

Tuning the drum: the mechanical basis for frequency discrimination in a Mediterranean cicada

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Summary

Cicadas are known to use sound to find a mate. While the mechanism employed by male cicadas to generate loud calling songs has been described in detail, little information exists to explain how their ears work. Using microscanning laser Doppler vibrometry, the tympanal vibrations in the cicada *Cicadatra atra* are measured in response to acoustic playbacks. The topographically accurate optical measurements reveal the vibrational behaviour of the anatomically complex tympanal membrane. Notably, the tympanal ridge, a distinct structural element of the tympanum that is a link to the receptor cells, undergoes mechanical vibrations reminiscent of a travelling wave. In effect, the frequency for which the maximum deflection amplitude is observed regularly decreases from the apex to the base of the ridge.

It is also shown that whilst female ears are mechanically tuned to the male's song, the male's tympanum is only partially tuned to its own song. This study establishes the presence of a peripheral auditory mechanism that can potentially process auditory frequency analysis. In view of the importance of acoustic signalling in cicadas, this unconventional tympanal mechanism may be employed in the context of species recognition and sexual selection.

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Key words: biomechanics, hearing, tympanum, travelling wave, frequency discrimination, cicada.

Introduction

Insects constantly receive a broad set of odours, images, sounds and vibrations. Hearing airborne vibrations, or sound, is a sense particularly important in many insects, being found in eight orders and playing a key role in both predator and prey detection and mate attraction (Greenfield, 2002). The reception of sound waves is mediated by two types of mechanical systems: particle velocity receivers, or air pressure receivers. In flies and mosquitoes, sound is detected using specialised antennae. Working as a simple harmonic oscillator driven by the velocity of air particles, the antenna oscillates with sound; this motion then mechanically activates neurones grouped in a sensory organ, Johnston's organ (Robert and Göpfert, 2002). Another, better known yet less frequent, way to capture sound energy is to build a very thin membrane backed by an air space. Due its low acoustic impedance, a thin and light structure can be easily deflected by sound pressure (Bennet-Clark, 1984). Such a membrane, or tympanum, operating in vertebrate and insect auditory systems, is a structurally heterogeneous structure (Stephen and Bennet-Clark, 1985; Yager, 1999; Yack, 2004) and the way it is actuated by incident sound energy has been investigated in only a few systems (Rodriguez et al., 2005;

Windmill et al., 2005). Recently, microscanning laser vibrometry experiments have investigated the patterns of vibration on the tympanal membranes of two grasshopper species (*Locusta migratoria* L. and *Schistocerca gregaria* Forskål). This analysis revealed the presence of distinct and frequency specific vibrational waves that can travel in the same time across the tympanal membrane. Remarkably, the travelling waves reach their peak amplitude at the tympanal locations where groups of sensory neurones attach (Windmill et al., 2005). Documenting that this eardrum does not undergo drum-skin-like motions, these results also demonstrated the mechanical process by which a complex sound wave can be decomposed into discrete frequency components.

In cicadas, another insect species with conspicuously well developed tympanal membranes, the mechanical basis of sound reception has not been investigated to such a degree. Initially identified by Duges, the cicada hearing system is located ventrally in the second segment of the abdomen (Duges, 1838). Vogel described the structure of the ear in detail (Vogel, 1921; Vogel, 1922; Vogel, 1923). The auditory system comprises two major elements: the tympanum and the sensory organ. The tympanum is a thin membrane of cuticle

backed by a tracheal air chamber. The tympanal membrane (TM) is a heterogeneous structure; it is partially crossed by a dark, spear-like structure called the tympanal ridge (TR). This ridge is extended by the tympanal apodeme, a cuticular attachment connecting directly to the sensory organ. The sensory organ is a typical chordotonal organ containing multicellular auditory receptors (scolopidia; type I monodynamal receptors). Each scolopidium is composed of five distinct cells: a bipolar sensory neuron, a cap cell, a scolopale cell, a distal attachment cell and a proximal attachment cell, the latter attaching to the tympanal apodeme (Chen, 1958; Michel, 1975; Young and Hill, 1977; Doolan and Young, 1981; Daws and Hennig, 1996). Exceptionally for insects, the sensory organ of the tympanal ears of cicadas contains a high number of auditory receptors, from 600 up to 2100 in each ear, rivalling or even exceeding the number observed in most lower vertebrates (Fonseca et al., 2000). The sensory axons of the mechanoreceptive neurons are grouped to form the auditory nerve that travels around the ventral edge of the tympanum. Numerous studies reporting averaged extracellular recordings of compound potentials along the auditory nerve have shown various degrees of matching between the hearing frequency range and the frequency spectrum of male calling songs (Enger et al., 1969; Simmons et al., 1971; Popov, 1981; Huber et al., 1990; Popov, 1990; Popov et al., 1991; Daws and Hennig, 1996). More accurately, ascending neurons recorded in *Magicicada cassini* (Fisher) responded selectively to the frequency of the calling song (1–3 kHz) while the auditory nerve was sensitive to sound stimulation up to 9 kHz (Huber et al., 1990). In addition, intracellular recordings made from eight interneurons of *Tettigetta josei* Boulard showed distinct frequency tuning that covered the conspecific song spectrum and may be involved in the process of auditory frequency discrimination (Fonseca et al., 2000).

While mechanisms for frequency discrimination have been intensively studied at the neuronal level, no work has been undertaken to explore the first stages of the chain of hearing, in particular the role of the tympanum, in the conversion of acoustic energy into mechanical energy. A recent study on the directionality of cicada hearing pointed out that vibrations of the tympanum seem to differ between the proximal and the distal part of the ridge (Fonseca and Hennig, 2004). To test the hypothesis that the mechanics of the cicada tympanum, and more particularly the TR, provide peripheral processing, and are possibly involved in frequency discrimination, we experimented with the vibrations of the tympanum of *Cicadatra atra* (Olivier), a medium-sized cicada from the Mediterranean Basin. By measuring the nanometre-scale tympanal vibrations elicited by both synthetic sounds and playback of sounds recorded in the field, we observed waves travelling from the tip to the base of the ridge, where it connects to the tympanal apodeme. Notably, these vibrations have deflection shapes similar to those described along the basilar membrane of the mammalian inner ear (von Békésy, 1960). In effect, these travelling waves displayed amplitude maxima

along the ridge at locations that were frequency dependent. Corroborating previous work that revealed the processing capacity of tympanal membranes in locusts (Windmill et al., 2005), this study documents the eardrum mechanics of – arguably – one of the most complex insect ears – that of a cicada. The mechanical response of both male and female eardrums is compared to the calling song of the male. This study also establishes the complex mechanics and preneural processing capacity of the tympanum of *C. atra*, a structure that, like the eardrum of many other animals, does not behave at all like the skin of a simple drum.

Materials and methods

Study site and calling song analysis

Cicadatra atra (Olivier) was studied during June 2005 in a fig tree field in Sales-le-Château (Pyrénées-Orientales, 66, France). The ambient temperature was 31–32°C. The calling song of four males was recorded at a distance of 1 m with a Sennheiser microphone (ME66, Wedemark, Germany) connected to a Marantz digital recorder (PMD 670, Eindhoven, The Netherlands). The recording sampling frequency was 44.1 kHz. The resulting .wav files were directly transferred with a Flash memory card to a PC computer. The frequency composition of the calling songs was analysed with the 'R' signal analysis package 'Seewave' (Sueur et al., 2006). A Fourier transform with a 43 Hz precision was calculated in the middle of the four calling songs. An average spectrum was then computed for the four calling songs.

Animals

Thirteen males and eight females were caught on the same study site (Fig. 1). Animals were cooled down to 8–10°C and immediately transferred to Bristol (UK) in an ice-box. Before measurements, animals were placed at 25–26°C in a plastic cage (0.53 m×0.22 m×0.22 m) provided with birch branches (*Betula* sp.). Both males and females sucked sap from the branches and males emitted typical calling song bouts, indicating a good general condition. The wings and the legs were cut back before mechanical measurements. The operculum and the meracanthus that ventrally cover the tympanal organs, but are not mechanically linked to them, were also removed. Animals were not anaesthetized during measurements but were firmly attached to a horizontal brass bar (6 mm wide, 1 mm thick and 16 mm long) using BLU-TACK (Bostik-Findley, Stafford, UK). The brass bar was connected to a metal rod (150 mm long, 8 mm diameter) *via* a thumbscrew, allowing the animal to be rotated and tilted into the required position. Only one ear was examined per animal. Tympanal vibrations were measured using a microscanning laser Doppler vibrometer (Polytec PSV-300-F; Waldbronn, Germany) with an OFV-056 scanning head. The animal was orientated such that the measuring Doppler vibrometer could scan the entire tympanum and that the tympanum was perpendicular to the direction of sound wave propagation. All experiments were carried out on a vibration isolation table



Fig. 1. Male *Cicadatra atra* emitting a calling song in a typical upside-down position. The white arrow on the male shows the localization of the auditory sensory organ. Scale=5 mm. Picture by Stéphane Puissant/OPIE-LR.

(TMC 784-443-12R, Technical Manufacturing Corp., Peabody, MA, USA) at room temperature (24–26°C) and relative humidity of 40–62%. The vibration isolation table with the animal and the laser vibrometry measurement head were located in a dedicated acoustic isolation booth (Industrial Acoustics IAC series 1204A, internal dimensions: length 4.50 m, width 2.25 m, height 1.98 m).

Mechanical measurements

Cicada ears are made of two main components: a tympanum and a sensory organ. The ears are symmetrically located in the second segment of the abdomen. Female ears are smaller than male ears (Fig. 2). The tympanum is a thin membrane partly crossed by a dark spear-like structure called the tympanal ridge (TR). This latter is extended by the tympanal apodeme, a cuticular attachment inside the sensory organ where the auditory receptors (scolopidia) are attached. The vibrations of the tympanum were studied following the same general procedure used in a previous study on migratory locusts (Windmill et al., 2005). The vibrations of the whole tympanum, of a focused area along the ridge and of a 35-point line along the ridge were examined in response to four different acoustic stimulations. The first stimulus consisted of a frequency modulated signal (duration=80 ms) sweeping at similar intensity all frequencies from 1 kHz to 30 kHz. The second stimulus was a typical *C. atra* calling song (duration=5 s, frequency range=1–22.05 kHz). This allowed observation of the response of the tympanum to a natural signal. The third stimulus was a short click, a square pulse (duration 186 μ s, output frequency bandwidth: 1–30 kHz) synthesized by the Polytec PSV300 sound generator. This

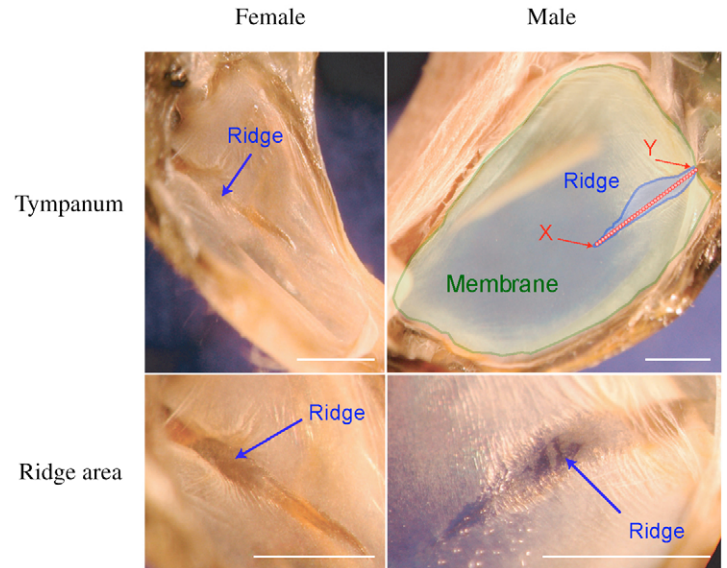


Fig. 2. Posterior view of female left tympanum and male right tympanum with close-up on the ridge. The membrane and the ridge are shown on male tympanum, the red dots indicating the laser positions where frequency measurements were made. X and Y, respectively, indicate the apex and the base of the ridge. Scale=0.5 mm.

stimulus was used to estimate, in the time domain, the first mode of vibration of the 35 scan points positioned along the ridge. The fourth stimulus consisted of a pure-tone signal increasing and then decreasing in amplitude from 1 mPa to 30 mPa sound pressure, i.e. from 34 dB to 63.5 dB SPL re 20 μ Pa (duration=10 s, frequency=11.7 kHz). This last stimulus provided a test for the potential non-linearity of the ridge in response to signals of different intensity. A non-linearity in the response might suggest an active process of audition.

All acoustic stimuli were amplified using a Sony Amplifier Model TAFE570 (Tokyo, Japan) and broadcast with a loudspeaker ESS AMT-1 (ESS Laboratory Inc., Sacramento, CA, USA) positioned at 0.25 m from the cicada. Thus, for the relevant frequency range (1–30 kHz), the animal was in the far-field of the sound source. The intensity of the acoustic stimulations was 77 dB SPL at the cicada position corresponding to the natural calling song pressure level at 1 m (Boulard, 1992).

As described in detail elsewhere (Windmill et al., 2005), the tympanal and ridge vibrations were analysed by simultaneous recording of the vibration velocity of the tympanum and the SPL adjacent to the tympanum. The laser vibrometer allowed accurate measurement (laser positioning \sim 1 μ m) of the topography of tympanal motion in the amplitude, time and frequency domains, in a contact-free way and without requiring the use of a reflective medium on TM. SPL was measured using a 1/8 inch (3.2 mm) precision pressure microphone (Bruel and Kjaer, 4138; Nærum, Denmark) and preamplifier (Bruel and Kjaer, 2633). The microphone has a linear response in the measured frequency range. The microphone's sensitivity was

calibrated using a Bruel and Kjaer sound level calibrator (4231, calibration at 1 kHz, 94 dB SPL). The microphone was positioned 10 mm from the tympanum, with its diaphragm parallel to the sound direction, thus maximizing the response. A calibrated stimulus sound level, at the tympanum, of 0.14 Pa (77 dB SPL) was used throughout these experiments.

Evaluation of tympanum response

Analyses of the tympanum velocity, U , and SPL were carried out by the vibrometer's control PC. The laser signals resulting from the FM sweep and natural sound stimuli were simultaneously sampled at 102.4 kHz. Sets of 15 data windows of 80 ms duration were acquired and averaged for each point across the membrane. Using an FFT (Fast Fourier Transform) with a rectangular window, which weights all sample points equally, a frequency spectrum was produced for each signal with a resolution of 12.5 Hz. The laser and microphone signals were then used to calculate different quantities, such as gain and phase responses. By combining the results from all the points scanned, oscillation profiles and animations of tympanal deflections were generated for specific frequencies.

Evaluation of ridge area response

Similarly, the ridge area vibrations induced with FM sweep and natural signals were reconstructed. More particularly, the frequency corresponding to a zero phase was measured for the vibration of each point of the 35 points line scanned along the ridge. This value obtained using the FM sweep stimulus indicates the frequency at which the tympanal point's velocity was maximal, corresponding to the resonant frequency at that position. We then obtained an estimation of the frequency sensitivity along from the apex (X) to the base (Y) of the ridge. In addition, the tympanum ridge velocity resulting from the broadcast of short clicks was recorded at a sampling frequency of 102.4 kHz for a time period of 1 ms. The vibrational response frequency was measured by the zero-crossing method.

We described the travelling waves running through the ridge area by three main characteristics (Robles and Ruggero, 2001). We estimated the travel time between two locations (δ_t), the wave velocity (V_{wave}) and the wavelength (L_{wave}), following:

$$\delta_t = \frac{\delta_\phi}{2\pi f}, \quad V_{\text{wave}} = \frac{\delta_x}{\delta_t}, \quad L_{\text{wave}} = \frac{2\pi\delta_x}{\delta_\phi}, \quad (1)$$

where f is the wave frequency, δ_ϕ is phase difference between the two locations, and δ_x is the distance between the two locations.

Data reliability

The data reliability was estimated by means of magnitude-squared coherence (C) functions between the laser and microphone signals following:

$$C = \frac{G_{ab}(f) \times G_{ba}(f)}{G_{aa}(f) \times G_{bb}(f)}, \quad (2)$$

where $G_{ab}(f)$ is the cross-spectrum of the velocity signal and reference signal (Fourier Transform of the cross correlation function between the two time series), $G_{ba}(f)$ is the cross-spectrum of the reference signal and velocity signal, $G_{aa}(f)$ and $G_{bb}(f)$ are, respectively, the auto-spectra of the reference signal and of the velocity signal. Only highly coherent measurements (> 85%) with minimal contamination by unrelated noise were used.

Statistics

Differences between male and female were tested using the non-parametric Wilcoxon test and differences between different driving frequencies were tested using the Kruskal–Wallis test. All statistics and graphics were processed using the base package of the software 'R' (R Development Core Team, 2004).

Results

Structure of the tympanum

Conspicuous differences in size, shape and iridescence were apparent between male and female tympana. Males typically showed larger tympana than females (Fig. 2). The central part of the male tympanum was iridescent but the peripheral part was opaque, indicating a heterogeneous material distribution. The entire surface of the female tympanum was opaque. Female TRs reached the centre of the membrane while the tip of a male TR was located at the lateral limit between the opaque periphery and the iridescent centre of the membrane (Fig. 2).

Frequency composition of the calling song

The calling song of *C. atra* consisted of a continuous buzz repeated at irregular intervals. In the frequency domain, the main energy of the signal was situated between 6 and 17 kHz with 50% of the energy concentrated between 9.1 and 11.3 kHz (Fig. 3). The dominant frequency was at 10.23 ± 0.78 kHz ($n=200$, $N=4$ males). There was no frequency modulation but a fast amplitude modulation at a rate of about 1000 Hz due to the pulsed structure of the signal. This introduced small secondary peaks every 1000 Hz around the dominant frequency.

Tympanal vibrations in response to a FM sweep signal and male calling song

To gather a complete topographic view of tympanum vibrations, the entire TM of 13 males and 8 females was successively stimulated with a FM sweep signal (Figs 4–6; supplementary material Movies 1–3) and a calling song (Fig. 7). Because of the larger size of the male TM, a higher resolution scan was also separately performed for the ridge area alone. For both male and female, the scans revealed that, for different incident sound frequencies, travelling waves are generated on the TM. For the smaller tympana of females, deflections affect the whole membrane, travelling across it from a medioventral position to a laterodorsal position. For a 10 kHz stimulus, a frequency corresponding to the dominant

frequency of the calling song, the displacement gain (=TM displacement/air displacement, expressed in nm Pa^{-1}) on the ridge was $244 \pm 168 \text{ nm Pa}^{-1}$ ($N=7$). In males, the displacement gain reached $261 \pm 117 \text{ nm Pa}^{-1}$ ($N=8$), a magnitude not significantly larger than that measured for females ($P=0.54$). Notably, the location of the point of maximal tympanal deflection varies with sound frequency. For females, the maximal deflection point occurs close to the laterodorsal part of the TM (base of the ridge) for low frequencies (5, 10 kHz) and close to the medioventral part of the TM (apex of the ridge) for high frequencies (15, 20 kHz) (Fig. 4; supplementary material, Movie 1). For males, the TM does not vibrate homogeneously. The central part of the TM, which is iridescent, vibrates less than the peripheral opaque part, in particular in the ridge area. This pattern was particularly evident for 10 kHz, the calling song dominant frequency (Fig. 5; supplementary material, Movie 2). A laser scan of the TR made at a higher spatial resolution reveals that a vibrational wave travels from a medioventral position to a laterodorsal position. As for females, the point of maximal deflection of the TR varies as a function of stimulus frequency. The maximal deflection point is similarly close to the base of the ridge for low frequencies (5, 10 kHz) and further apically for high frequencies (15, 20 kHz) (Fig. 6; supplementary material, Movie 3).

When stimulated with the male calling song, male and female TMs showed deflection shapes similar to those measured using frequency modulated chirps (Fig. 7). The same relationship was observed between localization of maximal deflection points and driving frequency. Finally, measurements done *post-mortem* immediately following an injection of ethanol showed the same results (data not shown).

Vibrations of the tympanal ridge in response to FM sweep frequency

The phase response along the TR whilst stimulating with FM sweep sound shows an increasing phase lag as a function of stimulus frequency (Fig. 8). This phase lag is substantial, exceeding the phase lag expected for the displacement response of a simple oscillator (maximum of -90° at frequencies above resonance). Lag increases as a function of frequency; at higher frequencies, such as 20 kHz, the phase lag reaches -300° for females and nearly -900° for males. The envelope of the wave in motion along the ridge becomes apparent when the response

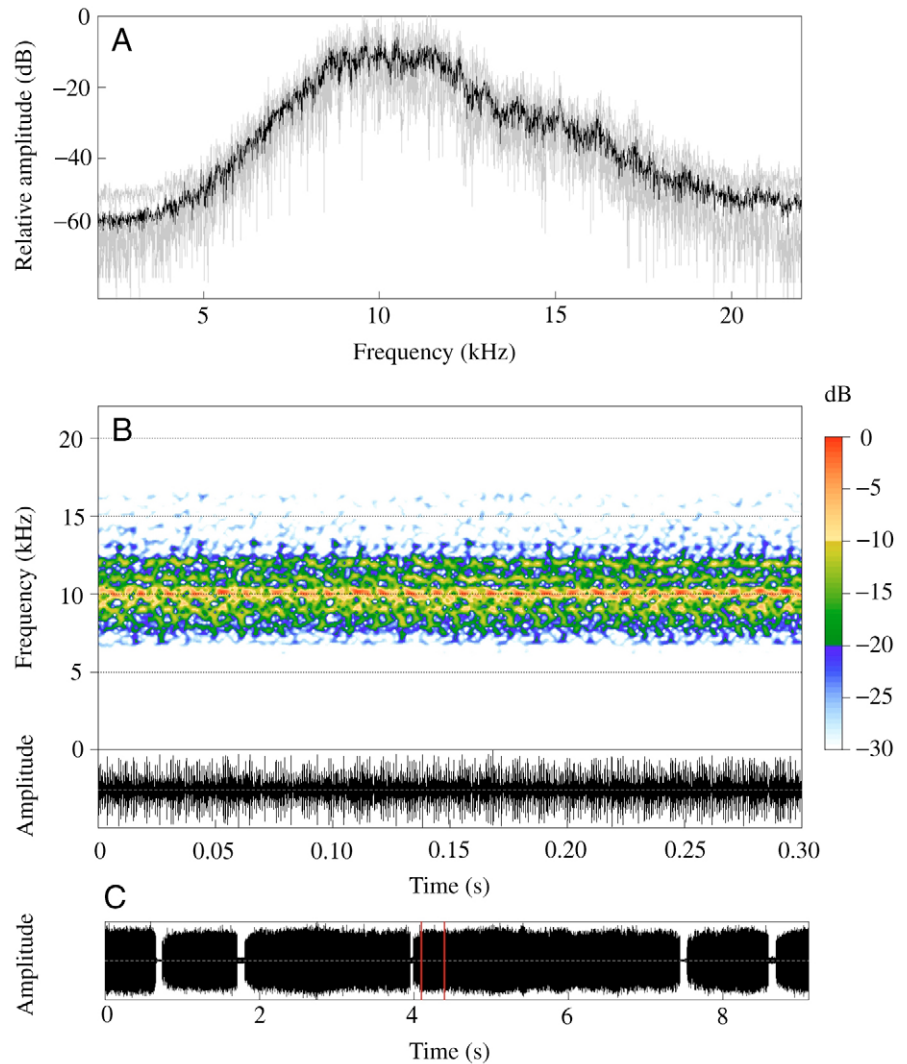


Fig. 3. Calling song of *Cicadatra atra*. (A) Spectra of four distinct males (grey lines) and their mean (black line). (B) Oscillogram and spectrogram corresponding to the zone delimited by the vertical red lines on the bottom oscillogram. (C) Oscillogram showing successive calls. Red lines show the part of the song enlarged in B.

is displayed for every 10° of phase angle in the full oscillation cycle (Fig. 9). At a frequency of 10 kHz, the deflection envelope of TR is thus shown every $2.78 \mu\text{s}$. The magnitude of the displacement envelopes is about four times higher for males than for females (displacement gain at 10 kHz: $5.84 \mu\text{m Pa}^{-1}$ for males; $1.34 \mu\text{m Pa}^{-1}$ for females). In addition, the frequency response of female TR differs from that of the male. Deflections of maximum magnitude are observed at 15 kHz for females, and 10 kHz for males. In other words, 15 and 10 kHz constitute the TR's mechanical 'best frequencies'. In effect, the deflection envelopes vary with the stimulus frequency, with deflection maxima occurring at different positions along the ridge. In the male, the measured deflection envelopes reveal that high frequencies deflect the apex of the ridge more than its base. This relationship between driving frequency and position along the ridge is less

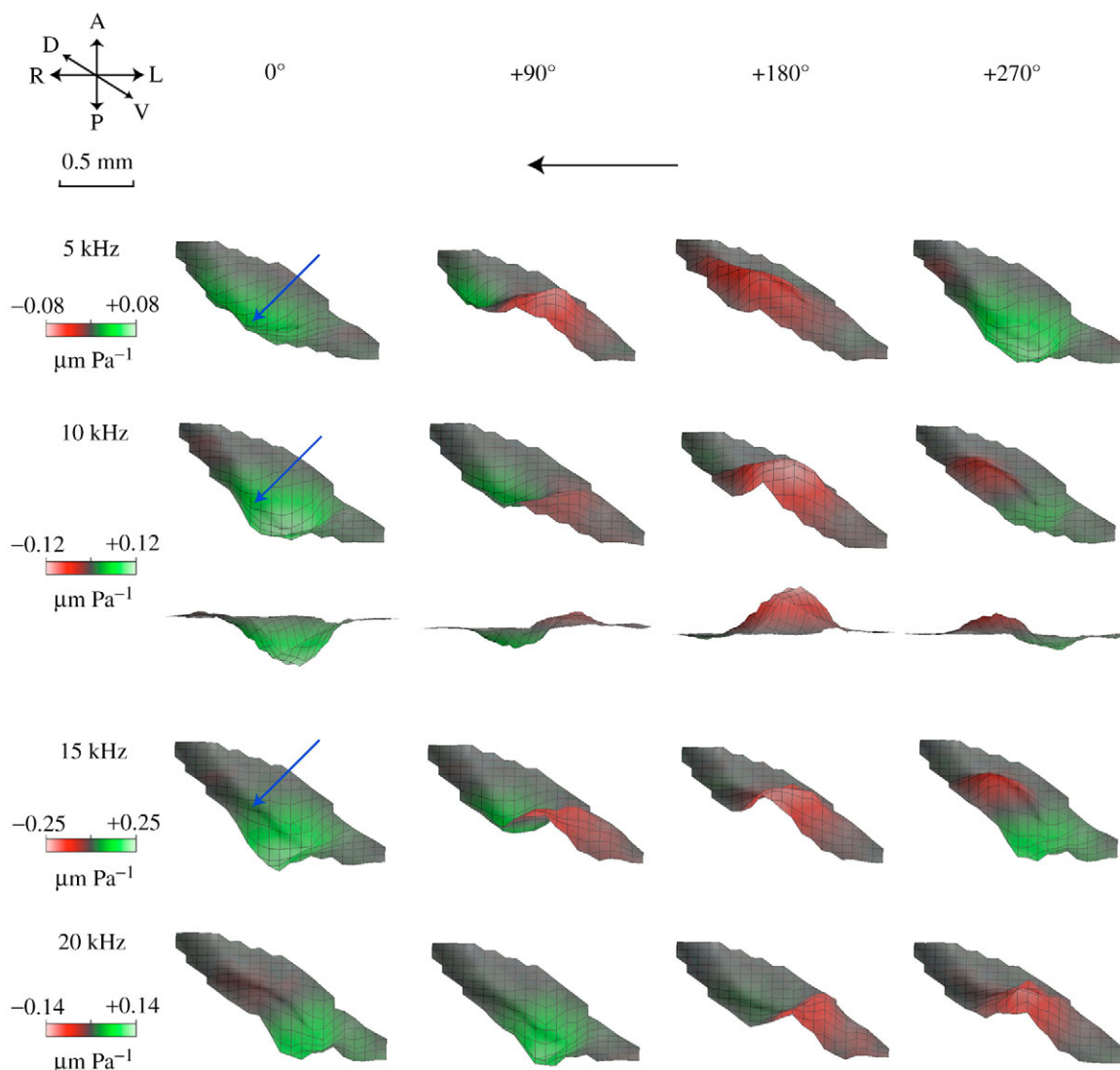


Fig. 4. Deflection shapes of a female right tympanic membrane. TM was stimulated with a FM sweep signal. The oscillations are shown for four different driving frequencies (5, 10, 15, 20 kHz) at four different phases (0, 90, 180, 270°) along the oscillation cycle. For 10 kHz, the dominant frequency of male calling song, deflections are shown as profiles, as if looking at the tympanum from its side. Deflections are expressed as displacement gain following the colour scale. Red indicates outward tympanal deflections and green inward tympanal deflections. Note the difference in scale for each driving frequency. Orientation is indicated by a 3D space reference (D, dorsal; V, ventral; P, posterior; A, anterior; L, left; R, right). The horizontal black arrow indicates the direction of wave propagation. The ridge is visible as a black elongated teardrop (blue arrow) on the scan map.

pronounced for females, where the maximum deflections driven by 5 and 20 kHz sound stimuli occur at similar locations along the ridge. Topographical positions for 5, 10 and 15 kHz are arranged in ascending order from the apex to the base of the ridge.

The differences in TR response with driving frequency were further assessed by computing the frequency spectrum at each of the 35 measurement points taken along the ridge (Fig. 2). Each point on the membrane can indeed be considered as an oscillator, like a mass-spring resonating system. These spectral responses were plotted as 2D density plots, in which the abscissa is the length along the TR (Fig. 10). This place spectrogram clearly shows a decrease in frequency from apex

to base, the female's 'best frequency' being overall higher than the male's.

Measurements of the resonant frequency at each point along the high-resolution line scan show a decrease in the frequency of resonance from the apex to the base (Fig. 11). This frequency range is significantly lower for males than for females ($P < 0.001$); from 9.8 ± 1.5 kHz to 3.8 ± 0.9 kHz for males ($N=9$) and from 13.7 ± 1.2 kHz to 5.7 ± 1.7 kHz for females ($N=6$). Therefore, the tympanal ridge's mechanical response varies with the frequency of the incoming sound. The female ridge is centrally tuned to the male's song (Fig. 10). The male ridge, however, has a resonant response that partially detuned away from the song's central frequency.

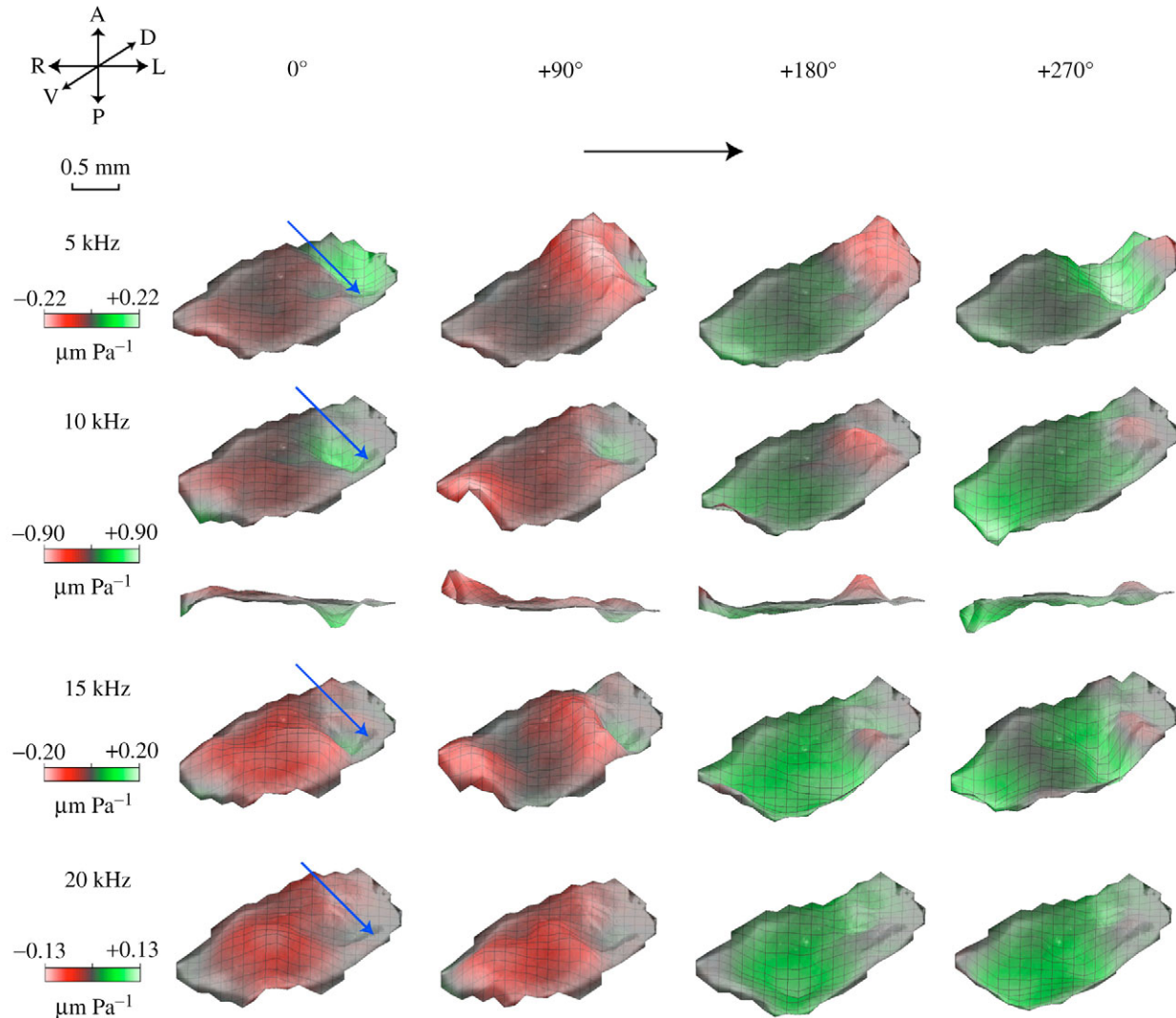


Fig. 5. Deflection shapes of a male left tympanal membrane. TM was stimulated with a FM sweep signal (0–30 kHz). The horizontal arrow indicates the direction of wave propagation. See Fig. 4 for details.

Finally, the travelling wave across the TR was characterised for different driving frequencies. For 9 males and 6 females, the travel time and phase difference across the ridge were estimated, as well as the wave velocity and the wavelength between the apex and the base (Fig. 12). For both males and females, the phase difference increases with the driving frequency (from $112.0 \pm 29.5^\circ$ to $515.0 \pm 104.6^\circ$ for males, $P < 0.001$; $138.3 \pm 51.0^\circ$ to $394.3 \pm 147.0^\circ$ for females, $P < 0.05$). There was no significant difference between males and females ($P = 0.388$). This corresponds to a decrease of the wavelength with increasing driving frequency for both sexes (from 1.62 ± 0.47 mm to 0.34 ± 0.17 mm for males, $P < 0.001$; from 1.35 ± 0.39 mm to 0.49 ± 0.20 mm for females, $P < 0.05$), with again no significant difference between them ($P = 0.388$). The travel time was significantly constant for males (between 57.04 ± 12.28 μs and 71.53 ± 13.72 μs , $P = 0.365$) and females (between 52.44 ± 18.33 μs and 76.85 ± 28.34 μs , $P = 0.715$). There was no significant difference between males and females

($P = 0.314$). This directly implies a constant wave velocity for males (between 6.87 ± 1.22 m s^{-1} and 8.68 ± 1.92 m s^{-1} , $P = 0.365$) and females (between 6.76 ± 1.94 m s^{-1} and 10.00 ± 3.59 m s^{-1} , $P = 0.715$) without significant differences between sexes ($P = 0.314$).

The impulse response of the tympanal ridge

The first vibration mode of the TR was estimated by measuring its response to short sound impulses (1 ms). An impulse stimulation generates a vibrational wave travelling across the ridge from its apex to its base. The frequency of the maximal vibrational response was estimated for each of the 35 scanning locations along the ridge (Figs 13, 14). This frequency decreases from the apex to the base. This frequency decrease spans from 9.35 ± 1.28 kHz to 5.19 ± 0.75 kHz for males ($N = 6$) and from 11.98 ± 2.16 kHz to 6.97 ± 2.09 kHz for females ($N = 3$). No statistical test could be run between male and female because of low female sample size, yet there was

a clear trend for the female TR to vibrate at a higher frequency than the male TR. This indicates that females are mechanically well tuned to the male calling song. By contrast, only the higher end of the male's response corresponds to the calling song.

Response of the tympanal ridge to stimulus amplitude

The response of the tympanal ridge to changes in the magnitude of sound pressure (34–63.5 dB SPL re 20 μPa) was examined. The tympana always showed a linear response to the variation in amplitude in the range tested (Fig. 15). Different frequencies, and amplitude ranges were investigated, consistently showing a linear response (data not shown). The level of linearity is shown by the high correlation between sound pressure and TM vibrations ($r=0.99$, Spearman's rank

correlation, $P<0.001$). Thus, based on this evidence, there is no indication of an active process in the cicada ear.

Discussion

Previous work on cicada hearing has focused on the relative role of the different sound inputs to the auditory system, and the sound diffraction by the cicada body (Fonseca, 1993; Fonseca and Popov, 1997; Fonseca and Hennig, 2004). Hence, while several important biophysical aspects of cicada audition have been studied, most notably directional hearing, the mechanical response of the tympanal membrane has remained elusive. Using a microscanning laser vibrometer and sound playback, we have measured and analysed the tympanal mechanics in the Mediterranean cicada *C. atra*. The results

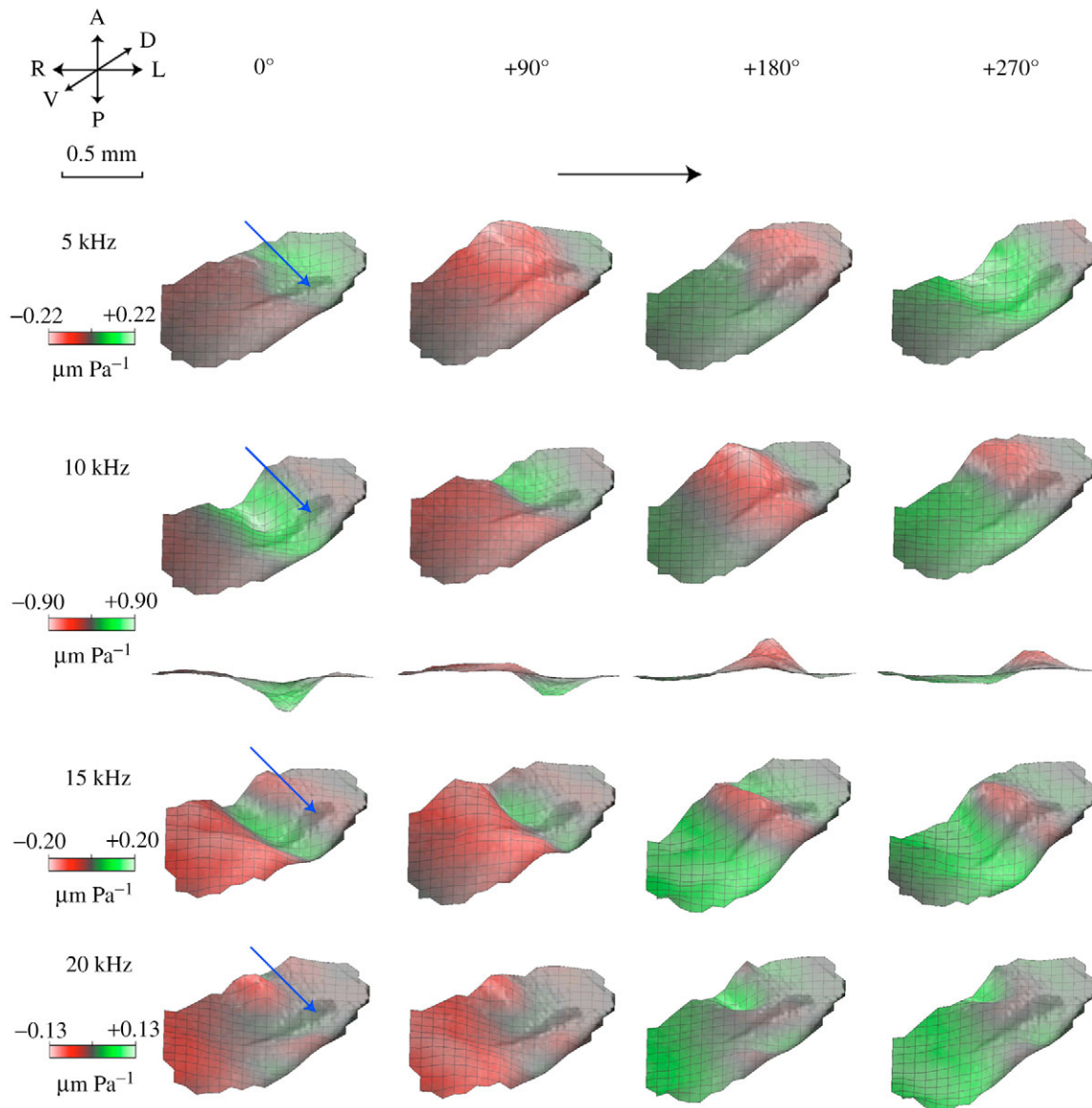


Fig. 6. Deflection shapes of a male left tympanal ridge as revealed by high resolution scanning. Stimulation was done with a FM sweep stimulus. The horizontal arrow indicates the direction of wave propagation. See Fig. 4 for details.

show that: (1) TM vibrations scale linearly with sound level, (2) a travelling wave propagates across the TM and more particularly across the TR, (3) this travelling wave varies in relation to the driving frequency, and (4) there are obvious differences between male and female TM vibrations. These results highlight the mechanical behaviour of the tympanum of an insect ear. Phenomenologically, the presence of a travelling wave suggests a peripheral mechanism supporting frequency discrimination.

Tympanal mechanics

When acoustically stimulated, the TM of *C. atra* vibrates in a complex way that is unlike the conventional resonant modes of a homogenous drum skin. In males, the peripheral part of

the TM, in particular the TR area, vibrates substantially more than the central tympanal area. In both males and females, the TR undergoes vibrations carried by a wave running from its apex to its base. This wave fulfils the three criteria of a travelling wave (von Békésy, 1960; Robles and Ruggero, 2001): (1) the magnitude of the oscillation shows an increasing phase lag along the propagating medium, (2) displacement magnitudes have an asymmetric envelope where the wave is seen to compress, (3) the wave is passive, resulting from the mechanical characteristics of the propagating medium. The travelling wave observed in *C. atra* can be compared to some extent with the travelling wave occurring in locust and vertebrate ears [see summary of travelling wave velocity and wavelength in different taxa (Windmill et al., 2005)]. As in the

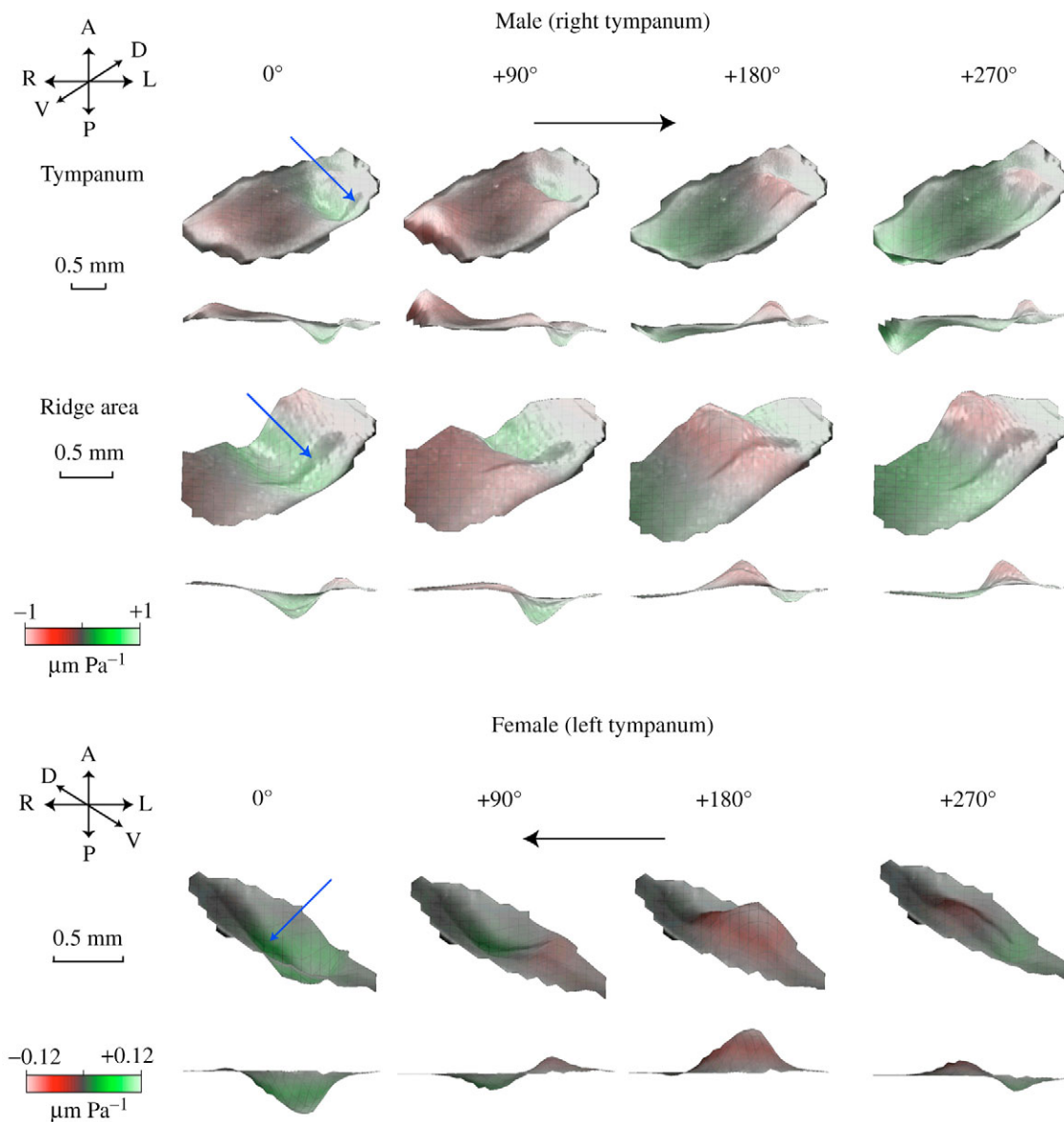


Fig. 7. Deflection shapes of a male left tympanal ridge and female right tympanal ridge. TM was stimulated with a natural male calling song. Deflections are shown for a 10 kHz frequency sine wave (calling song dominant frequency). Horizontal arrows indicate the direction of wave propagation. Note the difference in scale range between male and female. See Fig. 4 for details.

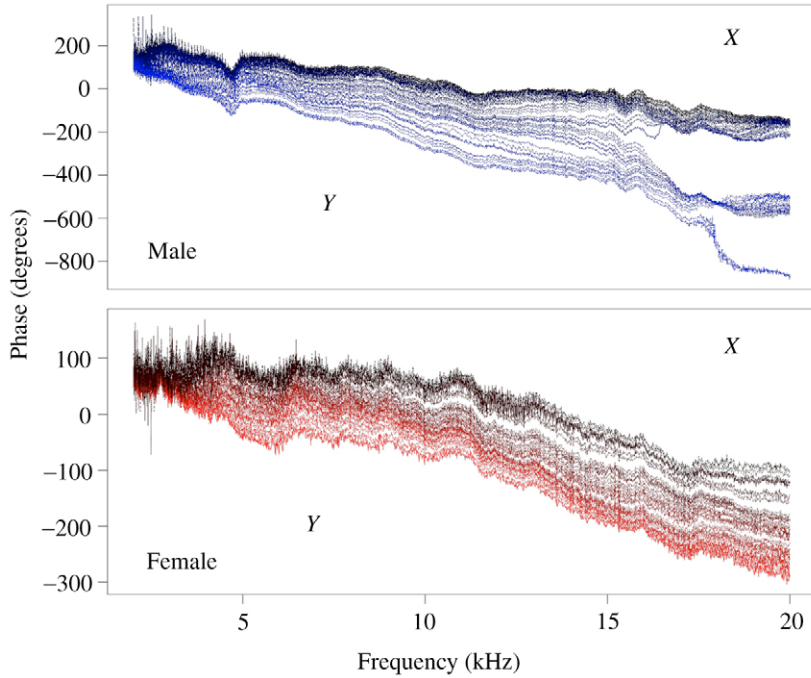


Fig. 8. Phase response along the ridge (TR). The phase response shows an increasing lag with both frequency and distance along the ridge. The phase lag increases to -300° for females and to -900° for males. X=TR apex, Y=TR base (see Fig. 2).

locust, but in contrast to vertebrates, the travelling wave velocity seems to be constant in cicadas. The lengths of the travelling waves are shorter in cicadas than in locusts, but show similar values to those of vertebrates (from 0.3 to 1.6 mm). Differences between locusts and cicadas might be due to the shorter length of the TR (around 1 mm) compared to the span of the locust tympanum (around 1.8 mm). In cicadas, as in vertebrates and locusts, the wavelength decreases with increasing forcing frequency. Yet, one important biophysical implementation distinguishes the travelling waves observed in cicadas and locusts to those reported for vertebrates. The medium of propagation and its surroundings are very different: both sides of insect tympana are directly in contact with air whereas the basilar membrane is set between two fluid filled cavities (the scalae media and tympani) (Olson, 1999; Robles and Ruggero, 2001). In locusts and vertebrates, the membrane is directly connected to groups of neurons that project towards central neural pathways. In cicadas, the TM is not directly in contact with mechanosensory nerve cells but through an

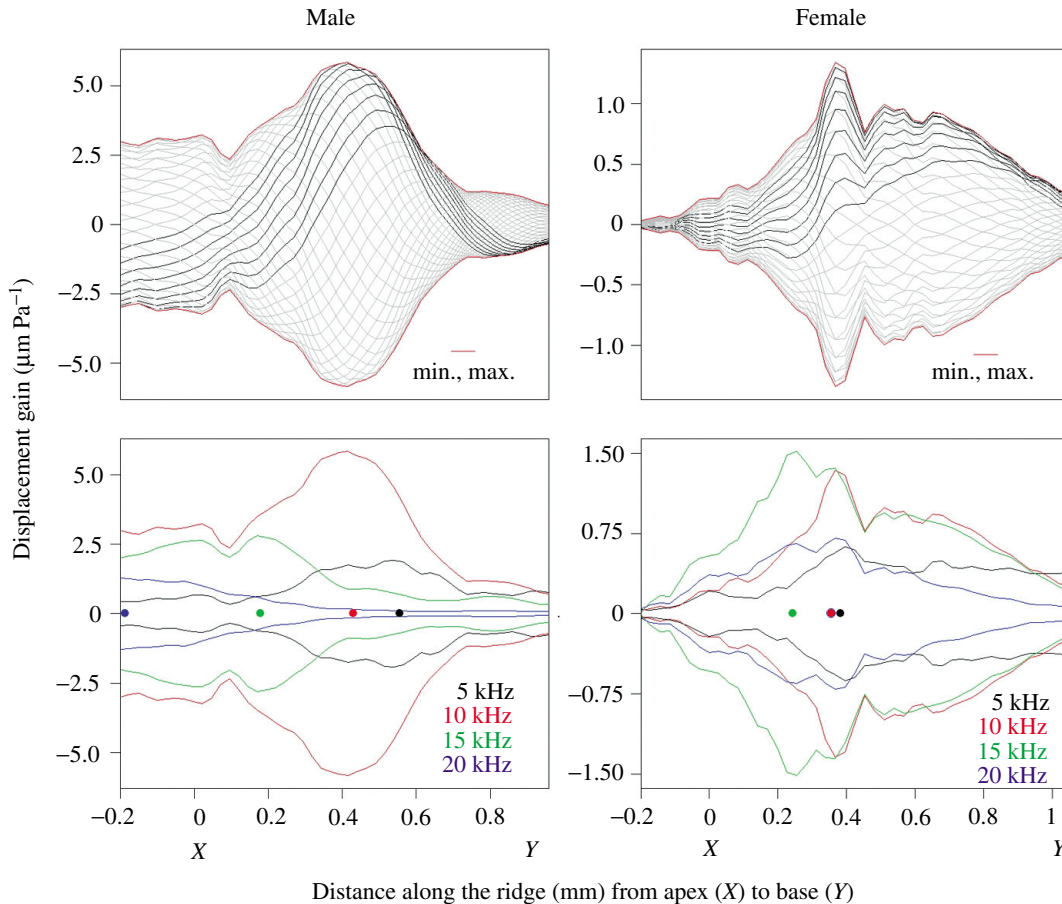


Fig. 9. Envelopes of mechanical deflections along the tympanal ridge. Top: for a 10 kHz driving frequency (calling song dominant frequency), deflections are shown for phase increments of 10° in the full oscillation cycle. Minimal and maximal values are plotted in red. Bottom: minimal and maximal deflection shapes for different driving frequencies: 5 kHz (black), 10 kHz (red), 15 kHz (green), 20 kHz (blue). Coloured dots indicate the position along the ridge where the deflection envelope is maximum for the frequency coding by colour. X, apex of the TR; Y, base of the TR.

Fig. 10. Frequency response of the ridge when stimulated with a FM sweep stimulus. Frequency spectra of ridge resonance were computed at each point of a 35-point line scan from the apex of the ridge to its base. Spectra were then plotted successively on a density plot ranging from 0 to -50 dB.

apodemal prolongation of the TR. It is plausible to postulate that travelling waves are transmitted from the TR to the apodeme.

Our measurements reveal clear differences between male and female TM vibrations. Female TMs show lower amplitude vibrations but sensitivity to higher frequencies. There are notable differences in size, shape and iridescence (material thickness) between male and female tympana. These differences are likely to generate variations in key mechanical properties of the tympanum, such as bulk and local stiffness and damping, and Poisson ratio of the TM, which all contribute to vibrational behaviour. Such properties are unknown for cicadas, as they are for all other insect auditory systems. Interestingly, it has been shown in other cicada species that the TM also acts as radiating surface during sound emission (Young, 1990). The enlarged and translucent central area of the TM, only present in males, which is now revealed to vibrate very little compared to the TR in *C. atra*, may also play a role in sound production. The cicada TM is a complex structure, allegedly serving, at least, the dual function of capturing sound energy from the acoustic pressure field and dissipating the large acoustic pressure generated by the animal's tymbal organ (Young, 1990). Different parts of the tympanal membrane may thus have distinct and specialised roles within that multifunctional scheme.

Frequency analysis

While many insect species show frequency tuning, few of them seem to be endowed with frequency discrimination. Frequency tuning, as the enhanced sensitivity to a particular frequency band, is usually situated around the dominant (or carrier) frequency of the relevant signal. This process has been shown to involve low-pass, high-pass or band-pass neuronal filtering (Hennig et al., 2004). Auditory nerve recordings in cicadas have shown typical V-shape tuning curves (Enger et al., 1969; Simmons et al., 1971; Popov, 1981; Huber et al., 1990; Popov, 1990; Popov et al., 1991; Daws and Hennig, 1996). It is shown in the present study that the female tympanum vibrates maximally at frequencies corresponding to the song's dominant frequencies. The males seem to be slightly detuned to their own song. Therefore, and different to that which has been put forward for the tympanum of the Australian cicada *Cyclochila australasiae* (Donovan) (Daws and Hennig, 1996), a mechanical frequency tuning through tympanal

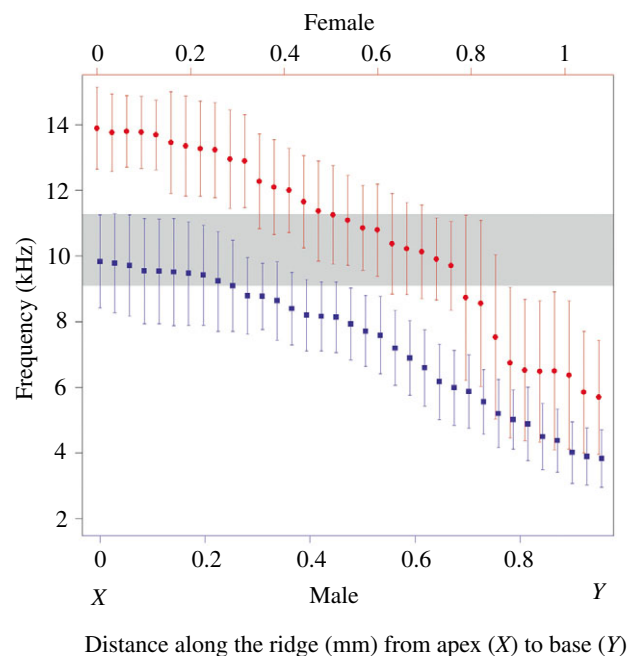
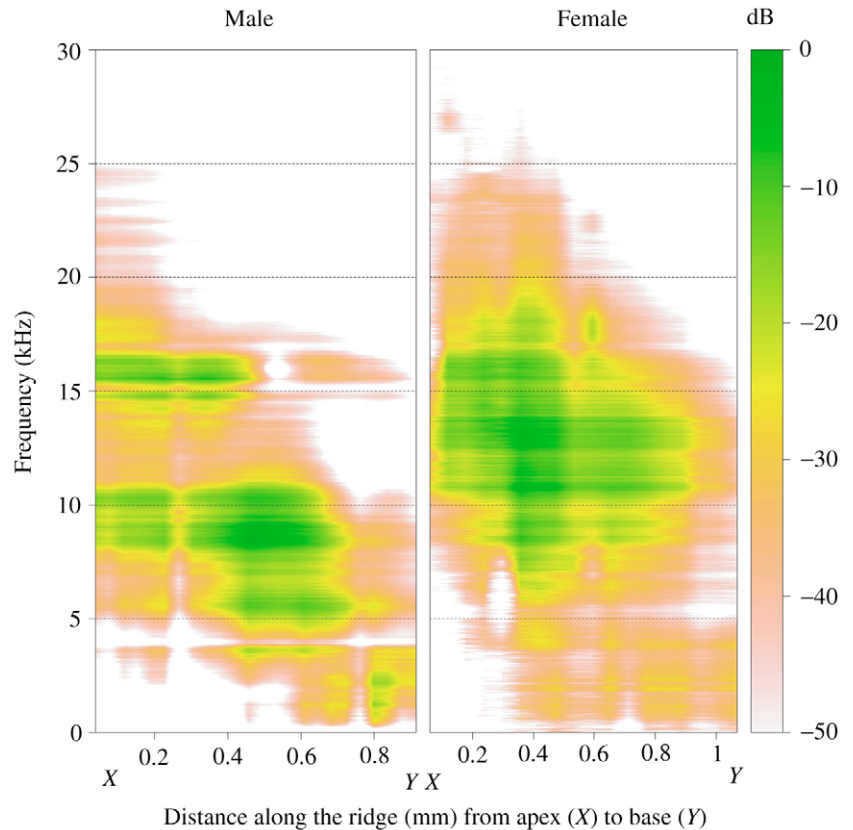


Fig. 11. Frequency of maximal mechanical response measured along the ridge, from apex to base, in response to FM sweep stimulus. Grey area corresponds to the frequency band containing 50% of calling song spectral energy. Values are means \pm s.d. for 9 males (blue) and 6 females (red).

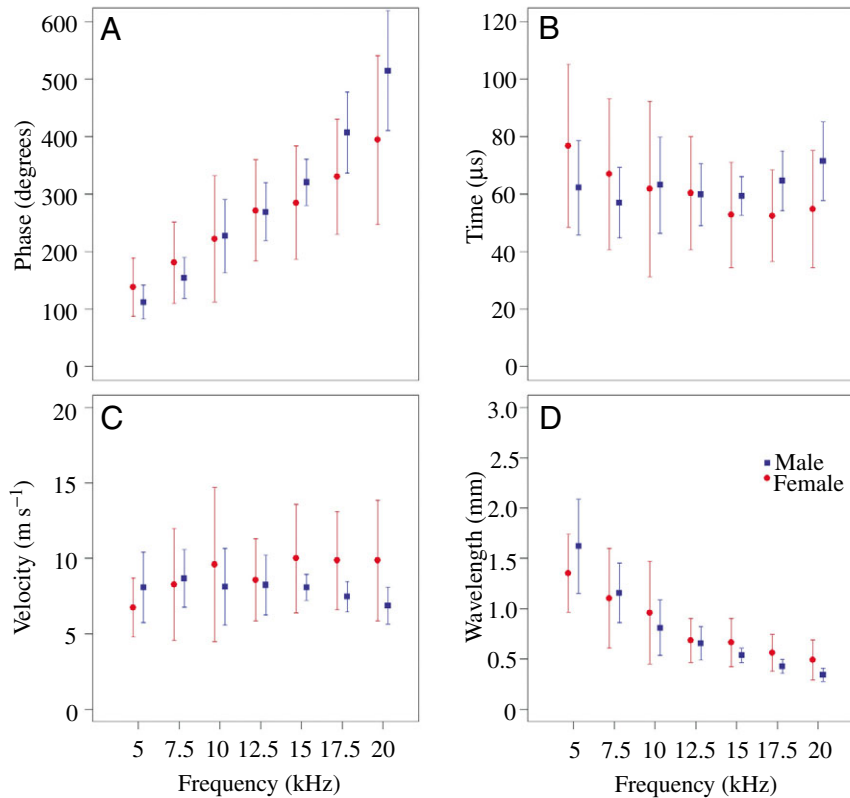


Fig. 12. Travelling wave parameters of the ridge: (A) Phase difference, (B) travel time, (C) wave velocity, (D) wavelength. Values are means \pm s.d. for 9 males and 6 females.

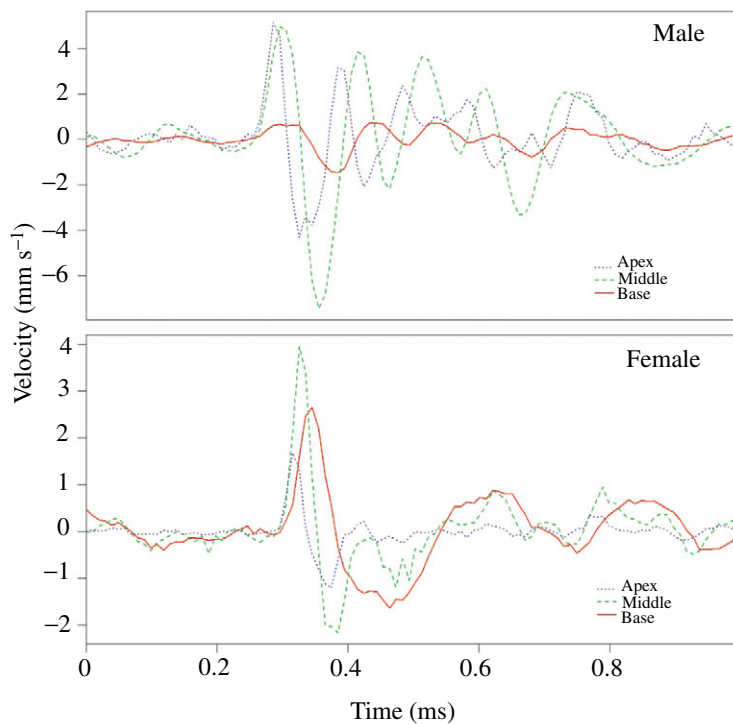


Fig. 13. Oscillations of the tympanum ridge after the broadcast of an intense short click. Oscillations are shown for one female and one male at three positions along the ridge: apex, middle and base. Note the difference in amplitude and period of the oscillations.

resonance is present prior to neuronal filtering.

Frequency discrimination implies that the receiving system is able to distinguish one frequency from another, whether present successively or simultaneously. While there is physiological evidence in insects for a fine frequency resolution at the level of receptor cells, only gross frequency discrimination has been behaviourally documented, and this only in very few species (Pollack and Imaizumi, 1999; Stumpner and von Helversen, 2001; Gerhardt and Huber, 2002; Hennig et al., 2004; Wyttenbach and Farris, 2004). Our data support the idea that cicadas could perform peripheral spectral processing with a resolution higher than most insects. As shown here, the pattern of TR vibrations varies with driving frequency. The vibrational wave travelling along the TR displays envelope peaks near the apex for high frequency tones and near the base for low frequencies. Also, the resonance frequency of the TM linearly decreases from the apex to the base of the TR (9.8 kHz–3.8 kHz for males; 13.7 kHz–5.7 kHz for females). Furthermore, the wavelength of the travelling wave decreases with the wavelength of the sound stimulus.

Altogether, these observations indicate that the TR can act as a frequency analyser between at least 4 kHz and 10 kHz for males and between 6 and 14 kHz for females, decomposing a sound wave in its different frequency components. This decomposition could be transmitted to the apodeme where the auditory receptor neurones are attached. It is, however, difficult to estimate the resolution of the frequency decomposition from the mechanical data presented here; but it is certain to exceed that of the locust, which encompasses four frequency components (Michelsen, 1968; Michelsen, 1971; Römer, 1976).

Behavioural implications

How do these results contribute to understanding the acoustic behaviour of cicadas? Females are tuned to the male calling song and thus they may show frequency tuning, as has been proposed for other cicada species (Daws et al., 1997; Fonseca and Revez, 2002; Sueur and Aubin, 2002). This probably ensures a species-specific recognition process. Frequency tuning can indeed ensure premating isolation from species calling in other frequency ranges [like *Lyristes plebejus* (Scopoli), *Cicada orni* L., *Tettigetta argentata* (Olivier), *Tibicicina garricola* Boulard, *T. corsica fairmairei* (Boulard), *T. tomentosa* (Olivier)]. If females were also able to resolve fine frequency differences based not only

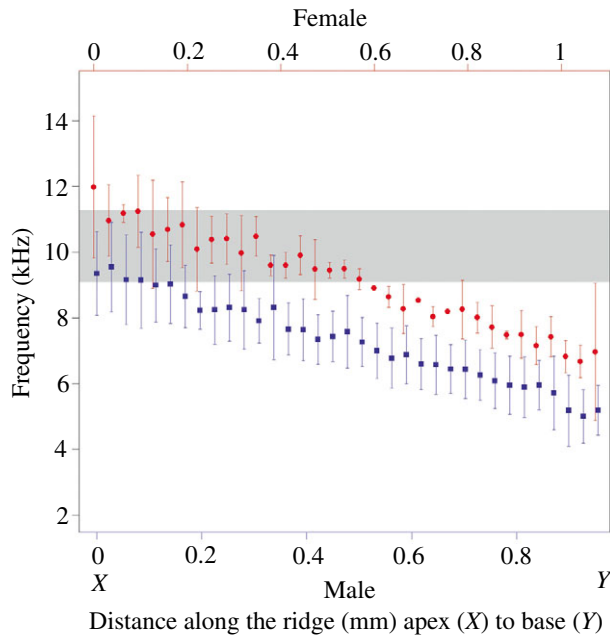


Fig. 14. Frequency response of the ridge from apex to base, stimulated with clicks. Grey area corresponds to 50% of calling song spectral energy. Values are means \pm s.d. for 6 males (blue) and 3 females (red).

on mechanical but also on neuronal mechanisms, they might be able to distinguish different conspecific calling songs in the background noise produced by other species with partially overlapping spectra (like *T. c. fairmairi*). Male spectral sensitivity seems to be shifted towards lower frequencies, implying some detuning. Because males produce intense

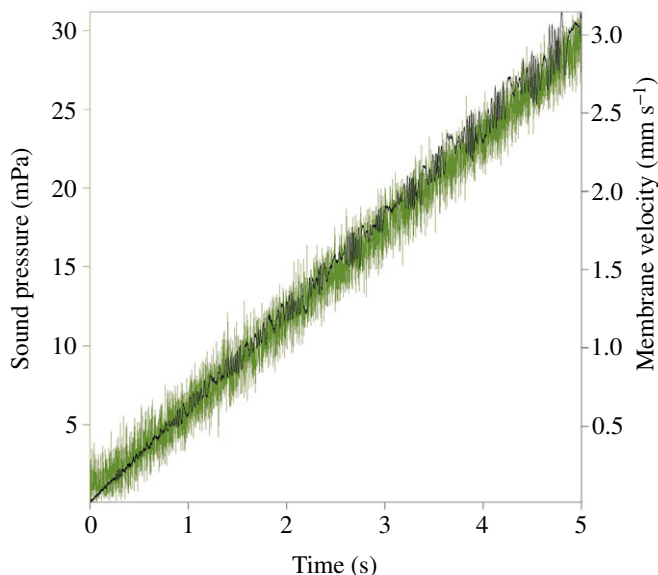


Fig. 15. Response velocity of the tympanal membrane at 11.7 kHz as a function of a continuous increase in forcing. Sound pressure was linearly increased from 1 mPa to 30 mPa in the time span of 5 s. Black curve: vibration velocity measured on the ridge (between the apex and the base). Green curve: time profile of sound stimulus.

signals [76–78 dB at 1 m (Boulard, 1992)], there is a risk of overdriving their own hearing system. To be slightly out-of-tune might reduce self-deafening risks, while still allowing the ability to listen to competitors when not calling. Injuries might also be reduced by a mechanical modification of TM tension. As already observed in other species (Pringle, 1954; Hennig et al., 1994; Yang et al., 1998), *C. atra* is able to fold its TM during sound production under the action of a tympanal muscle (data not shown).

The capacity for frequency analysis may have an important function in females in the context of male competition. Because, in their natural environment, males call in the vicinity of each other and actively compete for the acoustic space, females can hear several of them at the same time. Such competition results in the notoriously loud cacophony (at least to the human ear) of male songs. This situation makes a scheme of sexual selection by female choice plausible in *C. atra*. To our best knowledge, sexual selection exerted through preference of sound frequency has been clearly documented and tested in a few Orthopteran species, but never in cicadas (Gerhardt and Huber, 2002). Differences between males in the frequency composition of their calling have still to be precisely documented. However, tympanum mechanics combined with higher-level processes of frequency discrimination (auditory neurons, interneurons, brain neurons) might provide the basis for mating choice in female cicadas.

In addition, it may be crucial for both sexes to localise nearby singing males. Males need to know where their competitors are, and females, being the searching sex during pair formation, need to accurately localise and identify suitable courting males. The assessment of the distance to a sound source is usually based on the degradation of the signal's frequency composition (Naguib and Wiley, 2001). Sound propagation in air is frequency-dependent, greater attenuation affecting the higher frequencies contained in a signal. Spectral composition then varies with the distance between the emitter and the receiver. The capacity for frequency analysis may then help cicadas in estimating the distance to a sound source. It has been proposed that such a mechanism is at work in bushcrickets living in a highly scattering environment (Römer and Bailey, 1986; Römer, 1987). In this conjecture, male hearing tuned to lower frequencies, yet encompassing their own song, would enhance their sensitivity to distant acoustic competitors.

The apparent remarkable capacity of cicadas to process frequency information (Fonseca et al., 2000) needs to be further investigated, from the mechanical and physiological responses of the auditory system, to the behavioural and psychophysical capacity to respond to, and discriminate between, male songs of different qualities.

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