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# Sexual dimorphism in auditory mechanics: tympanal vibrations of *Cicada orni*

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## SUMMARY

**In cicadas, the tympanum is anatomically intricate and employs complex vibrations as a mechanism for auditory frequency analysis. Using microscanning laser Doppler vibrometry, the tympanal mechanics of *Cicada orni* can be characterized in controlled acoustical conditions. The tympanum of *C. orni* moves following a simple drum-like motion, rather than the travelling wave found in a previous study of *Cicadatra atra*. There is a clear sexual dimorphism in the tympanal mechanics. The large male tympanum is unexpectedly insensitive to the dominant frequency of its own calling song, possibly a reflection of its dual purpose as a sound emitter and receiver. The small female tympanum appears to be mechanically sensitive to the dominant frequency of the male calling song and to high-frequency sound, a capacity never suspected before in these insects. This sexual dimorphism probably results from a set of selective pressures acting in divergent directions, which are linked to the different role of the sexes in sound reception and production. These discoveries serve to indicate that there is far more to be learnt about the development of the cicada ear, its biomechanics and evolution, and the cicada's acoustic behaviour.**

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Key words: hearing, tympanum, biomechanics, laser vibrometry, high-frequency reception, predation, selective forces, cicada.

## INTRODUCTION

The production and reception of sound plays an important role in the life history of many insect species. For example, pair formation is ruled by the production and reception of sound between partners, and the orientation towards a prey or away from a predator can be elicited by acoustic cues (Greenfield, 2002). The success of such behaviour depends on the activation of complex auditory organs, either antennae working as near-field particle velocity detectors, or tympanal ears acting as far field pressure receivers (Robert and Göpfert, 2002; Robert and Hoy, 2007). In the latter case, the receiving organ is typically organized around a thin tympanal membrane (TM) made of cuticle, backed with air-filled tracheal sacs, and a set of sensory neurons connected to glial and support cells (for reviews, see Yager, 1999b; Yack, 2004). Such hearing organs have independently evolved in seven insect orders showing different degrees of development and organization (Hoy and Robert, 1996). Whatever the complexity of the hearing system is – from the 'cyclopean' ear of the praying mantis (Yager and Hoy, 1986), to the highly innervated pair of conspicuous cicada ears (Fonseca et al., 2000) – differences between male and female have been very rarely reported in tympanal structure and mechanics (Hoy and Robert, 1996). Sexual dimorphism has been documented in the ear anatomy of a small number of praying mantises (Yager, 1999a), bushcrickets (Bailey and Römer, 1991), flies (Robert et al., 1994) and moths (Minet and Surlykke, 2003), and was found to be obvious in cicadas (Pringle, 1954). Divergences in frequency tuning between sexes have been recorded in the ascending neurons of some crickets, bushcrickets, grasshoppers and cicadas (Gerhardt and Huber, 2002). However, little information has been made available on potential mechanical differences between male and female tympana (Meyer and Elsner, 1997). The origin of sexual dimorphism at the anatomical, mechanical or neuronal level may be explained by selective forces and constraints acting differently on the sexes. Several sex-linked factors can indeed be put forward: (1) sexes are exposed to different predators (Cardone and Fullard, 1988; Yager, 1990; Rydell et al., 1997); (2) prey or host detection is devoted to one sex only (Lakes-Harlan and Heller, 1992; Robert et al., 1994); (3) intra-sexual communication has been reduced, or disappeared in a single sex (Bailey and Römer, 1991; Mason and Bailey, 1998); (4) there is production of sex-specific signals in duetting species (Bailey, 2003); (5) the acoustic role of the sexes in pair formation is unbalanced; (6) each sex inhabits a specific niche implying different environmental constraints on sound propagation. To understand how one or several of these factors work at shaping the structural basis and functional diversity of insect auditory sexual dimorphism, it is necessary to study a model that shows an obvious sexual dimorphism and for which acoustic communication is well known.

In cicada, one of the noisiest animals in the world (Bennet-Clark and Young, 1992), there is extreme sexual dimorphism in the sound production system. Males possess a pair of abdominal tymbals fully dedicated to the generation of the calling song, a unique system that does not appear in the female (Pringle, 1954; Bennet-Clark and Young, 1992; Young and Bennet-Clark, 1995), hence the absence of any inter-female acoustic communication. Both male and female are nonetheless endowed with fully developed tympana whose differences in size and shape have been recognized since the middle of the nineteenth century (Dugès, 1838; Powell, 1873). These tympana are extended by a cuticular apodeme to which a set of sensory neurons (scolopidia; type I monodynal receptors) are attached. Tympana can therefore be considered as the first and necessary step of the mechanical chain that ensures audition in cicadas. The male tympanum is always larger, and is often coupled to a large air-filled abdomen. This dimorphism has been associated

with the mechanism of sound radiation through the tympana and abdomen (Young, 1990; Bennet-Clark and Young, 1992; Fonseca and Popov, 1994), but is undoubtedly involved in different auditory capacities of the sexes. Few attempts have been made to characterize the effects of dimorphism on auditory capability. In the Australian bladder cicada, *Cystosoma saundersii*, differences in tympanal and abdomen morphology drastically reduce the male's ability to localize a sound source, whereas the female exhibits accurate directional sensitivity (Young and Hill, 1977; Fletcher and Hill, 1978). In the Iberian cicada, *Tympanistalna gastrica*, larger tympanal membranes have been reported to impart a higher sensitivity to males (Fonseca, 1993) and in *Cicada barbara lusitanica* different tympanal structures imply different tuning and directionality (Fonseca and Popov, 1997). Tympanal membranes of both male and female *Cicadatra atra* vibrate with similar travelling waves, but males, with larger tympana, are slightly detuned to their own calling song, a system that might protect their auditory sensitivity (Sueur et al., 2006).

Do the morphological differences in cicada ears imply different auditory mechanics between sexes? What then could be the origin and consequences and diversity of sexual dimorphism in cicada audition? We analysed the mechanics of *Cicada orni*, an otherwise well investigated species with obvious sexual dimorphism affecting the hearing system. The histology of the chordotonal system has been studied previously in detail (Vogel, 1922; Michel, 1975) and the frequency tuning at the auditory nerve level has been measured (Popov et al., 1992), but nothing is known about the mechanics of the TM, where sound is transduced into a mechanical vibration. Using laser Doppler vibrometry, surface deflections of *C. orni* TM were reconstructed in three dimensions. This study reveals different deflection patterns than those previously observed in *C. atra* (Sueur et al., 2006), suggesting that different mechanical processes for filtering sound frequency content have evolved among cicadas.

## MATERIALS AND METHODS

### Animals for laser vibrometry experiments

Male and female *Cicada orni* L. were caught on the 9th July 2007 in Cuges-les-Pins, France (N43°16′18″ E5°41′24″). Animals were cooled down to 8–10°C and were immediately transferred to Bristol, UK in an ice-box. As previously described in detail (Sueur et al., 2006), animals were kept at this temperature but placed at 24–26°C before measurements. The wings, the legs, the operculum and the meracanthus, which are not mechanically linked to the tympanal organs, were cut back before mechanical measurements were made. 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 with 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 (TMC 784-443-12R; Technical Manufacturing Corp., Peabody, MA, USA) at 24–26°C and 40–62% relative humidity. 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).

### Calling song recordings

The calling song of *C. orni* could not be recorded in the same location (Cuges-les-Pins) because of the massive occurrence of two other singing cicada species, *Cicadatra atra* and *Lyristes plebejus*, that generated an important background noise. A previous study showed that the calling of *C. orni* song from western Europe, in particular from France, constituted an homogenous group (Pinto-Juma et al., 2005). We then used previous recordings made in two other locations (Peyriac-de-Mer, France, N43°05′14″ E2°57′33″; Molitg-les-Bains, France, N42°39′9″ E2°23′6″), at other dates (16th and 17th of July 2001) but at the same ambient temperature (26–27°C) of the sound-acoustic-proof room where laser experiments were carried out. Recordings were made using a Telinga Pro4PiP microphone (Telinga Microphones, Tobo, Sweden) (frequency response 40–18000 Hz ±1 dB) connected to a Sony TCD-D8 digital audiotape recorder (sampling frequency: 44.1 kHz, frequency response flat within the range 20–20000 Hz). The microphone was held at 50–60 cm dorsally from isolated singing males. One minute of each male calling song was analysed in the frequency domain using *Seewave* (Sueur et al., 2008). A mean frequency spectrum with a resolution of 12.5 Hz was computed for each individual using a Fourier transform with a Hamming window.

### Mechanical measurements

The vibrations of the tympanum were studied following the same general procedure used in a previous study (Sueur et al., 2006). The vibrations of the whole tympanum were examined in response to frequency modulated signals (duration=80 ms) sweeping at similar intensity all frequencies from 1 kHz to 22.05 kHz (low frequencies; LF), or all frequencies from 20 kHz to 80 kHz (high frequencies; HF). All acoustic stimuli were amplified with a Sony amplifier model TAFE570 (Tokyo, Japan) and were broadcast at 0.25 m from the cicada with a ESS AMT-1 loudspeaker (ESS Laboratory Inc., Sacramento, CA, USA) for LF, and with a SS-TW100ED loudspeaker (Sony) for HF. Thus, for both LF and HF ranges, the animal was in the far-field of the sound source. The vibrations of the tympanal ridge (TR), a dark spear-like structure connected to the apodeme where the sensory neurons (scolopidia) are attached, were studied in greater detail in six females using a line of scan points. The male TR was not examined in such a way as it was partially hidden by a cuticle sternal expansion that could not be removed without damaging the tympanum.

The intensity of the acoustic stimulations was 66 dB SPL at the cicada position. This corresponded to the sound pressure level (SPL) of a male calling at a distance of 4 m (Sueur and Aubin, 2003). This SPL was above auditory nerve threshold (Popov et al., 1992). The tympanal and female ridge vibrations were analysed by simultaneously recording the vibration velocity of the tympanum and the SPL adjacent to the tympanum. The laser vibrometer allowed accurate measurement (laser positioning ~1 μm) 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 the TM. SPL was measured using a 1/8 inch (3.2 mm) precision pressure microphone (Bruel & Kjaer, 4138; Nærum, Denmark) and preamplifier (Bruel & Kjaer, 2633). The microphone has a linear response in the measured frequency range. The sensitivity of the microphone was calibrated using a Bruel & 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.

The analysis of the tympanum displacement was carried out by the PC controlling the vibrometer. The laser signals resulting from the FM sweep were simultaneously sampled at 102.4 kHz for LF and at 204.8 kHz for HF. 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, 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.

Frequency spectra of the laser signal were normalized to those of the microphone signal by the computation of transfer functions, calculated as the cross-power spectrum of the laser and the microphone signals divided by the auto-power spectrum of the latter. In addition, the amount of unrelated noise was estimated by calculating the magnitude squared coherence (the ratio between the squared absolute value of the cross-power spectrum between the two signals divided by their auto-power spectra). Coherence values can range between zero and one, with a value of one indicating the absence of external, unrelated noise. Data were considered of sufficient quality when coherence exceeded 85%.

### Spectral analysis and statistics

To describe both calling song and tympanal frequency spectra, we used a measure of resonance quality at –3 dB around the dominant peak ($Q_{-3dB}$) (Bennet-Clark, 1999) and an estimation of spectral flatness (*SFM*; spectral flatness measure), which is the ratio of the geometric and arithmetic means of the frequency spectrum (Jayant and Noll, 1984). Values of $Q_{-3dB}$ increase with peak sharpness and values of *SFM* lay between 0 and 1, which respectively are indicative of a pure-tone signal and a random noise.

## RESULTS

### Tympanal anatomy

Like many other cicadas, the hearing system of *C. orni* resides ventrally in the second segment of the abdomen. The auditory system comprises two major elements: the tympanum and the sensory organ proper. 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, hidden in the auditory capsule, where the sensory neurons are attached. Conspicuous differences in size, shape and thickness are apparent between male and female tympana. Males have larger tympana than females surrounded by a larger cuticular frame, the dorsal rim being significantly stronger (Fig. 1). The male tympanum has three main zones. There are two opaque, white, thick zones that occupy medially and laterally three quarters of the TM surface (Fig. 1, blue and green lines). Between them lie one transparent thin central zone crossed by the ridge (Fig. 1, yellow dashed line). The latter is large and short, its apex reaching only one third of TM width. The female tympanum has only two zones, one is transparent, occupying three quarters of the TM surface, and crossed by a long, thin ridge, with its apex reaching around 80% of TM width (Fig. 1, yellow dashed line). The second zone, laterally located, is darker but not totally opaque (Fig. 1, red line).

### Spectral characteristics of the male calling song

The calling song of 10 males were recorded and analysed. The signal consists of short echemes regularly repeated [for a detailed

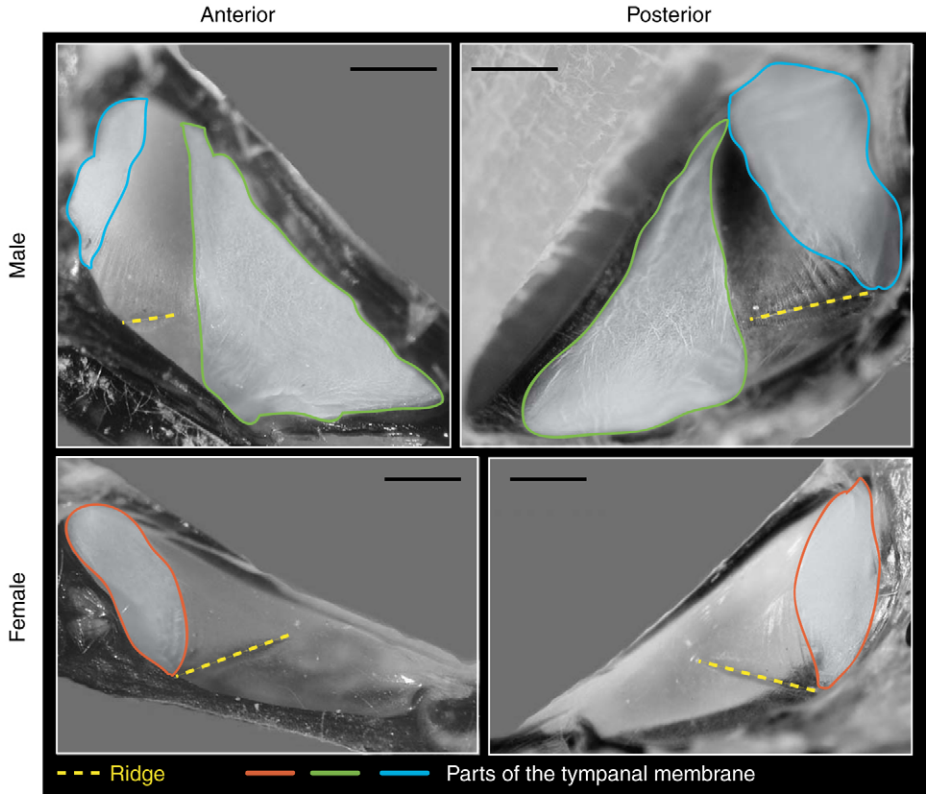


Fig. 1. Anterior and posterior (after removing the abdomen) views of male and female *C. orni* right tympanal membrane (TM). The male TM consists of three distinct parts, two of them are outlined with blue and green lines, respectively, and the third part is the area between. Female TM can be divided in two parts, one of them being shown in red. For both male and female the ridge area is indicated with a yellow dotted line. The shape of these parts differs slightly between anterior and posterior views as access and angle of view to the surface of the TM also differ. Scale bars, 0.5 mm.

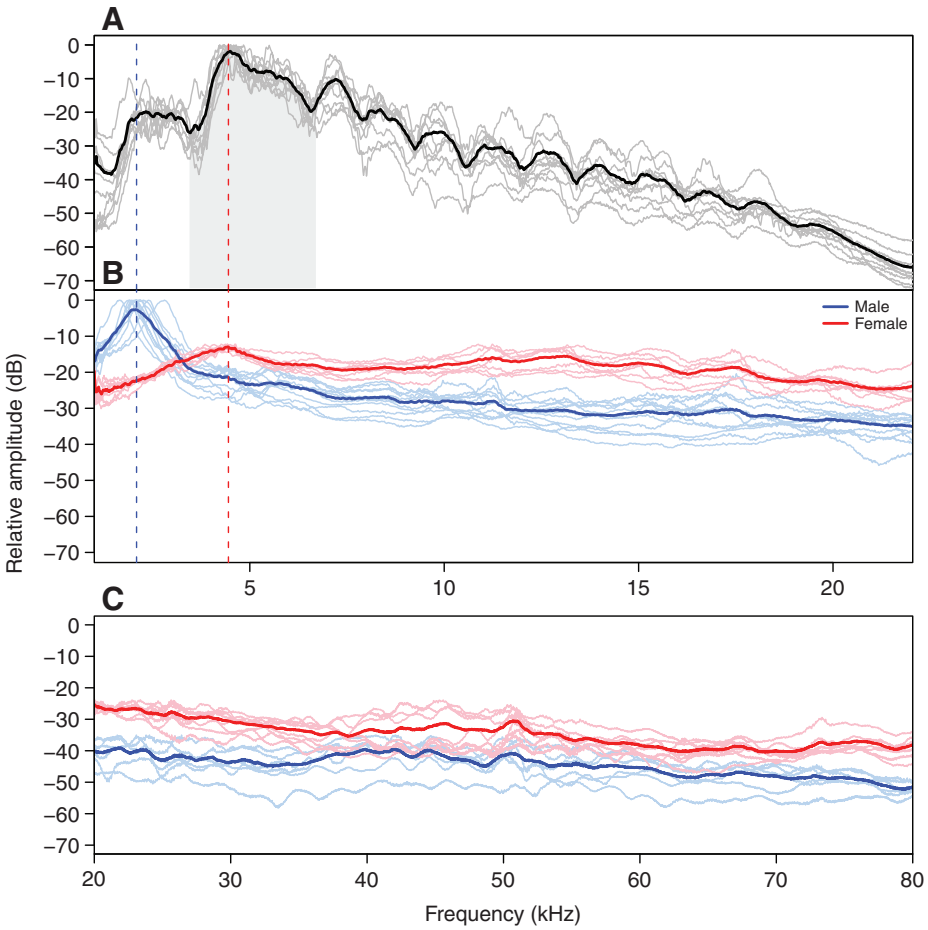


Fig. 2. Frequency magnitude spectra of the male calling song, and of the TM vibrations of both sexes. (A) Calling song spectra of 10 distinct males (grey lines) and their mean (black line), 50% of the male calling song energy is highlighted with a light grey shading. (B) TM vibrations spectra of 11 males and seven females (thin lines) and their respective mean (bold lines) at low frequencies (1–22.05 kHz), vertical dotted lines show the correspondence between maximal TM resonance and male calling song spectra. (C) TM vibrations spectra of six males and eight females and their respective mean (bold lines) at high frequencies (20–80 kHz). Originally expressed as amplitude data (mV for recorded songs) or gain data (nm Pa$^{-1}$ for TM vibrations), spectra were normalized between 0 and 1 and then transformed in decibels (dB) for the purpose of comparison.

analysis of the temporal pattern see Pinto-Juma et al. (Pinto-Juma et al., 2005) (Supplementary material Audio 1)]. In the frequency domain, the calling song covers a wide band, from around 1.5 to 19 kHz with 50% of the energy between 4.46±0.21 kHz (mean ± s.d.) and 6.73±0.74 kHz (Fig. 2A). The dominant frequency is 4.5±0.17 kHz with a resonance quality factor, $Q_{-3dB}$=10.04±1.66. The peak of the first frequency band is 2.27±0.18 kHz, its relative amplitude compared to the dominant frequency being at –17.05±4.19 dB. The signal is not modulated in frequency but a fast amplitude modulation at a rate of about 1 kHz is present due to the pulsed structure of the signal introducing secondary peaks every 1 kHz (Fig. 2A).

### Spectral characteristics of male and female entire TM

Scanning the entire TM surface with the laser Doppler vibrometer with meshes of 151±23 (females), 164±27 (males) points allows the measurement of the mechanical response of the cicada hearing system in the frequency domain. Averaging all points measured, it is then possible to obtain a frequency magnitude spectrum that indicates for which driving frequency the whole TM vibrates the most and thus reveals the first step of mechanical filter processes. In the low frequency range (LF, 1–20 kHz), the TM response of 11 males showed a sharp dominant peak at 2.13±0.30 kHz with a $Q_{-3dB}$ factor at 2.92±0.86 (Fig. 2B). The concentration of energy around this dominant peak is confirmed by intermediate *SFM* values (0.542±0.072). The male TM is therefore sharply tuned to the lowest frequency component of the male calling song (Fig. 2A,B, vertical dashed blue line). The frequency response between 1 and 20 kHz is broader for the seven females as shown by significantly higher *SFM* values at 0.93±0.022 (Welch *t*-test: $t$=–16.6096, d.f.=12.648, $P$=5.778×10$^{-10}$) (Fig. 2B). The dominant peak is higher at 4.35±0.29 kHz (Welch *t*-test, $t$=–15.655, d.f.=13.456, $P$=5.052×10$^{-10}$) with a similar $Q_{-3dB}$ at 2.71±1.11 (Welch *t*-test, $t$=0.453, d.f.=10.884, $P$=0.659). Thus the female TM vibrates over a wide frequency band, but has a sharp maximal resonance exactly matching the male's calling song dominant frequency (Fig. 2A,B, vertical dashed red line). Displacement gain at the frequency peak is 486±153 nm Pa$^{-1}$ for males and 119±46 nm Pa$^{-1}$ for females (Mann–Whitney test: $W$=0, $P$=6.285×10$^{-5}$). At their best resonant frequency, the male TM is then moving 4.08 (=12.2 dB) times more than female TM. This partly compensates for the relative amplitude difference between the 2.1 kHz and 4.5 kHz frequency bands of the calling song.

In the high frequency domain (HF, 20–80 kHz), neither the six males nor the eight females tested show specific frequency selectivity (Fig. 2C). The frequency spectra are similarly broad (*SFM*: males=0.87±0.08, females=0.86±0.05, Welch *t*-test: $t$=0.1698, d.f.=7.848, $P$=0.87) and no single dominant peak could be identified. Mean of displacement gain is 5.49±4.49 nm Pa$^{-1}$ for males and 17.1±13.8 nm Pa$^{-1}$ for females (Mann–Whitney test: $W$=153897118, $P$<2.2×10$^{-16}$).

### Motion patterns of male and female tympana

Three-dimensional reconstruction of the laser Doppler data reveals the patterns of motion of the tympanal system (Fig. 3A).

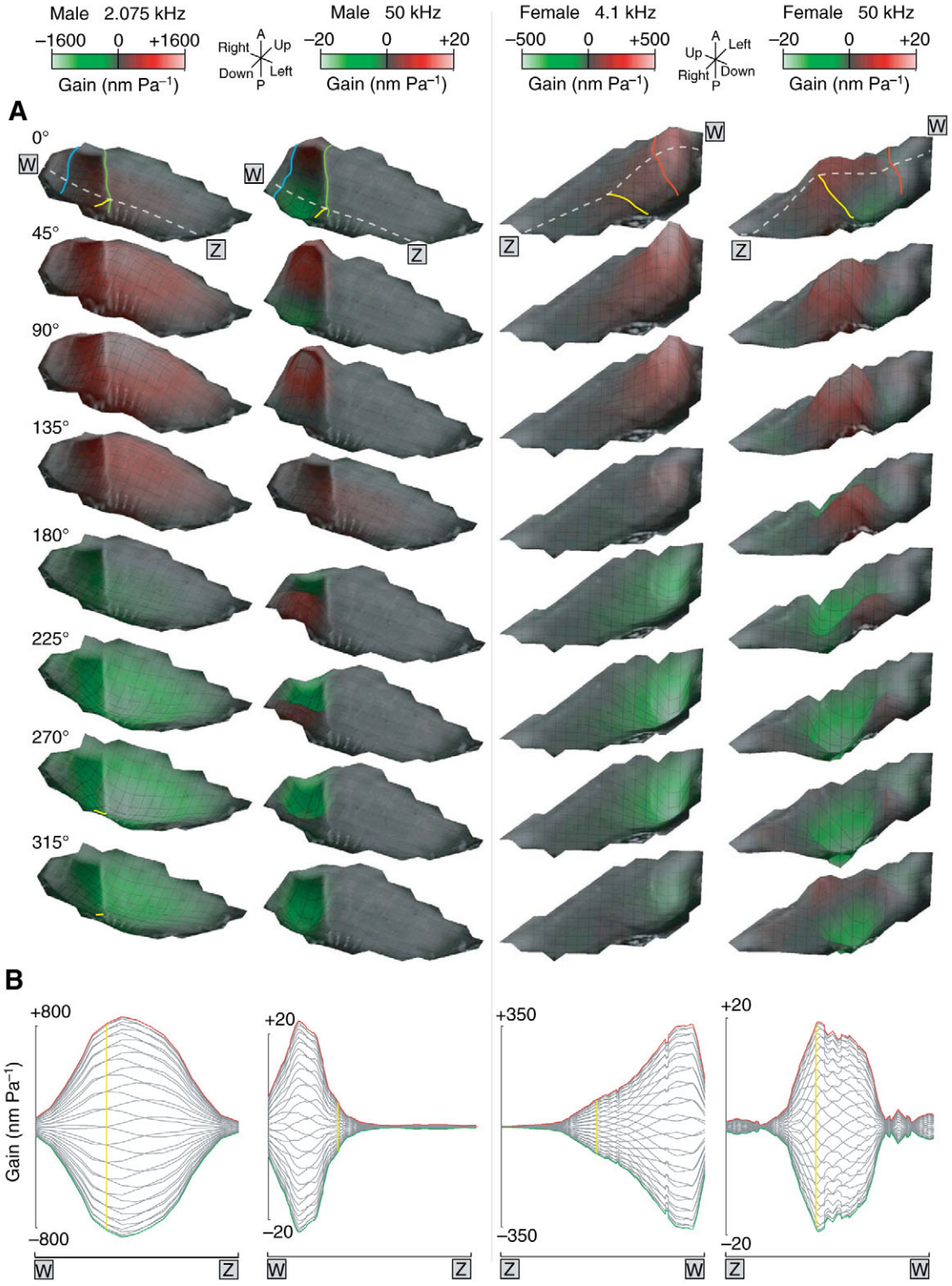


Fig. 3. Deflection shapes of a male right TM and of a female left TM. The TM was stimulated with a FM sweep signal. (A) Oscillations are shown at eight different phases (45° increment) along the oscillation cycle at the best resonance frequency in the low frequency domain (2.075 kHz for the male, 4.1 kHz for the female) and at 50 kHz. Deflections are expressed as displacement gain following the colour scale (nm $Pa^{-1}$). Red indicates outward tympanal deflections and green inward tympanal deflections. Note the difference in scale for each sex, and each driving frequency. Orientation is indicated by a 3D space reference (P, post; A, ant). The yellow line indicates the approximate position of the ridge and the green, blue and red lines show the limits of the different TM parts (see Fig. 1). (B) Corresponding envelopes of mechanical deflections (nm $Pa^{-1}$) across TM along the W–Z transect line. The position of the ridge apex is indicated by a vertical yellow line. Green and red curves are minimum and maximum values, respectively. Note the difference in scale.

At low frequencies, notably around the resonance peak at 2 kHz, the entire male TM vibrates in a simple oscillatory motion (Supplementary material Movie 1). This is particularly clear when looking at the envelopes of deflection shapes across the TM (Fig. 3B). The point of maximum deflection is located at the centre of the TM, close to the apex of the ridge. When stimulating male TMs at high frequency (50 kHz), only the transparent middle zone is vibrating, the two other zones remaining still (Supplementary material Movie 2). In this case the ridge is almost not driven by the TM, its apex being outside the area of maximal TM motion. The female tympanal system shows different patterns of motion (Fig. 3A). Around the frequency peak at 4 kHz, the female TM moves up and down asymmetrically but in phase. The lateral opaque zone is notably moving more than the rest of the membrane (Supplementary material Movie 3). This generates asymmetric deflection shape envelopes across the TM (Fig. 3B). Lying in the central part of the TM, the ridge is away from the maximal deflection point. Driven with HF, female TM showed a different pattern as seen for a 50 kHz stimulus in Fig. 3 (Supplementary material Movie 4). The membrane was moving up and down maximally in its middle part exactly where the ridge is found. This motion is organized, as not all TM points were moving exactly in phase.

### Mechanics of the female tympanal ridge

We studied in more detail the mechanics of the tympanal ridge (TR) of six females. We limited this analysis to the LF domain where frequency discrimination for male calling song is expected to occur. The male TR is unfortunately not accessible to the beam of the laser vibrometer. The differences in TR response with driving frequency are further assessed by computing the frequency spectrum at each of the measurement points taken along the ridge. The frequency response of the TR is characterized by two main peaks, the lowest at 5.53±1.05 kHz ($N$=155 points for six females) and the highest at 16.65±2.42 kHz ($N$=155 points for six females; Fig. 4A). The first peak matches 50% of the male calling song spectrum. There is no frequency modulation along the ridge (Fig. 4B), but the amplitude of the peaks changes from the apex to the base of the TR (Fig. 4C). When looking at a normalized frequency response, it appears that the relative amplitude of the 16.65 kHz peak is maximal and linear along the ridge. Indeed, this frequency shows the highest relative amplitude for 97.4% of the measurement points. At the same time, the relative amplitude of the 5.53 kHz peak is significantly increasing from the apex (0.46±0.15 relative amplitude, $N$=6) to the base of the TR (0.83±0.20 relative amplitude, $N$=6). However, absolute measurements show that the displacement of the TR is the same for the 5.53 kHz peak (apex: 102.7±42 nm Pa$^{-1}$, $N$=6; base: 97.6±36.2 nm Pa$^{-1}$, $N$=6; Welch $t$-test: $t$=0.2249, d.f.=9.786, $P$=0.8267), but decreases for the 16.65 kHz peak (apex: 223.8±66.4 nm Pa$^{-1}$, $N$=6; base: 127.2±74.3 nm Pa$^{-1}$, $N$=6; Welch $t$-test: $t$=2.3752, d.f.=9.876, $P$=0.03923). Altogether, this suggests that the TR acts as a low-pass filter: its base is less sensitive than its apex to frequencies around 16.65 kHz, but is equally

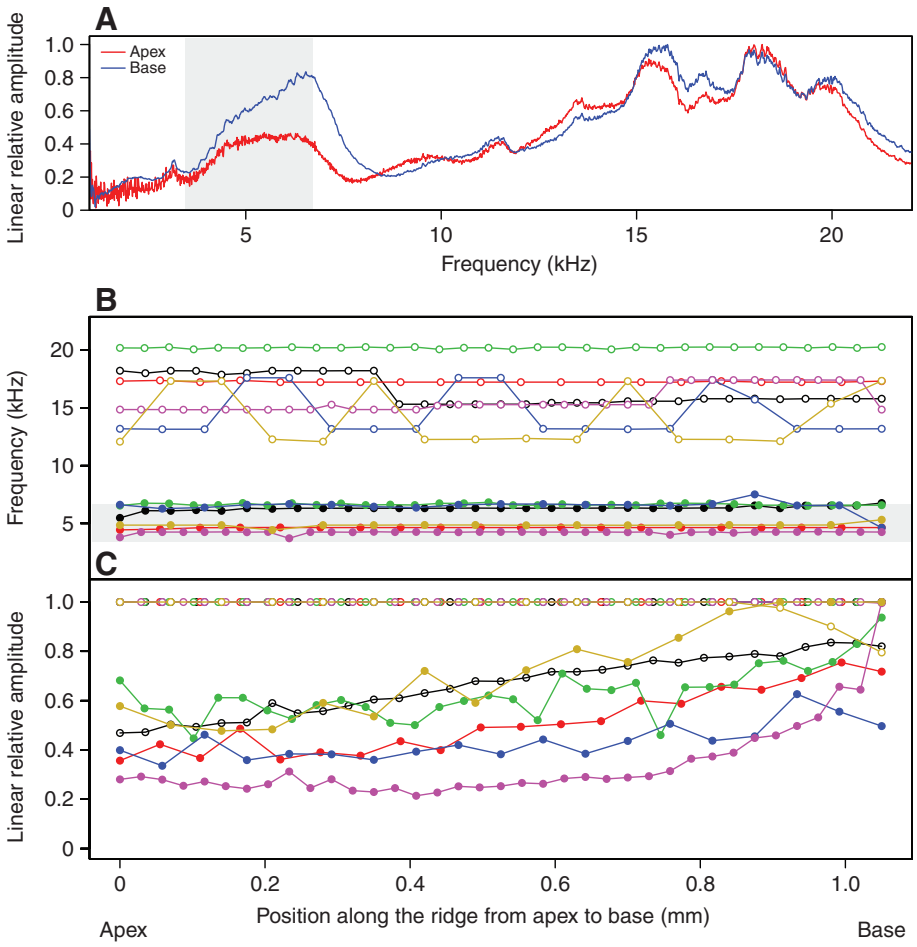


Fig. 4. Frequency response of the female tympanal ridge (TR). A line of scan points was taken along the ridge, and the frequency response was measured at each point. (A) A typical frequency response of the TR at its apex (red) and at its base (blue). Both frequency spectra show two main bands, one around 6 kHz, one around 17 kHz. Grey shading highlights 50% of the male calling song energy to show the match with the lowest frequency peak. Spectra were normalized between 0 and 1 to allow profile comparison. (B) Frequency of the two main spectra peaks. Filled circle indicate the lowest frequency peak and open circles, the highest peak. Different colours indicate different females. The number of scan points was not the same for all females. Grey area as in A. (C) Variation of the relative amplitude of both peaks on a linear scale normalized between 0 and 1. The relative amplitude of 17 kHz peak is linear and maximal whereas the relative amplitude of the 6 kHz peak increases along the ridge.

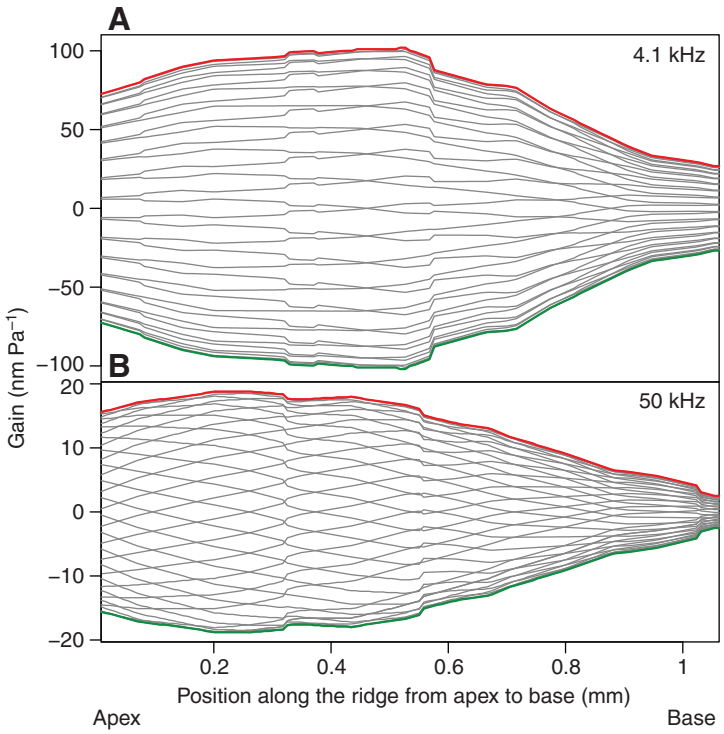


Fig. 5. Deflection shapes along the female TR from its apex to its base. The ridge was driven at its best resonance frequency in the low frequency domain (4.1 kHz) and at 50 kHz for the high frequency domain. Green and red curves are minimum and maximum values, respectively.

sensitive for frequencies around 6 kHz. Deflection shapes show steady waves with a drum-like motion, the base of the TR moving less than its apex (Fig. 5). The phase response along the TR does not show a significant increasing lag as a function of stimulus frequency (Fig. 6A). There is no phase lag either between the apex and the base as shown (Fig. 6B). This differs drastically from the phase response of the TR of another species, *Cicadatra atra*, in which travelling waves generate phase lags with both frequency and position along the ridge (Sueur et al., 2006).

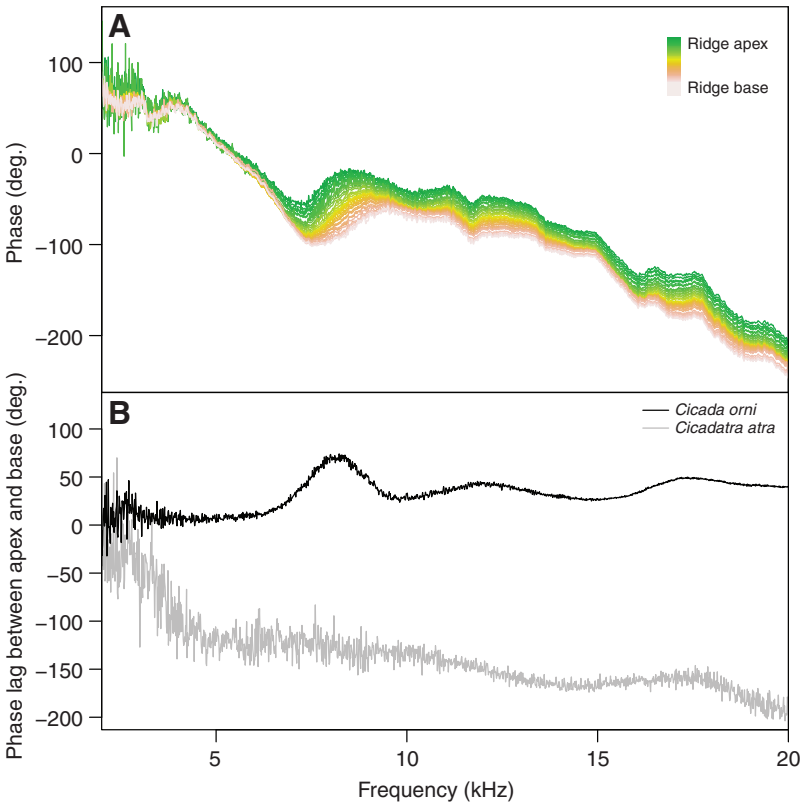


Fig. 6. Phase response of the female TR. (A) Phase response along the ridge from its apex to its base. (B) Difference between the phase response measured at the apex and at the base. There is no significant increasing phase lag with frequency, the maximal difference being 74° at 8.225 kHz. For comparison similar data measured for a *Cicadatra atra* female are shown, where travelling waves occur along the ridge. In this case the phase lag reaches 204° at 19.912 kHz. *C. atra* data are modified from Sueur et al. (Sueur et al., 2006).

## DISCUSSION

Believing that the cicada hearing system is architecturally homogeneous, we expected to observe similar tympanal mechanics between *C. orni* and *C. atra*, as both species belong to the same tribe Cicadini. The vibration pattern of the *C. atra* membrane is complex, characterized by waves travelling across the ridge, the phase and wavelength of which vary with the driving frequency, and with male and female showing slight tuning differences (Sueur et al., 2006). Surprisingly, we found significant differences in *C. orni*, revealing a further aspect to insect auditory diversity. *C. orni* seems to have developed a distinct mechanical strategy that filters out frequencies not relevant to acoustic communication. The *C. orni* tympanum indeed moves with a simple drum-like motion similar to the membrane of a microphone (Windmill et al., 2007) and show important sex-specific characteristics, which we hereafter compare in more detail.

### Male acoustic reception

Scanning the whole male TM indicates that the system is designed to receive one specific frequency band around 2.1 kHz. The resonance of the TM is sharply tuned around this frequency, and the ridge apex is not driven significantly at higher frequencies, in particular at ultrasonic frequencies. This tuning seems to be conserved, but slightly broader, when recording the summed excitation of the auditory receptors in the auditory nerve (Popov et al., 1992). However, more recent intracellular recordings from auditory interneurons of another species (*Tettigetta josei*) suggest that the cicada ear uses differential tuning of the auditory receptors for frequency discrimination (Fonseca et al., 2000).

Frequency selection is accompanied by high sensitivity as indicated by a maximal displacement gain around 880 nm $Pa^{-1}$. In other words, males seem to be able to listen efficiently to a narrow frequency band centred around 2.1 kHz. Surprisingly, this selectivity is not congruent with the maximal song energy around 4.5 kHz, but to the lowest component of the emission spectrum, some 17 dB lower in intensity. This discrepancy between emission and reception spectra is probably linked to the large size of the tympanum, knowing that the frequency of the first mode of vibration is inversely proportional to the square root of the area of the membrane (Fletcher, 1992). Such apparent detuning can, however, confer some advantages. With such a high sensitivity a perfect tuning with the calling song dominant frequency would probably overdrive the system during self-generated calling. If the auditory threshold can be reduced by the tympana folding through the action of an accessory muscle (Hennig et al., 1994), frequency detuning may also provide some protection of sensitivity, and prevent deafening. It is also important to note that a mismatch between mechanics and calling song might disappear when testing the behavioural response to stimuli with different frequencies. This is, for instance, the case of the sibling species *C. barbara lusitanica*, as the males have an auditory nerve that responds best to 3–4 kHz tones, but behaviourally have a more sensitive response to 6 kHz sound (Fonseca and Revez, 2002). It would be interesting to conduct playback experiments with *C. orni* to know whether a correlation between mechanics and behaviour does exist.

In addition, cicada male tympana work like passive radiators of a simple Helmholtz resonator, whose cavity is the abdomen and drivers are the tymbals (Young, 1990). Variation in tympanal structure is likely to modify the quality of the sound produced. Sound frequency and energy increase with the size of the tympanum and, inversely, resonant frequency shifts down when thickness augments (Bennet-Clark and Young, 1992). The large size of the male tympana might then facilitate a good transmission of high frequency sound. By contrast, tympana appear to be particularly thick in their median and lateral parts and thus probably shift the calling song to lower frequencies than it would have been with only thinner membranes. Because they are involved in sound emission and reception in the same time, male cicada tympana work as dual structures, and as such must be the result of a trade-off between several sets of selective forces.

### Female acoustic reception

The female tympanum is precisely tuned to the dominant frequency of the calling song, presumably maximizing the detection of the species-specific song, and its recognition. This sharp tuning is probably the result of sexual selection forces through female choice. It is highly probable that the temporal pattern of the song, made of the regular repetition of echemes (Pinto-Juma et al., 2005), also participates in song identification as was suggested to occur in *C. barbara lusitanica* (Fonseca and Revez, 2002). As in males, the female TM works like a simple membrane, but the pattern is asymmetric at low frequencies. For the apex of the ridge, the deflection is maximal at high frequency. A precise examination of the TR deflection shape reveals that the response amplitude to high frequency components decreases in amplitude from the apex to the base. It appears that the TR works as a low-pass filter focussing low frequency components, around the dominant frequency of the calling song, to its base, which is directly connected to the internal apodeme where sensory neurons attach. Because the TR is a part of the TM and not an independent structure, the vibrations of the other parts of the TM probably contribute to this mechanical filter. Again, TR deflections follow a simple oscillatory pattern very different from the complex travelling waves observed in *C. atra*, indicating that these two species use different passive frequency filters.

The resonance quality factor ($Q_{-3dB}$) around maximal resonance is similar in male and female tympana, but the spectral flatness measure (*SFM*) indicates that the female tympanum has a broader frequency sensitivity, being able to move significantly at frequencies higher than 6 kHz. This result indicates that the sensitivity of the female might then cover the whole spectrum of the male calling song. Females are the searching sex and need to precisely locate singing males. As shown in *C. barbara lusitanica*, which is extremely similar in size and morphology to *C. orni*, phase and amplitude differences between left and right tympana due to diffraction around the body are significant only above 10 kHz and tympanal directionality also increases with frequency (Fonseca and Popov, 1997). To be able to listen to a broad frequency spectrum ensures that the females receive more cues on the localization of the source. Our data reveal that female auditory capacity not only encompasses the highest frequency part of the calling song, but might extend into the ultrasound domain. This aptitude might also be linked to the small size of the tympanum. Although ultrasound use has never been reported in cicadas, many insects are known to exploit high frequency sound for mating (Mason and Bailey, 1998; Skals and Surlykke, 1999; Montealegre-Z et al., 2006; Nakano et al., 2006) or during prey–predator interactions (Lakes-Harlan and Heller, 1992; Yack and Fullard, 2000; Ratcliffe and Fullard, 2005; Höbel and Schul, 2007). It is now necessary to conduct behavioural observations and experiments to determine in which context – reproduction or predator avoidance – cicadas might use ultrasound. This would also encompass recordings of auditory neurons to ensure that ultrasound is integrated by the neuronal system.

## LIST OF ABBREVIATIONS

| | |
|---|---|
| HF | high frequency |
| LF | low frequency |
| $Q_{-3dB}$ | resonance quality factor at –3dB |
| SFM | spectral flatness measure |
| TM | tympanal membrane |
| TR | tympanal ridge |

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