lasting for 402 s (Fig. 1a).

In trials in which an acoustic stimulus accompanied a dynamic visual signal, crossmodality synchrony was accomplished by manually inflating and deflating the VS once for each call, or 10 times per bout. We adjusted the VS inflation magnitude of the EMF to approximate that obtained from videotaped images of a naturally calling male. Before the playback experiment, two logs were placed on the forest floor 2 m from each other so that each was located at the vertex of an equilateral triangle, including the focal male (Fig. 1b). A complete experimental trial was presented from one log; successive trials alternated between logs. Playback experiments were conducted during the hours of intense calling activity by *E. femoralis* males in the Arataï population, between 1400 hours, 00 min and 1800 hours, 00 min. All trials were run during rainless conditions and at temperatures of 23.5–26.0°C.

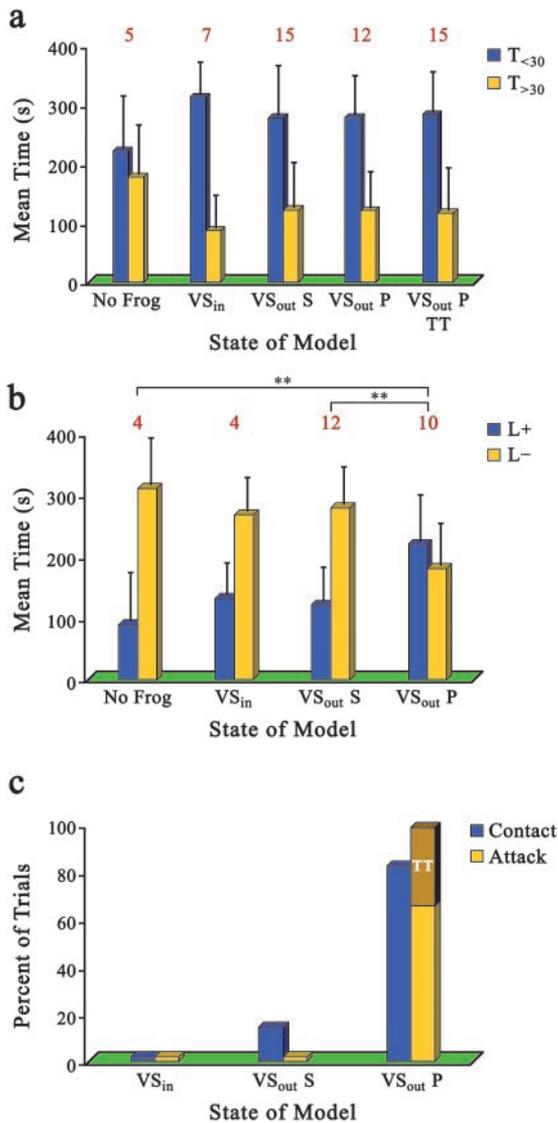
## Results and Discussion

Unimodal control trials in which the EMF was present but unaccompanied by call playback ( $n = 6$ ) never resulted in a successful approach (i.e., crossing a 30-cm perimeter around the log during the trial period) by the test male (data not shown). In another set of unimodal control trials in which calls were broadcast in the absence of the EMF, five of nine males successfully approached but never exhibited aggressive behavior



**Fig. 1.** (a) Schematic diagram of the spectrogram of the synthetic call used as the acoustic stimulus in playback experiments. Each call consists of four frequency-modulated notes sweeping upward in frequency (see text for call note parameters) followed by an intercall interval (ICI) of 458 ms. The call and ICI are repeated 10 times to form a bout. After an interbout interval (IBI) of 8.2 s, the bout and IBI are repeated 25 times to comprise a 402-s trial. (b) Configuration of stimulus logs and test male during playback experiments. The logs are placed at two vertices of a 2-m equilateral triangle in which the frog forms the third vertex. Trials were run alternately from the two logs.

toward the loudspeaker. In most (54 of 74) of the bimodal playback trials (EMF present and accompanied by call playback) test males oriented toward and closely approached the sound source. The time required for a successful approach was model-state-independent (Fig. 2a), strongly implicating the species-specific call alone as the long-distance attractant. Mean approach velocity for all males making a successful approach to the model ( $n = 54$  trials, 13 males) was 1.4 cm·s<sup>-1</sup>; maximum approach velocity was 6.9 cm·s<sup>-1</sup>. Having made a successful approach, males spent significantly more time on the log during trials in which the EMFs VS was inflated and pulsating than during those in which either (i) the VS was inflated and stationary or (ii) the EMF was removed from the log [ $P < 0.01$ , Fisher's pairwise least significant difference (PLSD) test in both cases], and marginally significantly more time on the log than when the VS was deflated ( $P = 0.07$ , Fisher's PLSD test). Thus, the dynamic calling model captures the attention of the resident male to a significantly greater extent than either the acoustic cues presented in the absence of the model, or the static model accompanied by call playback (Fig. 2b). Unexpectedly, we observed that of the seven VS-deflated trials that resulted in a successful approach to the log, none evoked any contact or attacks by the resident male. Moreover, 16% of the contacts with the model were observed during trials in which the VS was inflated but stationary. In contrast, 84% of the contacts observed and 100% of the physical attacks on the model (wrestling, pushing, kicking, etc.) occurred during trials in which the VS was inflated and pulsating (Figs. 2c and 3). Back-and-forth rotation of the model on its turntable neither enhanced nor inhibited aggressive behavior (Fig. 2c).



**Fig. 2.** Responses of test *E. femoralis* males in acoustic playback experiments for different states of the EMF. No Frog, EMF removed from the log; the EMF was present in all other states. VS<sub>in</sub>, EMF VS deflated; VS<sub>out</sub> S, EMF VS inflated and stationary; VS<sub>out</sub> P, EMF VS inflated and pulsating (see *Methods* for details); VS<sub>out</sub> P TT, EMF VS inflated and pulsating accompanied by back-and-forth rotation of the EMF on the turntable. For each state of the model, the number of animals tested varied from 9 to 15. The number of successful trials run for each model state is indicated above the bars. (a) Mean time test males spent inside ( $T_{<30}$ ) and outside ( $T_{>30}$ ) a 30-cm perimeter around the log ( $T_{<30} + T_{>30} = \text{trial duration} = 402 \text{ s}$ ). None of the  $T_{<30}$  or  $T_{>30}$  values are significantly different from the others (ANOVA;  $P > 0.05$ ). (b) Mean time test males spent on (L+) and off (L-) the log. \*\*,  $P < 0.01$ ; Fisher's pairwise least significant difference (PLSD) test. (c) Percent of trials in which either contact with the model ( $n = 7$  trials, 8 bouts) or physical attack ( $n = 6$  trials, 30.4 bouts) occurred for each state of the model. TT, trials in which the turntable was activated. Error bars are standard deviations about the mean.

Two previous studies have used models to examine aggression in frogs. Male bullfrogs (*Rana catesbeiana*) have been shown to attack a stationary ceramic model of a bullfrog after a loud-speaker stopped broadcasting calls (27). Moreover, males of this species would attack stationary models in the water, but not those positioned on land close to the speaker playing the calls (27). These observations suggest that movement may not be necessary to elicit aggression in bullfrogs, although the sample size in this study was small. In contrast, territorial male green



**Fig. 3.** A rendering from video frame illustrating aggressive (fighting) behavior of an *E. femoralis* male (right) toward the EMF (left) placed in his territory, 2 m from his initial calling position. In all experimental trials that evoked fighting, the model's VS was inflated and pulsating, and was accompanied by playback of the male's species-specific territorial call.

frogs (*Rana clamitans*) sometimes attacked latex models of frogs that were moved up and down in the water, but they did not attack stationary models (28). These results suggest that in this species, movement alone may be sufficient to elicit aggressive behavior.

Animal acoustic signals play seminal roles in mate attraction and regulation of male spacing, maintenance of pairbonds, localization of hosts by parasites, and feeding behavior (1, 29–32). Among vertebrate signals, it is becoming clear that no single stereotyped signal feature reliably elicits species-specific behavior, but rather, that a suite of characters is involved (33–35). Evidence that bimodal cues may also play a key role in amphibian mate choice comes from a recent playback study in which female túngara frogs, *Physalaemus pustulosus*, significantly preferred advertisement calls accompanied by a video playback of VS inflation to calls with no inflation playback (G. Rosenthal, S. Rand, and M. Ryan, unpublished data). Thus, in this species, the female's attraction to an acoustic cue is modulated by the presence of the inflating VS.

With our EMF, we were able to evoke fighting behavior and determine the communication cues necessary for eliciting both physical contact and combative behavior by an anuran amphibian. This system affords the possibility for future studies in which multimodal communication signals may be systematically manipulated to reveal the relative contribution of each modality. In further experiments with *E. femoralis* the acoustic and visual components of its advertisement call can be separated, both temporally and spatially, to quantify the degree of crossmodality chunking (36) of the stimulus components required to evoke aggression.

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