

RESEARCH ARTICLE

A novel acoustic-vibratory multimodal duet

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ABSTRACT

The communication strategy of most crickets and bushcrickets typically consists of males broadcasting loud acoustic calling songs, while females perform phonotaxis, moving towards the source of the call. Males of the pseudophylline bushcricket species *Onomarchus uninotatus* produce an unusually low-pitched call, and we found that the immediate and most robust response of females to the male acoustic call was a bodily vibration, or tremulation, following each syllable of the call. We hypothesized that these bodily oscillations might send out a vibrational signal along the substrate on which the female stands, which males could use to localize her position. We quantified these vibrational signals using a laser vibrometer and found a clear phase relationship of alternation between the chirps of the male acoustic call and the female vibrational response. This system therefore constitutes a novel multimodal duet with a reliable temporal structure. We also found that males could localize the source of vibration but only if both the acoustic and vibratory components of the duet were played back. This unique multimodal duetting system may have evolved in response to higher levels of bat predation on searching bushcricket females than calling males, shifting part of the risk associated with partner localization onto the male. This is the first known example of bushcricket female tremulation in response to a long-range male acoustic signal and the first known example of a multimodal duet among animals.

KEY WORDS: Tremulation, Phonotaxis, *Onomarchus*, Vibration, Bushcricket, Katydid

INTRODUCTION

The acoustic chorus of insects at dusk is mostly produced by male crickets and bushcrickets to advertise their identity and position to potential mates (Alexander, 1967). Females typically do not produce acoustic signals; they perform phonotaxis, moving towards the source of the call (Robinson and Hall, 2002). This paradigm is modified in some bushcricket genera, where females produce acoustic signals, either spontaneously as in the case of some ephippigerine bushcrickets (*Platystolus obvius*: Korsunovskaya, 2008) or in response to the male call, resulting in acoustic duets. A duet can be defined as a dialogue between two signalers (Bailey and Hammond, 2004), with a stereotyped temporal relationship between the signal from one individual and the reply from the other individual (Bailey, 2003). Duets are common among some ephippigerine bushcrickets such as *Steropleurus stali*, *Steropleurus nobrei*, *Platystolus obvius* (Hartley et al., 1974; Hartley, 1993),

Ephippiger ephippiger (Ritchie, 1991) and *Deracantha onos* (Korsunovskaya, 2008); zaprochiline bushcrickets such as *Meconoma thalassina* (Robinson, 1990); and phaneropterine bushcrickets such as *Leptophyes punctissima* (Robinson et al., 1986), *Elephantodeta nobilis* (Bailey and Field, 2000), *Scudderia curvicauda* (Spoonner, 1968), *Phaneroptera nana* (Tauber et al., 2001), *Metaplastes* spp., *Euconocercus iris*, *Amblycorypha uhleri* (Korsunovskaya, 2008), *Barbitistes* spp., (Stumpner and Meyer, 2001), *Caedicia* spp. (Bailey and Hammond, 2004), *Andreiniimon nuptialis*, *Ancistrura nigrovittata*, *Isophya lemone* and several *Poecilimon* species (Heller and von Helversen, 1986).

Insect duets typically start with a male call, and the female responds with a fixed latency relative to the male call, that latency being important for species recognition (Bailey, 2003). In many bushcricket species that acoustically duet, the timing of the female's call relative to the male's call is species specific and crucial in eliciting male phonotactic behavior (Heller and von Helversen, 1986; Robinson et al., 1986; Hartley, 1993; Stumpner and Meyer, 2001; Bailey and Hammond, 2004). Such duetting can result either in phonotaxis by both sexes that engage in the duet, or in stationary female replies and male-only movement. Exclusively male phonotaxis exists among many phaneropterine genera, including *Ancistura*, *Andreiniimon*, *Leptophyes*, *Isophya* and *Poecilimon* spp. (Hartley and Robinson, 1976; Heller and von Helversen, 1986). In some ephippigerine species such as *S. stali* and *S. nobrei*, where both sexes perform phonotaxis, male phonotaxis has been shown to outperform female phonotaxis in speed and accuracy (Hartley, 1993). Sometimes the female call stimulates an increase in the male's calling rate, whether phonotaxis is performed exclusively by males (Robinson, 1980) or by both sexes (Hartley et al., 1974).

In contrast to the acoustically duetting species described above, males of some neotropical pseudophylline (Belwood and Morris, 1987; Mason et al., 1991; Morris et al., 1994; Römer et al., 2010) and conocephaline (Morris, 1980; Belwood and Morris, 1987; Morris et al., 1994) species switch between the use of acoustic and vibrational signals to advertise their location to females. This is distinct from the phenomenon where a vibrational by-product of acoustic stridulation gets transmitted along the substratum (Keuper and Kuhne, 1983). Such vibratory components of the stridulatory signal may enhance the ability of the female to recognize the conspecific song (Kalmring and Kuhne, 1980), or to localize the male (Latimer and Schatral, 1983; Wiedmann and Keuper, 1987). Males can broadcast vibrational signals independently of acoustic stridulation by tremulating, i.e. by shaking their bodies in the vertical plane while all their legs remain rooted to the substratum (de Luca and Morris, 1998), thereby vibrating the substrate they sit on. Calling tremulations are observed in the absence of females and are thus distinguished from courtship tremulations that manifest themselves after members of a courtship pair find each other through acoustic signaling (Gwynne, 1977; Mason et al., 1991; Korsunovskaya, 2008). Tremulation as a complex, calling signal has mostly been observed among males of neotropical bushcricket

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species (Belwood and Morris, 1987; Morris et al., 1994), although males of a few temperate species such as *Conocephalus nigropleurum* also tremulate (de Luca and Morris, 1998). In some cases, female tremulation has also been observed in response to male tremulations at close range, although as a faint signal relative to the male tremulatory signal (Belwood and Morris, 1987). This female tremulatory response was reported to increase the male tremulation rate and to decrease the use of acoustic calling in at least one species (*Balboa tibialis*) (Belwood and Morris, 1987).

The prevalence of reduced calling rates and tremulation as a mode of non-acoustic signaling among male neotropical pseudophyllines is thought to reflect adaptation to predation pressure by bats that are known to use calling songs to locate singing males (Heller, 1995; Belwood and Morris, 1987). The paleotropics, however, are reported to have fewer numbers of species of primitive echolocator insectivorous bats that use acoustic and visual cues to locate their prey (Heller and Volleth, 1995). In turn, paleotropical pseudophylline males typically have high calling rates and no instances of male tremulation as a vibrational calling signal are known (Heller, 1995). This might reflect differences in bat predation pressure on calling pseudophylline males in the paleotropics as compared with the neotropics.

Here, we report on a unique departure from the paradigm of male acoustic calls and female phonotaxis. *Onomarchus uninotatus* (Serville 1838), a pseudophylline bushcricket that has been described in Malaysia (Heller, 1995) and Southwestern India (Diwakar and Balakrishnan, 2007), has an unusually low male acoustic call frequency of 3.2 kHz (Diwakar and Balakrishnan, 2007). In this study, we report that the primary female response to the male acoustic call consists of bouts of tremulation that emit a measurable vibration signal, whose timing bears a specific temporal relationship to the chirps of the male acoustic call. Males perform vibrotaxis to the source of vibration, but only if the acoustic component of the duet is played back along with the tremulation. This constitutes the first known case of a multimodal acoustic-vibratory duet.

RESULTS

Onomarchus uninotatus females responded to the onset of the conspecific male call by tremulating: executing a whole-body shake that vibrates the substrate upon which the insect sits (supplementary material Movie 1). This tremulation produced a vibrational signal measurable off an *Artocarpus heterophyllus* (jackfruit) branch, the natural substrate for this species (Fig. 1A). The insect's feet are the only point of contact with the substratum; the insect's abdomen does not make contact with the substratum. Each tremulatory event followed and alternated with chirps of the conspecific male call (Fig. 1A). The amplitude of tremulation varied across tremulatory events, as did the duration (0.258 ± 0.189 s, $N=6$ females with 10 tremulations each; data are means \pm s.d. unless marked otherwise). Not every chirp elicited a tremulatory response; $44 \pm 29\%$ ($N=5$) of chirps elicited a tremulatory response in the 90 s following the first tremulation. The female tremulation signal had maximum power at 48.13 ± 7.41 Hz (10 tremulations each in $N=5$ individuals; the frequency spectrum of 10 tremulations in one individual is shown in Fig. 1B), compared with the 3.2 kHz peak on average for the male acoustic call (Diwakar and Balakrishnan, 2007) (the frequency spectrum of a sample chirp of the male acoustic call can be seen in Fig. 1B).

The onset of tremulation occurred reliably at an individual-specific phase relative to the time period between the onset of consecutive male chirps. All females tested had an average phase

vector angle of the onset of tremulation relative to the time period between consecutive chirps of the male call in the range between 137.3 and 216.3 deg, so that the tremulation bout began 1/4 to 3/4 of the way through the time period between chirps of the male call (Fig. 2). All females tested had an average vector length in the range 0.88–0.99, suggesting each individual had its own specific, reliable and robust phase of tremulation relative to the conspecific call. The male call and the female tremulation therefore occurred in an alternating sequence in the form of a multimodal acoustic-vibratory duet.

Tremulation commenced 3 ± 2.5 s (median \pm semi-interquartile range, $N=19$ females) after the onset of playback of the conspecific call (Fig. 3A). Animals sometimes attempted phonotaxis after a period of tremulation (supplementary material Movie 1). The average latency for the animal to initiate movement was 119 ± 95 s ($N=15$) after the onset of the conspecific call playback. The latency of onset of movement was significantly greater than the latency of onset of tremulation after the commencement of the male call ($t=-4.64$, d.f.=14, $P=0.00019$) for the 15 animals that initiated movement (Fig. 3A). Animals that did not move continued tremulating on the spot, sometimes for the entire 5 min of the video recording.

Tremulation was a clear, immediate and robust response to the conspecific call, occurring in all 19 animals tested (Fig. 3B). Animals sometimes attempted phonotaxis after a period of tremulation (Fig. 3B). In contrast, only 15 animals attempted to move in response to the call. Of these, two animals moved along the branch in response to the male call but did not complete phonotaxis, and only 13 animals managed to reach a speaker (Fig. 3B). Complete phonotaxis was a significantly less likely response than tremulation ($\chi^2=2.25$, d.f.=1, $P=0.041$). Four animals flew instead of walking: after a long period of tremulation and, in three cases out of four, no phonotaxis, the animals took short flights and landed around half a meter away. Starting around 215 ± 53 s ($N=4$) on average (Fig. 3A), the flights typically lasted 1 s and never took the animals towards a speaker; after landing, the animals walked rapidly rather than sitting still (Fig. 3B). Flight was a significantly less frequently observed behavior than either tremulation ($\chi^2=13.07$, d.f.=1, $P=0.0003$) or phonotaxis ($\chi^2=5.79$, d.f.=1, $P=0.0055$).

Tremulation was not observed in silent control trials or in response to a sympatric heterospecific *Gryllacropsis* call (Diwakar and Balakrishnan, 2007) played back at the same sound pressure level (SPL) as the conspecific call (Fig. 4A). The number of animals tremulating in response to the conspecific call was significantly greater than the number responding either to silence or to the heterospecific call (in both cases, $\chi^2=17.053$, d.f.=1, $P=0.000036$).

Phonotaxis was a more ambiguous response, with two animals out of 19 walking to a speaker in response to silence and two (other) animals doing so in response to the heterospecific call (Fig. 4B). Still, the probability of complete phonotaxis in response to the conspecific call was significantly different from the probability in response to silence or the heterospecific call (in both cases, $\chi^2=9.09$, d.f.=1, $P=0.0026$). Accurate phonotaxis, or walking to the correct speaker playing the call, was only achieved in $N=9$ animals out of the 13 that reached a speaker (Fig. 4B). This is a significant response compared with zero animals performing accurate phonotaxis in silence ($\chi^2=9.09$, d.f.=1, $P=0.0026$), and is significant in comparison to the single animal that performed accurate phonotaxis in response to the heterospecific call ($\chi^2=4.9$, d.f.=1, $P=0.027$).

The standard Orthopteran communication paradigm of male acoustic calling and female phonotaxis therefore operates with some

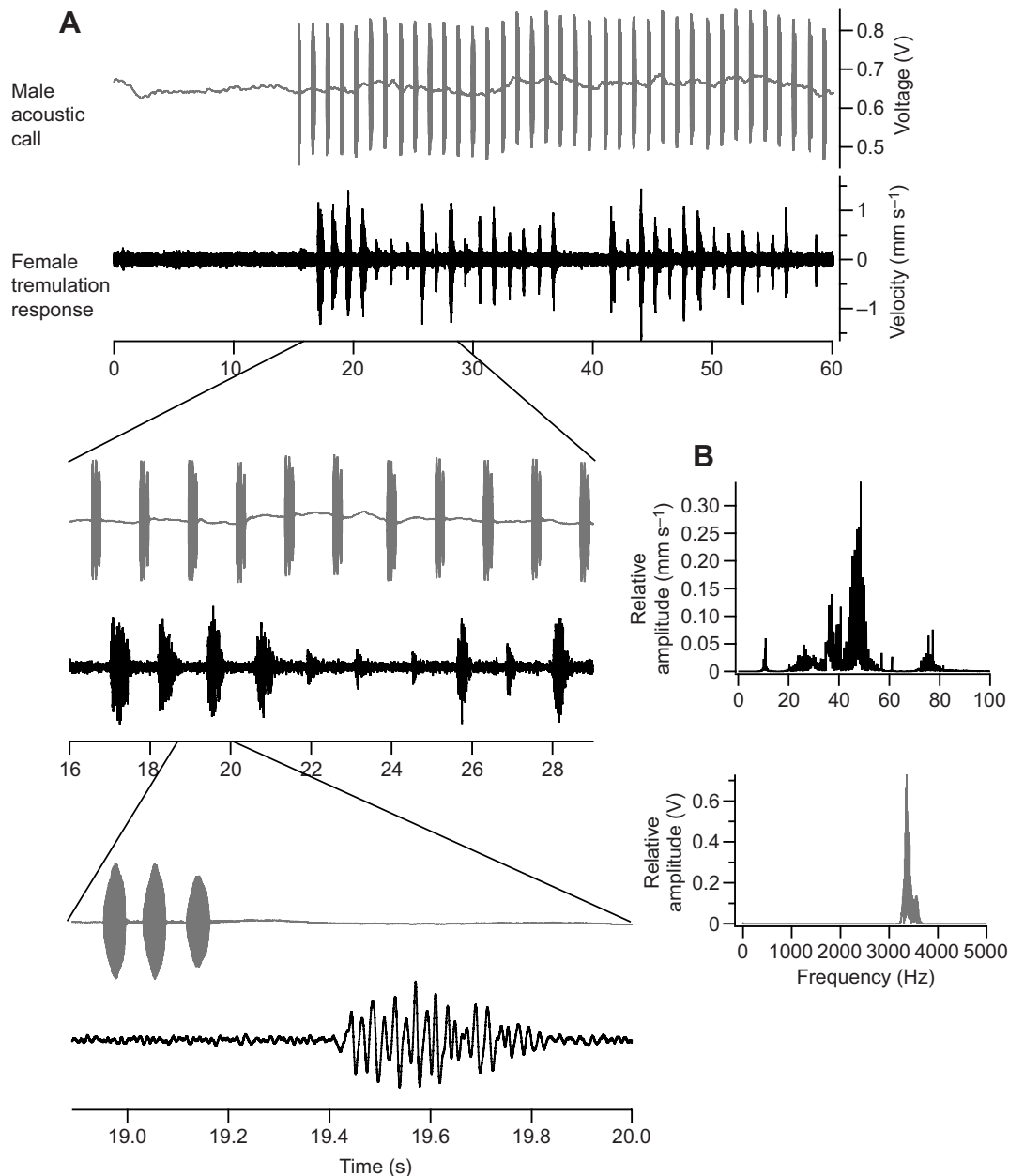


Fig. 1. The female tremulation signal. (A) A sample series of female tremulations (black trace) in response to acoustic chirps of the conspecific male acoustic call (gray trace), at three levels of temporal resolution. (B) The frequency spectrum of a full time series of 10 female tremulations (black) compared with the frequency spectrum of a chirp of the male acoustic call (gray).

delay and uncertainty in *O. uninotatus* relative to female tremulation. As the tremulation emits vibrational signals, we hypothesized that a potential second loop of communication could exist, requiring the males to be able to localize females based on ‘vibrotaxis’, or movement towards the source of vibration.

Males were able to localize females tremulating in boxes coupled to a Y-shaped jackfruit branch ($N=9$) (Fig. 5A). Female tremulation was seen both in response to the playback of a male call and in response to isolated calls made by the male as he walked towards the female. In silence, females did not tremulate and males typically did not move (Fig. 5A). When the branch was disconnected from the box containing the tremulating female, the males did not move towards the female (Fig. 5A). This shows that a direct coupling between the female’s container and the branch was necessary to elicit vibrotaxis. Along with the fact that the females were kept in

sealed containers, this makes it unlikely that males followed olfactory or other airborne cues emitted by tremulating females.

To confirm that males performed vibrotaxis rather than relying on other cues to localize the female (e.g. olfactory cues such as pheromones that might be released by tremulatory shaking), the male’s response to a simulated female tremulatory signal was investigated. When both the acoustic and vibratory components of the duet were played back, all males tested performed vibrotaxis towards the source of vibration (supplementary material Movie 2; Fig. 5B) ($N=14$), typically placing a foot on each branch at the fork or sequentially sampling each branch with both feet before making a decision. Most males (11 out of 14) also occasionally called in the intervals following the cessation of the played-back duet. Male stridulation did not produce any discernible vibratory signal.

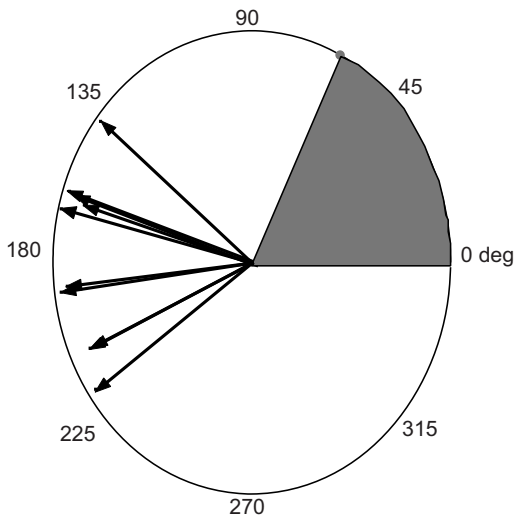


Fig. 2. The phase of the female tremulation signal relative to the duration between male chirps. The individual-specific average phase vector of the timing of onset of tremulation relative to the time period between the onset of consecutive chirps of the conspecific call ($N=9$ females). The shaded area is the duration of each male acoustic chirp.

Interestingly, when the vibrational component of the duet was played in the absence of the acoustic component, not a single male performed vibrotaxis (Fig. 5B). The difference between the responses of the males to both components of the duet played simultaneously and to the vibration alone was highly significant

($\chi^2=12.0714$, d.f.=1, $P=0.0005$, $N=14$). Males therefore do not respond just to the vibratory signal from the female's tremulation, but require both components of the multimodal acoustic-vibratory duet to perform vibrotaxis.

When the source of vibration was disconnected from the branch, but the bimodal duet continued to be played back, males did not move towards the source of vibration (Fig. 5C). They also did not move when only the acoustic signal was played or when neither signal was played back (Fig. 5C). Significantly more males performed vibrotaxis in response to the vibratory-acoustic duet ($N=9$) than to the above three controls ($\chi^2=7.1$, d.f.=1, $P=0.0077$) (Fig. 5C). All of these experiments unambiguously showed that a direct coupling between an active source of vibration and the branch was necessary to elicit male responses.

DISCUSSION

A novel multimodal duetting system

The alternation of a female tremulation vibratory signal with the chirps of a male acoustic call constitutes a novel multimodal duetting system with a precise temporal structure. Unimodal acoustic duets and temporally specific patterns of antiphonal calling by males and females have been described among some bushcrickets (Hartley et al., 1974; Heller and von Helversen, 1986; Robinson et al., 1986; Stumpner and Meyer, 2001; Bailey and Hammond, 2004), grasshoppers (von Helversen et al., 2004), mosquitos (Cator et al., 2009), flies (Donegan and Ewing, 1980), frogs (Tobias et al., 1998), mammals (Mitani, 1985; Nietsch, 1999) and birds (Thorpe et al., 1972). Unimodal vibratory courtship duets have likewise been described among spiders (Baurecht and Barth,

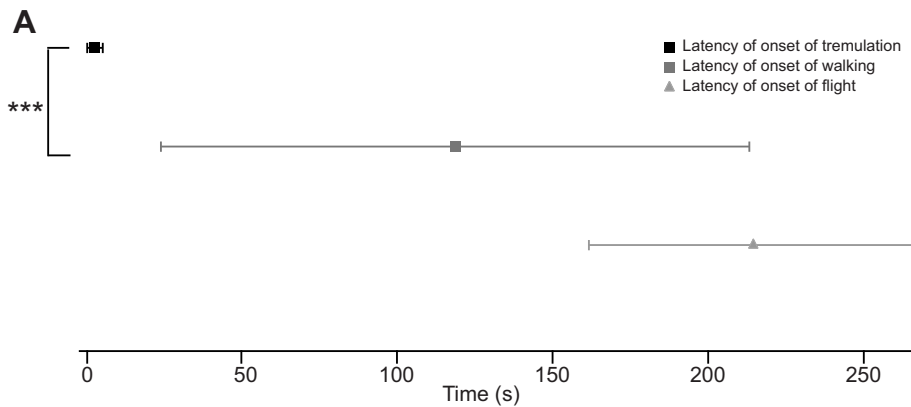
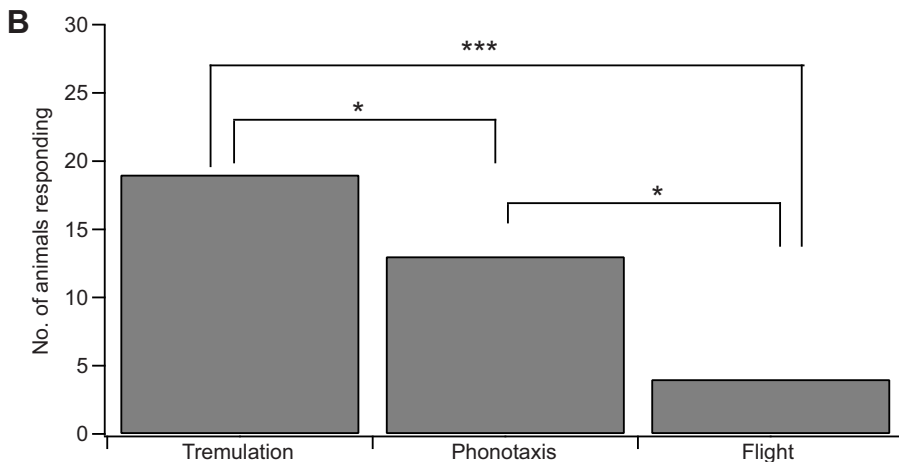


Fig. 3. Timing and probability of events in the female's response to the male's acoustic call. (A) Average time after the onset of the conspecific call when tremulation begins ($N=19$); when walking begins ($N=15$); and when the animal makes short flights ($N=4$). (B) The number of animals out of a total of 19 showing each kind of response. * $P<0.05$, *** $P<0.001$ for pair-wise comparisons.



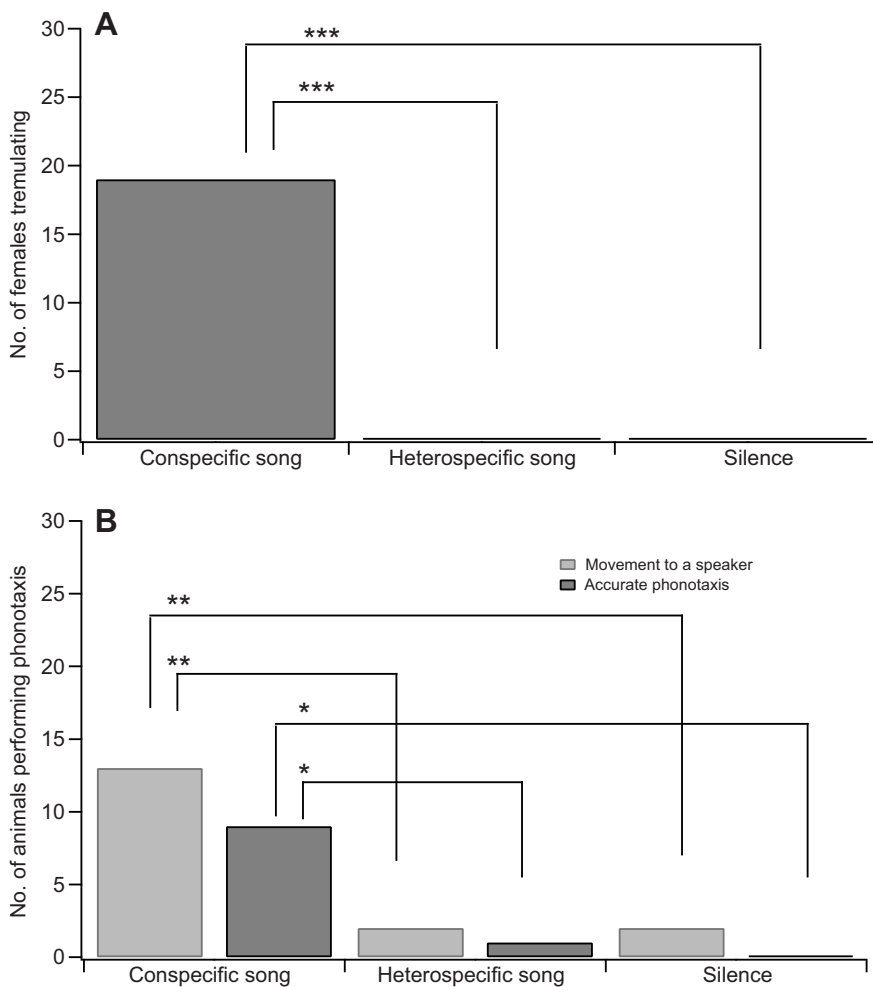


Fig. 4. The specificity of tremulation versus phonotaxis. (A) The number of animals ($N=19$) that responded to the conspecific call stimulus by tremulating, compared with the number tremulating in response to a heterospecific call at an equivalent sound pressure level (SPL), or silence. (B) The number of animals walking to a speaker and the subset that accurately walked to the speaker playing the stimulus, in response to the conspecific call, the heterospecific song, or silence. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ for pair-wise comparisons.

1992), planthoppers (Ichikawa, 1976), stoneflies (Ziegler and Stewart, 1977), stinkbugs (Cokl and Virant-Doberlet, 2003) and lacewings (Henry, 1980). However, multimodal duets have not previously been described.

Multimodal cues are often used by animals during mate search. Adult female tree crickets are known to follow chemical, acoustic and vibratory cues to find males (Bell, 1980). Visual and vibratory mixed courtship displays have been described among spiders (Gibson and Uetz, 2008; Elias et al., 2012). Multimodal courtship displays are also known among birds, with a visible wing-stroke by female cowbirds in response to specific acoustic patterns in the male calls inducing increased use of the specific call type (West and King, 1988). However, these kinds of multimodal interactions do not constitute a calling duet, such as that seen in *O. unnotatus*, where long-range male calls in one modality alternate with female calls in another modality, for each to advertise their position.

Both modes of the multimodal duet are necessary for vibrotaxis-based localization

The fact that *O. unnotatus* males perform vibrotaxis in response to the vibrational signal only if both the vibrational and acoustic components of the duet are played back together suggests that multimodality plays a role in the specificity of the vibrotaxis response. At a neurophysiological level, the male's decision to perform vibrotaxis could be dependent upon the integration of information from the auditory and vibratory sensory pathways. As

the timing of female tremulation relative to the male acoustic call is quite temporally precise, male vibrotaxis might also be contingent upon the female's signal arriving within a certain time window of the male acoustic call, as is the case in some acoustically duetting bushcricket species (Hartley et al., 1974; Heller and von Helversen, 1986; Robinson et al., 1986). Temporal features of the male vibrational signal have also been shown to accurately indicate male size, and affect female mate choice in the meadow katydid (de Luca and Morris, 1998), so in this case temporal aspects of the female vibrational signal might play a role in male mate choice. However, the fact that males only perform vibrotaxis when both components of the duet are played back suggests that the relationship of the vibratory and acoustic signals might serve as a confirmation of conspecific signaler identity, in the context of vibratory noise from heterospecific signalers (such as spiders).

In a natural situation, males probably perform vibrotaxis when females tremulate in response to the male's own calls (as also seen occasionally in our experiments when males themselves called). Some males produced occasional chirps while performing vibrotaxis, moving as soon as a female tremulated in response. It remains to be seen whether males process the timing of their own calls relative to the female's vibration signal by listening to their own calls, or through an additional circuit comparing an efference copy (Webb, 2004) of the motor signal with the incoming vibratory information. The first possibility, that multimodal integration is performed using acoustic feedback, could explain the fact that males perform vibrotaxis towards females that respond to acoustic signals

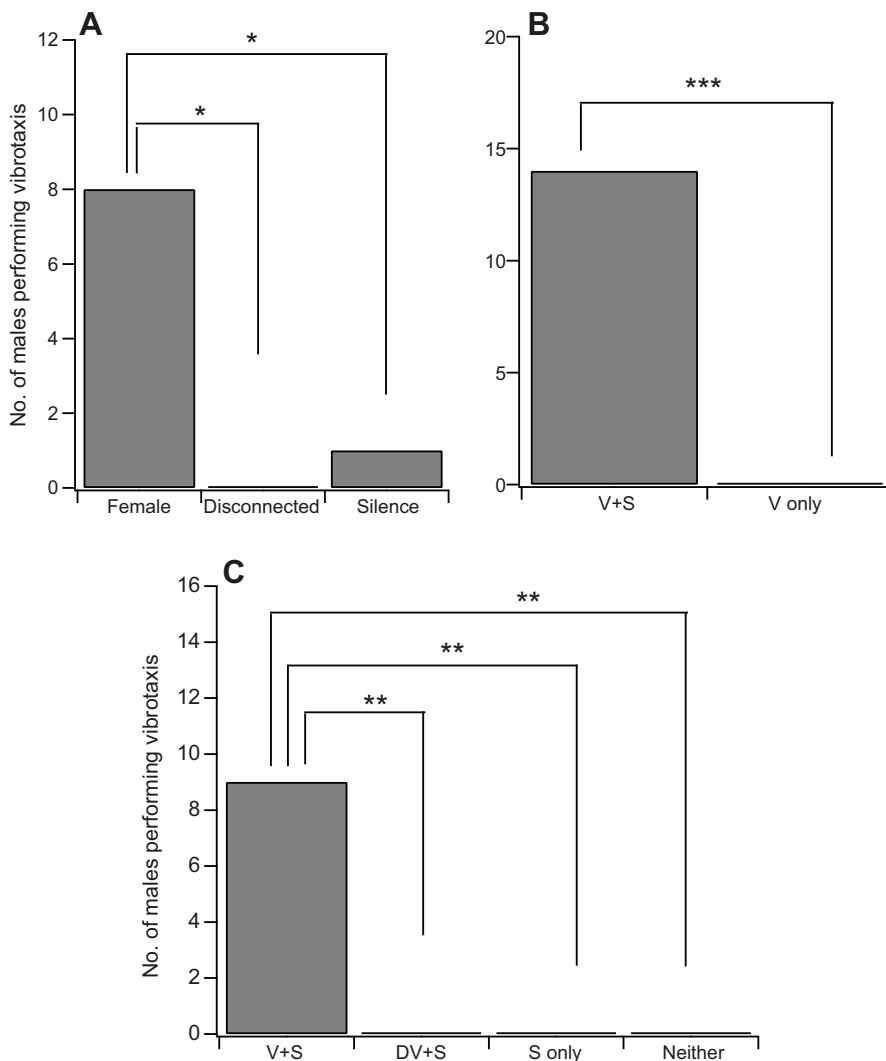


Fig. 5. Male vibrotaxis. (A) The number of males that performed vibrotaxis toward a tremulating female in a box coupled to a branch, compared with the disconnected box control and a silent control ($N=9$). (B) The number of males that performed vibrotaxis in response to the playback of both components of the acoustic-vibratory duet (V+S), as compared with just the vibratory component (V) ($N=14$). (C) The number of males that performed vibrotaxis in response to the duet (V+S), versus only the acoustic component (S); in the absence of all stimulation (Neither); and in response to the duet with the vibrational source disconnected from the branch on which the male sat (DV+S) ($N=9$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$ for pair-wise comparisons.

generated by other males, as seen in the playback experiments. The other possibility is that males are capable of using both acoustic feedback and efference copy mechanisms, so that vibrotaxis in response to tremulation triggered by other males' calls in fact constitutes a satellite strategy (Bailey and Field, 2000) to exploit other calling males. In the laboratory, among a group of 10 males, typically only one would continuously call and, when the calling male was removed from the room, another male would take over as the continuous caller. This suggests the possibility of dominance interactions wherein some males may be callers and others silent at a given point in time. The silent males could well benefit from approaching the vibratory replies of females to the calls of rivals; the fact that males initiated calls after the played-back call ceased could also indicate an attempt to engage a female in a duet when another male duetting with her ceases to call.

The fact that females perform phonotaxis after a period of tremulation suggests that phonotaxis still plays a useful role in enabling male–female co-localization. If a calling male sits on a vibrationally isolated substrate from the female, such as another disconnected tree, her tremulation signals are not likely to reach him. Tremulation is therefore an insufficient cue in some circumstances, and despite being delayed, uncertain and sometimes inaccurate, female phonotaxis may be the only means by which males and females can co-localize in those circumstances.

A single tremulation event has also been shown to be almost 7-fold more energetically costly than producing an acoustic call, which might serve as a limiting factor in the use of vibratory communication (Römer et al., 2010).

Female vibrational signaling as a novel response to predation pressure

Among pseudophylline bushcrickets, tremulation as a calling signal has previously only been reported for males of neotropical species (Morris et al., 1994). This is the first known instance of paleotropical pseudophylline tremulation. This is also the first known instance of female tremulation being used as a calling vibrational signal in response to the male acoustic call.

The use of tremulation as an alternative channel of vibratory communication to acoustic calls by males has been reported as a strategy to minimize predation risks in the ecological context of the neotropics (Morris, 1980; Belwood and Morris, 1987; Mason et al., 1991; Morris et al., 1994). In the neotropics, bats have been shown to locate male katydids by eavesdropping on their calls, and this has been hypothesized to drive low acoustic calling rates and the preferential use of an alternative channel of vibrational communication by neotropical pseudophylline males (Belwood and Morris, 1987; Morris et al., 1994; Heller, 1995; ter Hofstede et al., 2008; Römer et al., 2010).

However, neither low call rates nor vibrational signaling has been reported in paleotropical pseudophylline males. In fact, playbacks of acoustic calls of male *O. uninotatus* were found to attract the predatory bat *Megaderma spasma* in only 30% of trials, compared with a 100% approach rate towards flying, non-stridulating females (Raghuram et al., 2015). This, combined with the fact that significantly more female bushcricket forewings were found in *M. spasma*'s diet than male forewings, suggests higher predator pressure upon moving *O. uninotatus* females than calling males. In this ecological context, female tremulation and male vibrotaxis may serve to shift the risk of mate localization away from females. This unique case of acoustic-vibratory duetting between *O. uninotatus* males and females may therefore be an evolutionary response to the higher predation risk faced by flying bushcricket females over calling males in paleotropical assemblages (Raghuram et al., 2015).

MATERIALS AND METHODS

Study system

Experiments were carried out on 19 *O. uninotatus* virgin females caught as nymphs, and 17 males caught at various stages of their life cycle from *A. heterophyllus* (jackfruit) plantations in Kadari village, Karnataka (latitude 13°13'N, longitude 75°5'E), in Southwestern India, between December 2011 and May 2014. This species is mostly found on *Artocarpus* trees and subsists on its leaves. The animals were reared in individual plastic containers (10×6×4 cm) at room temperature (18–24°C) in the laboratory on a 12 h:12 h light:dark cycle, with *ad libitum* access to fresh jackfruit leaves and water. Animals were not acoustically or vibrationally isolated from one another, nor were they acoustically isolated from calling heterospecifics in the same room. Females showed responses to male calls a minimum of 3 weeks after the final molt and were therefore used for experiments 3–6 weeks after the final molt. All experiments were carried out in complete darkness between 21:00 h and 06:00 h, which coincides with the natural calling period of *O. uninotatus* in the wild (Diwakar and Balakrishnan, 2007).

Female response to male acoustic call

A T-shaped setup was constructed by nailing a 1 m-long branch of an *A. heterophyllus* jackfruit tree to the center of another 2 m-long jackfruit tree branch. The setup was elevated off the ground, placed such that each of the three ends rested on a block of black acoustic foam (Monarch Tapes and Foams Ltd, Bangalore, India) and the setup (Fig. 6A) was placed in a dark anechoic room (dimensions 3×2.75×3 m l×w×h). The female was then placed on the free end of the 1 m-long branch and, once the animal was still, a pre-recorded conspecific male call (Jain and Balakrishnan, 2012) was played back via a loudspeaker (X-Mini, v1.1, XMI Pvt Ltd, Singapore) with a frequency range from 120 Hz to 20 kHz, located at one of the two ends of the 2 m-long T-branch. The call was broadcast from a CD by a mobile CD player (Sony WM-D6C Professional Walkman, Japan) at a sampling rate of 44.1 kHz, such that the call SPL was 66 dB SPL (re. 2×10^{-5} N m⁻²) measured at the center of the T-junction with a handheld analyzer (Bruel & Kjaer 2250, Denmark), using the LZF setting to evenly weight spectral contributions to the SPL reading, fitted with a 1/2 in microphone (Bruel and Kjaer 4155, Denmark) with a frequency range from 4 Hz to 16 kHz. The location of the speaker was randomized between the two ends of the T-branch to avoid positional bias.

The female response to the male acoustic call was recorded using a video camera (Sony Handycam DCR-HC96E, Japan) in the infra-red Nightshot mode and digitized onto a laptop computer (Compaq nx6320, Hewlett-Packard, USA) using Microsoft Windows Movie Maker software (version 5.1, Microsoft Corporation, USA). The female's responses to a control sympatric heterospecific call (*Gryllacropsis* sp.; Diwakar and Balakrishnan, 2007) and a silent control treatment, presented in random order with the conspecific call to each female, were also recorded. All video recordings were made for a minimum of 5 min, and females were considered to have successfully performed phonotaxis if they walked along the branch or flew directly to the speaker playing a call. They were considered to have

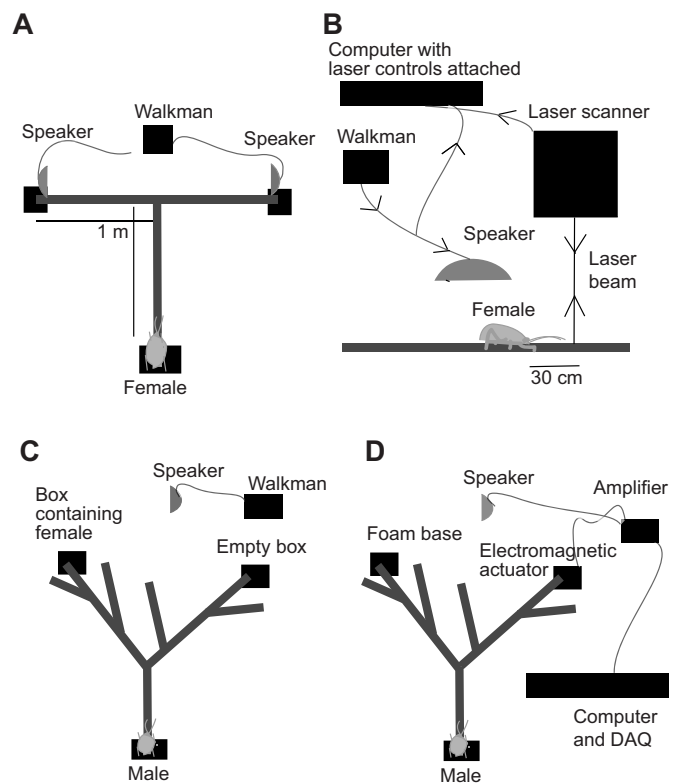


Fig. 6. The experimental setup. (A) The male call was played from a loudspeaker at one end of a T-shaped branch, with the female on the third branch facing the T-junction. (B) The tremulation signal of the female was assessed using a vibrometer facing downward onto the branch, 30 cm away from the female's front legs. A loudspeaker suspended above her was used to playback the male call. (C) The setup used to test male vibrotaxis consisted of a Y-shaped branch, one end of which was coupled to a sealed plastic box containing a female. The male call was played back from a loudspeaker suspended above the central branching point. (D) The setup used to test male vibrotaxis to an artificial source of vibration consisted of an electromagnetic actuator coupled to one terminal end of the Y-shaped branch. The actuator was driven with the vibrational component of the duet by a computer through a DAQ and an amplifier while the acoustic component was played out as before through a loudspeaker.

performed inaccurate but complete phonotaxis if they walked or flew to either terminal end of the T-shaped branch. All video recordings are stored at the Center for Ecological Sciences (CES), Indian Institute of Science (IISc), Bangalore, India, for reference.

Female tremulation: the vibrational signal

A female was introduced to one end of the 2 m-long branch. A portable laser Doppler vibrometer (Polytec PDV-100, Waldbronn, Germany) was placed so that the beam was directed downwards and was maximally reflected off the most perpendicular surface of the same branch, at a distance of 30 cm from the animal (Fig. 6B). After the animal sat still on the branch, the conspecific male call was played back from a laptop (MacBook 4.1, Apple, USA) at a sampling rate of 44.1 kHz via the X-Mini loudspeaker suspended above the animal, at 66 dB SPL measured as described above at the location of the animal. A copy of the audio input to the loudspeaker was acquired simultaneously with the signal from the vibrometer using a Polytec Scanning Vibrometer data acquisition unit (VIB-E-220, Polytec GmbH, Waldbronn, Germany) controlled by the vibrometer software (Vibsoft version 4.8, Polytec GmbH). The time difference between the onset of each bout of tremulation and the onset of the male acoustic chirp preceding it was computed for 10 bouts of tremulation each for nine individuals. A vector representing the average phase delay was computed for each individual where the angle of the vector represents the average timing of that

individual's tremulation, relative to the time period between the onset of consecutive chirps of the male call. The time period between chirps of the male call corresponds to a full circle of phase or 360 deg, and a unit vector of phase 0 deg (or 360 deg) would represent an animal whose onset of tremulation exactly coincides with the onset of every male acoustic chirp, while a unit vector of phase 180 deg would represent an animal tremulating exactly out of phase with chirps of the male acoustic call. The length of the average phase delay vector represents the strength of clustering of the phase delays of different bouts of tremulation by the animal relative to each preceding chirp, and the vector fully extends to the unit circle if the phase delay for each bout of tremulation relative to the preceding acoustic chirp of the male call is precisely repeatable.

Male vibrotaxis to female tremulation

A portion of an *A. heterophyllus* tree consisting of a Y-shaped primary branch with secondary branching on either side of the primary branch was chosen for vibrotaxis (Fig. 6C). The branch terminals rested on acoustic foam blocks in the dark anechoic chamber such that no part of the branch was in contact with the ground. All experiments presented the vibrational stimulus randomly to one of the two most widely separated terminal ends, to avoid positional bias.

In the experiment testing the ability of the male to perform vibrotaxis towards a tremulating female, one of the terminal ends of the branch rested on a cylindrical plastic box (15 cm diameter, 16 cm height) with a sealed flat top lid containing a mature female while the other terminal rested on an identical but empty box (Fig. 6C). The tremulation signal from the boxed-in female was measured using the laser vibrometer at the primary branching node of the Y-branch and it was found to be similar to the vibrational signal from a tremulating female sitting directly on the terminal end of the branch, although noisier and diminished in intensity (supplementary material Fig. S1). Both boxes were sealed to ensure that no chemical signals that might have been released by tremulation could waft towards the male; all experiments were performed in the dark to ensure that the female's tremulation was not visible to the male. We performed a control treatment to check whether any non-vibrational component of the female's tremulation could function as an attractant for the male by placing both ends of the branch on empty boxes, with a female in a box disconnected from the branch near one of these terminals, and an empty box disconnected from the branch near the other terminal. The laser vibrometer was used to confirm that no vibrational signals were transmitted onto the branch when the tremulating female was located in a box disconnected from the branch (supplementary material Fig. S1).

Onomarchus unnotatus males were placed near the base of the branch, about 15 cm before the first fork, and a small X-Mini speaker (described above) suspended vertically above the starting position of the male was used to broadcast the male call in order to elicit a tremulation response from the female. A male was considered to have performed successful vibrotaxis if it walked to the lid of the box containing the tremulating female. Males sometimes wandered around the branch in silence before sitting still, and the male call was only played once males stopped moving. Males were subjected in random order on a single night to each of the following treatments: the experimental treatment consisting of a female tremulating as a response to the played-back male call in a box connected to the branch, a control treatment with the female tremulating in response to the male call in a disconnected box, and a silent control treatment eliciting no tremulation. The tremulating female was visible through the plastic box and was videotaped with the same video camera–laptop system described above, and male vibrotaxis was simultaneously recorded using an infra-red DVR system (Ziacom 540PTZ IR Speed dome camera Z30XSP830 with 4-channel DVR system, Mumbai, India) for a duration of 5 min.

Male vibrotaxis to simulated tremulation

Responses of the male to a simulated vibrational signal without a female present were also assessed. A recording of the acoustic and vibrational input that had been simultaneously acquired by the vibrometer software was played out from a laptop (Acer Aspire S3), through a data acquisition (DAQ) card (NIDAQ 6215, National Instruments, USA) controlled by LabView software (version 10.0, 2010, National Instruments). The vibrational component of the signal was amplified (model 7602, Krohn-Hite

Corporation, USA), and sent to an electromagnetic actuator (10 in audio woofer, frequency range 5–500 Hz, Jenstar, India), which was coupled in each trial to one randomly chosen end of the two most widely separated terminal ends of the Y-shaped branch. The other terminal end of the Y-branch was placed upon an empty circular box that mimicked the shape and size of the speaker (Fig. 6D). The simulated signal traveling down the branch was recorded using the laser vibrometer and was found to be comparable to the signal recorded directly on the branch from a tremulating female (supplementary material Fig. S1). The acoustic component of the signal was reproduced by feeding the DAQ output signal to the X-Mini speaker suspended above the first branching node of the Y-shaped branch.

The signal consisted of a 30 s-long segment of tremulation and song, followed by 15 s of silence, with this sequence repeating. For each animal, control treatments were performed of playing only the vibration component of the signal; only the acoustic component; silence; and both components of the signal while disconnecting the vibrating speaker from the branch. Each male exposed to the duet was subjected to all control treatments, in random order. *Onomarchus unnotatus* males were placed 15 cm before the first branching node of the twig and vibrotaxis was considered to have successfully taken place only if the first choices made at both the first and second branching nodes were correct. Responses were recorded for a minimum of 5 min with the video camera as described above.

Statistical analysis

A two-tailed paired *t*-test was used to assess the difference between the latency of tremulation and phonotaxis, after checking that the differences in latency formed a normal distribution using a Shapiro–Wilk test. The null hypothesis was that there was no difference in the latency of tremulation and phonotaxis. If timing data were normally distributed, means and s.d. were used to describe them; otherwise median and the semi-interquartile range were used. A McNemar chi-squared test applying the Yates correction was used to assess the significance of the difference between the number of animals showing particular types of responses to the male acoustic call; this was assessed in paired comparisons between the following response types: tremulation, phonotaxis and flight. The null hypothesis was that the same number of animals showed each type of response. A McNemar chi-squared test applying the Yates correction was also used to test the significance for the number of individuals showing a particular response type to different treatments. The null hypothesis in all these cases was that there was no difference in the number of individuals that responded to experimental versus control treatments. When testing the specificity of the tremulation or phonotaxis response to the conspecific call, two control treatments were used: silence and a heterospecific call, and the significance of the difference in the number of individuals showing a tremulation response to the conspecific call was assessed relative to each control treatment. When testing the specificity of vibrotaxis in response to the acoustic-vibratory duet, several control treatments were used as described above: vibration alone, sound alone, neither signal, and sound with a disconnected source of vibration. The significance of the difference in the number of animals responding to the duet compared with each control treatment was calculated.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

K.R. performed all experiments and analyses and wrote the paper. R.B. participated in experimental design and writing of the manuscript. V.G. and R.P. provided and set up the laser vibrometer and participated in the vibrational signal measurement experiments. All authors gave final approval for publication.

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Supplementary material

Supplementary material available online at
<http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.122911/-DC1>

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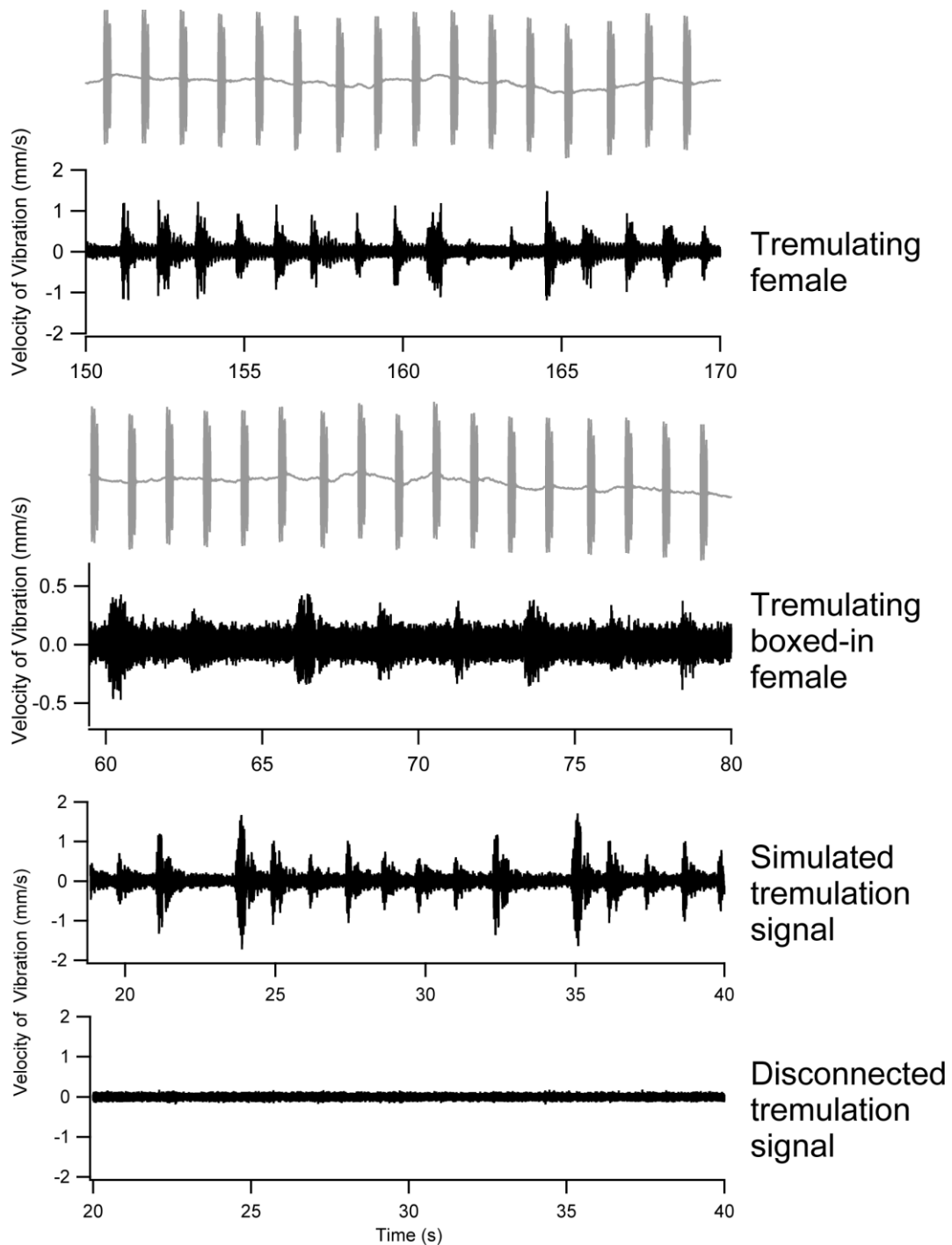


Fig. S1. The tremulation signal (upper black trace) measured 30 cm away from a *O. unnotatus* female sitting directly on a branch in response to the male call (upper grey trace), compared to the signal measured at the central branching point on the Y-shaped branch from the same female tremulating in a box coupled to the left terminal of the branch (as in Fig. 6C) (second black trace from above) in response to the male call (second grey trace from above); the simulated tremulation signal from an electromagnetic actuator coupled to the left terminal of the Y-shaped branch (third black trace from above); and the signal from a disconnected source of vibration (lowermost black trace), all measured 4 cm before the central branching point on the Y-shaped branch.



Movie 1. Tremulation. This video demonstrates a female tremulating briefly and then performing phonotaxis in response to chirps of the male call played back from a loudspeaker.



Movie 2. Vibrotaxis, i.e. male localization of a tremulation signal. The male is able to localize a played back vibratory signal simulating female tremulation, by localizing the branch attached to the electromagnetic actuator from which the vibratory signal is played.