Vibrational Signaling in the Agonistic Interactions of Red-Eyed Treefrogs

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Summary

Sensitivity to substrate-borne vibrations is widespread in animals and evolutionarily precedes hearing but, compared with other sensory modalities, we know little about vibrational communication, particularly in vertebrates [1]. For plant-dwelling arthropods, vibrations are likely as important as sound [1–3]. Arboreal vertebrates excite plant vibrations with most movements [4], but the behavioral relevance of these vibrations has not been tested experimentally [5, 6]. In playback experiments using a robotic model frog and an electrodynamic shaker, we demonstrate that plant-borne vibrations generated by the shaking (tremulation) display of male red-eyed treefrogs (Agalychnis callidryas) are a vibrational signal, necessary and sufficient to elicit tremulations in response. A trend toward increased aggression during visual playbacks suggests that the visual component of tremulations may also convey information. In male-male contests, tremulations were the most frequent aggressive display and their use and vibrational characteristics varied with male size and conflict context. Nearly all of A. callidryas’ signaling behaviors, including tremulations and acoustic calls, excite strong, stereotyped vibrations that travel through plants and could be informative to receivers. Our results demonstrate that vibrational signals serve a key role in the biology of one well-known arboreal frog and suggest that consideration of the vibrational modality may significantly broaden our appreciation of the behavior and evolution of arboreal vertebrates.

Results

Red-eyed treefrogs, Agalychnis callidryas, form nocturnal mating aggregations in vegetation over Mesoamerican wet forest ponds. Males defend calling sites, maintaining a spacing of at least 0.5 m [7]. In addition to “chack” advertisement calls, males issue staccato “chuckle” calls, apparently to reinforce the boundaries of calling territories [7]. Most females pair with one male, but multimale amplexus and paternity occur, and aggression between amplexant and unpaired males is common [7, 8]. During contests over females, competing males issue chuckle calls and perform a display wherein the signaler raises his body off the plant and then rapidly contracts and extends his hindlimbs, shaking his hind end (tremulation) [7]. Because tremulating males likely excite strong, stereotyped vibrations in plants, we hypothesized that this display generates a vibrational signal, or possibly a bimodal signal with both visual and vibrational components.

We observed natural interactions between males at choruses to characterize the contexts of aggressive behaviors and to construct an ethogram for these interactions. We conducted staged contests between calling males to quantify the relationships between morphology, behavior, tremulation characteristics, and conflict outcomes. Finally, we used playback experiments to test whether the visual and vibrational components of tremulations are behaviorally relevant to receivers.

Observations

We observed frogs via red light or infrared-capable cameras to minimize disturbance. A previous study, using broad-spectrum lights, observed male-male aggression only in the presence of females [7]. We found that male-male aggression was common regardless of the presence of females. We observed aggressive behavior during 12 of 88 15 min focal samples and outside of focal sampling on over 90 separate occasions. Males jumped or walked toward each other with their bodies raised off the plant and made aggressive vocalizations, including high- and low-amplitude chuckles and low-amplitude chacks (henceforth “half-chacks”). Males tremulated in all aggressive interactions observed in focal sampling (Figure 1; see also Movie S1 available online), directing tremulations toward both lone (n = 10) and amplexant (n = 2) males. Tremulating frogs were 0.06–0.40 m from the nearest male (0.14 ± 0.09 m, mean ± standard deviation, here and throughout text), closer than noninteracting males (1.17 ± 0.78 m, range 0.02–3.40 m, n = 76; Mann-Whitney U = 38.5, p < 0.001). Occasionally, males slowly extended or made kicking gestures with their hindlimbs. Hindlimb displays resembled some described for other anurans in aggressive contexts [9]. Some interactions escalated to wrestling (4 of 12 in focal sampling). During intense bouts of wrestling, males produced a previously undescribed “groaning” acoustic call and what appeared to be a typical anuran release signal, vibrating their flanks and emitting low clucking sounds. Submissive males either fled the plant or remained silent and motionless, sometimes with their body flat against the substrate. Dominant males usually resumed advertisement calls soon after their opponent submitted. Some males retreated after a single chuckle or tremulation. Three pairs wrestled continuously for >4 hr, eliminating any opportunity for copulation that night. We videotaped two instances where females in amplexus appeared to tremulate in response to an intervening male and frequently observed females kick aggressively at second males. Prior work, using white light, reported no female aggression in such contexts [7].

Staged Contests

We conducted 38 staged contests to quantify behaviors during agonistic interactions. For each contest, two calling
males captured from separate areas of the pond were simultaneously introduced to a single pondside sapling. We recorded video, audio, and substrate vibrations from their interactions until one male fled the plant or was silent and motionless for 5 min while the other male issued advertisement calls. Calling males did not tolerate other active males on the same sapling. Males displayed the full range of aggressive behaviors that we observed in natural interactions, and 31 of 38 contests escalated to wrestling. Contest length was highly variable (18.1 ± 15.4 min, range 0.9–56.0 min). Victorious males produced more calls and more tremulations than did defeated males (Figure 2; Movie S2). The frequencies excited by tremulations were always well above the dominant vibrational modes excited in plants by common physical disturbances (2.53 ± 1.10 Hz, n = 15 plants), indicating that *A. callidryas* does not exploit these modes while signaling.

Tremulations are clearly an important aggressive signal. They were common, occurring in 37 of 38 staged contests; their use correlated with conflict outcome; and variation in tremulation properties was correlated with male size and conflict context. Victors tremulated for a greater fraction of total contest time than did losers (paired t test, t = 3.92, df = 37, p < 0.001), their tremulations were longer (t = 2.438, df = 19, p = 0.015), and they were more likely to produce the final tremulation (χ² (1) = 16.89, p < 0.001). Tremulations were the most common aggressive display.

Of victorious males, those that were smaller or more closely size matched to their opponents produced tremulations with higher dominant frequencies (Figure S1, multiple regression: overall model: R² = 0.36, n = 28 contests, F = 7.16, p = 0.003; SVL: t = −2.65, p = 0.014; difference in SVL: t = −2.34, p = 0.028). Unlike dominant frequency, the fundamental frequency of tremulations did not vary with male size, nor did that of victors vary with competitor size difference (linear regressions: SVL: R² = 0.23, n = 42 males, F = 0.96, p = 0.333; difference in SVL: R² = 0.08, n = 28 contests, a mean fundamental frequency of 12.13 ± 1.93 Hz (n = 44 males) and a mean dominant frequency of 13.28 ± 3.52 Hz (n = 44). Usually the fundamental dominated the signal and there was also some energy at the first harmonic, ~24 Hz. Occasionally this harmonic dominated the signal. Tremulations had a mean duration of 2.95 ± 1.57 s (range 0.3–12.2 s, n = 57 males) and held consistently high amplitude throughout their duration. Occasionally males tremulated with one hindlimb hanging off the plant. This did not affect the spectral properties of the vibrations produced (dominant, χ² (1) = 0.67, p = 0.414). The frequencies excited by tremulations were always well above the dominant vibrational modes excited in plants by common physical disturbances (2.53 ± 1.10 Hz, n = 15 plants), indicating that *A. callidryas* does not exploit these modes while signaling.

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their acoustic counterparts (see [10]). While chuckling, the broad-band spectral properties closely resembling those of tations produced by chacks were particularly intense, with 2 away from the signaler or obscured by vegetation (paired t more likely to be facing them than to be visually oriented When males tremulated, however, their opponents were no males to be more likely to tremulate when in view of a receiver. F = 2.11, p = 0.158). Male size also did not affect tremulation during Four A. callidryas

Figure 3. Waveforms and Spectrograms of Substrate Vibrations Excited during Four A. callidryas Signaling Behaviors
(A and B) A chuckle aggressive call. (C and D) A release signal produced during wrestling. (E and F) A chack advertisement call. (G and H) A plucking kick with the hindlimb. Each exemplar was recorded from the substrate at ~ 0.5 m distance.

F = 2.11, p = 0.158). Male size also did not affect tremulation duration. If tremulations were solely a visual signal, we might expect males to be more likely to tremulate when in view of a receiver. When males tremulated, however, their opponents were no more likely to be facing them than to be visually oriented away from the signaler or obscured by vegetation (paired t test: t = −3.08, df = 75, p = 0.380).

Figure 3. Waveforms and Spectrograms of Substrate Vibrations Excited during Four A. callidryas Signaling Behaviors
(A and B) A chuckle aggressive call. (C and D) A release signal produced during wrestling. (E and F) A chack advertisement call. (G and H) A plucking kick with the hindlimb. Each exemplar was recorded from the substrate at ~ 0.5 m distance.

Like tremulations, chack, chuckle, and release calls produced vibrations in the plant (Figure 3). Substrate vibrations produced by chacks were particularly intense, with broad-band spectral properties closely resembling those of their acoustic counterparts (see [10]). While chuckling, the torso of the signaling male jerked slightly with each call pulse. The acoustic pulse rate of chuckles, and corresponding fundamental frequency of substrate vibration, was 10.43 ± 0.83 Hz (n = 19 males). Strong substrate vibrations were also produced by some hindlimb kicks (Figures 3G and 3H). Videos revealed that males began rapid hindlimb extension with their feet still in contact with the plant. Their feet deflected, then suddenly slipped off the stem, much as a musician plucks a guitar string. It was not clear whether this “plucking” was an intentional signaling behavior or was incidental to kicking movements.

Visual and Vibrational Playbacks
To test whether visual and vibrational components of the tremulation display convey behaviorally relevant information, we varied these components independently in pondside playback trials with 66 male A. callidryas. We used an electrodynamic shaker and a robotic frog model to present (1) a no-model, no-vibration control; (2) a static model frog; (3) simultaneous visual and vibrational playback of recorded tremulations; (4) tremulation vibrations with a static model frog; (5) the visibly tremulating model with no substrate vibration; and (6) white-noise vibrations, matched in amplitude and duration to tremulations, with a static model frog.

Males responded aggressively to tremulation playbacks, but not to the static model frog when presented alone or to vibrational white noise (Figure 4; Movie S3). We only observed tremulations in response to stimuli that included tremulation vibrations. There was also a nonsignificant trend toward other aggressive behaviors in response to stimuli that contained the visual component of tremulations.

Discussion
The tremulation display is an important signal in the agonistic interactions of red-eyed treefrogs and transmits information to receivers through vibrations in the plant substrate. Plant-borne vibrations are thus an information channel relevant to vertebrates. Tremulating frogs produce both conspicuous substrate vibrations and visually conspicuous movements. Our playbacks demonstrate that receivers respond to the substrate vibrations excited by tremulations. Males did not respond to vibrational noise; the aggression we saw was specific to tremulations, not a general response to vibration. The role of the visual component of tremulations is not as clear. Nonetheless, visual and vibrational components may interact as a bimodal signal. Males tremulated frequently in response to stimuli that included tremulation vibrations, but never in response to visual-only playbacks, suggesting that the vibrational component of tremulations carries information that is absent from the visual component. The large variation in male response to visual playbacks may reflect the fact that males could not always see the tremulating model, as a result of their body orientation or intervening vegetation. These factors likely also interfere with the efficacy of visual signals in nature. Tremulation vibrations, however, do not suffer the same constraints. Indeed, during staged contests, males tremulated in interactions both with visible rivals and with those obscured by vegetation.

What Information Do Tremulations Carry?
Tremulations seem to carry information about both the relative status or motivation of a signaler and his size. Information may be coded in temporal, frequency, or amplitude properties of tremulation vibrations. The temporal properties of tremulation were correlated with dominance: victorious males tremulated more often and produced longer tremulations. Male size did not affect temporal characteristics of the signal.

Tremulation vibration frequency varied with both conflict context and male size. Smaller victorious males and victors more closely size matched to their rivals produced tremulations that, on average, had higher dominant frequencies. However, the fundamental frequencies of tremulations were remarkably constant. Higher-frequency tremulations occurred when the first harmonic came to dominate the signal. A possible explanation for this pattern is that a male’s ability to produce a tonal 12 Hz signal degrades as he reaches some amplitude or energetic threshold determined by his body size, where the biomechanics of signal production begin to exceed their range of linear response [4]. We might expect both highly motivated males and smaller males to push the limits of their abilities to generate tonal signals in order to
remain within an area of a few meters, often returning to the same calling sites several nights in a row (M.S.C. and G.R.J., unpublished data). Males fiercely defend their calling sites via a series of loosely graded displays and physical combat in a manner consistent with closely related species [12–15]. Whereas males commonly issue territorial chuckle calls even when the nearest conspecific is several meters away, we only observed hindlimb displays, tremulations, and half-chucks when two males shared the same small plant or were within 2 m of each other. Although tremulation appeared to be the highest escalation of aggressive signaling, often immediately preceding wrestling, signals could occur in any order.

Male size did not predict conflict outcome. Overall, the importance of male size to anuran mating success is highly variable [16]. The fact that male A. callidryas occasionally forgo advertisement calling for the remainder of the night while contesting a calling site suggests that territorial defense may be part of a mult infield reproductive strategy. Indeed, chorus tenure is the best predictor of mating success in many anuran species with extended breeding seasons [17].

Putative Vibrational Signals

Male A. callidryas produce a rich assortment of displays during agonistic interactions, nearly all of which produce distinctive substrate vibrations. Because this species clearly communicates via plant-borne vibrations, it is plausible that vibrations from any of these displays could convey behaviorally relevant information. Two signals are particularly promising candidates. First, plucking hindlimb kicks produce distinctive and intense substrate vibrations unlike those we might expect from a visual signal; their role deserves further attention. Second, along with their acoustic component, aggressive chuckle calls produce strong substrate vibrations. The acoustic pulse rate and substrate fundamental frequency of chuckles (10.4 Hz) closely resembles the fundamental frequency of tremulations (12.1 Hz). Although some physiological or environmental constraint may account for this similarity, these two aggressive signals may be vibrationally similar as a result of selection by receivers. Of further interest is the fact that males sometimes produce both chuckle and chack calls (half-chucks) with extremely low acoustic amplitudes ([18]; M.S.C., unpublished data). Low-amplitude calls such as these are common during close-range communication in anurans [19] and could represent a shift in the relative importance of acoustic and substrate components of a signal. Such multimodal plasticity may be beneficial when substrate vibrations reach fewer unintended receivers, such as predators, than do their acoustic counterparts. In arboreal environments, the active space of vibrational signals is largely confined to individual plants [20]. During courtship or agonistic interactions, conspecifics on a plant may be the receivers most relevant to a signaler.

Whether or not A. callidryas use vibrational signals apart from the tremulation display, our recordings demonstrate that most of their signaling behaviors excite strong, stereotyped vibrations in the plant substrate. Because of their relatively massive bodies, the movements of other arboreal vertebrates are likely to produce strong substrate vibrations as well.
vibrations in a vertebrate, other arboreal vertebrates almost certainly use this communication channel. Sensitivity to substrate vibrations has been demonstrated in the ground-dwelling relatives of each major group of arboreal vertebrates, including mammals [21–23], frogs [24], reptiles [25, 26], and birds [27, 28]. There are already several likely candidates for arboreal vertebrates that use vibrational signals. Male veiled chameleons, *Chamaeleo calyptratus*, produce at least three distinct patterns of substrate vibration accompanied by very faint sound when in the presence of a female [6]. Likewise, female South Asian common treefrogs, *Polypedates leuco- mystax*, rhythmically tap their toes on the vegetation above breeding sites, which appears to attract males [5]. The vibrational components of signals are likely important in both of these species, but receiver responses have not been experimentally tested. In the vast majority of cases, however, substrate vibration has not been considered in the study of arboreal vertebrate behavior. Indeed, probable vibrational signals have sometimes been assigned to other, more familiar, modalities. Wogel et al. [12] describe a “visual display” in *Phylomedusa rhodei*, another phyllomedusine treefrog, in which a victorious male “oriented towards the loser and shook his body up and down.” This signal may well convey information via substrate vibration. Similarly, pedal luring, wherein frogs tap the substrate with their toes, apparently to provoke movements from invertebrate prey, has been observed in many anuran taxa, including several arboreal species. Although it is traditionally described as a visual display, Sloggett and Zeilstra [29] make a compelling case that it is the substrate vibrations excited by this tapping that influence the behavior of its heterospecific receivers.

Moreover, signals in other modalities often excite substrate vibrations as well. For instance, many visual signals involve a behavioral component [9, 30], and these movements inevitably produce vibrations in any substrate mechanically coupled with the signaler [4]. This is equally true for acoustic signals. Studies on elephants’ use of seismic information have revealed substantial interplay between acoustic and substrate-borne vibrational signals [23]. The advertisement calls of the frogs *Physalaemus pustulosus* and *Dendropsophus ebraccatus* calling from the mud or plants several meters away were faint but clearly identifiable in some of our substrate recordings from saplings. For animals such as frogs, which transduce acoustic and vibrational stimuli via the same peripheral sensors [24], substrate vibrations generated by acoustic calls could be an important source of information. Indeed, while calling to attract females, males of the ground-dwelling white-lipped frog, *Leptodactylus albilabris*, generate seismic “thumps” when their vocal sacs impact the ground. In acoustically noisy environments, other males use these thumps to adjust their call timing [31].

**Conclusions**

We have shown that substrate vibrations serve an important role in the aggressive interactions of red-eyed treefrogs. We do not, however, believe that the tremulation display is an isolated phenomenon; vibrational signaling may be common among arboreal vertebrates. It is impossible to understand the function or evolution of communication systems without first recognizing the sensory modalities that they employ. Not only does each modality carry information vital to understanding animal interactions, but each is subject to unique constraints that act to shape the evolution of these behaviors. Although vibrational sensitivity is widespread across animal taxa, substrate vibrations have received little attention as a channel for information transfer, particularly among arboreal vertebrates. This group includes many frogs and lizards and the vast majority of birds and primates, taxa that have formed the core of our understanding of vertebrate communication. Yet we know almost nothing about vibrational signaling in these species. The further study of vibrational communication among arboreal vertebrates presents important unexplored opportunities to improve our comprehension of the behavioral ecology of these species, and of animal communication as a whole.

**Supplemental Information**

Supplemental Information includes one figure, Supplemental Experimental Procedures, and three movies and can be found with this article online at doi:10.1016/j.cub.2010.03.069.

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**References**


Figure S1. Effects of Body Size and Conflict Context on Tremulation Dominant Frequencies

(A) Mean dominant frequency of tremulations of victorious males as a function of their body size (snout-vent length). In most tremulations, the fundamental frequency was dominant. However, the first harmonic dominated vibrations of smaller males during some tremulations, resulting in higher frequency tremulations on average.

(B) Mean dominant frequency of victor tremulations vs. the absolute difference in body size between competitors. Victorious males tremulated at higher frequencies in more closely matched contests.
Supplementary Experimental Procedures

Observations of Natural Interactions
We observed male behavior in breeding aggregations at Ocelot Pond, ~2 km southeast of Gamboa, Panama. We conducted 15-min focal observations of 88 calling males in June, 2000. A few males climbed out of view in as little as 10 min, truncating their observations. If a male began aggressive interaction with a conspecific, we extended observation up to 60 min. We noted all tremulation displays and bouts of wrestling. When a focal male tremulated, we recorded his distance from the closest conspecific male. On the same nights, we also recorded nearest-neighbor distances for 64 additional males that were not engaged in agonistic interactions. In 2006–7 we made >90 additional opportunistic observations of males interacting aggressively.

Staged Contests
We conducted staged contests between male *A. callidryas* at Ocelot Pond and Experimental Pond in Gamboa. For each contest, we affixed an accelerometer (AP32, mass 2.0 g, flat frequency response 0.5–18,000 Hz, powered by an APC7 charge amplifier, AP Technologies, Oosterhout, Netherlands) to a pondside sapling (1–3 m in height) using modeling clay and plastic tape. The accelerometer was attached halfway up the main trunk of the sapling (~1–2 cm in diameter); thus, signaling frogs were up to 1.5 m from the sensor. We removed any resident frogs from the plant, and mounted an infrared-capable camcorder nearby (Sony TRV350, New York, USA). Vibrations were digitized using Canary bioacoustics software (v.1.2.4, Cornell University Laboratory of Ornithology, Ithaca, USA) on a Macintosh G4 laptop computer equipped with external sound card (Onkyo MSE-U33HB, Saddle River, USA). All ~25 saplings used in this experiment were naturally used by *A. callidryas* as calling sites. Two calling males chosen at random from separate areas of the pond were transferred to the focal sapling. To control for resident advantage, males were introduced simultaneously. Aggressive interactions usually began within a few minutes. We recorded vibrations, infrared video, and audio of interactions until one male either fled from the plant or remained silent and motionless for >5 min while the other male issued chack advertisement calls. We consider males who fled or remained silent losers and males who remained calling on the plant victors. Following each contest (n = 38), we recorded mass and snout–vent length (SVL) of both males and returned them to their collection sites, except eight who escaped without measurement. We recorded individual skin markings (white spots and scars) for identification. Identifiable males were used in one contest each. Males without distinctive markings (n = 17) may have been used in >1 contest, but the large populations at both ponds make this unlikely.

Analysis of Contest Recordings
Aggressive signals, advertisement calls, and wrestling bouts were quantified from videotapes using JWatcher Video v 1.0 [S1].

To assess if males preferentially tremulate when their rivals can see them, we scored visibility during each tremulation. There is no published information about *A. callidryas*’ field of view; we operationally defined signalers as ‘visible’ if they were neither obscured by intervening vegetation nor within the ~60° arc behind their opponent where its eyeshine is not visible.

We measured tremulation durations with JWatcher and frequency characteristics of substrate vibrations produced by chuckles and tremulations using Canary. We measured vibration properties from up to five examples of each behavior per male, recording quality
permitting, calculated mean property values for each male, and used these to calculate overall means. We generated a power spectrum for each male’s tremulations using Canary (filter bandwidth, 2.69 Hz; frame length, 743 ms; grid resolution, 1.36 Hz) and used these to calculate a mean spectrum for tremulations.

Water drop impacts generate strong vibrations in the plants on which *A. callidryas* signals. We measured frequencies of plant free vibrations from the periods of sinusoidal ringdown that followed drop impacts, recorded during staged contests, using Canary.

Male mass and SVL were highly correlated (Pearson’s correlation, n = 68, p < 0.001). We use SVL for size comparisons as mass changed if frogs urinated during handling. Because mass changes with urination were relatively large compared to among-male mass variation, we could not use regressions of mass on body size to estimate male condition. We use absolute difference in SVL to measure how closely opponents were size-matched. All statistical analyses were conducted in SPSS (v.16, SPSS Inc., Chicago, USA).

**Playbacks**

We conducted all playbacks at Experimental Pond with local males. All trials used the same 2 m tall potted sapling 0.5 m from the pond’s edge. Male *A. callidryas* routinely called from this sapling, and were removed before playbacks began each night. We recorded infrared video, audio, and vibration of the trials as in staged contests. We played visual and vibrational stimuli from Canary on a Macintosh G4 laptop equipped with an Onkyo MSE-U33HB soundcard. Vibrational stimuli were played through a TIRA 51075e electrodynamic shaker powered by a BAA120 amplifier (TIRA, Schalkau, Germany). The shaker was coupled to the sapling 1 m from the base of the plant via a thin but rigid metal rod (stinger) approximately 25 cm in length, and 0.5 cm in diameter. The stinger was bent 90° at one end to facilitate attachment to the sapling, and was bound tightly to the plant with plastic tape. We used custom Matlab scripts to equalize playback stimuli and compensate for frequency-dependant filtering inherent to our apparatus. We checked all stimuli for fidelity by rerecording vibrations from the sapling. To reproduce the visual component of tremulations, we used a model *A. callidryas* with articulated joints custom-built based on measurements and photographs of Gamboa frogs. We attached the model’s feet to a wooden frame adjacent to, but not touching, the sapling. The model appeared to be sitting on stems extending from the sapling, but no vibrations could propagate from it to the sapling. We animated the model using a rigid metal stinger attached to a B&K 4810 minishaker (Bruel and Kjaer, Nærum, Denmark) powered by a custom-built amplifier (E. Hazen, Boston University Electronic Design Facility). Video recordings of visual playback confirmed that movements of the model closely matched the frequency and torso displacements of the original display.

Before each trial, the model was hidden under a leaf. We then transferred a randomly selected calling male to the sapling. If the male did not resume chack advertisement calls within 15 min, a new male was selected. If the male began calling, we started the trial and began infrared video, audio, and vibration recordings. After 5 min, we uncovered the model. At 10 min, we began playback. Each male was used for one trial, returned to his original calling site, and not used again for this experiment. Stimuli played every 5 min, up to four times (i.e. at 10, 15, 20, and 25 min after the trial began). If a male left the plant, the trial was ended. Most males remained on the plant for all four presentations, and we stopped recording at 30 min. Only trials in which males experienced ≥2 stimulus presentations were analyzed. Each male was presented with one of six stimulus types, as follows. 1) No model, no playback control (n = 9): At 5 min we removed the leaf but no model frog was present. 2) Model alone (n = 12): We uncovered the
model and it remained static. 3) Visual-vibrational tremulation playback (n = 12): We used tremulation vibration recordings from staged contests to generate synchronous visual and vibrational stimuli. A unique stimulus file, containing four unique tremulation exemplars, was used for each playback trial. These stimulus files were also used for vibration-only and visual-only tremulation stimuli. No recorded exemplar was used for multiple presentations or trials within a single treatment. 4) Tremulation vibrations alone (n = 12): We played tremulation vibrations through the sapling. The model frog was present but static. 5) Tremulation visual alone (n = 10): We animated the model frog using recorded tremulation vibrations. No vibrations were played through the plant. 6) White noise vibrations (n = 11): We played band-limited 0-1 kHz vibrational white noise through the plant, with amplitude and duration matched to the means for tremulation exemplars used in the experiment. The model was present but static.

**Analysis of Playbacks**

To assess if visual or vibrational components of tremulations are behaviorally relevant, we compared aggressive responses of males to playback of tremulation vibrations, visual tremulations, and both components combined, with male responses to the static model. We quantified advertisement calls and aggressive half chucks, chuckles, hindlimb displays, and tremulations from the videotapes using JWatcher Video. For statistical analyses, we used the count of aggressive behaviors per stimulus presentation as a measure of male response. To test whether playback treatment affected the overall level of aggressive behaviors, we calculated exact tie-corrected probabilities using Mann-Whitney U tests in SPSS. We also specifically tested if males were more likely to tremulate in response to tremulation playbacks than controls using Fisher’s exact tests. Statements of significance reflect corrections made using Holm’s sequential Bonferroni method.

**Supplemental References**