

RESEARCH ARTICLE

Low-pass filters and differential tympanal tuning in a paleotropical bushcricket with an unusually low frequency call

Kaveri Rajaraman^{1,*}, Natasha Mhatre², Manjari Jain¹, Mathew Postles², Rohini Balakrishnan¹ and Daniel Robert²

¹Center for Ecological Sciences, Indian Institute of Science, Bangalore 560012, India and ²School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG, UK

*Author for correspondence (kaveri.indira@gmail.com)

SUMMARY

Low-frequency sounds are advantageous for long-range acoustic signal transmission, but for small animals they constitute a challenge for signal detection and localization. The efficient detection of sound in insects is enhanced by mechanical resonance either in the tracheal or tympanal system before subsequent neuronal amplification. Making small structures resonant at low sound frequencies poses challenges for insects and has not been adequately studied. Similarly, detecting the direction of long-wavelength sound using interaural signal amplitude and/or phase differences is difficult for small animals. Pseudophylline bushcrickets predominantly call at high, often ultrasonic frequencies, but a few paleotropical species use lower frequencies. We investigated the mechanical frequency tuning of the tympana of one such species, *Onomarchus uninotatus*, a large bushcricket that produces a narrow bandwidth call at an unusually low carrier frequency of 3.2 kHz. *Onomarchus uninotatus*, like most bushcrickets, has two large tympanal membranes on each fore-tibia. We found that both these membranes vibrate like hinged flaps anchored at the dorsal wall and do not show higher modes of vibration in the frequency range investigated (1.5–20 kHz). The anterior tympanal membrane acts as a low-pass filter, attenuating sounds at frequencies above 3.5 kHz, in contrast to the high-pass filter characteristic of other bushcricket tympana. Responses to higher frequencies are partitioned to the posterior tympanal membrane, which shows maximal sensitivity at several broad frequency ranges, peaking at 3.1, 7.4 and 14.4 kHz. This partitioning between the two tympanal membranes constitutes an unusual feature of peripheral auditory processing in insects. The complex tracheal shape of *O. uninotatus* also deviates from the known tube or horn shapes associated with simple band-pass or high-pass amplification of tracheal input to the tympana. Interestingly, while the anterior tympanal membrane shows directional sensitivity at conspecific call frequencies, the posterior tympanal membrane is not directional at conspecific frequencies and instead shows directionality at higher frequencies.

Key words: auditory, tympanum, katydid, tettigoniid, Orthoptera, laser vibrometry, trachea, pseudophylline, *Onomarchus uninotatus*.

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INTRODUCTION

Tettigoniid (bushcricket) acoustic communication is under several different evolutionary selection pressures. To improve mate attraction by broadcasting their songs over a large area, males should ideally produce loud and low-frequency calls, which transmit better in the environment (Forrest, 1994) and are harder for predators such as bats to detect (Belwood and Morris, 1987). However, several other forces drive them towards producing higher frequencies. The smaller the size of the insect, the less efficient it is at producing low-frequency song (Bennet-Clark, 1998), an already energetically expensive activity (Prestwich and O'Sullivan, 2005). On the receiver end, for females attempting to find the males, low-frequency calls can be difficult to localize by an animal whose body size may be smaller than the wavelength of the incoming sound (Robert, 2005; Robinson and Hall, 2002). In addition, the evolution of call frequency is also driven by the acoustic community, which competes for bandwidth within the available acoustic space (Schmidt et al., 2011).

Pseudophylline bushcrickets present an interesting system with which to study the evolution of call frequency and auditory tuning in response to these disparate physical and ecological pressures. The

acoustic features of tettigoniid calls have been studied in detail relative to ecological pressures from eavesdropping parasitoid flies (Lakes-Harlan and Heller, 1992; Walker, 1993; Lehmann and Heller, 1998) and predatory bats that use this acoustic information to locate their targets (Tuttle et al., 1985; Belwood and Morris, 1987; Belwood, 1990). Specifically, it has been suggested that low calling activity and the use of pure tones and very high ultrasonic frequencies by neotropical pseudophyllines (Rentz, 1975; Morris and Beier, 1982; Belwood and Morris, 1987; Morris et al., 1989; Morris et al., 1994; Heller, 1995) makes them difficult to localize by mammalian ears (Belwood and Morris, 1987) and limits the spread of the sound due to frequency-dependent attenuation (Morris et al., 1994), thereby reducing detection by mammalian predators (Heller, 1995). In contrast, paleotropical pseudophyllines have significantly lower-frequency calls and call more frequently than those from the neotropics (Heller, 1995). A majority (66%) of the ca. 400 pseudophylline species investigated so far produce calls with dominant frequencies in the ultrasonic range (Montealegre-Z et al., 2006), and a majority (75%) of 66 pseudophyllines reviewed by Montealegre-Z and Morris (Montealegre-Z and Morris, 1999) produce narrow-bandwidth calls. Of these, the only two with carrier

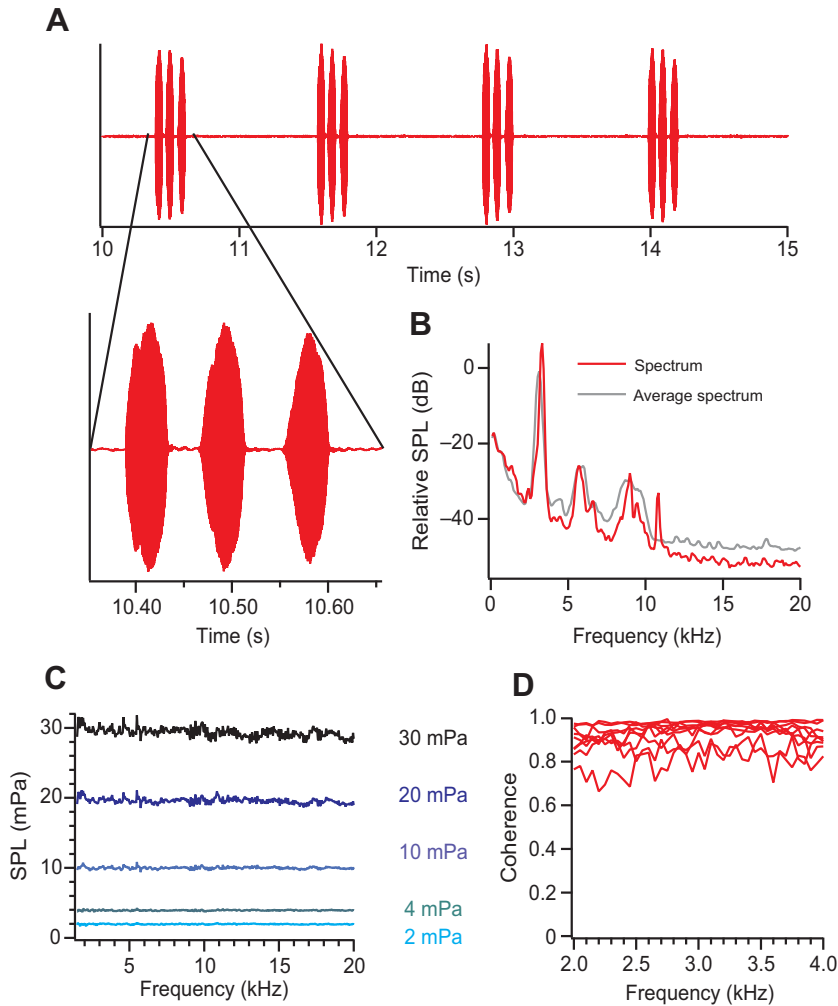


Fig. 1. The calling song of *Onomarchus uninotatus*, the experimental stimulus and response coherence. (A) Time series of the call of a sample male, with a close-up of a single chirp, and (B) the frequency spectrum of the song of a sample male (red) alongside the average spectrum for 12 animals (grey). (C) The stimulus sound pressure level (SPL) across frequencies as measured near the preparation and (D) the high coherence of ATM responses with respect to the stimulus SPL in the 2–4 kHz frequency range.

frequencies below 5 kHz were discovered in the paleotropics. One is *Tympanophyllum arcuifolium*, which calls at 600 Hz, the lowest airborne sound frequency at which any insect is known to communicate, and the other is *Onomarchus uninotatus*, which produces low-frequency calls around 2 kHz when handled, presumably protest calls (Heller, 1995).

In the tropical evergreen forests of Kudremukh National Park in southwestern India, *O. uninotatus* (Serville 1838) has been shown to call (Fig. 1A) at 3.23 ± 0.1 kHz ($N=10$) under natural conditions (Diwakar and Balakrishnan, 2007). Extensive studies have been carried out on the acoustic community of Kudremukh, which includes pseudophyllines calling at higher frequencies (Diwakar and Balakrishnan, 2007) and predatory bats (H. Raghuram and R.B., unpublished). In addition, the signal transmission characteristics of different calls within this habitat have been well characterized (Jain and Balakrishnan, 2012). This provides us with an excellent opportunity to study and interpret the biomechanics of the auditory system of a species with a low-frequency call within the well-documented context of its habitat.

The most commonly used mechanism to improve call detection and discrimination is to have an auditory system with a frequency response corresponding, or matched, to the conspecific carrier frequency. In insects, this correspondence is often achieved by exploiting the resonant properties of the auditory system. Such a match can be seen in the receiver's tympanum vibrating with the highest amplitude at the sender's carrier frequency (Lewis et al.,

1975; Paton et al., 1977; Larsen and Michelsen, 1978; Michelsen et al., 1994; Nowotny et al., 2010), and is enhanced by an abundantly documented correspondence between the call carrier frequency and the spectral sensitivity of a majority of the receiver's auditory neurons (Nocke, 1975; Zaretsky and Eibl, 1978; Esch et al., 1980; Hill and Oldfield, 1981; Hutchings and Lewis, 1981; Oldfield et al., 1986; Ball et al., 1989; Larsen et al., 1989; Mason et al., 1991; Lin et al., 1993; Stumpner, 1996; Imaizumi and Pollack, 1999; Kostarakos et al., 2009; Hummel et al., 2011; Schmidt et al., 2011).

In bushcrickets, two tympanal membranes positioned on each proximal tibia are set into vibration by incident sound pressure. These tympanal membranes are backed by a tracheal tube, which is closed distally but extends proximally through the leg and into the animal's thorax, opening out into a spiracle on the lateral side of the body (Bailey, 1991; Kalmring et al., 2003). Each tympanum of a bushcricket ear can have sound impinging on both its exterior surface and its interior surface through spiracular input and transmission through the tracheal tube. At frequencies below 3 kHz, the sound pressures upon the internal and external surfaces of the ear in two bushcricket species of the genus *Tettigonia* have been shown to be numerically almost equal, such that the ear behaves as an ideal pressure difference receiver (Michelsen and Larsen, 1978). The tracheal system of these species was described as horn-like, with reference to the cross-sectional area expansion of the trachea going from ear to spiracle (Michelsen and Larsen, 1978). At higher frequencies (ca. >10 kHz), the contribution of sound pressure conducted by the tracheal tube is much greater than

the sound pressure acting directly upon the external tympanal surface, a difference that was attributed to the frequency-dependent amplification of the sound conducted through the spiracle and trachea (Michelsen and Larsen, 1978; Lewis, 1974a; Lewis, 1974b; Lewis, 1974c; Nocke, 1975). This amplification has been shown to be high-pass in tracheae that show an exponential flare in their cross-sectional area between the tympanum and the spiracle, a shape hypothesized to produce the amplification expected from a finite exponential horn (Lewis, 1974a; Hoffmann and Jatho, 1995). In a few other cases, band-pass amplification has been associated with tracheae that show a non-exponential increase in cross-sectional area with distance from the tympanum, and in these cases the tracheae are described as behaving like resonating cylindrical tubes whose resonance frequency depends inversely on the length of the tube (Nocke, 1975).

Thus, the size and shape of the tracheae and spiracular openings have been shown to contribute most to the frequency tuning, directionality and threshold of tympanal responses (Heinrich et al., 1993; Shen, 1993; Kalmring and Jatho, 1994; Kalmring et al., 1995; Hoffmann and Jatho, 1995; Bangert et al., 1998), although the tympanal membranes have also been shown to play a role (Lewis, 1974b; Lewis, 1974c; Mason et al., 1991; Michelsen et al., 1994; Bangert et al., 1998; Nowotny et al., 2010; Hummel et al., 2011). In all these cases, however, low sensitivity to frequencies below 5 kHz is observed, implying that none of these auditory systems would respond to the low-frequency calls (Fig. 1B) of *O. uninotatus*.

In this study, we used X-ray microtomography (μ -CT) to reconstruct the geometry of the tracheal system of *O. uninotatus*. Microscanning laser Doppler vibrometry was then used to investigate the match between the mechanical response of the two tympanal membranes of *O. uninotatus* and the power spectrum of its narrow-band calling song. Ultimately, efficient acoustic communication depends on the mechanical sensitivity of the tympanal membranes; therefore, the relationship between tympanal responses and incident sound pressure level (SPL) was also investigated. Finally, the directionality of the tympanal mechanical response was quantified, providing evidence that *O. uninotatus* is capable of localizing the low-frequency conspecific calls.

MATERIALS AND METHODS

Experiments were carried out using nine wild-caught *O. uninotatus* females. The animals were maintained individually in the laboratory on a 12h:12h light:dark cycle at 26°C, with *ad libitum* access to water, pollen, apple and banana.

Morphology

The anatomy of the ear and tracheal system was examined using μ -CT and three-dimensional (3-D) reconstruction. Each female was killed using a formalin injection and then mounted in a plastic tube holder and scanned using a Skyscan 1172 μ -CT workstation (Skyscan, Kontich, Belgium) at a layer resolution of 8.7 μ m. The μ -CT images were then reconstructed using the software CTan (Version 1.10, provided by Skyscan) into a series of slice images. The 3-D model was then constructed from these slice images using AMIRA 4.1 software (Visage Imaging, San Diego, CA, USA).

Vibrometry

Each animal was anaesthetized with carbon dioxide and glued dorsally to a rectangular metal plate (8×4×1 cm) using a combination of liquid latex (Magnacraft, Midhurst, UK) and Blu-Tack (Bostik, Leicester, UK) such that its fore-tibia could not move or vibrate. The animal was placed on the metal plate such that the fore-tibia bearing the tympanal membranes faced forward, similar to the

configuration sketched by Mhatre et al. (Mhatre et al., 2009), except that its feet were not glued at the tarsi but rather were immobilized at all leg joints by an extension of its dorsal scaffolding made of Blu-Tack. The metal plate was connected to a horizontal metal rod (150 mm long, 8 mm diameter) held by a micromanipulator clamped onto a vibration isolation air table (TMC 784-443-12R, Technical Manufacturing Corp., Peabody, MA, USA) in an acoustic isolation booth (IAC series 1204A, 4.50×2.25×1.98 m, Industrial Acoustics, Bronx, NY, USA).

The animal was then positioned in the beam path of a scanning laser Doppler vibrometer (Polytec PSV-300-F, OFV-056 scanning head with close-up attachment; Polytec, Waldbronn, Germany). The tympanal membrane being measured was positioned so that the flat portion of its surface was as close as possible to perpendicular to the incident laser beam. Measurements were made 20 times at each point on the lattice and averaged. Scanning measurements were made using a lattice of 20–60 points spread across the surface of the membrane (Polytec Scanning Vibrometer software, version 7.4). Recordings on three animals were made at extremely high resolution (1100 to 1600 scan points) to allow us to sensitively measure the deflection shapes produced at different frequencies. These measurements validated those made with lattices of fewer points. These three recordings were made with the animals held upside down, such that their legs faced upward.

The vibration velocity of the tympanal membranes was measured in response to sound stimuli. The stimulus was relayed through a data acquisition board (PCI-4451, National Instruments, Austin, TX, USA) and amplifier (Sony Amplifier Model TA-FE570, Sony, Tokyo, Japan) to a loudspeaker (ESS AMT-1, ESS Laboratories, South El Monte, CA, USA). Measurements were made with this loudspeaker placed ~25 cm from the animal, first from the ipsilateral position at 90 deg relative to the animal's longitudinal body axis, and then from a contralateral position at 270 deg. The acoustic stimulus played to the animal was recorded simultaneously using a 1/8 inch (3.2 cm) precision pressure microphone (model 4138, Brüel and Kjær, Naerum, Denmark) positioned just above the laser path, ~5 mm away from the animal's ears. This microphone has a flat response to stimuli within the frequency range measured, and was connected *via* a preamplifier (model 2633, Brüel and Kjær) to the Polytec data management system (PSV-Z-040-F).

The stimulus used was a periodic chirp from 1.5 to 20 kHz generated by the Polytec Scanning Vibrometer software and D/A card. For each position of the loudspeaker, measurements were repeated at five SPLs: 2, 4, 10, 20 and 30 mPa [respectively, 40, 46, 54, 60 and 63.5 dB SPL (re. 2×10^{-5} N m⁻²)]. The digitally synthesized stimulus was adjusted using correction files, so as to equalize output amplitudes and produce a constant SPL across the frequency band (1.5–20 kHz; Fig. 1C). Both the acoustic stimulus and the tympanal membrane velocity were sampled at 81.92 kHz. The coherence of the membrane displacement with respect to the acoustic signal, the velocity (mms⁻¹ Pa⁻¹) and displacement (mPa⁻¹) transfer functions of the tympanal membrane response relative to stimulus SPL (Pa), and the phase of the response were calculated to a 50 Hz resolution using a fast Fourier transform with a rectangular window, as described in Windmill et al. (Windmill et al., 2005). In this case, the frequency of the male call at 3.23±0.10 kHz (Diwakar and Balakrishnan, 2007) defines the bandwidth of interest. Hence, only data in response to stimulation at 20 mPa, with a minimum coherence over 0.75 in frequencies ranging from 3 to 3.5 kHz, were considered (Fig. 1D). Directionality estimates were performed in response to stimulation at 30 mPa, with the application of the same cut-off criterion for coherence. At lower stimulation intensities, coherence fell below this

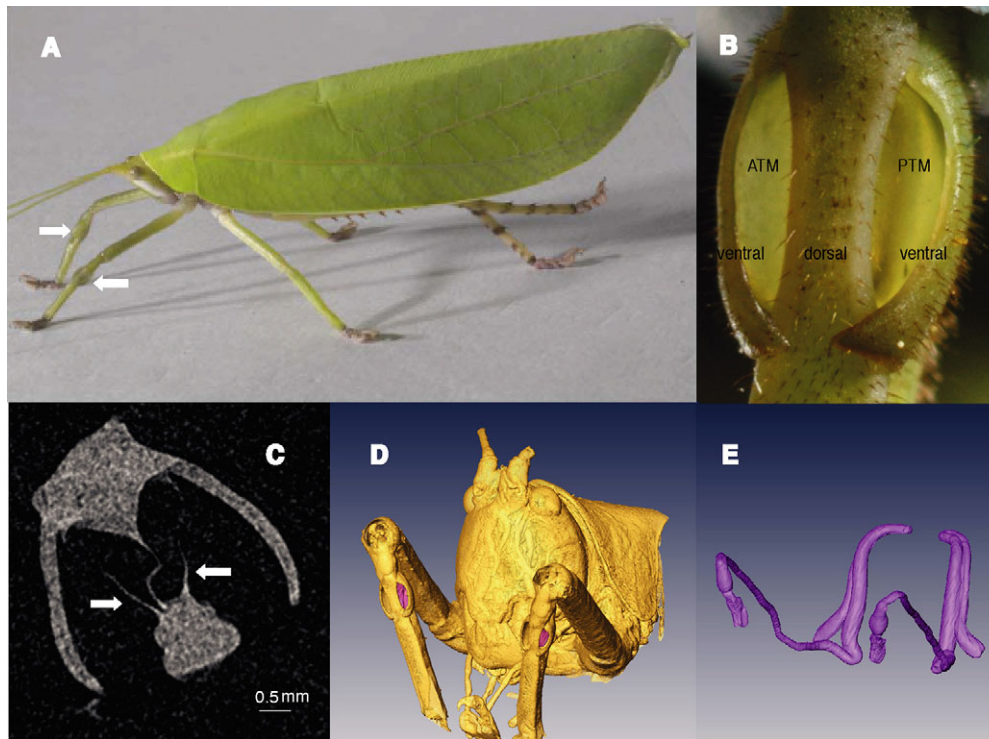


Fig. 2. (A) Side view of *O. unnotatus* with white arrows marking the tympanal organ on each leg. (B) Close-up of the tympanal organ showing the tympanal slits with the anterior and posterior tympanal membrane's dorsal and ventral sides labelled. (C) A cross-section through the tympanal organ (scale bar, 0.5 mm), with white arrows marking the thin tympana. (D,E) μ -CT scan images of the tympana contiguous with the tracheal system from an (D) external and (E) internal view.

level, as expected. This stringent criterion is not applicable to the study of intensity dependence. Responses were fitted with a simple harmonic oscillator model where the transfer function varied with angular frequency (ω) as described in Mhatre et al. (Mhatre et al., 2011):

$$\text{Amplitude}(\omega) = A v^2 / [(v^2 - \omega^2)^2 + (2\gamma\omega v)^2]^{1/2}, \quad (1)$$

where v is the resonant frequency, γ is the damping ratio and A is the zero value of the transfer function.

The relationship of phase to frequency (ω) was modelled as:

$$\text{Phase}(\omega) = \sin^{-1} \{ (v^2 - \omega^2) / [(v^2 - \omega^2)^2 + (2\gamma\omega v)^2]^{1/2} \}. \quad (2)$$

RESULTS

Morphology

The auditory system of *O. unnotatus* is similar to that of other described bushcrickets in most respects (Schumacher, 1975; Rössler et al., 1994). The ears are located on the dorsal proximal part of the fore-tibiae (Fig. 2A), presenting an anterior tympanal membrane (ATM) and a posterior tympanal membrane (PTM), each tethered to either side of a medial wall and protected by crescent-shaped covers (Fig. 2B). The tympanal membranes can be viewed through wide tympanal slits, which appear symmetrically placed with respect to each other (Fig. 2B). In section, the tympana form a 70 deg angle with respect to each other where they meet the cuticle of the hemolymph channel (Fig. 2C). Each membrane has a thicker dorsal portion near the point at which it is tethered to the central dorsal cuticle, and a thinner ventral surround extending to the point of attachment near the posterior part of the leg (Fig. 2C). Each membrane is backed by a separate tracheal cavity, which constitutes part of the anterior and posterior tracheal branch, respectively (Fig. 2D,E). The two tracheal branches are separate up to the tibial organ, after which they fuse into a single tracheal tube. This trachea then extends through the proximal part of the leg into the prothorax, ending at the spiracular atrium and spiracular opening. The trachea

increases in diameter within the animal's prothorax, where it forms a large U-bend (Fig. 2E). It then enlarges even further, forming the atrium, just before joining the relatively smaller spiracles. Like the tympana and the spiracular opening, the tracheae present an oval cross-section with a ratio of approximately 2:1 between the maximum and minimum diameter throughout their length (Table 1). The tracheae on the two sides of the body are not connected to each other (Fig. 2D,E).

Frequency responses of the tympanal membranes

In order to investigate the tuning of the ATM's response to acoustic stimuli, we examined the average transfer function at the point on the ATM where the tympanal response showed the maximum coherence. The mechanical response is approximately flat at a mean (\pm s.d.) value of $21.14 \pm 8.21 \text{ nm Pa}^{-1}$ ($N=10$) for frequencies from 1.5 to 3.5 kHz, exhibiting a maximum at 3.2 kHz at $22.91 \pm 8.62 \text{ nm Pa}^{-1}$ (mean \pm s.d., $N=10$). The response then drops steeply between 3.5 and 4.5 kHz, to a mean (\pm s.d.) value of

Table 1. Dimensions of the tympanal membranes, spiracular openings and tracheal tubes on either side of the body of one specimen *Onomarchus unnotatus*

Structure measured	Metric (mm)	Left side	Right side
ATM	Length	1.724	1.722
ATM	Width	0.755	0.767
PTM	Length	1.762	1.716
PTM	Width	0.762	0.764
Spiracle	Length	0.815	0.829
Spiracle	Width	0.385	0.364
Trachea (post spiracle)	Max. diameter	0.995	0.883
Trachea (thoracic)	Max. diameter	0.949	0.900
Trachea (femural)	Max. diameter	0.401	0.413
Trachea (post femural expansion)	Max. diameter	0.933	0.968

ATM, anterior tympanal membrane; PTM, posterior tympanal membrane.

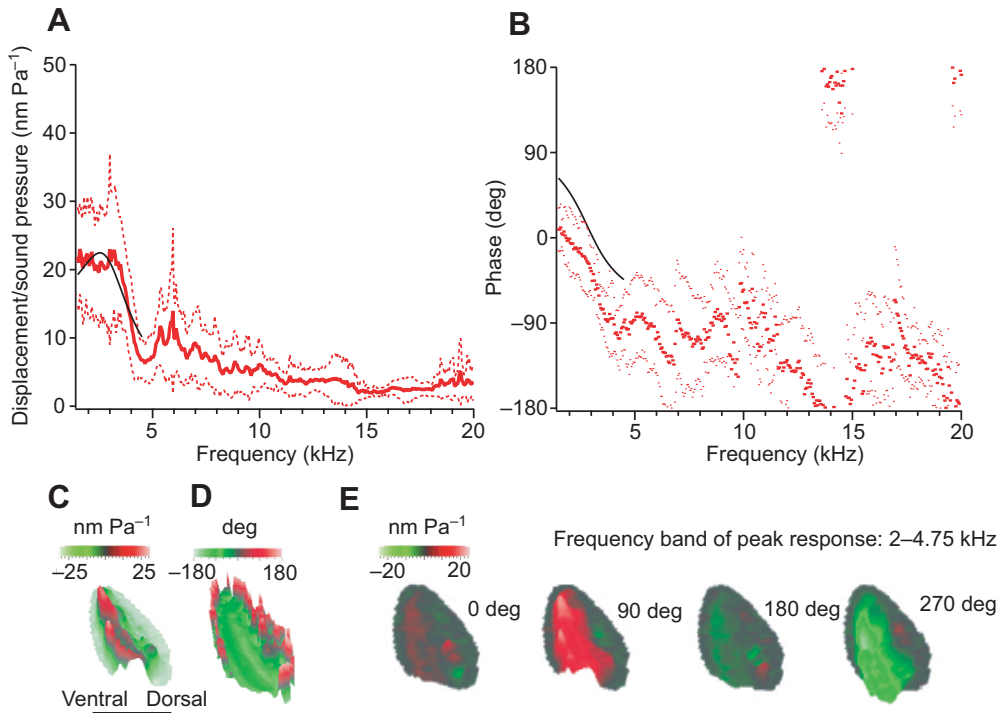


Fig. 3. Frequency response of the anterior tympanal membrane (ATM). (A) Displacement per unit sound pressure, or the transfer function of the ATM relative to the auditory stimulus played ipsilaterally, plotted relative to frequency. The mean membrane response of 10 individuals is indicated by the solid red line and the standard deviation envelope is indicated by the dotted red lines. The average amplitude response is fitted to a simple harmonic oscillator model (SHO) shown in black (parameters are given in Table 2). (B) Mean (\pm s.d.) phase of the vibration of the ATM relative to the sound stimulus as recorded near the animal's tympanal organ, plotted with respect to frequency (red) and compared with the expected relationship derived from the SHO model for the transfer function's relationship to stimulus frequency shown in A. The spatial distribution across a sample ATM membrane of (C) its displacement per unit pressure and (D) the phase of vibration relative to the sound stimulus are also shown, along with (E) the map of displacement of the membrane at different phases of the sound stimulus: 0, 90, 180 and 270 deg.

$4.73 \pm 2.70 \text{ nm Pa}^{-1}$ ($N=10$) from 4.5 to 20 kHz (Fig. 3A). The ATM's range of maximum sensitivity includes the carrier frequency of male call broadcast at $3.23 \pm 0.10 \text{ kHz}$ (mean \pm s.e.m., $N=10$) and sharply filters out frequencies above 3.5 kHz. The magnitude of the ATM transfer function between 1.5 and 4.5 kHz can be fitted by a simple harmonic oscillator (SHO) model with a resonant frequency, ν , of $3.08 \pm 0.12 \text{ kHz}$ (mean \pm s.d., $N=10$) and an R^2 value of 0.83 for the fit (Fig. 3A). The other parameters of the SHO model are described in Table 2. However, in this frequency range, the phase transition in the transfer function does not cross from 90 to -90 deg as would be expected from an SHO model (Fig. 3B).

In terms of its spatial response, the ventral part of the ATM responds with higher displacement than the dorsal part (Fig. 3C). The phase response of the membrane does not vary over its surface (Fig. 3D, phase map). The displacement map (deflection shape map) of the membrane at different phases across one stimulus cycle reveals that the ATM vibrates like a hinged flap (Fig. 3E).

Unlike the ATM, the PTM responds to more than one frequency band (Fig. 4A). The transfer function of the PTM is nearly flat from 1.5 to 4 kHz. Unlike the ATM, the PTM does not show a steep decline in response to higher frequencies. Instead, the transfer

function exhibits a broad peak from 4.5 to 11.5 kHz, reaching a maximum value of $21.47 \pm 15.73 \text{ nm Pa}^{-1}$ (mean \pm s.d., $N=6$) at 7.35 kHz. A third peak is also seen between 11.5 and 18.5 kHz, with a maximum value of $14.23 \pm 17.57 \text{ nm Pa}^{-1}$ (mean \pm s.d., $N=6$) at 14.35 kHz. Each of these peaks can be described by an independently fit SHO model. Similar to the ATM, the first peak of the PTM amplitude response can be fitted to an SHO model with a resonant frequency of $3.50 \pm 0.13 \text{ kHz}$ (mean \pm s.d., $N=6$), but the fit is poor with a R^2 value of 0.51. The second peak is fitted to a second SHO with a resonant frequency of $8.13 \pm 0.07 \text{ kHz}$ (mean \pm s.d., $N=6$) and a R^2 value of 0.88, while the third peak is fitted by an SHO exhibiting a resonance at $14.65 \pm 0.08 \text{ kHz}$ (mean \pm s.d., $N=6$) and a R^2 value of 0.91 (Fig. 4A). The parameter values used to define SHO fits are listed in Table 2. The measured phase of the PTM response, however, shows several transitions that differ from the phase transitions predicted by the three independent SHO models (Fig. 4B).

Spatial maps of membrane displacement amplitude and phase were plotted for the three frequency bands with significant responses (Fig. 4C–E). If these peaks represented distinct deflection modes, clear nodes and antinodes would be expected to form within the displacement map of the membrane. This would be associated with sharp transitions in the phase response and its spatial

Table 2. Parameters of the simple harmonic oscillatory model fit to the transfer function of the tympanal membranes, within each frequency range where peaks in the response to auditory stimuli were observed

	ATM		PTM	
	1.5–4.5 kHz	1.5–4.5 kHz	4.5–11.5 kHz	11.5–18.5 kHz
Resonant frequency, ν (kHz)	3.08 ± 0.12	3.50 ± 0.13	8.13 ± 0.07	14.65 ± 0.08
Damping ratio, γ	0.40 ± 0.06	0.41 ± 0.04	0.18 ± 0.01	0.09 ± 0.01
Transfer function zero value, A (nm Pa^{-1})	16.52 ± 1.55	12.36 ± 0.82	6.57 ± 0.29	1.89 ± 0.10
R^2	0.83	0.51	0.88	0.91

ATM, anterior tympanal membrane; PTM, posterior tympanal membrane. Values are means \pm s.d.

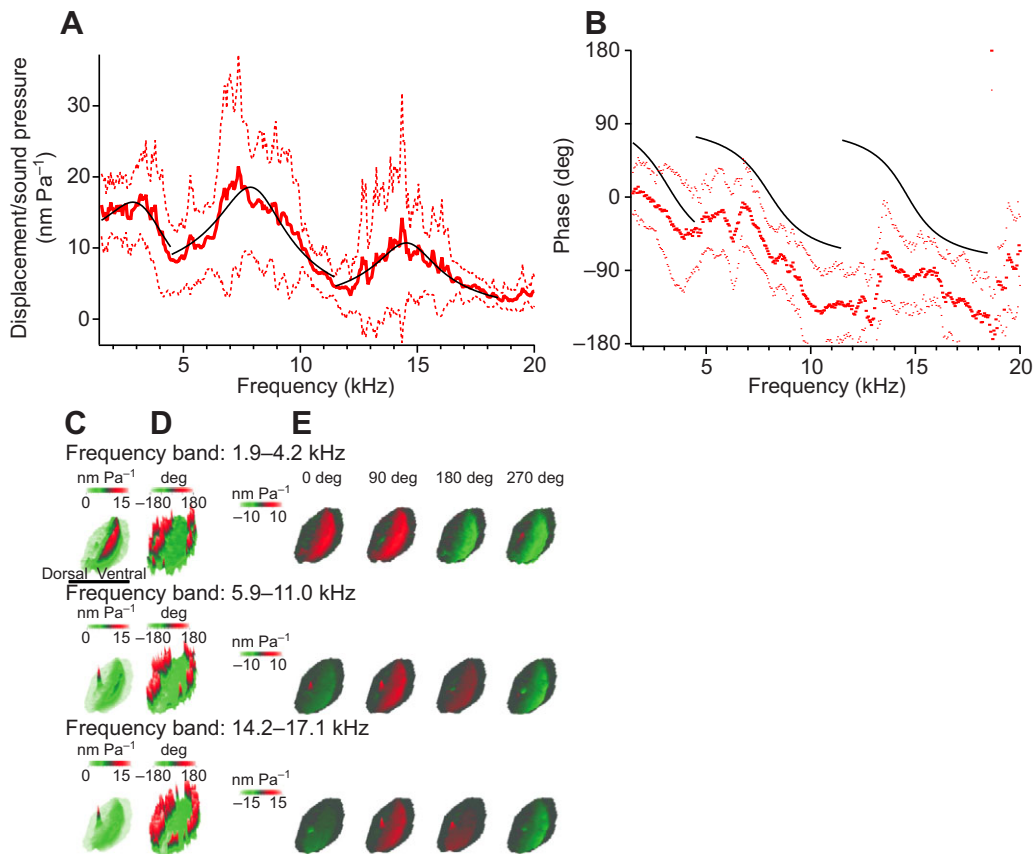


Fig. 4. Frequency response of the posterior tympanal membrane (PTM). (A) Amplitude and (B) phase of vibration of the PTM relative to the frequency of sound stimulus played ipsilaterally to the animal. The means \pm s.d. are depicted as in Fig. 3 ($N=6$). The SHO models depicted in black are fitted to each of the three peaks of the average amplitude response (parameters in Table 2) and then applied to the SHO model prediction for the phase relationship. The spatial distribution of the (C) displacement per unit sound pressure, (D) the phase of vibration relative to the stimulus and (E) the displacement of the membrane at various phases of the sound stimulus (0, 90, 180 and 270 deg) are also shown at three frequency bands that roughly correspond to the three peaks seen in A (2–4.75 kHz, 5.9–11.0 kHz and 14.2–17.1 kHz, respectively).

representation (Fig. 4E). No clear antinodes or sharp phase transitions were observed at any of the three frequency ranges (Fig. 4C,D). This provides evidence that the three peaks in the PTM response do not represent membrane modes. The instantaneous displacement maps at the three frequencies (Fig. 4E) clearly demonstrate that most of the PTM moves in phase with no bending observed within the membrane. Like the ATM, the PTM behaves like a hinged flap with larger displacement magnitude dorsally than ventrally (Fig. 4C–E).

Although most of the membrane moves in unison, a small portion near the dorsal anchor of the membrane can be observed to move in anti-phase (Fig. 4C,E). While the amplitude of the displacement of this region is more pronounced at higher frequencies, it can also be observed at low frequencies. A similar region moving in anti-phase at low amplitudes is present in the ATM (Fig. 3C,E). A dorsal region of the inner plate with similar ‘wobbling’ behaviour, out of phase with the rest of the membrane at higher frequencies, has also been observed in another bushcricket species, *Mecopoda elongata* (Nowotny et al., 2010).

In order to assess how the ATM and PTM move with respect to each other, difference spectra of the ATM relative to the PTM were obtained for ipsilateral stimulation. The difference spectra showed that, on average, the ATM responds more strongly to sound in the frequency range of 1 to 3.5 kHz whereas the PTM responds relatively more to higher frequencies (Fig. 5A). The phase difference between ATM and PTM is clear for low frequencies, between 1.5 and 3.5 kHz, whereby the ATM lags behind the PTM by 14.64 ± 41.44 deg (mean \pm s.d., $N=6$; Fig. 5B). At higher frequencies, the ATM oscillates much less and the phase difference is not as well defined (Fig. 3A,B, Fig. 4A,B), yet the tendency is that the ATM leads the PTM (Fig. 5B).

Response linearity, or the lack thereof, carries important information about the mechanisms of auditory processing (Michelsen and Larsen, 1978; Göpfert and Robert, 2001). The magnitude of the tympanal mechanical response as a function of incident SPLs was evaluated. The mean response magnitudes were measured for the ATM (Fig. 6A) and the PTM (Fig. 6B) at five levels of acoustic stimulation. The frequency response of both membranes remains the same at different sound intensities, with membrane displacement changing monotonically with stimulus SPL. The magnitude transfer functions for different SPLs for both the ATM (Fig. 6C) and the PTM (Fig. 6D) do not reveal any significant increase in gain as stimulus magnitude decreases. A change of gain would indicate a non-linearity, which, in other insects such as mosquitoes, has been linked to active auditory mechanics (Göpfert and Robert, 2001).

Directionality

The directionality of the mechanical response of each of the tympanal membranes was quantified by subtracting the frequency response of each tympanal membrane to contralateral acoustic stimulation from the response to ipsilateral stimulation. A mean of these individual difference spectra was then calculated. For the ATM, the mean difference spectrum remained near 0 nm Pa^{-1} , except between 1.5 and 4 kHz (Fig. 7A). In this frequency band, the mean (\pm s.d.) difference between the ATM’s response between ipsilateral and contralateral stimulation at 30 mPa SPL was $7.72 \pm 7.23 \text{ nm Pa}^{-1}$ ($N=6$). At 3.25 kHz, close to the conspecific song carrier frequency of 3.23 kHz, the difference between the ATM’s response between ipsilateral and contralateral stimulation was $10.90 \pm 12.89 \text{ nm Pa}^{-1}$ (mean \pm s.d., $N=6$). This corresponds to a difference of ~ 7.24 dB SPL between ipsilateral and contralateral

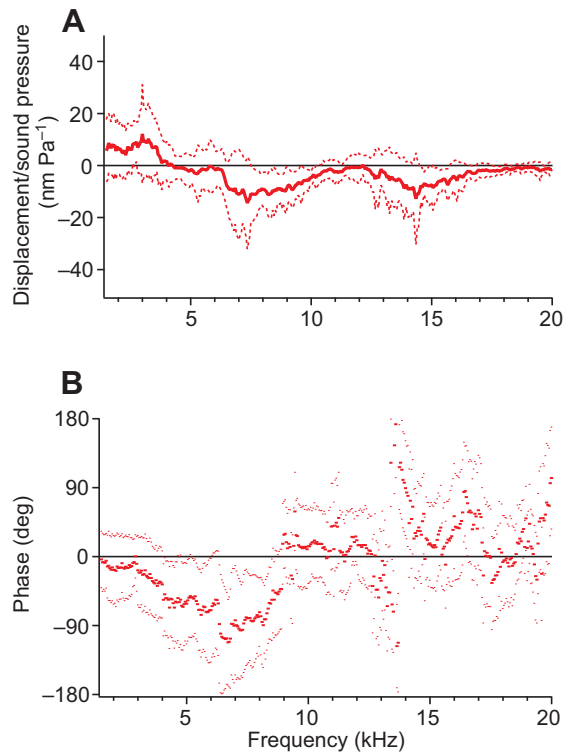


Fig. 5. Difference spectra between the ATM and PTM response to ipsilateral stimulation ($N=6$). (A) The difference between the mean amplitude (bold red line) \pm s.d. (dashed lines) and (B) the mean phase (bold red dotted trace) \pm s.d. (small dotted traces) of vibration of the ATM and PTM relative to the sound stimulus at different frequencies of stimulation.

stimulation. Therefore, we can conclude that the ATM exhibits a directional response to low frequencies around the conspecific song carrier frequency (Fig. 7A).

The mean difference spectrum for the PTM (Fig. 7B) was close to 0 nm Pa^{-1} around the song frequency but exhibited broad peaks with maximum values at 6.9 and 14.35 kHz. In the frequency range from 6.5 to 10 kHz, the mean (\pm s.d.) difference was $10.46 \pm 11.50 \text{ nm Pa}^{-1}$ ($N=4$). At 6.9 kHz, the maximal difference was $18.53 \pm 18.90 \text{ nm Pa}^{-1}$, corresponding to a 9.29 dB SPL difference between ipsilateral and contralateral stimulation. In the frequency range from 12.5 to 15 kHz, the mean (\pm s.d.) displacement per unit sound pressure of the PTM in response to ipsilateral stimulation minus contralateral stimulation was $7.12 \pm 8.05 \text{ nm Pa}^{-1}$ ($N=4$). The peak value of this difference at 14.35 kHz was $13.71 \pm 19.17 \text{ nm Pa}^{-1}$, corresponding to a difference of 17.91 dB between ipsilateral and contralateral stimulation. Thus the PTM shows poor directionality near conspecific song frequency, but produces a directional response to higher-frequency stimuli (Fig. 7B).

DISCUSSION

Matched filters

The ATM of the ear of *O. unnotatus* responds to sound between 1.5 to 3.5 kHz, which is well matched with the male calling song at $3.23 \pm 0.10 \text{ kHz}$ (mean \pm s.e.m.) (Diwakar and Balakrishnan, 2007). Interestingly, there is a sharp drop in the sensitivity of the tympanum to frequencies higher than song carrier frequency, a characteristic resembling a high-quality low-pass filter. This response suggests that there may be selective pressures to evolve relatively high

mechanical sensitivity to frequencies of the male calling song, but not so for high frequencies. Other studies (Kostarakos et al., 2009) have shown similar patterns of steep filters in the context of acoustic competition with sympatric species. Likewise, in the rainforest cricket *Paroecanthus podagrosus*, Schmidt et al. (Schmidt et al., 2011) have observed the presence of a steeper filter at frequencies above that of the male call but not at frequencies below the call. It was suggested that this is because more species producing competing calls in tropical rainforests utilize carrier frequencies above 4 kHz rather than below. Sixteen of the 19 other identified members of the orthopteran acoustic community syntopic with *O. unnotatus* in Kudremukh call at frequencies higher than 4 kHz (Diwakar and Balakrishnan, 2007), and two other species call in the frequency range between 3.2 and 4 kHz. This supports the suggestion that the low-pass filter characteristics of the ATM of *O. unnotatus* are driven by the pressure of masking interference from the acoustic community, predominantly at frequencies above its own call carrier frequency (Fig. 1B).

The high sensitivity of the ATM of *O. unnotatus* in the range of 1.5 to 3.5 kHz, within which the male call lies (Fig. 1B), is also consonant with the notion of matched filtering observed among other orthopteran species, such as field crickets (Larsen and Michelsen, 1978; Paton et al., 1977), wetas (Lomas et al., 2011) and bushcrickets (Lewis et al., 1975; Hill et al., 1981; Mason et al., 1991; Lin et al., 1993; Nowotny et al., 2010). However, mismatches in auditory tuning relative to call frequencies have been found in many ensiferan species including bushcrickets (Suga, 1966; Bailey and Römer, 1991; Mason, 1991) and prothalangopsids (Mason et al., 1999). So far, the auditory tuning of pseudophyllines has been studied only in species that call at high audio and ultrasonic frequencies (Mason et al., 1991), where call frequency was matched to tympanal nerve tuning. The present investigation in a pseudophylline calling at low frequencies likewise reveals a match between tympanal mechanical sensitivity and call frequency, albeit with a low-pass rather than a band-pass filter characteristic. A band-pass characteristic of the tympanal tuning might be revealed upon sampling at frequencies below 1.5 kHz, and additional band-pass filtering may also take place in the course of neuronal processing of auditory input. However, it is noteworthy that a very small number of sympatric and acoustically coactive species call at frequencies below the range in which we sampled [only one gryllacridoid species calling at 1.7 kHz (Diwakar and Balakrishnan, 2007)].

Differential frequency tuning of the ATM and PTM

The ATM behaves like an oscillator with a resonant frequency of $3.08 \pm 0.12 \text{ kHz}$ (mean \pm s.d., $N=10$). The amplitude and phase maps of membrane displacement confirm that the membrane vibrates in basic mode because no antinodes or sharp phase transitions are observed in the membrane. The PTM exhibits a more complicated response, with several resonant peaks at higher frequencies. Responding much like the ATM at lower frequencies, the PTM shows additional response maxima revealing the presence of independent oscillators with resonant frequencies at 3.50 ± 0.13 , 8.13 ± 0.07 and $14.65 \pm 0.0 \text{ kHz}$ (mean \pm s.d., $N=6$). Notably, as for the ATM, higher vibrational modes are never observed in the PTM; the entire surface area of the membrane vibrates in phase at all frequencies tested. Such mechanical behaviour suggests that the high-frequency response is related to transmission modes of the tracheal tube, rather than to intrinsic tympanal mechanical characteristics. The tracheal system is known in katydids to alter the gain of the pressure upon the tympanal membranes (Michelsen and Larsen, 1978). Slight differences in the mechanical properties

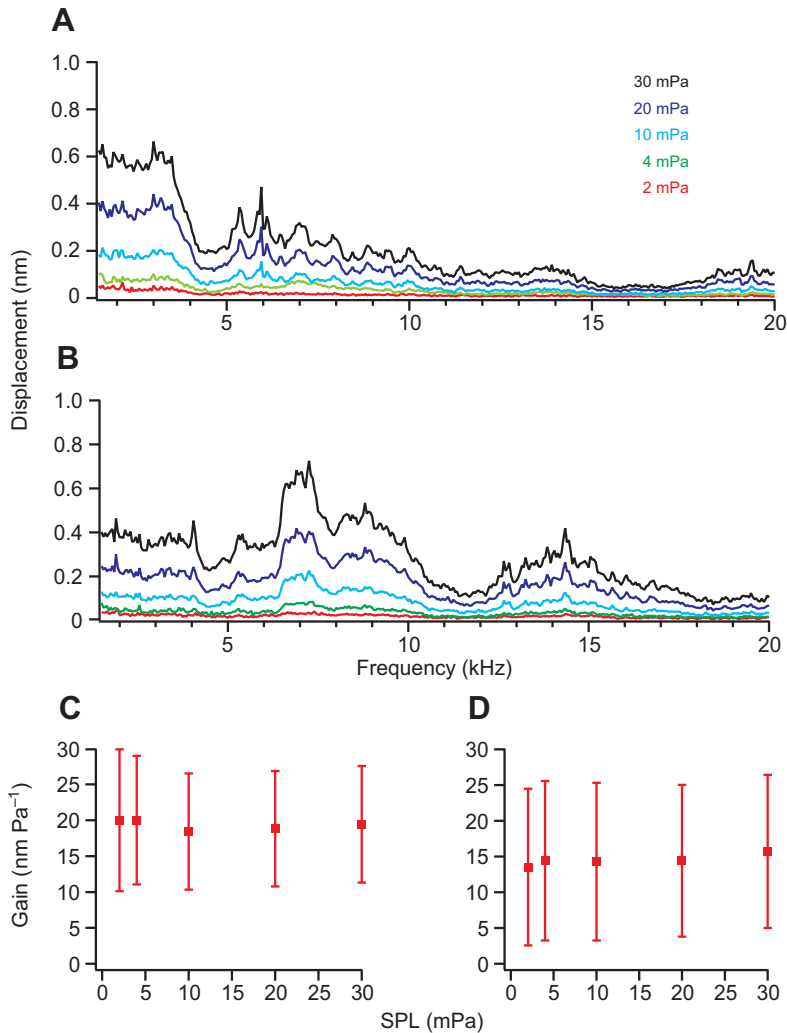


Fig. 6. Intensity dependence of the ATM and PTM responses. Absolute displacement of the (A) ATM and (B) PTM in response to different frequencies of ipsilateral stimulation, at increasing SPLs of stimulation ranging from 2, 4, 10, 20 to 30 mPa. Each trace is a mean calculated from six individuals. (C) The gain of the transfer function of the ATM and (D) PTM in terms of displacement per unit sound pressure, measured at different SPLs of stimulation. Data in C and D are means \pm s.d.

of the tympanal membranes coupled with tracheal modes would be capable of producing the multiple resonances reported here.

The difference spectra between the ATM and PTM responses suggest that the ATM responds more than the PTM to sound in the conspecific frequency range. The PTM responds more to higher-frequency acoustic input. This suggests that this acoustic system partitions responsiveness to the bandwidth of male calls to the ATM, and higher frequencies to the PTM. This partitioning of frequency responsiveness bands between tympana is similar to the phenomenon observed in some species of hymenoptid mantid, where the neural responses from the metathoracic ear were sensitive to ultrasound frequencies (25–50 kHz) while a serially homologous mesothoracic ear specific to these hymenoptids shows neural responses to very low frequencies between 2 and 4 kHz (Yager, 1996). Neither ear in this case was directionally sensitive. The ecological significance of the high-frequency responses appears to be predator avoidance, because the observed motor response to ultrasound pulses consists of a steep dive, based on a change in wing beat and body posture. However, the low-frequency responses seemed unlikely to be involved in predator response, as they have a high latency and do not show a monotonic increase in response strength with stimulus intensity. The low-frequency response was postulated to be involved in intraspecific communication, although acoustic communication has not been observed in these mantids. Conversely, in our case with *O. unnotatus*, the ecological significance of the low-frequency

response of the ATM is clear, because it is selective for the conspecific call, while the ecological significance of the peaks in the high-frequency responses of the PTM is unclear. The other known case of asymmetric tuning between two tympana is found in corixids, or water bugs – where laser vibrometry and electrophysiology have both been used to determine different threshold curves for the two tympana and the underlying receptor cell for each (Prager 1976; Prager and Larsen, 1981). However, the difference between threshold minima for these two tympana was smaller, with the sensitivity of the left receptor cell A1 peaking at 2.35 kHz and that of the right A1 cell peaking at 1.73 kHz. The two tympana also showed a difference in tympanal sensitivity at the threshold minimum, the left A1 cell being more sensitive with a higher vibration velocity at all frequencies of stimulation (Prager and Larsen, 1981). This is quite unlike what we found in *O. unnotatus* with one tympanum showing maximal sensitivity for low frequencies and another for high frequencies.

The only previous assessment of the responsiveness of both the tympanal membranes of a bushcricket suggested no difference between the ATM and the PTM (Bangert et al., 1998). Most other results on bushcricket tympanal transfer functions have only examined the behaviour of one of the two tympanal membranes present on each leg (Michelsen et al., 1994; Michelsen and Larsen, 1978). Vastly dissimilar ATM and PTM responses to sound have also been observed in field crickets (Larsen and Michelsen, 1978),

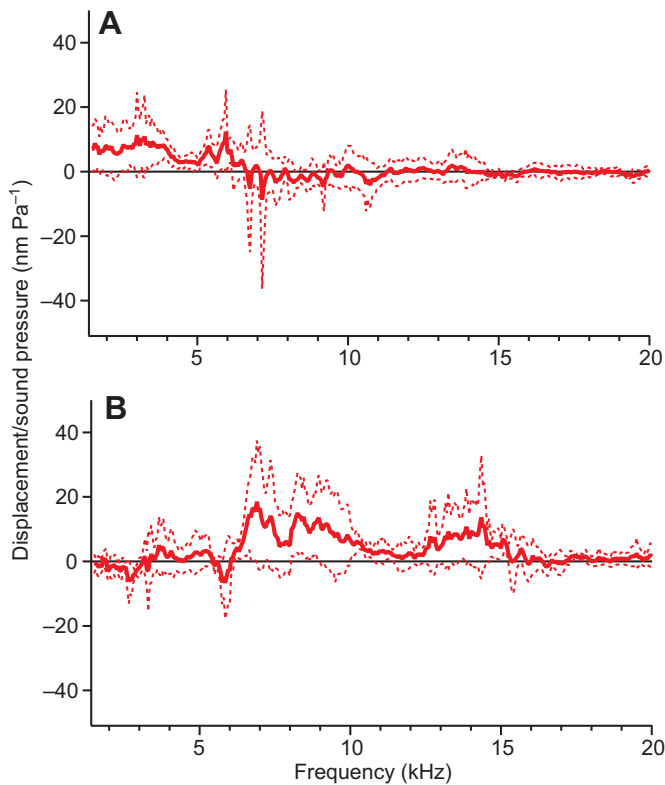


Fig. 7. Directionality of the ATM and PTM responses. Difference in the amplitude of the (A) ATM ($N=6$) and (B) PTM response ($N=4$) between ipsilateral (90 deg) and contralateral stimulation (270 deg). Mean traces (thick line) and standard error envelopes (dashed lines) are shown.

although the two tympanal membranes of tree crickets and wetas have been shown to have similar amplitude and phase spectral responses (Mhatre et al., 2009; Lomas et al., 2011). Our results suggest that the auditory abilities of the animal can only be comprehensively assessed by observing the behaviour of both tympanal membranes.

Both the ATM and the PTM move with the same phase over most of their surface area, and in phase with the acoustic stimulation near the conspecific call frequency. This mechanical behaviour effectively doubles the input to the auditory system, similar to the tree cricket system (Mhatre et al., 2009) and unlike the field cricket system (Larsen and Michelsen, 1978). Each membrane also behaves like a hinged flap, with greater ventral than dorsal displacement. As with other bushcricket tympanal membranes (Bangert et al., 1998; Nowotny et al., 2010), the ventral portion of the membrane is thinner than the dorsal portion near the point where they are jointly tethered to the dorsal wall (Fig. 2C). In further detail, it is worth mentioning that both the ATM and the PTM exhibit oscillations, within a small dorsal area, that are in anti-phase with the rest of the membrane (Fig. 3C,E, Fig. 4C,E). This effect is stronger at higher frequencies (Fig. 4C,E) and is similar to the high-frequency ‘wobbling’ in the dorsal ‘inner plate’ of the ATM of the bushcricket *M. elongata* (Nowotny et al., 2010).

Implications of tracheal morphology

Input from the auditory trachea has been shown to be a contributor to bushcricket auditory responses, usually at frequencies above those of interest to *O. unnotatus* (Nocke, 1975; Michelsen and Larsen, 1978; Mason et al., 1991; Shen, 1993; Kalmring et al.,

1995; Bangert et al., 1998). The frequency tuning of bushcricket tympana is therefore related to the frequency characteristics of tracheal transmission, depending on tracheal geometry. Some tracheae are shaped like an exponential horn (Bangert et al., 1998), a shape expected and found to produce high-pass amplification in the course of tracheal transmission of sound from the spiracle to the tympana (Hoffmann and Jatho, 1995). In other species where tympanal resonances are reported (Nowotny et al., 2010), the resonance frequencies have been attributed to amplification through the cylinder-shaped tracheal tube (Nocke, 1975). For the low-frequency *O. unnotatus*, μ -CT analysis reveals an unusual tracheal anatomy (Fig. 2D), which cannot be readily reduced to either a horn or a tube. *Onomarchus unnotatus* has an unusually small oval spiracular opening (0.364–0.385 mm diameter) leading to a large tracheal tube of nearly uniform diameter (ca. 1 mm; Table 1). This tracheal tube then narrows and remains narrow before expanding into a large cavity and splitting into two branches, each of which backs one of the two eardrums. In addition, the trachea forms a U-bend where the two arms of the bend can potentially interact with each other. As a result, deriving the transfer function between the forces at the front and back face of the tympanum will not be trivial and is certainly not possible from existing models. It is worth noting that the tracheal geometry of another bushcricket, *M. elongata*, also defies simplistic description, being exponentially horn-shaped at either end and tube-shaped in the middle (Nowotny et al., 2010).

The exact contribution of the tracheal input to both ATM and PTM mechanical responses is not yet entirely understood. Fitting a driven SHO model to these transfer function data assumes that sound is delivered only directly to the tympanum, i.e. that it is a simple pressure receiver. When amplitude data are fitted to such a model, the phase of the ATM relative to the impinging sound does not match or show behaviour like a typical sub-critically damped SHO, i.e. a linear decrease from 90 to -90 deg around resonance frequency. The spatial maps exclude the possibility that these modes are membrane modes. Therefore, the difference of the measured phase response from the phase predicted by the SHO model suggests the presence of an additional driving force upon the tympanum. This is consistent with the idea that these ears operate as pressure difference receivers, with sound impinging directly on the front of the membrane as well as on the back of the membrane due to tracheal transmission, resulting in a difference between the phase and amplitude of the effective force on the tympanum and the phase and amplitude of the incident sound. The changing impedance of an oddly shaped trachea is likely to change the amplitude and phase of the force impinging on the membrane. Thus, the amplitude and phase of the membrane’s oscillations will show driven SHO-like behaviour not with respect to the direct sound, but rather with respect to this altered force function. While this force function is non-trivial to derive, the mismatch between the phase of the SHO model fit and the direct measurement strongly implicates the involvement of the tracheal system.

Directionality of tympanal responses

Auditory directionality is an interesting problem in *O. unnotatus*. The wavelength of the conspecific song is 10.6 cm, i.e. much larger than the distance between the ears positioned on the two forelegs (ca. 1 cm). Thus, interaural intensity differences based on diffraction alone would hardly be distinguishable in this system, as in many insect species (Robert, 2005). Temporal cues, although not excluded, are also limited. Because the tracheal system is not connected across the body and does not allow for tracheal

contralateral input, the four-input model proposed for crickets (Larsen and Michelsen, 1978) is an unlikely candidate for the generation of directionality. Further research is required to fully establish the biophysical basis of directional hearing in these katydids.

The ATM exhibits a directional mechanical response to frequencies in the range of the male call, while the PTM's response is not directional in that same range. For the PTM, directionality arises only for higher frequencies, ca. 7 and 14kHz. The conspecific song does contain some energy near 7kHz; however, the relative amplitude is more than 20dB SPL lower. The behavioural and ecological relevance of these higher frequencies, in terms of hearing predators and perhaps other members of its syntopic species ensemble, constitutes an outstanding question in this species and will be the subject of future studies. As a morphologically cryptic species with beautiful leaf-like wings, *O. oninotatus* constitutes an excellent and amenable system with which to study the influence of predator-prey interactions upon the complexity of multimodal communication.

LIST OF SYMBOLS AND ABBREVIATIONS

<i>A</i>	amplitude of transfer function in SHO
ATM	anterior tympanal membrane
PTM	posterior tympanal membrane
SHO	simple harmonic oscillator
SPL	sound pressure level
γ	damping ratio of SHO
ν	resonant frequency of SHO
ω	angular frequency

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