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ORIGINAL PAPER

# Mechanical response of the tympanal membranes of the tree cricket *Oecanthus henryi*

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**Abstract** Crickets have two tympanal membranes on the tibiae of each foreleg. Among several field cricket species of the genus *Gryllus* (Gryllinae), the posterior tympanal membrane (PTM) is significantly larger than the anterior membrane (ATM). Laser Doppler vibrometric measurements have shown that the smaller ATM does not respond as much as the PTM to sound. Hence the PTM has been suggested to be the principal tympanal acoustic input to the auditory organ. In tree crickets (Oecanthinae), the ATM is slightly larger than the PTM. Both membranes are structurally complex, presenting a series of transverse folds on their surface, which are more pronounced on the ATM than on the PTM. The mechanical response of both membranes to acoustic stimulation was investigated using microscanning laser Doppler vibrometry. Only a small portion of the membrane surface deflects in response to sound. Both membranes exhibit similar frequency responses, and move out of phase with each other, producing compressions and rarefactions of the tracheal volume backing the tympanum. Therefore, unlike field crickets, tree crickets may have four instead of two functional tympanal membranes. This is interesting in the context of the outstanding question of the role of spiracular inputs in the auditory system of tree crickets.

**Keywords** Auditory organ · Cricket · Orthoptera · Eardrum · Tree cricket

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## Introduction

The mechanism of hearing in field crickets has been the subject of extensive investigation (Larsen et al. 1989; Michelsen 1998) and forms the basis of our knowledge about the entire gryllid family (true crickets). However, there are several distinctive groups within this family which use acoustic communication, but have different body types, ecological niches and behavioural repertoires (Zuk and Simmons 1997). Each of these groups may have found distinct solutions to the problems of hearing the songs of their conspecific males and localizing them.

Field crickets and tree crickets (Fig. 1a) fall within the family Gryllidae, but belong to two different subfamilies: Gryllinae and Oecanthinae, respectively (Chopard 1969). Tree crickets are quite different from field crickets. Apart from other ecological differences, the calling songs of tree crickets have, on average, lower carrier frequencies (2–4 kHz) (Metrani and Balakrishnan 2005) compared to field crickets (3–8 kHz) (Otte 1992). In addition, calling song carrier frequency in tree crickets changes dramatically with temperature, a feature not observed in most field crickets (Walker 1962a, b; Metrani and Balakrishnan 2005).

These differences between the two sub-families raise interesting questions about the possible mechanisms underlying hearing among their members. A damped resonance of the tympanal membrane close to conspecific carrier frequency has been reported in field crickets (Paton et al. 1977; Larsen and Michelsen 1978). A fixed resonant system would be maladaptive in the Oecanthinae, where the carrier frequency varies with temperature by as much as 100–200 Hz/°C (Walker 1962a, b; Metrani and Balakrishnan 2005). Among field crickets, directionality is produced by the diffraction of sound and the phase differences due to distance between the different sound inputs in a pressure

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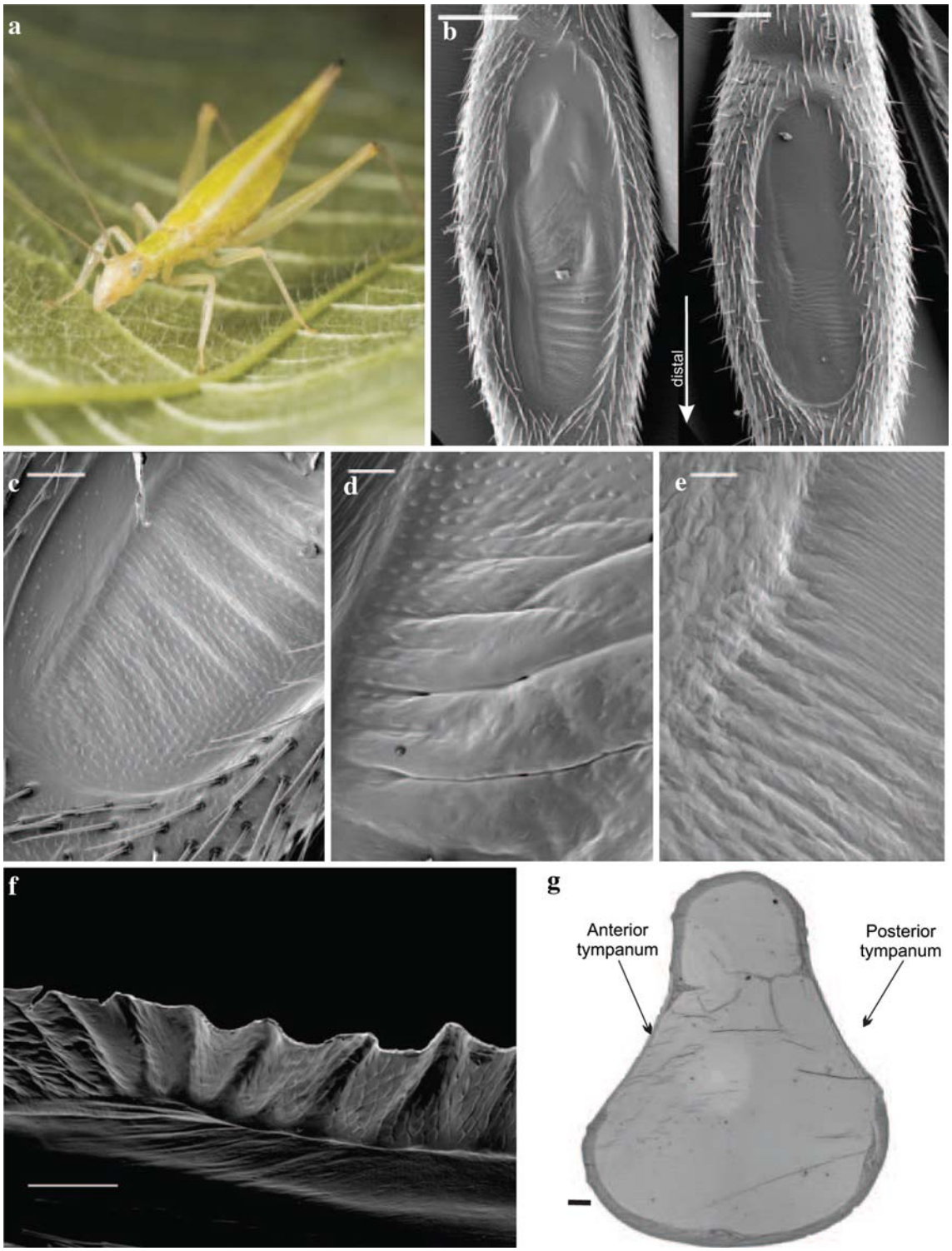


**Fig. 1** Anatomy of the tree cricket ear. **a** A female *Oecanthus henryi*. Average body length 12.5 ± 0.7 mm (Metrani and Balakrishnan 2005). **b** SEM images of the anterior and posterior tympanal membranes of an *O. henryi* female (*scale bar* 200 μm). **c** A SEM of the ridges on the distal portion of the anterior tympanal membrane (*scale bar* 50 μm). **d** A SEM of the ridges on the central part of the anterior tympanal membrane (*scale bar* 20 μm). **e** A SEM of the ridges on the distal portion of the posterior tympanal membrane (*scale bar* 20 μm). **f** A SEM of a vertical section of the anterior TM (*scale bar* 50 μm). **g** A light transmission microscope image of a transverse section through the leg of a *O. henryi* female showing the orientation of the two tympanal membranes with respect to each other (*scale bar* 20 μm)

difference receiver system (Michelsen et al. 1994). However in an animal the size of a tree cricket, significant diffraction of calling song is not expected since the song has a low carrier frequency and hence a long wavelength. Similarly, there will be very small phase differences in calling song at the four putative inputs. Hence, questions about the mechanisms underlying directionality in this system remain unanswered.

Field crickets possess two tympanal membranes (TMs) on each fore-tibia, the anterior tympanal membrane (ATM) and the posterior tympanal membrane (PTM). The two membranes are markedly asymmetric in several properties. The ATM is significantly smaller than the PTM and responds significantly less than the PTM to acoustic stimulation (Larsen and Michelsen 1978; Larsen 1987). Behaviourally, the occlusion of the ATM with wax does not significantly disrupt phonotaxis (Bailey and Thomson 1977), whereas when the PTM is occluded, the threshold for this behaviour is raised significantly (Huber et al. 1984). It has been shown that if the vibrations of the PTM are prevented, neuronal responses to the sound are eliminated (Kleindienst et al. 1983). Hence, it is believed that the PTMs are principally responsible for hearing among field crickets, while the ATMs play a minor role when the PTMs are occluded (Huber et al. 1984; Schmitz 1985; Larsen 1987).

The TMs of tree crickets however do not show a marked size difference; the ATM is slightly larger than the PTM, whereas in field crickets, the PTM is significantly larger than the ATM. The two membranes are also structurally more complex than field cricket membranes. These structural dissimilarities suggest that there might be differences in the functions of the ATM and PTM between field crickets and tree crickets.

In this paper, we have thus investigated the mechanical responses of the ATM and PTM of a tree cricket (*Oecanthus henryi*) to sound using microscanning laser Doppler vibrometry. We have focused on the magnitude of the response of the ATM and PTM at different sound frequencies and the phase of the motion of the two membranes with respect to each other.

## Materials and methods

### Animals

Experiments were carried out using nine wild-caught adult *O. henryi* females. *O. henryi* is a tree cricket species found in southern India and Sri Lanka (Chopard 1969; Metrani and Balakrishnan 2005). The animals were maintained individually in plastic vials with foam caps in the laboratory under standard conditions of 12:12 h light:dark cycles at room temperature (26–28°C). Food (apple pieces) and water were provided ad libitum. In order to make tympanic vibration measurements, each animal was immobilized ventrum down, and dorsally glued to a rectangular brass bar (5 × 1 × 60 mm) using liquid latex (Magnacraft, Midhurst, UK). The brass bar was connected to a metal rod (150 mm long, 8 mm diameter) by a thumbscrew, allowing the animal to be manipulated into required position. The legs of the animal were placed in a standing posture, ATM forward, and the tarsi glued to a wooden balsa block, which was also mounted on a brass bar connected to a rod with a thumbscrew. See Fig. 2 for details of the preparation.

Measurements were made on both the ATM and PTM of one ear. The animal was oriented such that the laser vibrometer could be repositioned around it and was able to scan each tympanal membrane separately. The laser beam was perpendicular to the tympanal membrane being measured and could scan its entire surface. Ipsilateral stimulation was used in all of the experiments described in this paper.

All experiments were carried out on a vibration isolation table (TMC 784-443-12R; Technical Manufacturing Corp., Peabody, MA, USA) at room temperature (26–28°C). The vibration isolation table and the experimental setup on it

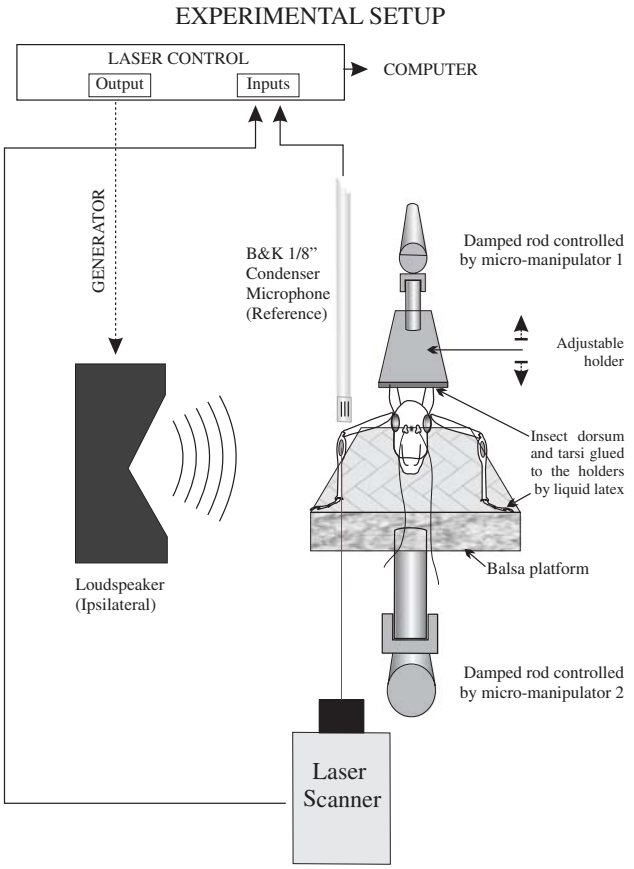


**Fig. 2** Design of experimental arena and preparation. Not to scale

were placed in an acoustic isolation booth (IAC series 1204A; 4.50 × 2.25 × 1.98 m; Industrial Acoustics, Bronx, NY, USA).

## Morphology

For morphological studies of the tympanum, the fore-tibiae were removed from two freshly killed females and tympanal structure observed using scanning electron microscopy (SEM). Specimens mounted on stubs were gold-coated and studied using a Philips 501B Scanning Microscope (Netherlands, Eindhoven). SEM images were digitized with a Keithley DAS 1202 plug in card (Keithley Instruments, Tauton, MA, USA), and the software SEM 1.2 (A. Gebert & G. Preiss, Medical School, Lab of Cell and Electron Microscopy, Hannover, Germany). Details of the tympanal orientation were studied using transverse sections of the fore-tibia according to the process described by Di Sant'Agnese and De Mesy Jensen (1984). Microtome sections were mounted on slides and examined in a light transmission microscope (JEOL, 1200 EX, Tokyo, Japan).

## Acoustic stimulation and mechanical measurements

The mechanical response of the two membranes to sound was investigated using both broadband acoustic stimuli (periodic chirps) and pure-tone stimuli. In particular, we concentrated on the magnitude of response shown by both membranes and the phase of the motion of the two membranes with respect to each other. The SPLs of the stimuli used for our experiments were based on both calling song SPLs recorded from males in the field and on SPLs known to be sufficient to produce localization behaviour in females (personal observation R. Balakrishnan). The wideband signals were always presented at 40 mPa [66 dB SPL (re $2 \times 10^{-5}$ N/m$^2$), from 2 to 7 kHz] and 15 mPa [57 dB SPL (re $2 \times 10^{-5}$ N/m$^2$), from 2 to 20 kHz]. The amplitude of the signals was kept constant over the entire frequency range (Fig. 3a). The sinusoidal signals were presented at 4 Pa peak to peak (Fig. 3b).

Vibrations of the ATM and PTM were examined in response to acoustic stimulation by periodic chirp signals of frequency range 2–7 and 2–20 kHz (Fig. 3a). The wideband signals were generated by the Polytec Scanning Vibrometer

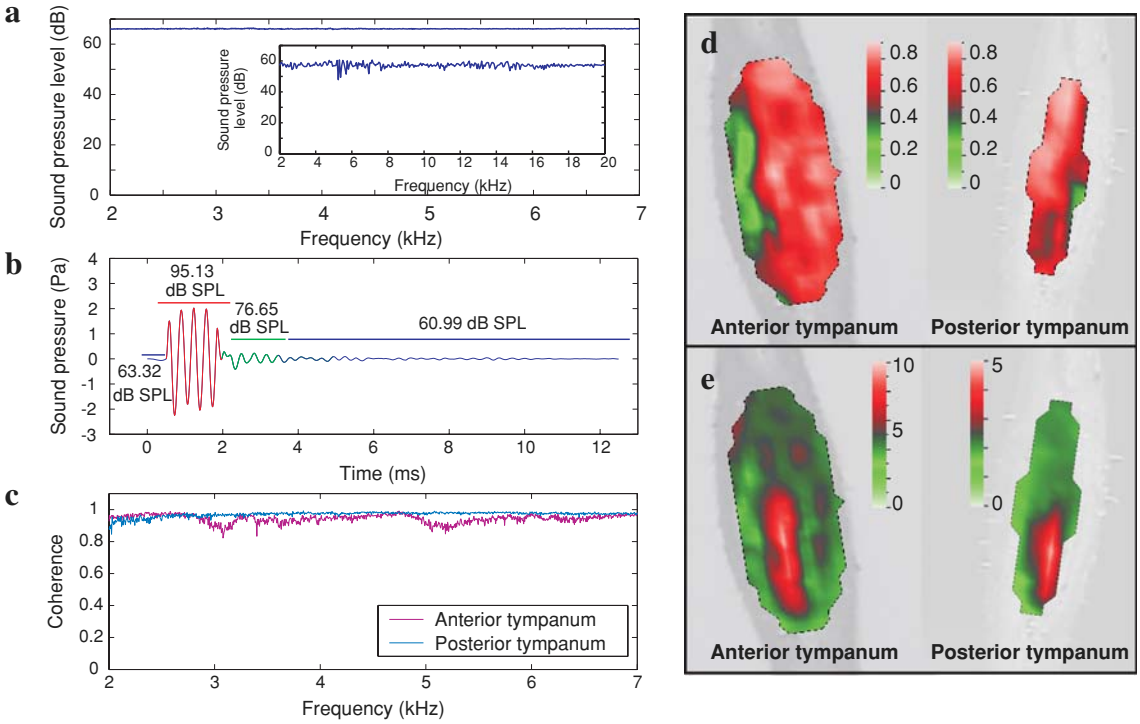


**Fig. 3** Acoustic stimuli and mechanical response of the anterior and posterior tympanal membranes. **a** Frequency spectrum of the incident stimulus, the reference signal over the two bandwidths used for stimulating the specimen. **b** An example of the stimuli (at 3 kHz) used for examining the time domain responses of the tympanal membranes. The RMS sound pressure levels of the different parts of the stimulus are indicated in the figure. **c** The coherence between the vibrometer and microphone signals of the parts of the two tympanal membranes that show the maximal deflection when stimulated with sound. **d** Coherence maps of the two tympanal membranes of the right leg of an individual between 2.3 and 3.3 kHz, the range of frequencies at which *O. henryi* males are known to call (optimal coherence obtained at 1). **e** The displacement of different parts of the same tympanal membranes in nm/Pa when stimulated by sound within the same bandwidth

software (version 7.4, Polytec Gmbh, Waldbronn, Germany) through a data acquisition board (National Instruments, PCI-4451; Austin, TX, USA), amplified (Sony Amplifier Model TA-FE570; Tokyo, Japan) and passed to a loudspeaker (ESS AMT-1; ESS Laboratory, Inc., Sacramento, CA, USA) positioned 13 cm from the animal. The TMs were also stimulated using sinusoidal sound waves at different frequencies (2.5, 3, 4, 4.5, 5 and 7 kHz) presented either in one or four cycle bursts (Fig. 3b) produced using a signal generator (Agilent 33120A, Agilent Technologies, Santa Clara, California, USA), amplified and broadcast in the same way as the periodic chirps. Acoustic stimuli were measured and recorded simultaneously with the vibrometric measurements using a 1/8 inch precision pressure microphone (Brüel and Kjær, 4138; Naerum, Denmark) and preamplifier (Brüel and Kjær, 2633). The microphone has a flat response in the measured frequency range. The microphone was positioned approximately 2 mm directly above the thorax of the animal.

Vibration velocities at different points on the TMs on a scanning lattice were measured simultaneously with the acoustic stimuli. Vibration velocities were measured using a microscanning laser Doppler vibrometer (Polytec PSV-300-F; Waldbronn, Germany) with an OFV-056 scanning head fitted with a close-up attachment. This allows the laser spot (~5 μm diameter) to be positioned with an accuracy of ~1 μm. Measurements across an entire TM could be taken without readjusting the position of any component in the experiment. The laser spot position was monitored via a live video feed to the vibrometer's controlling computer. This allowed us to correlate the motion of the two TMs with their morphological structure.

### Evaluation of data

The analysis of membrane velocity and the SPL was carried out by the laser control software (Polytec Scanning Vibrometer, version 7.4, Polytec GmbH, Waldbronn, Germany) in the vibrometer's control PC. The velocity of vibration of the membrane was sampled simultaneously with the acoustic signal at a minimum sampling rate of 82 kHz. Averages of 20–50 responses were made at each point in the scanning lattice. Using an Fast Fourier transform (FFT) with a rectangular window, a frequency spectrum was produced with a resolution of 6.25 Hz for the smaller bandwidth signal and a resolution of 62.5 Hz for the larger bandwidth. The laser and microphone signals were then used to calculate different quantities such as the gain and phase of the responses. Data from all points across the lattice were used to generate profiles and animations of tympanal deflections in the bandwidth of interest, i.e. the bandwidth of the frequencies of calling song of *O. henryi* as recorded in the field (Metrani and Balakrishnan 2005). For this study, the transfer function (*m*/Pa) of the membrane displacement (*m*) with respect to the reference sound level of the acoustic stimulus (Pa) and the coherence between the vibrometer and the microphone signals was calculated as in Windmill et al. (2005). When coherence exceeded 85% in parts of the membrane that showed significant displacement, the data were considered reliable (Fig. 3c).

## Results

### Tree cricket tympanal membranes: anatomy

The TMs are present on the proximal part of the fore-tibiae (Fig. 1a). The part of the leg that accommodates these membranes is flattened along the antero-posterior axis, but is inflated laterally (Fig. 1a, g). The ATM is slightly larger than the PTM (Fig. 1b) and is structurally more complex, having more pronounced transverse folds in the central and distal regions, and a pit-like structure at the proximal end (Fig. 1b, c, d). The PTM in comparison has transverse folds that are much less pronounced only on the distal part of the membrane (Fig. 1e). The ATM has a thickness of approximately 2 μm in section (Fig. 1f). The PTM appears to have a similar thickness (Fig. 1g). The two membranes form an angle of circa 60° (each membrane is oriented at 30° from the sagittal plane that longitudinally divides the leg in two halves) (Fig. 1g).

### Tree cricket tympanal membranes: mechanical responses

The calling songs of *O. henryi* males recorded in the field varied with changing temperature between 2.3 and 3.3 kHz (Metrani and Balakrishnan 2005). When stimulated with sound within this band of frequencies, a large proportion of the two membranes moved coherently with sound (Fig. 3d). Significant motion in the two TMs, however, was restricted to small portions of the membranes (Fig. 3e). High coherence in the bandwidth of interest, i.e. above 85% (Fig. 3c), was recorded only within these areas of the membrane. Since the positions of the scanning lattice on the membranes were aligned with a live video feed of the membrane, the mobile areas could be identified with respect to the microstructure of the membranes. These areas were found to lie close to the region of the TMs, where the transverse folds are. In the ATM, the part of the membrane that showed maximal deflection appeared to lie on the pronounced folds in the central region (Figs. 1b, 3e). The mobile areas of the ATM and the PTM, on the basis of the orientation of the two membranes with respect to each other and their positions on the TMs, appeared to be aligned with each other (Fig. 3e).

The absolute amount of displacement in the mobile areas of the two membranes was, however, quite small. In the

band of frequencies of interest, at the behaviourally relevant stimulus SPL used, i.e. 66 dB SPL, for the exemplar female in Fig. 3, the point that showed maximal deflection on the ATM moved about 0.4 nm (Fig. 3e). The corresponding average value across all animals was 0.24 ± 0.08 nm (mean ± SE, $N = 9$). On the PTM, the corresponding value for the exemplar membrane was 0.2 nm (Fig. 3e) and the average across all animals was 0.20 ± 0.09 nm (mean ± SE, $N = 9$). In comparison, the TMs were approximately 2,000 nm in thickness (Fig. 1f).

In order to study the deflection envelopes of the mobile areas, two transect lines were drawn across them. One was drawn along the length of the mobile area in the longitudinal direction and the other along the width in the latitudinal direction (Fig. 4a). A stimulus of 3 kHz was used to investigate the deflection envelopes of the membrane, since this was the approximate frequency at which *O. henryi* males would call at the temperatures recorded in the anechoic chamber (Metrani and Balakrishnan 2005). The instantaneous velocities of the membrane at all points along the transects were recorded and plotted at 13° intervals. The deflection envelopes of the membrane (Fig. 4b) in response to a single cycle of acoustic stimulus were recorded (Fig. 4c). The instantaneous velocity through the entire stimulation cycle as recorded at the point that shows maximal velocity is also shown (Fig. 4d). The part of the membrane response that is depicted in Fig. 4b is highlighted in Fig. 4d.

At this frequency, the TMs appeared to show simple standing wave-like behaviour (Fig. 4b). The nodes of the standing wave lay within the membrane and did not coincide with the part of the membrane that was attached to the leg cuticle. The anti-node of the standing wave, the point on the membrane that showed the maximal deflection, also

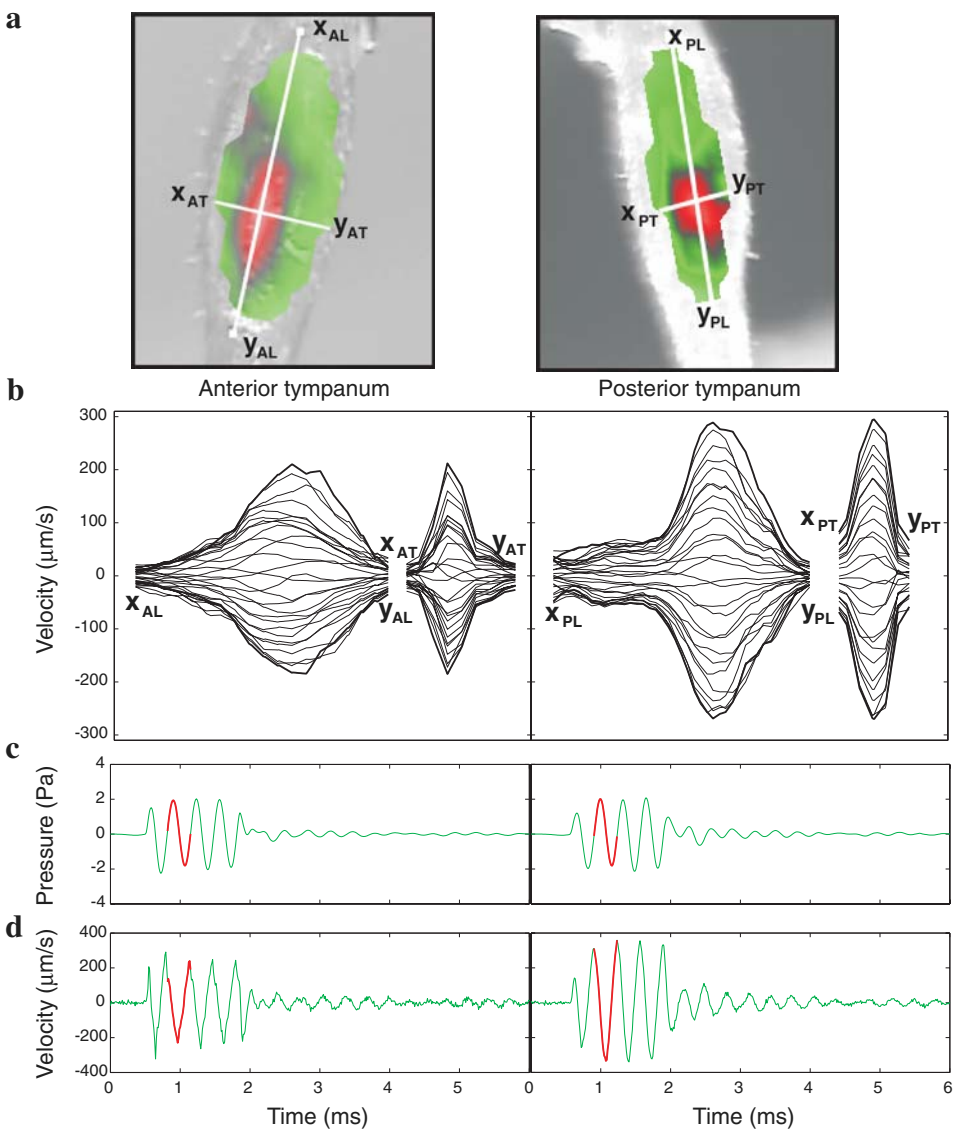


**Fig. 4** Envelopes of the deflections of the tympanal membranes (right leg) along transect lines in response to a sinusoidal stimulus at 3 kHz. **a** The positions of the transect lines are shown on a displacement map on the anterior and posterior tympanal membrane. **b** Deflection envelopes showing instantaneous velocities along the transects on both tympanal membranes at different phases in a complete stimulus cycle. Since the laser assembly was moved to record from both tympanal membranes, the distances on the *X* axis between the two tympanal membranes are not comparable, but are only comparable within each tympanal membrane plot. **c** The stimulus used to excite the tympanal membranes. The cycle of the stimulus, which was used to construct the deflection envelopes, is highlighted in *red*. **d** The response of the membrane to the presented stimulus as measured at the point on the membrane that deflects with the greatest velocity. The part of the response, which corresponds to the stimulus in time, is highlighted in *red*

remained nearly constant throughout the cycle (Fig. 4b). The maximal velocity shown by the TMs in response to the stimulation was quite low. For the exemplar female in Fig. 4, the maximal velocity on both TMs was only about 0.3 mm/s, which is comparable to the maximal velocity of 'non-functional' ATM and cuticle in *Gryllus campestris* (Larsen and Michelsen 1978). The average maximal velocity across females for the ATM was $0.37 \pm 0.07$ mm/s (mean $\pm$ SD, $N = 6$) and for the PTM was $0.40 \pm 0.2$ mm/s (mean $\pm$SD, $N = 6$).

## Differential responses of the anterior and posterior tympanal membranes

The response gains of the maximal deflection points on ATM and PTM across a broad range of frequencies were calculated with respect to the acoustic stimuli (Fig. 5a, c). Similarly, the phases of the mechanical vibrations with respect to the acoustical stimuli at these points on the TMs were also calculated (ATM: Fig. 5b; PTM: Fig. 5d). Both the ATM and the PTM showed similar levels of displacement in response to sound across a range of frequencies (Fig. 5a, c). There were also no marked phase transitions in the range of 2–7 kHz (with the exception of one animal; Fig. 5b, d). The phase of the membrane response gradually drifted away from the 0° baseline with an increase in frequency. This drift is probably due to the small time delay between the signal reaching the microphone and the tympanal membrane. This delay will increase in terms of phase at higher frequencies causing the pattern seen here (Fig. 5b, d). In the broader frequency band, we saw the phase spectrum drifting below −180° and the phase plot becomes noisier thereafter, but yet does not show marked phase transition behaviour.

When the response gain of each ATM was subtracted from the response gain of the PTM of the same female, difference spectra were obtained (green traces in Fig. 5e). The average difference spectra for response gain showed that the difference between the two TMs was very close to 0 nm/Pa across a wide range of frequencies (red trace in Fig. 5e). This suggests that both membranes are equally displaced by acoustic stimulation.

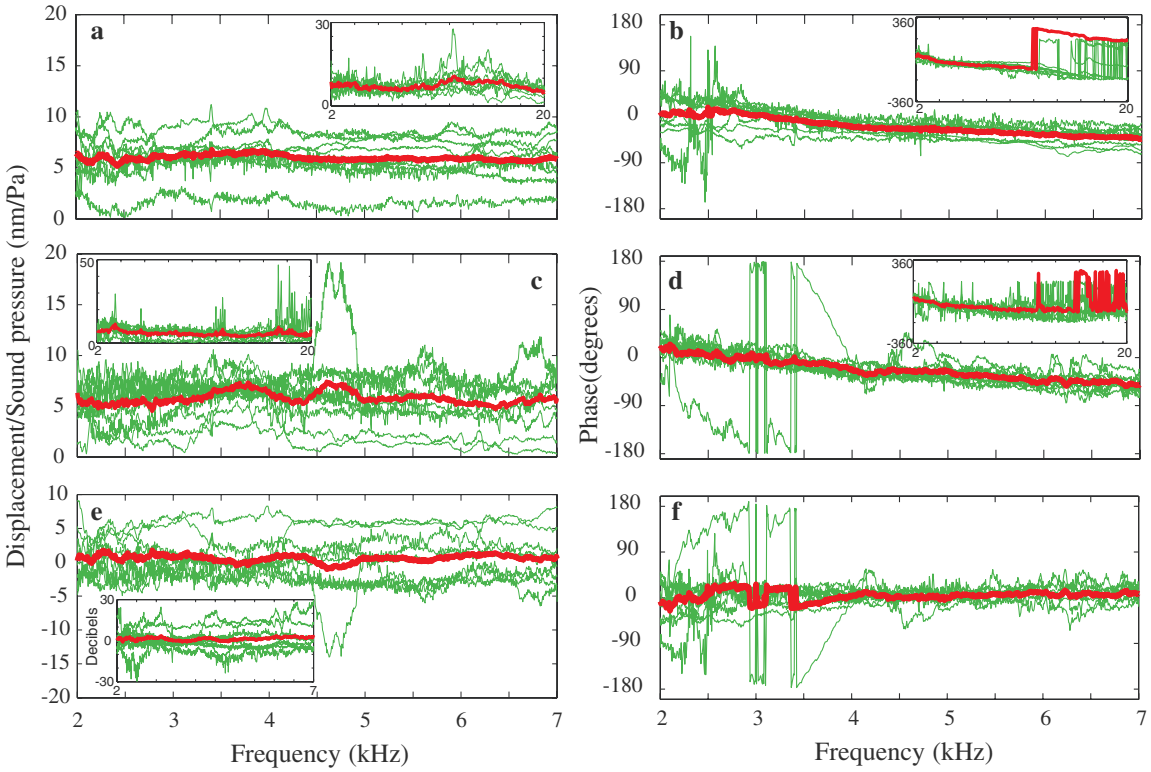


**Fig. 5** Mechanical response of the ATM and the PTM at the point of maximal deflection in each membrane. Data are based on nine females each plotted in *green*. The average responses are plotted in *red*. **a** Response gain and **b** phase of membrane vibrations of the anterior tympanal membranes. The inset shows data for the broader bandwidth between 2 and 20 kHz. **c** Response gain and **d** phase of membrane vibrations of the posterior tympanal membranes. The *inset* shows data for the broader bandwidth between 2 and 20 kHz. **e** Difference between the response gain of the ATM and the PTM. The *inset* shows the same data on a decibel scale. **f** Difference between the response phase of the ATM and the PTM. The response gains and the phases of the membrane vibrations are based on the transfer functions between the measured vibrations and the measured incident acoustic stimuli

To determine the direction of displacement of the two membranes with respect to each other, the phases of the mechanical vibrations of the TMs with respect to the acoustic stimuli were subtracted from each other. The difference spectra between the phases of the two membranes tended to 0° both in individual samples, (with a single exception, green traces in Fig. 5f) and on average (red trace in Fig. 5f).

In order to interpret these data, let us consider two possibilities. Either the membranes move in the same direction or they move in opposite directions (Fig. 6a). If the membranes move in the same direction, one of the membranes will move out of phase with respect to the external pressure, i.e. the acoustic stimuli (Fig. 6a). If the membranes move in opposite directions, each membrane will move in phase with respect to external pressure, i.e. the acoustic stimuli (Fig. 6a). Hence, when the vibrations of the ATM and the PTM are in phase with acoustic stimuli, it means that the two membranes are moving in opposite directions (Fig. 6a). As we can see, in this case (Fig. 6a), the phase of vibrometric measurements of the two TMs will be the same and the phase difference spectrum will tend to 0°. In contrast, if one of the membranes moves out of phase with the acoustic stimuli, then the two membranes are moving in the same direction (Fig. 6a) and the vibrometric measurements of the two TMs will be different and the difference spectrum will tend to 180°. Since the difference spectrum of the two membranes of *O. henryi* tend to 0° (Fig. 5f), it appears that the ATM and PTM in *O. henryi* move in opposite directions with respect to each other, causing compressions and rarefactions of the tracheal volume backing the tympanal system.

To confirm the expectations derived from this data, the membrane responses to sinusoidal sound stimuli were examined. The acoustic stimuli used to stimulate the ATM and PTM were aligned in time in order to account for small differences in when the stimulus appears in the recording window (Fig. 6b). The responses of the two membranes were then aligned in time to their respective stimuli in order to account for transmission delays between the microphone and vibrometer signals. When these time-corrected signals were viewed with respect to each other, the ATM and PTM vibrometer traces were seen to be in phase with their acoustic stimuli (Fig. 6c) and hence in opposite directions with respect to each other (Fig. 6a), confirming the inference made from Fig. 5f.

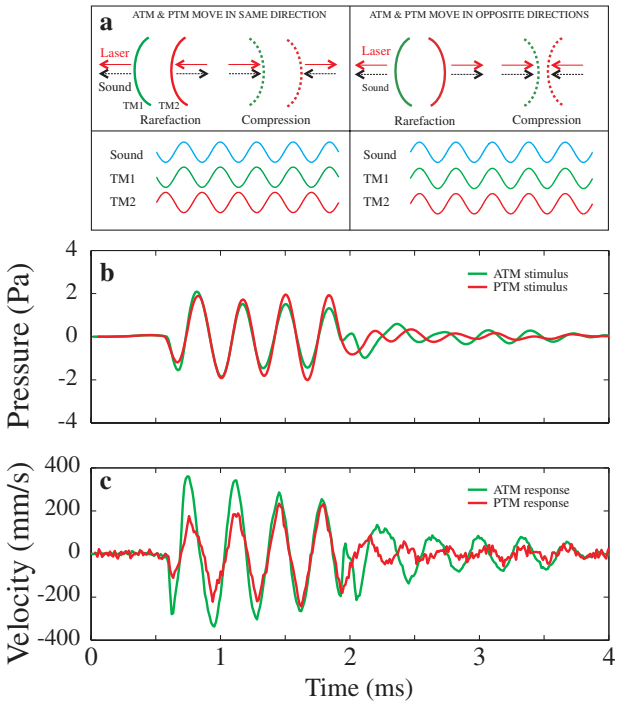


**Fig. 6** Response of ATM and PTM of an animal in the time domain when stimulated with sound at 3 kHz. **a** Cartoons depicting the motion of the membranes and the corresponding vibrometer and microphone recordings that would be obtained if the two membranes moved in the same or opposite directions. The *stippled arrows* in the *upper panels* represent the direction of the forces applied by sound pressure on the tympanal membranes in the rarefaction and compression phases of a cycle of sound. The *red arrows* indicate the direction of vibration of the membrane as measured by the laser Doppler vibrometer. The waveforms shown below depict the waveforms that will be obtained by the microphone and those obtained by the vibrometer for TM1 and TM2. See text for details. **b** Incident acoustic stimuli recorded when measuring the mechanical vibrations of the ATM and the PTM. **c** Recorded response of the ATM and the PTM at the point of maximal velocity on each membrane

## Discussion

### Tree cricket tympanal membranes

The TMs of *O. henryi* are not only anatomically different and more structurally complex compared to those of gryllid membranes, but also quite distinct in vibrational behaviour. The entire TMs of *G. pennsylvanicus* appear to vibrate in response to sound (Paton et al. 1977). Our observations of the tympanal membrane of *G. bimaculatus* using the same setup as described in this paper show that the entire TM vibrates. The parts of the membrane attached to the leg cuticle behave like the nodes and the central portion like the anti-node (Fig. 7). The *O. henryi* TMs, however, show quite distinct behaviour. Only small portions of the TMs were set in motion in response to sound. Both the nodes and the anti-nodes of the vibrating portion lay within the TMs (Fig. 3e).

The velocity of the *G. bimaculatus* ATM near conspecific song frequency at a stimulation SPL of 100 dB is reported to be 0.3 mm/s, the PTM under presumably similar stimulus conditions being about 5 mm/s (Larsen and Michelsen 1978). In contrast, both the ATM and PTM of *O. henryi* show a vibration velocity similar to the

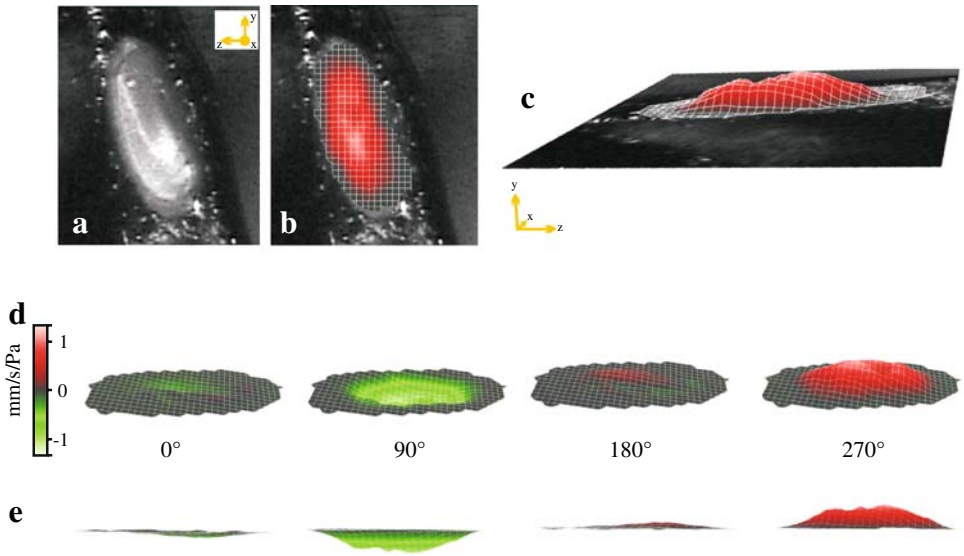


**Fig. 7** Area scan and deflection shapes of the PTM in *Gryllus bimaculatus*. Orientation image relating tympanal topography (**a**) to the position of the scanning lattice (**b**, **c**). (**d–e**) Area scans of tympanal deflections at 5 kHz. The deflections are shown as two perspectives, looking at the tympanum from its side, and each time for four different phases. Note that maximal deflection occurs in the central region of the membrane. *Red* indicates positive displacement (or outward tympanal deflections), and *green* indicates negative displacement (or inward tympanal deflections)

*G. bimaculatus* ATM velocity, which was believed to be too small to be physiologically relevant, at least when the PTM was intact (Larsen and Michelsen 1978; Larsen 1987). Also, the average displacement of the *O. henryi* ATM and PTM (0.2–0.25 nm) at behaviourally relevant stimulus intensities (Rohini Balakrishnan and Monisha Bhattacharya, personal communication) was only a small fraction of the apparent membrane thickness (2,000 nm). However, mechano-sensory neurons are capable of responding to similar deflection levels usually using active auditory processes. Mosquito antennae when deflected by near-field sound by as little as 7 nm at the tip and 0.3 nm at the base of the Johnston's organ generate a neuronal response (Robert and Göpfert 2002). It is unknown whether *O. henryi* TM thickness is spatially variable and whether the membrane becomes thinner in the region that shows displacement. A more complete histological investigation of the tympanal organ of *O. henryi* will be required to investigate this possibility.

## Differential response of the two TMs

The two *O. henryi* TMs are not only similar in size, but also similar in response amplitude, unlike the *G. bimaculatus* TMs which show a 20- to 50-fold or 20 dB difference in response velocity (Larsen and Michelsen 1978; Larsen 1987). The positions of the responsive parts of the *O. henryi* TMs appear to lie close to each other in space. The antinodes of the two areas move out of phase with each other, thus creating compressions and rarefactions in the tracheal volume backing the tympanal organ, in phase with the external sound pressure. Thus, since the TMs are moving at circa equal amplitudes in opposite directions, the total displacement affecting the stretch receptors in the tympanal organ could be effectively doubled if the receptors were placed between these two mobile regions of the TMs. This would provide a larger signal than if the TMs moved in the same direction. In contrast, the two *G. bimaculatus* TMs at 5 kHz appear to move in the same direction (since one of them moves out of phase with its acoustic stimuli) (observed in Figs. 4, 5 in Larsen and Michelsen 1978). In this situation, it is possible that the larger PTM forces the motion of the smaller ATM.

Finally, it remains to be investigated whether the two membranes are mechanically coupled in any way and directly interact with each other. This is especially interesting in view of directionality generated by mechanically coupled TMs that have been discovered in other small insects (Robert et al. 1996, 1999; Robert 2005). *O. henryi* and tree crickets in general as mentioned before are much smaller than *G. bimaculatus*. The mechanism shown to produce directionality in the field cricket system remains to be examined in the tree cricket system. The mechanism suggested for producing directional cues in the field cricket system would be insufficient to produce such cues in the tree cricket system, leaving open the question of whether and how directionality is produced in the ears of these insects. The distinctive behaviour of the ATM and PTM of this insect suggests interesting new possibilities particularly vis-à-vis directionality producing mechanisms in other small insects that have been investigated (Miles and Hoy 2006).

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## References


Bailey W, Thomson P (1977) Acoustic orientation in the cricket *Teleogryllus oceanicus* (Le Guillou). J Exp Biol 67:61–75

Chopard L (1969) The fauna of India and adjacent countries: Orthoptera. Baptist Mission Press, Calcutta

Di Sant'Agnese PA, De Mesy Jensen KL (1984) Dibasic staining of large epoxy tissue sections and applications to surgical pathology. Am J Clin Pathol 81:25–29

Huber F, Kleindienst H-U, Weber T, Thorson J (1984) Auditory behavior of the cricket. J Comp Physiol A 155:725–738

Kleindienst H-U, Wohlers DW, Larsen ON (1983) Tympanal membrane motion is necessary for hearing in crickets. J Comp Physiol A 151:397–400

Larsen ON (1987) The cricket's anterior tympanum revisited. Naturwissenschaften 74:92–94

Larsen ON, Michelsen A (1978) Biophysics of the ensiferan ear. III. The cricket ear as a four input system. J Comp Physiol A 123:217–227

Larsen ON, Kleindienst H-U, Michelsen A (1989) Biophysical aspects of sound reception. In: Huber F, Moore T, Loher W (eds) Cricket behavior and neurobiology. Cornell University Press, Ithaca, pp 365–390

Metrani S, Balakrishnan R (2005) The utility of song and morphological characters in delineating species boundaries among sympatric tree crickets of the genus *Oecanthus* (Orthoptera: Gryllidae: Oecanthinae): a numerical taxonomic approach. J Orth Res 14:1–16

Michelsen A (1998) The tuned cricket. New Physiol Sci 13:32–38

Michelsen A, Popov AV, Lewis B (1994) Physics of directional hearing in the cricket *Gryllus bimaculatus*. J Comp Physiol A 175:153–164

Miles RN, Hoy RR (2006) The development of a biologically-inspired directional microphone for hearing aids. Audiol Neurootol 11:86–94

Otte D (1992) Evolution of cricket songs. J Orth Res 1:25–49

Paton JA, Capranica RR, Dragsten PR, Webb WW (1977) Physical basis for auditory frequency analysis in field crickets (Gryllidae). J Comp Physiol A 119:221–240

Robert D (2005) Directional hearing in insects. In: Popper AN, Fay RR (eds) Sound source localization. Springer, NY, pp 6–36

Robert D, Göpfert M (2002) Novel schemes for hearing and orientation in insects. Curr Opin Neurobiol 12(6):715–720

Robert D, Miles RN, Hoy RR (1996) Directional hearing by mechanical coupling in the parasitoid fly *Ormia ochracea*. J Comp Physiol A 179:29–44

Robert D, Miles R, Hoy R (1999) Tympanal hearing in the sarcophagid parasitoid fly *Emblemasoma* sp.: the biomechanics of directional hearing. J Exp Biol 202:1865–1876

Schmitz B (1985) Phonotaxis in *Gryllus campestris* L. (Orthoptera, Gryllidae) III. Intensity dependence of the behavioural performance and relative importance of tympana and spiracles in directional hearing. J Comp Physiol A 156:165–180

Walker TJ (1962a) Factors responsible for intraspecific variation in the calling songs of crickets. Evolution 16:407–428

Walker TJ (1962b) The taxonomy and calling songs of United States tree crickets (Orthoptera: Gryllidae: Oecanthinae). I. The genus Neoxabea and the niveus and varicornis groups of the genus Oecanthus. Ann Entomol Soc Am 55:303–322

Windmill J, Göpfert M, Robert D (2005) Tympanal travelling waves in migratory locusts. J Exp Biol 208:157–168

Zuk M, Simmons LW (1997) Reproductive strategies of the crickets (Orthoptera: Gryllidae). In: Choe JC, Crespi BJ (eds) The Evolution of mating systems in insects and arachnids. Cambridge University Press, Cambridge, pp 89–109