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RESEARCH ARTICLE

Beyond sound: bimodal acoustic calls used in mate-choice and aggression by red-eyed treefrogs

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ABSTRACT

Airborne sound signals function as key mediators of mate-choice, aggression and other social interactions in a wide range of vertebrate and invertebrate animals. Calling animals produce more than sound, however. When displaying on or near a solid substrate, such as vegetation or soil, they also unavoidably excite substrate vibrations because of the physics of sound production and of acoustic propagation, and these vibrations can propagate to receivers. Despite their near ubiquity, these vibrational signal components have received very little research attention and in vertebrates it is unknown whether they are relevant to mate-choice, an important driver of evolutionary divergence. Here, we show that female red-eyed treefrogs are more than twice as likely to choose a male mating call when airborne sound is paired with its corresponding substrate vibrations. Furthermore, males of the same species are more aggressive towards and display a greater range of aggressive behaviors in response to bimodal (sound and vibration) versus unimodal (sound or vibration alone) calls. In aggressive contexts, at least, air- and substrate-borne signal components function non-redundantly. These results are a clear demonstration that vibrations produced by a calling animal can function together with airborne sound to markedly enhance the function of a signal. If this phenomenon proves widespread, this finding has the potential to substantially influence our understanding of the function and evolution of acoustic signals.

KEY WORDS: Sexual selection, Animal communication, Multimodal, Substrate vibration, Biotremology, Anura

INTRODUCTION

Acoustic signals play key roles in determining mating success in many animals, both as a focus of mate-choice and as mediators of agonistic interactions between competing individuals. Selection on these signals is, therefore, an important driver of evolution, and understanding their function is vital in teasing apart the mechanisms that lead to the elaboration of sexual traits, and to the diversification and maintenance of evolutionary lineages (Podos, 2022; Wilkins et al., 2013). There exists a massive body of work exploring the function of acoustic signals in sexual and other contexts (Gerhardt and Huber, 2002; Marler and Slabbekoorn, 2004; Simmons et al., 2003). The vast majority of these studies focus on animal sounds or substrate vibrations in a unimodal context (Gerhardt and Huber, 2002; Marler and Slabbekoorn, 2004) or as bimodal signals with a visual component (Elias et al., 2005; Laird et al., 2016; Narins et al., 2003).

The two acoustic modalities – sound that travels through air or water and vibrations that propagate through or along the surface of solid substrates – are however, inexorably linked. When calling near or perched upon vegetation or the ground, signalers produce not only sound, but also unavoidably produce substrate vibrations that in some cases can propagate to receivers (Caldwell, 2014) (Fig. 1). This is because some portion of the acoustic energy reaching a boundary between two media will propagate across that boundary (Cremer et al., 2005). Airborne sound, for example, will excite vibrations in solid structures, such as vegetation, and vibrations generated by vocal folds, stridulatory structures, tymbal mechanisms and other sound-producing organs travel through the bodies of signalers, passing into the substrate on which they are perched (Caldwell, 2014). Moreover, the impedance mismatch between the matrix of liquids and solids that make up a signaler's body and the substrate on which it is perched will, in most cases, be far less than the mismatch between that body and the air (Bennet-Clark, 2001; Cremer et al., 2005). This works to facilitate the generation of substrate vibrations without elaborate impedance-matching structures.

As animals from a broad range of vertebrate and invertebrate taxa are capable of transducing substrate vibrations (Hill, 2009), the fact that sound and vibration are commonly excited together sets up the intriguing possibility that in some cases acoustic signals function simultaneously through both air- and substrate-borne information channels.

The existence of such bimodal acoustic calls has been demonstrated in a handful of species. In at least two species of katydid, the airborne component of male calls attracts conspecifics from afar, while the substrate-borne component aids rivals and potential mates in localizing the signaler at close distances (Keuper and Kuhne, 1983; Latimer and Schatral, 1983; Stiedl and Kalmring, 1989). African elephants (*Loxodonta africana*) respond defensively to playback of either the air- or substrate-borne components of their powerful low frequency rumble calls (O'Connell-Rodwell et al., 2006, 2000) and can use the seismic component of these calls to discriminate between familiar and unfamiliar signalers (O'Connell-Rodwell et al., 2007). Male mole crickets (*Gryllotalpa major*) and male white-lipped frogs (*Leptodactylus albilabris*: Leptodactylidae) adjust the relative timing of their sexual advertisement calls in response to the substrate-borne components of neighboring males' calls (Hill and Shadley, 2001; Lewis et al., 2001). Male túngara frogs (*Engystomops pustulosus*: Leptodactylidae) likewise increase the rate and complexity of their calls in response to water ripples produced by calling conspecifics or rainfall (Halfwerk et al., 2014, 2016). Similarly, it is likely that the calls of male golden rocket frogs (*Anomaloglossus beebei*: Dendrobatidae) function bimodally, as males alter the rate and structure of their own calls and are more

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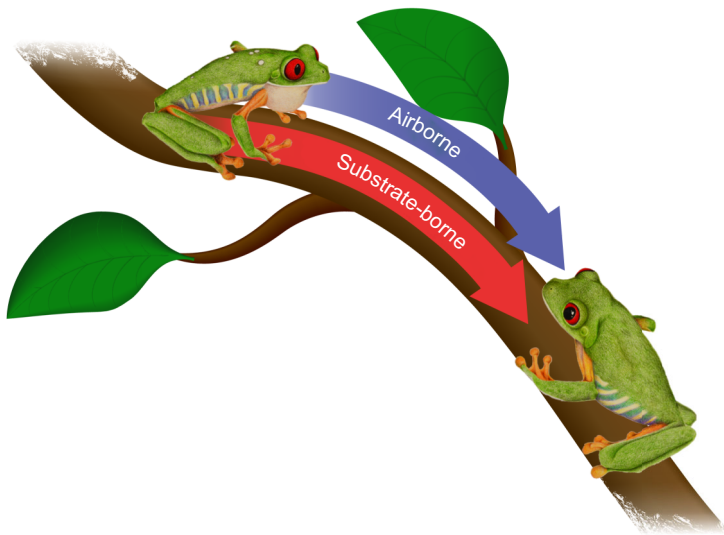
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Fig. 1. Parallel paths of acoustic signal propagation. Vibrations generated by animal acoustic calls propagate to receivers through both airborne sound and substrate-borne vibrations. Illustration by Ana Ospina.

likely to move towards the source acoustic playback during unimodal presentation of call vibrations (Narins et al., 2018).

Still, considering the near ubiquity of vibration sensitivity across taxa (Hill, 2009) and that airborne calls produced by insects, anurans and birds are often mechanically constrained to excite substrate vibrations (Caldwell, 2014), bimodal acoustic calls have received very little research attention. It is completely unknown, for example, whether bimodal acoustic calls play a role during mate choice in vertebrates. Given that selection on either component of a bimodal acoustic call will necessarily act on the other, mechanistically intertwined signal component, the high potential for interplay between these two communication channels to influence the evolutionary trajectory of acoustic signals should not be overlooked.

In this study, we used acoustic playback experiments in the field to test whether the vocalizations of red-eyed treefrogs [*Agalychnis callidryas*: Hylidae (Cope 1862)], previously assumed to function via their airborne sound component alone, function as bimodal acoustic calls with both air- and substrate-borne components. We found that substrate vibrations excited by these calls strongly influence their function, both in the contexts of female mate-choice and during aggressive interactions between males.

MATERIALS AND METHODS

Both mate-choice and aggression trials were conducted at the Smithsonian Tropical Research Institute's (STRI) Experimental Pond facility in Gamboa, Panama. This study proceeded under research permits granted by the Autoridad Nacional del Ambiente of Panama (SE/A-83-10 & SE/A-57-12) and STRI IACUC protocols (20100816-1012-16 & 2012-0715-2015). Live animals were handled in accordance with guidelines published by the American Society of Ichthyologists and Herpetologists (Beaupre et al., 2004).

Call recordings and analysis

Female red-eyed treefrogs choose mates, in part, based on the airborne component of male 'chack' advertisement calls (Kaiser et al., 2018; Robertson et al., 2017) and the same call has an important role in mediating agonistic interactions between males (Caldwell et al., 2010). These calls produce strong substrate vibrations in the plants from which males display and are detectable at distances of over 1 m from the source (Caldwell, 2014). Males produce either single-note 'chack' or similar two-note

'double-chack' advertisement calls (Pyburn, 1970). Call analyses reported here compare mean call characteristics for single-chack calls from 63 males at natural calling sites around Experimental Pond (1–15 calls per individual; median, 4 calls). Audio from these calling males was recorded in 44.1 kHz, 24-bit .wav format, using a shotgun microphone at ∼1 m (model NTG2, Rode Microphones, Sydney, Australia) connected to a solid-state recorder (model PMD661, Marantz, Kanagawa, Japan). Substrate vibrations produced during these calls were simultaneously recorded into the same recorder using a 2 g miniature accelerometer (model APT32, AP Technology, Oosterhout, Netherlands) affixed to a plant stem contiguous with, and within 100 cm of, each calling male. Ensuring separation between the air- and substrate-borne acoustic modalities was critical to the experiments reported here. Most multi-channel recording devices, however, are subject to 'crosstalk', where signals from one channel can be inadvertently recorded onto other channels at low amplitudes. We, therefore, examined the recording equipment used to rule out significant electrical contamination between the stereo channels. Signal-to-crosstalk noise ratios during these tests were all greater than 40 dB.

We measured the dominant frequency for both the air- and substrate-borne components of each call, as well as the range of frequencies with energy within 10 dB of the dominant (10 dB bandwidth). For substrate vibrations, this bandwidth measurement excluded energy within a secondary low-frequency peak, below 500 Hz. All measurements were taken from spectrograms (2157 sample Hann window, with 50% overlap) in Raven Pro (v.1.5, Cornell Lab of Ornithology, Ithaca, NY, USA). We compared dominant frequencies and bandwidths for the air- and substrate-borne call components using Wilcoxen match-pairs signed rank tests.

Stimulus construction

Playback stimuli for trials with both males and females were constructed from exemplars of *A. callidryas* chack and double-chack advertisement calls recorded using the methods described above. While call timing for the male and female experiments varied (as described below), they shared a set of 14 base stimuli containing synchronized air-and substrate-borne call components. Each base stimulus included 5 separate calls from a single male, with calls from unique males being used for each of the 14 base stimuli. During behavioural trials, calls were played from a solid-state

recorder (Marantz PMD-660 or PMD-661). Airborne call components were played through a portable field speaker (model 7-100, Pignose Amps, Las Vegas, USA) at 70 dB SPL re. 20 µPa, measured at 1 m (C-weighted, max, fast response; model 33-025, Radioshack, Fort Worth, USA), which is a typical amplitude within the range of natural variation for this population (mean 64 dB, range 60–71 dB, N=11 males and 52 calls) and similar to the reported amplitude for *A. callidryas* calls in northern Costa Rica (Kaiser et al., 2018). Substrate-borne call components were played through a custom amplifier (E. Hazen, Boston University Electronic Design Facility) and an electrodynamic shaker (model 4810, Brüel & Kjær, Nærum, Denmark) attached to the playback plant via a stiff metal rod. Because both the playback equipment and the substrate through which vibrational signals propagate introduce substantial spectral filtering (Cocroft et al., 2014), we used a custom script in MATLAB (R2010a, MathWorks, Natick, USA) and an iterative series of re-recordings with the original recording equipment to equalize each playback stimulus to match the spectral properties of the original recordings at 50 cm from the attachment of the shaker. Stimulus equalization was performed separately for the female and male experiments, as the substrate for these two experiments differed. Vibrational components were played at a peak amplitude of 2 m s^{-2} measured at 50 cm from the point of playback, a level typical of and within the range of those recorded from the local population (mean±s.d.: 3.46±2.93 m s^{-2}; range: 0.67–9.17; N=63 males, 254 calls, recorded 10–100 cm from the source).

Female choice trials

Gravid female *A. callidryas* either in amplexus (N=28) or alone (N=13) were collected between 20:00 h and 00:00 h from the vegetation surrounding Experimental Pond. While awaiting behavioral trials, the frogs were held for up to 1 h in inflated zip closure bags that each included several wet leaves in a dark location approximately 10 m from the active chorus at the pond. Because it was not clear whether females collected outside of amplexus were actively searching for mates, we report analyses both with and without these individuals. Once released at their point of collection, all females captured, in or out of amplexus were, however, observed mating later the same night, suggesting their readiness to breed.

The arena for mate-choice trials was a Y-shaped branch approximately 2 m in height, which bifurcated approximately 1 m above the ground. Through the course of the experiment, two such branches were used, and results from trials with each branch were congruent. The base of the branch was embedded in a 5-gallon pot filled with soil and rocks and placed at the edge of Experimental Pond. During trials, the airborne call component was played from speakers near the distal tip of each side of the Y-shaped branch, such that there was a 2 m separation between speakers. Speakers did not touch the branch, and were separated from the branch by a distance of 30 cm. Identical airborne call components were presented antiphonally from the two speakers, with a call period of 30 s for each speaker. For each trial, the substrate-borne call component was played in synchrony with the airborne component produced by one of the two speakers through a shaker coupled to the branch at a point adjacent to that speaker. At the start of each trial, the focal female was placed on the base of the Y-shaped branch, 50 cm below the branching point. Thus, the female was presented with a choice, between the airborne component of a male call played alone on one side of the Y, and that same call played bimodally, with air- and substrate-borne components, on the other side of the Y. We alternated the side of the branch from which vibrations were played

between trials, and each stimulus was played an equal number of times from the left and right sides.

We filmed infrared video (model HDR-SR11, Sony, New York, USA) of the females' behavior during the trials, and kept recording until either the female had hopped from the branch or 10 min had elapsed. Females that did not move during the trial, that hopped away before reaching the branching point of the Y, or that climbed past the branching point before experiencing 4 call exemplars (2 min) were excluded from the final analysis.

To reduce the ability of females to use any sound produced by the electrodynamic shaker to inform their choices, we enclosed the shaker in a plastic box lined with butyl rubber and acoustic foam. Other than a small opening through which the rod attached to the playback branch extended, this enclosure was airtight. No sound from the shaker was audible to the experimenter's ear during field playbacks (M.S.C., personal observations), and the sound of vibration playback was not detectable above background within a semi-anechoic chamber using a SPL meter at 1 m (ANSI Type 1, GenRad model 1982). To determine whether the mere presence of the electrodynamic shaker on one side of the playback setup altered female behavior, we conducted a series of control trials where the speakers and shaker played as normal, but where the shaker was mechanically decoupled from the playback branch. Females showed no bias, visiting the side of the setup with the shaker in exactly half of control trials (10 of 20 trials).

Male–male aggression trials

To test the relevance of the vibrational component of calls to aggressive interactions between male *A. callidryas*, we presented males with one of 4 stimuli: (1) bimodal 'chack' calls containing both their air- and substrate-borne call components; (2) the airborne component alone; (3) the substrate-borne component alone; or 4) a control where no stimulus was played. In a previous playback study, red-eyed treefrogs responded to a non-vocal aggressive vibrational signal (tremulation) but not to vibrational noise (Caldwell et al., 2010). It is therefore likely that any response to vibrational playback seen in the current study was similarly specific to call vibrations. The experimental arena for male–male trials consisted of a potted sapling ~1.5 m in height placed at the edge of Experimental Pond. A similar sound and vibration playback set-up was used in trials with males as was used with females, except that the point of playback was ~30 cm from the base of the sapling and only a single speaker was used. Playback amplitude for air- and substrate-borne call components was the same as that played during the experiment with female subjects.

For each trial, a unique male was transplanted from its calling site around Experimental Pond to the top of the playback sapling. Most males eventually began calling from the playback sapling and were then subsequently given a 5 min acclamation period before a playback trial began. Trials lasted 20 min each, during which one of the 4 stimuli was played every 3 min. We recorded audio and infrared video of each acclamation period and trial (Sony HDR-SR11). Males that left the plant without calling or before experiencing 3 stimulus presentations were excluded from the study. Following each trial, males were measured, weighed, and the pattern of spots and scars along their dorsal surface was recorded to enable individual identification. These measurements and dorsal marks were used in combination to select unique, naïve males for each trial. Over the 2 year period in which this study was conducted, approximately 10% of male *A. callidryas* at Experimental Pond had no uniquely identifiable dorsal marks. It is possible that a small number of these males may have participated

in more than one of the 50 playback trials included in the final analysis.

Video scoring and analysis of playback trials

Videos of behavioral trials were scored without their accompanying audio by observers blind to treatment using the behavioral analysis software BORIS v.3.0 (Friard and Gamba, 2016). For trials with females, frogs were considered to have 'chosen' a stimulus when they climbed more than 4 body lengths past the branching point of the Y on the side from which that stimulus was presented. Female red-eyed treefrogs in breeding condition are known to exhibit directed phonotaxis towards playback of airborne call components (Kaiser et al., 2018; Robertson et al., 2017). Nevertheless, some females may have climbed the branch motivated by something other than a desire to mate. Unless these females were also attracted to vibrational call components, such behaviour would only serve to obscure any patterns of interest to this study. For trials with males, observers scored the number of aggressive behaviours observed during the 20 min playback period, including chuckle calls, very low amplitude chack calls used in aggressive contexts ('half-chacks'), groaning acoustic calls and vibratory tremulation displays (Caldwell et al., 2010; Pyburn, 1970).

All statistical analyses were conducted in SPSS (v.27, IBM Corp., Armonk, NY, USA). We report two-tailed P-values throughout, and include Bonferroni adjusted significances for *post hoc* Dunn pairwise comparisons of Kruskal–Wallis results.

RESULTS

Sound and vibrational characteristics of red-eyed treefrog calls

Comparison of the air- and substrate-borne components of single chack calls from 63 unique males (283 individual calls) revealed both broad similarities and some consistent differences. Overall, air- and substrate-borne signal components had similar temporal and spectral properties (Fig. 2). The airborne component of chack calls had a slightly higher mean (±s.d) dominant frequency (airborne: 1831±225 Hz; substrate-borne: 1581±240 Hz; Wilcoxon match-

pairs signed rank test: Z=5.4, P<0.001, N=63) and a wider 10 dB bandwidth (airborne: 1461±267 Hz; substrate-borne: 975±451 Hz; Z=5.5, P<0.001, N=63) than did their substrate-borne counterparts. Substrate-borne vibrations also included substantial low-frequency energy, below 500 Hz, that was not present in the airborne component (Fig. 2). Large displacements of the substrate associated with these low frequencies are visible in trial videos and appear to be due to a shift in the weight of the frog as it called. A more targeted investigation of call mechanics would be necessary to confirm this, however.

Bimodal acoustic calls in mate-choice

During mate-choice trials, a higher proportion of females approached the bimodal, sound and vibration, playback source than approached unimodal sound playback (68.3% choosing bimodal playback; Exact binomial test: N=41, P=0.029; Cohen's g=0.183; Fig. 3A). Females also spent a larger percentage of time during trials on the side of the Y-shaped branch with bimodal, rather than unimodal, playback (64.2±6.9% mean±s.e.m.; one-sample Wilcoxon signed rank test: Z=1.95, P=0.052, N=41). This pattern of preference for the bimodal stimulus strengthened when unpaired females were excluded from the analysis, and only females collected in amplexus were considered (75.0% choosing bimodal playback, N=28, P=0.014; Cohen's g=0.250; Fig. 3A; 70.7±8.3% of time on side of bimodal playback, Z=2.2, P=0.026, N=28). While all females tested were later observed mating on the same night, it is possible that females collected in amplexus were more highly motivated to find a mate. Overall, females strongly preferred to approach bimodal male calls, as they naturally occur in the wild, over calls presented as airborne sound alone.

Bimodal acoustic calls in agonistic interactions

Again, the presence of the substrate-borne call component drastically altered male responses. Males were quantitatively more aggressive when airborne components were played in conjunction with their substrate-borne counterparts, as opposed to when airborne components were played alone (Table 1, Fig. 3B). The
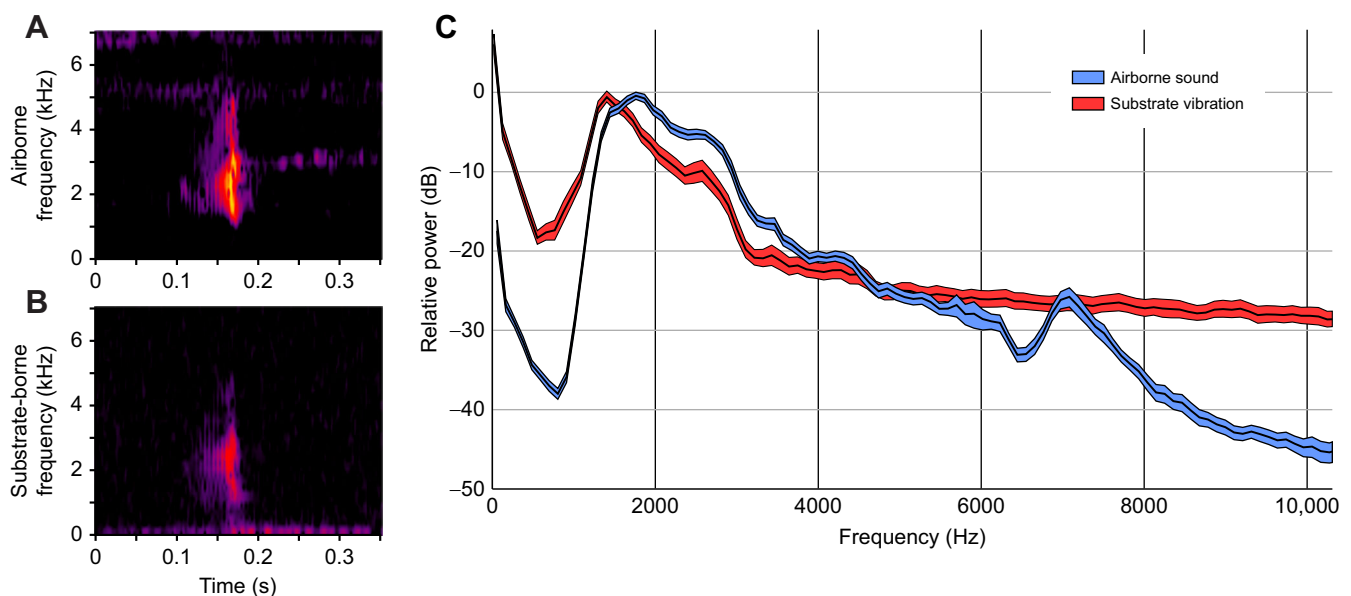

Fig. 2. Air- and substrate-borne components of red-eyed treefrog advertisement call. (A,B) Spectrograms for the two call components of a typical 'chack' advertisement call. (C) Peak standardized average power spectra for the advisement call. Shown are mean values±s.e.m for 283 calls from 63 unique males.

Table 1. Comparison of the number of individual aggressive signals displayed by males per presentation of bimodal and unimodal playback of chack call components

Pairwise comparisons	Z	P
None versus airborne	1.19	1.000
None versus substrate-borne	0.37	1.000
None versus bimodal	3.50	0.003*
Substrate-borne versus airborne	1.61	0.649
Substrate-borne versus bimodal	3.91	0.001*
Airborne versus bimodal	−2.71	0.040*

Overall Kruskal–Wallis model: H=20.5; d.f.=3; N=50; *P<0.001; ε^2=0.419

Table 2. Comparison of the number of different types of aggressive signal displayed by males in response to bimodal and unimodal playback of chack call components

Pairwise comparisons	Z	P
None versus airborne	0.68	1.000
None versus substrate-borne	0.49	1.000
None versus bimodal	3.05	0.014*
Substrate-borne versus airborne	1.23	1.000
Substrate-borne versus bimodal	3.61	0.002*
Airborne versus bimodal	−2.80	0.031*

Overall Kruskal–Wallis model: H=17.3; d.f.=3; N=50; *P<0.001; ε^2=0.352

aggressive response was also qualitatively different during bimodal playback, and males responded with a greater range of different aggressive signals when call vibrations were present, as compared to unimodal playback of the airborne component alone (Table 2, Fig. 3C). Highly aggressive vibrational tremulation displays were seen in response to unimodal playback of the airborne component or bimodal playback of chack calls, but were considerably more

common during bimodal playback trials (Mann–Whitney exact test: U=214, N=32, P<0.001). In general males were not strongly aggressive towards the airborne call component of chack calls played alone, nor did they respond to playback of the substrate-borne component of calls when played in isolation (Tables 1 and 2; Fig. 3B,C). Our results indicate that the 'chack' advertisement call also functions as a bimodal acoustic signal in agonistic interactions
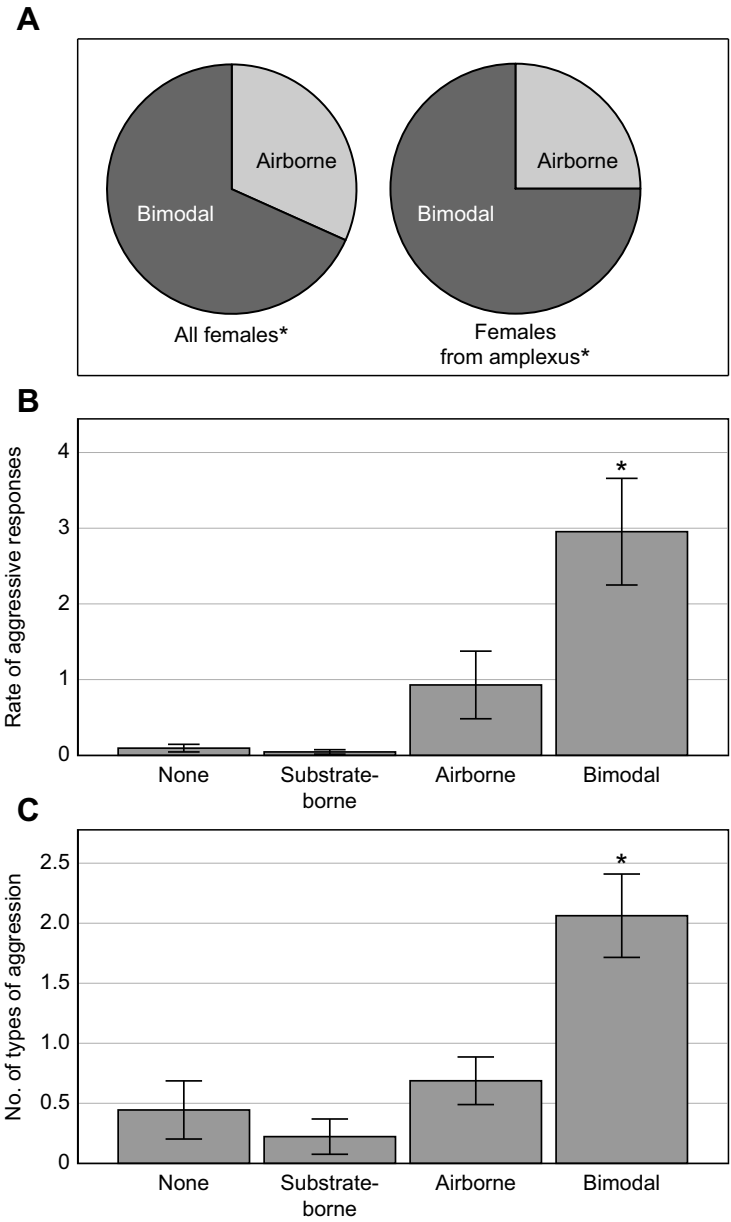

A

B

C

Fig. 3. Response to bimodal playback of advertisement calls in the contexts of mate-choice and aggression. (A) Female preference in two-choice trials between unimodal presentation of the airborne call component and presentation of the full bimodal call, including both the air- and substrate-borne components. In all females (left, N=41) and females collected while in amplexus (right, N=28). (B) Mean rate of male aggressive behaviors elicited per presentation of unimodal and bimodal call stimuli (N=50 males). (C) Mean number of different types of male aggressive behaviors elicited per playback trial (N=50 males). Asterisks indicate significant differences from all other groups (*P<0.05). Error bars represent ±1 s.e.m.

between male red-eyed treefrogs, with both the air- and substrate-borne components necessary to elicit the full range of aggressive responses from rivals.

DISCUSSION

Both female call preferences and the aggressive responses of male red-eyed treefrogs were strongly influenced by substrate vibrations excited by conspecific advertisement calls. When airborne call components were presented bimodally in conjunction with their corresponding substrate-borne components, females were more than twice as likely to choose a call over the same call presented as airborne sound alone. Likewise, males were more aggressive in response to, and showed a greater range of aggressive behaviors towards, bimodal call playback, as opposed to unimodal presentation of either the air- or substrate-borne call components.

Do air- and substrate-borne call components function redundantly?

A major outstanding question about the function of bimodal acoustic calls is whether air- and substrate-borne components carry any non-redundant information to receivers (Caldwell, 2014; Partan and Marler, 2005). Our results indicate that in agonistic contexts, the air- and substrate-borne components of red-eyed treefrog advertisement calls do not function redundantly. While the airborne component played alone provoked slight, non-significant, aggressive response from males, this response was both qualitatively different and substantially more intense when airborne sound was played in conjunction with its substrate-borne counterpart. The vibrational component of advertisement calls played alone provoked no discernable response from males. This is, perhaps, not surprising, as the air- and substrate-borne components of this signal are presumably mechanistically linked and are always produced simultaneously in nature. While males on vegetation non-contiguous with the signaler commonly experience airborne sound produced by the 'chack' call without the accompanying substrate-borne component, it is unlikely that they experience naturally produced call vibrations in isolation. Interestingly, substrate vibrations produced during another of the red-eyed treefrog's aggressive behaviors – tremulation displays – are in themselves sufficient to elicit strong responses (Caldwell et al., 2010). Like bimodal acoustic calls, the tremulation display appears to be bimodal, but with visual and vibrational components carrying the message to receivers. In contrast to bimodal acoustic calls, however, the visual component of tremulation displays is often blocked by intervening vegetation, leaving the substrate-borne component to reach the receiver alone. Overall, our results are in line with non-redundant aggressive and competitive responses of males to bimodal call presentation that have been observed in both the dendrobatid frog *Allobates femoralis* and the túngara frog, *Engystomops pustulosus* (Halfwerk et al., 2014; Narins et al., 2003). Future work will be necessary to determine whether females similarly extract non-redundant information from the two simultaneously produced components of the red-eyed treefrog's advertisement call.

The function of vibrational call components

Our study was designed to test whether red-eyed treefrogs use bimodal acoustic signals. Further investigation will be necessary to determine what information is carried by the substrate-borne component of the red-eyed treefrog's advertisement call or how receivers integrate across the air- and substrate-borne information channels. Much as airborne call components function in a wide range of anurans (Gerhardt and Huber, 2002), substrate vibrations may communicate information about the species identity, physical properties, location or motivational state of the signaler (e.g. Caldwell, 2014; Virant-Doberlet and Cokl, 2004). Indeed, the spectral and temporal properties of the two components of the red-eyed treefrog's bimodal acoustic call are strikingly similar (Fig. 2) and are likely to encode much of the same information. These call components are, however, filtered differently as they are introduced into and propagate through media with distinct acoustic properties, and this filtering has the potential to introduce information useful to receivers (Caldwell, 2014). Substrate vibrations, for example, had lower dominant frequencies than their airborne counterparts. The substrate-borne call component also included low frequency vibrations below 500 Hz, absent from the airborne component. At this point, it is not known whether receivers in our study responded to low or high frequency energy in call vibrations. Teasing apart which aspects of the substrate component of advertisement calls are most relevant to receivers was beyond the scope of our current study. Future playback studies, however, should resolve this question.

It is also not known whether red-eyed treefrogs perceive air- and substrate-borne call components separately. In fact, high frequency energy in both call components is likely transduced by the same sensory epithelia in the inner ear (Lewis et al., 2001). This sets up the intriguing possibility that the bimodal acoustic call is simply perceived as louder, and perhaps of lower frequency, when the signaler is on contiguous substrate and the vibrational component is available to receivers. Such a shift in amplitude or spectral properties could be perceived as indicating physical qualities of the signaler (Gerhardt and Huber, 2002).

Alternatively, amplitude or spectral properties of the substrate-borne signal component may be used to determine the relative location of the signaler. We currently do not have any evidence that red-eyed treefrogs can use substate vibrations to determine the direction from which a signal originated along a branch. In contrast to the propagation of airborne sound, the amplitude of substrate vibrations cannot be expected to decrease monotonically with distance from a signal source, especially in plant substrates, where standing waves are common (Mazzoni et al., 2014). This considerably complicates, but does not preclude (Cocroft et al., 2000; Virant-Doberlet and Cokl, 2004), the use of vibrations for localization and ranging. Females in our study were able to localize the source of the call playback, but they may have done so by virtue of the airborne signal components.

At the very least, the presence of substrate vibrations encodes key, non-redundant information about the presence of a conspecific male on substrate contiguous with that touching the receiver. When strong call vibrations are detected by a receiver, the signaling male is calling from the same plant. Such information is not trivial to arboreal animals attempting to communicate in contexts where intervening vegetation can obscure visual cues and rapidly degrade airborne sound signals. Moreover, our results suggest that a male red-eyed treefrog will enjoy a competitive advantage in attracting females within the active space of his call vibrations, which are largely confined to the plant on which he is perched. This result is consistent with the pattern of enhanced male aggression towards playback of calls including substrate vibrations. If vibrations produced by a conspecific male are detectable, then this rival is likely calling from the same plant, and therefore poses a greater threat, as it enjoys the same advantage in attracting females also on the plant.

Potential evolutionary consequences of bimodal acoustic calls

Because the production of air- and substrate-borne components of bimodal acoustic calls are mechanistically linked, selection on either component will necessarily affect the function and evolutionary trajectory of the other. Thus, to more fully understand the evolution of sound signals, we would benefit by determining if and how the vibrational components they produce function during communication. Selection on the airborne component of acoustic calls is exceedingly well studied (Gerhardt and Huber, 2002). While they have received very little research attention, opportunities for selection to shape the corresponding substrate component are abundant. The simple physics of calling on, or near, a substrate, results in some acoustic energy entering into that medium (Caldwell, 2014). It is inevitable, therefore, that intended receivers and potential eavesdroppers across a broad range of taxa experience vibrations produced by signalers calling nearby. Furthermore, solid substrates are more heterogeneous in their acoustic properties than is air. This results in a high potential for selection leading to improved signal transmission of substrate call components in the local environment to drive divergence of signals in allopatric populations.

Conclusions

The red-eyed treefrog's advertisement call was previously assumed to function unimodally through airborne sound, as have the acoustic calls of most frogs, birds and many insects. Our results indicate, however, that in the context of both mate-choice and male–male aggression, this display functions simultaneously through airborne sound and through substrate vibration as a bimodal acoustic call. There is little reason to label the red-eyed treefrog as a 'special case'. Indeed, several distantly related anurans appear to communicate with bimodal acoustic calls during intrasexual interactions (Halfwerk et al., 2016; Lewis et al., 2001; Narins et al., 2018) and the red-eyed treefrog is not known to have any peculiar morphology or calling behavior that would predispose it to bimodal acoustic signaling. Likewise, it seems probable that other species known to communicate through airborne sound may do so as part of currently undescribed bimodal acoustic calls. It may also be generally prudent to refine playback methodologies to consider the potential influence of substrate vibrations inadvertently excited during the presentation of airborne sound stimuli. Altogether, these new findings suggest that by broadening our perspective to regularly consider substrate vibrations, we would gain the potential to substantially improve our understanding of the function and evolution of acoustic communication systems in a wide range of taxa.

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