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RESEARCH ARTICLE

Mechanical filtering for narrow-band hearing in the weta

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SUMMARY

This paper constitutes a major attempt to associate tympanic deflections with the mechanoreceptor organ location in an acoustic insect. The New Zealand tree weta (*Hemideina thoracica*) has tympanal ears located on each of the prothoracic tibiae. The tympana exhibit a sclerotized oval plate, membranous processes bulging out from the tibial cuticle and many loosely suspended ripples. We used microscanning laser Doppler vibrometry to determine how such a tympanal membrane vibrates in response to sound and whether the sclerotized region plays a role in hearing. The tympanum displays a single resonance at the calling frequency of the male, an unusual example of an insect tympana acting as a narrow bandpass filter. Both tympana resonate in phase with the stimulus and with each other. Histological sections show that the tympanal area is divided into two distinct regions, as in other ensiferans. An oval plate lies in the middle of a thickened region and is surrounded by a transparent and uniformly thin region. It is hinged dorsally to the tympanal rim and thus resembles the model of a 'hinged flap'. The thickened region appears to act as a damping mass on the oscillation of the thin region, and vibration displacement is reduced in this area. The thinner area vibrates with higher amplitude, inducing mechanical pressure on the dorsal area adjacent to the crista acustica. We present a new model showing how the thickened region might confer a mechanical gain onto the activation of the crista acustica sensory neurons during the sound-induced oscillations.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/214/5/778/DC1

Key words: weta, laser vibrometry, ensiferan ear, tympanum vibration, mechanical tuning.

INTRODUCTION

The auditory systems of insects are typically adapted to be most sensitive to sounds that are significant for survival and/or reproductive success. Such sounds include conspecific signals and incidental noises generated by predators and/or prey. The range of frequencies detectable by insect species can be relatively broad (Hill and Oldfield, 1981; Hoy et al., 1982; Mhatre et al., 2009; Yager and Hoy, 1986) or quite narrow (Field et al., 1980; Mason, 1991) (present study). The ultimate reasons favouring broad or narrow frequency selectivity and the biophysical and neural mechanisms subtending such selectivity are still only partially understood. It is remarkable that insects with narrow-band intraspecific communication signals (such as field crickets) do not necessarily display narrow-band tuning in their auditory or mechanical sensitivity.

The endemic tree weta of New Zealand (Orthoptera, Ensifera, Anostostomatidae, genus *Hemideina*) has typical ensiferan prothoracic tibial ears. Superficially, the morphology and anatomy of their ears resembles the relatively well-known tympanal ear of the Tettigoniidae and the lesser-known ear of the Haglidae (Mason, 1991). All three groups have two equally sized tympana located on each prothoracic tibia. Comparison between the better-known Tettigoniidae and the tree weta shows that they also have similar underlying tracheal chambers (tympanal vesicles) and associated receptor organs. However, a marked difference exists in the size and thickness of the tympanal membranes, with weta having a larger, thicker membrane than the Tettigoniidae (Ball and Field, 1981; Bangert et al., 1998). A further difference is that the tympanum of tree weta does not appear to be taut, and conspicuous ripples radiate out from a sclerotized thickened area (Ball and Field, 1981), referred here to as the inner plate. The tettigoniid tympanum tends to be taut, as are those of most other insects. Given the size and thickness of the weta tympanal membranes, they might be expected to have low responsiveness for the transmission of sound waves (Beranek, 1954).

Although it has been established that tree weta hear well [peak sensitivity 2–3 kHz, threshold at 34 dB sound pressure level (SPL)] (Field et al., 1980), it is still unclear how sound is transduced given the suggested large compliance of the tympanal membrane. A model that may apply to this system has been described by Bangert et al. (Bangert et al., 1998) for the tettigoniids. Their hinge-door model describes equally sized membranes, both moving in anti-phase to each other. Sound pressure in the underlying tracheal chambers pushes out the inner plates of the tympana (acting as hinged flaps), causing stretching of the crista acustica (CA).

The anatomical observations of Ball and Field (Ball and Field, 1981) and the model proposed by Bangert et al. (Bangert et al., 1998) together suggest a functional arrangement – to be tested in the weta – that would rely on a putatively similar hinge-like mechanical response. To document the mechanics of the weta tympanal system with sufficient accuracy and evaluate its function in hearing, we undertook a renewed morphological and physiological

study and performed high-resolution vibration analysis. The weta tympanum displays a tuned mechanical response that is distinct from that of a homogenous membrane. Tympanal deflection shapes reveal non-symmetrical patterns introduced by the presence of the stiff inner plate. The auditory function of this unusually thick, loosely suspended and heterogeneous tympanal membrane is discussed in the context of the acoustic communication of the weta.

MATERIALS AND METHODS

Animals

Adult male and female *Hemideina thoracia* (White 1846) were collected from around Auckland, New Zealand, in October 2008. Animals were kept in a 1×1×1 m enclosure and provided with a diet of New Zealand native plant material.

Tympanal morphology

To examine the morphological characteristics of the tympana, examinations were carried out at the University of Auckland, New Zealand. The two tympana are known as the anterior tympanic membrane (ATM) and the posterior tympanic membrane (PTM). The surface structure of each tympanum was examined by scanning electron microscopy (SEM). Specimens were prepared by removing the forelegs and preserving them in 90% ethanol. They were then mounted on stubs, dried in a critical point dryer and gold-coated before being scanned using an environmental scanning electron microscope (FEI Quanta 200 F, FEI, Hillsboro, OR, USA). SEM images were digitised using FEI's standard hardware and software.

Details of the tympanum structure and thickness were studied using transverse and longitudinal sections under a 10× light microscope. Four tibia were removed and immediately fixed in Bouin's medium overnight, then transferred to cold 10% sucrose (in Millonig's buffer) for 2 h (4°C), transferred to cold 30% sucrose (in Millonig's buffer) and kept at 4°C until they sank to the bottom of the vials (Field, 1993). Specimens were embedded in optimal cutting temperature (OCT) compound and sliced into 20 µm sections using a freezing microtome. Measurements from the tympana were means of the four membranes measured. For resin sections, foreleg tibia were removed and fixed in 4% formaldehyde for 4–6 h. The tissue was then embedded in Histocryl resin with a polymerization time of 10 min. The Histocryl blocks were sectioned at 5 µm intervals on a standard microtome. All sections were post-stained with Toluidine blue and imaged on a Leica DMR fluorescence microscope.

Auditory threshold curve

Male and female *H. thoracica* were mounted dorsal-side-up on a platform of sticky wax. The legs were secured with metal clamps and the tympanal nerve was exposed by removing the cuticle overlaying the forefemur. A well made from dental wax was constructed around the region of the exposed nerve. The connection to the hemolymph was broken by lifting the nerve out of the well with the hooked electrode. The exposed nerve was covered with a 1:1 Vaseline®:paraffin oil mix to prevent it from drying out. Sound-evoked responses were recorded from the tympanal nerve of the foreleg with a silver hooked electrode (0.250 mm). Summed action potentials were amplified and recorded with a TDT RA16 amplifier (high pass 300 Hz, low pass 10,000 Hz; Tucker Davis Technologies, Alachua, FL, USA). To define threshold levels, the number of summed action potentials was counted at 1 ms time intervals; responses were classified using post-stimulus time histograms (PSTHs) generated in TDT OpenEx software. Thresholds were defined as the mean number of summed action potentials of the spontaneous rate, plus one deviation in two out of three recordings. The spontaneous rate was determined from recordings 0–5 ms pre-stimulus. Thresholds were confirmed using the headphone method described by Autrum (Autrum, 1941).

Stimuli presented were 40 ms tones, decreasing in intensity, with 0.4 ms rise and fall times, produced with a TDT RA6 processor in 1 kHz increments between 1 and 60 kHz. Stimuli were presented ipsilateral to the recorded nerve in random order. Amplitude was measured by recording tones at position of preparation using a 0.25 inch condenser microphone (Brüel & Kjær 4939, Nærum, Denmark) and calibrating with a Brüel & Kjær acoustical calibrator (type 421; 90 dB SPL at 1000 Hz).

Mechanical measurements

Thirty-two *H. thoracica* were transported to the University of Bristol, UK, where the biomechanical experiments were carried out. Once in the UK, animals were placed in plastic boxes and provided with a substitute diet of apple and carrot with some lettuce. The temperature was kept at an optimal range for weta (10–20°C). The experiments did not commence until 1 week after the animals arrived, allowing them to acclimatise to their new environment.

Movement of the animals was restricted during laser scans by firmly attaching the meso-thoracic, meta-thoracic and dorsal side of the thorax to a horizontal brass plate (6×1×16 mm) using liquid latex (Magnacraft, Midhurst, UK). The brass plate was connected to a metal rod (150 mm long, 8 mm diameter) *via* a thumbscrew, allowing the preparation to be rotated and tilted into a position where the tympanum was perpendicular to the laser beam. Blu-Tack® (Bostik-Findley, Stafford, UK) was wrapped around the rod to minimise residual resonant vibrations of the tethering system. The forelegs of the animal were secured to a wooden perch that placed them in a natural standing posture. Animals were not anaesthetised during measurements. However, in some cases it was necessary to further restrict movement of the animals. To achieve this, an injection of 2.5 g 10 ml^{-1} of glutamic acid/insect ringer mix was used to relax the animal. All animals survived this procedure and returned to their individual cages.

The sound-induced mechanical response of each tympanum (ATM and PTM) was measured across the entire membrane. Preliminary experiments carried out with a wide-band stimulation of 1–60 kHz (a sampling rate of 500,000 samples s^{-1} was used in this case) showed that the tympanal response to frequencies higher than 8 kHz was extremely small, and immeasurable for higher frequencies. Therefore, to reduce measurement time and therefore possible errors due to movement of the animal, acoustic stimuli were reduced to 1–25 kHz sweeps. Microphone and laser signals were simultaneously sampled at 102,400 samples s^{-1} in these experiments; the 25 kHz wide-band chirps lasted 80 ms. The spectrum of the stimulus was corrected to be flat (±1.5 dB) at 55 dB SPL (re. 20 µPa), well above the auditory nerve threshold of 34 dB SPL. The other stimulus was a 3.6 kHz four-cycle tone at 55 dB SPL. Acoustic signals were generated using a data acquisition board (PCI-4451, National Instruments, Austin, TX, USA), amplified (Sony Amplifier Model TA-FE570, Tokyo, Japan) and presented through a loudspeaker (ESS AMT – 1; ESS Laboratory Inc., Sacramento, CA, USA) positioned *ca.* 200 mm from the tympanum in contralateral position. The speaker position was contralateral because acoustic conditions were better on that side, facilitating spectral correction and flattening. Amplitude was measured using a 0.125 in (3.2 mm) condenser microphone (Brüel & Kjær 4138) and preamplifier (Brüel & Kjær 2633), positioned 1–2 mm from the tympanum. The microphone had a linear

response in the measured frequency range. The microphone's sensitivity was calibrated against a sound level calibrator (Brüel & Kjær 4231, producing 94 dB SPL at 1 kHz).

Vibrational responses to sound were measured using a micro-scanning laser Doppler vibrometer (Polytec PSV-300-F, Waldbronn, Germany) with an OFV-056 scanning head fitted with a close-up attachment. This allowed the laser spot (~5 µm diameter) to be positioned with an accuracy of ~1 µm. The laser spot was positioned and monitored *via* a live video feed to the vibrometer's controlling computer located outside the acoustic isolation booth. The tympanum was oriented to allow the laser beam to scan the entire membrane. All experiments were carried out on a vibration isolation table (TMC 784-443-12R, Technical Manufacturing Corp., Peabody, MA, USA) at a room temperature of 18–21°C and relative humidity of 40–62%. The entire preparation was located in an acoustic isolation booth (internal dimensions: length 4.50 m, width 2.25 m, height 1.98 m; Industrial Acoustics IAC series 1204A, Winchester, UK). All components for the experiment were positioned to allow measurements to be taken without having to readjust the position of any equipment.

Analytical and response signals were processed, analysed, stored and displayed using the vibrometer's software (PSV v.7.4). Sets of 25 data windows of 80 ms duration were acquired and averaged for each point across the membrane. For each signal, a frequency spectrum was generated using a fast Fourier transform (FFT) with a rectangular window and a resolution of 12.5 Hz. From this, the transfer function of the membrane velocity to SPL (Pa) was calculated to produce the amplitude gain and phase response of the system at different frequencies.

To determine the direction of displacement of the two membranes with respect to each other, the phases of their mechanical vibrations with respect to the acoustic stimuli were subtracted from each other ($Phase_{ATM} - Phase_{PTM}$). The method for estimating phase and direction of vibration between two adjacent tympanic membranes is discussed and explained in more detail in Mhatre et al. (Mhatre et al., 2009).

Differences between membrane responses from males and females were tested using two-way ANOVA in JMP statistical software (SAS, Cary, NC, USA). *Q*-factors that describe the resonance quality at 3 dB below the dominant peak (Q_{-3dB}) (Bennet-Clark, 1999) were calculated in MATLAB (The MathWorks, Inc., Natick, MA, USA).

The magnitude-squared coherence between the vibrometer and microphone signals was also computed for each data point to assess data quality for the entire data set [this method is briefly described in Windmill et al. (Windmill et al., 2005)]. Coherence values range between 0 and 1, with a value of 1 indicating the absence of external, unrelated noise. Data were considered of sufficient quality when coherence exceeded 0.85.

RESULTS

Tympanum anatomy

Tympanal membranes of *H. thoracica* are identical in both size and structure. They are oval-shaped, ~1.5 mm wide and 2.5 mm long, with a total surface area of ~3.75 mm^2. Notably, the membranes are not stretched tight but loosely suspended, giving a rippled appearance (Fig. 1A,B). The tympana are divided into two distinct zones. The first is a thickened region with a tanned inner plate area (Fig. 1B). A particularly striking feature of this region is its thickness, an average of 151 µm at the thickest point, with a maximum of 185 µm in one individual. Longitudinal sections show that the thickened zone is asymmetrical lengthwise; the thickest point is near the dorsal hinge, tapering distally down to *ca.* 77 µm. In the dorsal centre of the thickened region is the inner plate, which has a thick outer layer of tanned cuticle (Fig. 1B). The inner plate is *ca.* 13 µm thick and acts to stiffen the thickened region of the membrane. The second region is thin and loosely suspended, and surrounds the thickened region (Fig. 1B). The mean width of the thinner region was 22 µm. Histologically, the thinned region is not a separate entity; it has the same laminate structure as the thickened region (Fig. 2).

The loosely suspended ripples of the thinned region enable the membrane to expand out from the surrounding cuticle by ~100 µm (Fig. 1C, Fig. 2). Because of sample preparation, the SEM scans (Fig. 1D) do not show these inflated properties. The scans do, however, show the full size of the thickened region that extends beyond the inner plate area (inner plate area: width 0.3 mm, height 0.5 mm, total area ~0.15 mm^2; thickened region: width 0.7 mm, height 1.5 mm, total area ~1.05 mm^2; Fig. 1D).

Threshold curve

The tympanal organ of *H. thoracica* has a sensitivity peak to acoustic frequencies of ~3 kHz (Fig. 3). At best sensitivity, the threshold was 39.11±1.12 dB SPL (*N*=7 animals). At frequencies below 3 kHz, sensitivity was reduced by ~21 dB per octave. At frequencies above

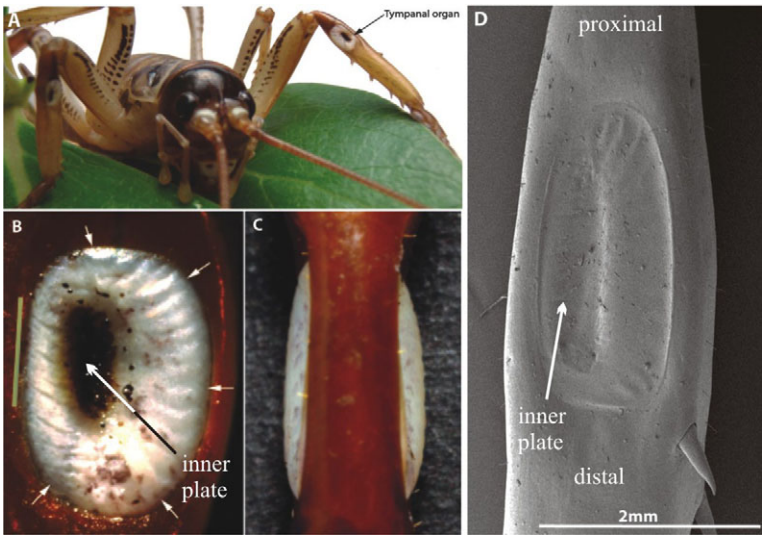

Fig. 1. External anatomy of the hearing organ of *Hemideina thoracica*. (A) Female perched on a leaf showing the large tympanal membrane located on the tibia. (B) Close-up of the anterior tympanal membrane (ATM) showing the inner plate (long arrow) and a loosely suspended, lightly coloured rippled zone (short arrows). (C) Close-up of both tympana in frontal view, showing the bulging of the tympanal membranes from the surrounding cuticle. (D) SEM of the left posterior tympanal membrane (PTM). The depressed area on the right of the membrane (arrow) is the inner plate.

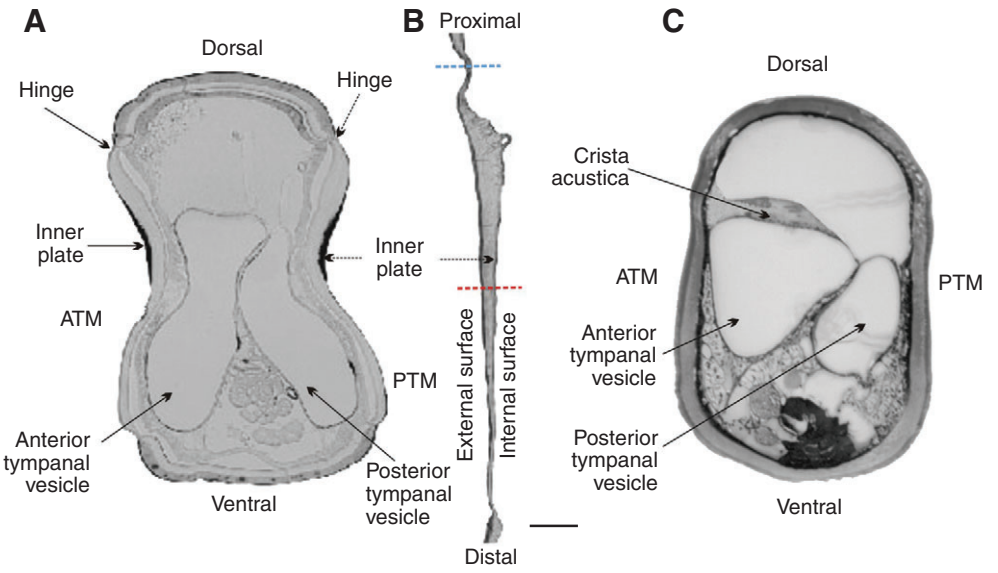

Fig. 2. Transverse and longitudinal sections of the tympanal hearing organ of *H. thoracica*. (A) Transverse section of the left leg showing the inner plates as thicker, tanned cuticle. (B) Longitudinal section of the tympanal membrane inner plate, thicker at its centre. The red dashed line indicates where the cross-section shown in A was taken. The blue dashed line indicates where the cross-section shown in C was taken. Proximal, towards the femur; distal, towards the tarsus. The hinge is the area where the inner plate is attached to the surrounding cuticle and moves in a cantilever manner. (C) Transverse section of the left leg showing the position of the crista acustica. Scale bar, 200 μm.

3 kHz, the roll off was ~31 dB per octave. No response was recorded to frequencies over 8 kHz, with the exception of one male who showed a response to 10 kHz above 81 dB SPL. These results agree with the auditory threshold curve obtained by Field et al. (Field et al., 1980).

Mechanical response of tympanum

The mechanical response of ATM and PTM was characterised for males and females to assess possible sexual dimorphism of the auditory apparatus. No significant difference in the resonant peaks of either the ATM or the PTM was found between males and females ($F_{3,16}$=1.46, P=0.27; Fig. 4A–D). Responses to broad-band frequency sweeps (1–25 kHz) showed a sharp peak for the ATM in males at 3.4±0.3 kHz (Q_{-3dB}=2.4±0.5) and in females at 3.4±0.2 kHz (Q_{-3dB}=2.3±0.2). The PTM showed a maximum amplitude peak at 3.8±0.6 kHz in males (Q_{-3dB}=2.5±0.4) and at 3.3±0.2 kHz in females (Q_{-3dB}=2.8±0.6). The Q_{-3dB} factors of the ATM and PTM were not significantly different between sexes ($F_{3,12}$=0.77, P>0.05). The phase of the response, relative to the driving acoustic input, changed by almost 180 deg between 1 and 6 kHz. The phase change in both membranes took place near the resonance peak (Fig. 5A,B), and high coherence was recorded for the narrow bandwidth around this peak (3–4 kHz) (Fig. 4F, Fig. 5C). This highlights the high fidelity of the mechanical response. Together, this evidence shows that the membranes of both males and females are mechanically sharply tuned to a narrow frequency range, that of the conspecific calling song. However, computation of the resonance difference function ($f_{o,females} - f_{o,males}$) for the mean vectors of the ATM and the PTM between males and females, respectively, shows that the ATM was slightly more sensitive in females than in males (Fig. 4E). The mechanical response in the ultrasonic range was weak. The ATM of one female vibrated at 51.6 μm s^{-1} Pa^{-1} at 20 kHz, 46.94 μm s^{-1} Pa^{-1} at 30 kHz, 28.2 μm s^{-1} Pa^{-1} at 40 kHz and 36.1 μm s^{-1} Pa^{-1} at 50 kHz.

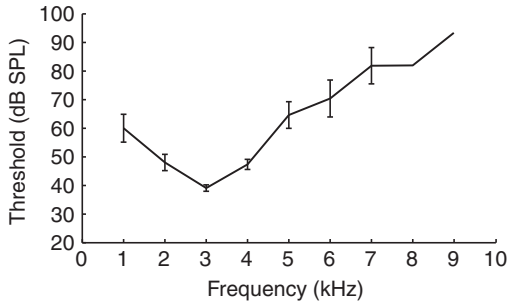

Fig. 3. Neural auditory tuning of the tympanal organ of *H. thoracica*. The frequency of best sensitivity is at 3 kHz, with a threshold at 39 dB SPL (±s.d., N=7). No response was recorded for frequencies over 10 kHz.

A three-dimensional reconstruction of the laser Doppler data was used to reveal the deflection patterns of the tympanal membranes. At the resonant frequency (3–4 kHz), the entire membrane oscillated in a simple single mode (Fig. 6A). Because the mechanical response consistently showed only one distinct peak in the frequency range investigated (up to 60 kHz), it appears that only the fundamental resonance mode is relevant to hearing (Fig. 6B,C). The thinner regions of the ATM and PTM consistently exhibited a larger response magnitude than the thickened region. This was confirmed by analyses of transverse and longitudinal transects across the tympana that revealed the deflection envelopes in more detail (Fig. 7). Deflection envelopes through a complete stimulus cycle show that the area of maximum deflection coincides with the thinner region in the middle of the tympanum (Fig. 7D,H). Importantly, deflection of the thinner region became convex towards maximum deflection, whereas the inner plate did not undergo such bending. This plate-like deflection pattern reveals the stiffer nature of the tanned, thicker area. The observed asymmetry of the deflection patterns suggest that the thin region is leading, i.e. bulging out slightly ahead of the thickened area, as it reaches maximal outward and inward deflection. The ATM undergoes greater displacement and displays a higher response gain than the PTM (although statistics do not suggest significant differences, see below). This correlates with the closer proximity of the ATM to the CA, which is located on the dorsal side of the anterior vesicle (Fig. 2C, Fig. 7).

The maximum deflection magnitude at the resonant frequency was not different between the ATM and PTM (males and females pooled, as there is no difference between sexes; two-tailed Wilcoxon, Z=–0.889, P=0.374, N=9; Fig. 5A), although the mean deflection was higher for the ATM (11.36±1.79 nm Pa^{-1}, N=9) than for the PTM (10.34±2.07 nm Pa^{-1}, N=9). Note that the mean deflection values given here are lower than those shown in the deflection

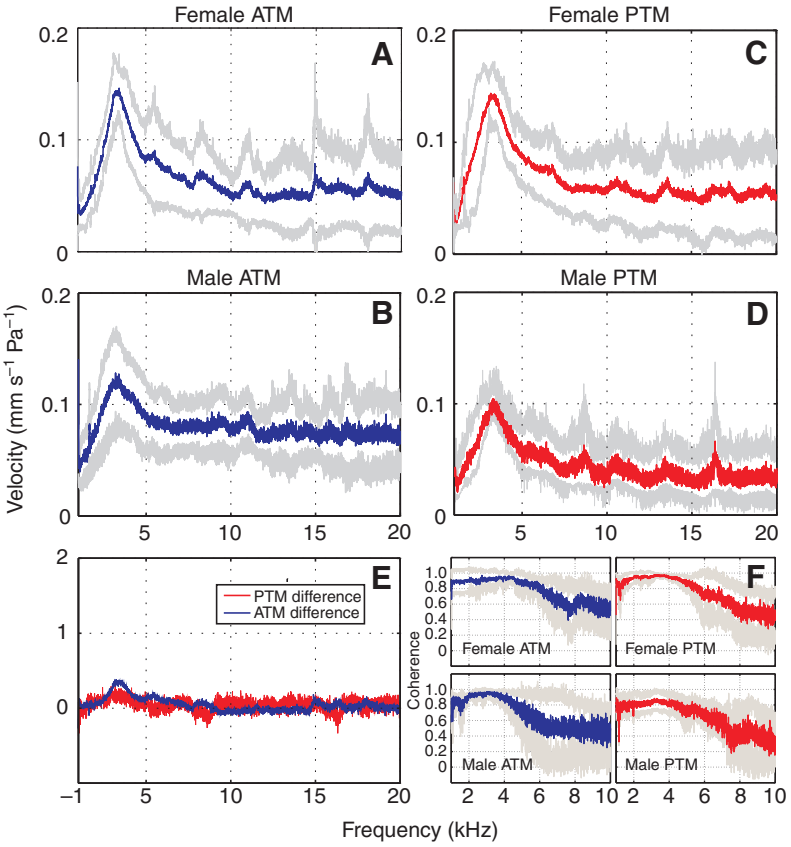

Fig. 4. Male and female *H. thoracica* mechanical response. (A,B) Mean mechanical response of the ATM (N=5). (C,D) Mean mechanical response of the PTM (N=4). (E) Sex-specific differences in the mean mechanical responses of both tympanic membranes. (F) Coherence signal across the frequency range for the measurements shown in A–D. Blue and red traces represent mean vectors; grey traces represent ±s.d.

envelopes taken from profiles across the tympanum (Fig. 7). This is because the profile deflection is made with the real values of points touched by the bisecting line whereas the statistics include the mean values of all points on the entire membrane. Similarly, the maximum velocity of the tympana peaks at ~3.5 kHz and is similar for both the ATM and the PTM (two-tailed Wilcoxon, Z=–1.481, P=0.139, N=9). The mean maximum across all animals was 138.09±49.18 µm s Pa^{-1} for the ATM and 126.57±52.14 µm s Pa^{-1} for the PTM. Membrane velocity was greatly reduced for other frequencies.

Phase response of the tympanal membranes

The ATM and PTM are of equal size and structure and are located at the same relative proximal position on the prothoracic tibia (Fig. 1C). Both tympana have very similar temporal responses to incident sound pressure; their phase responses are nearly identical (Fig. 6B,C). The phase difference function ($Phase_{ATM} - Phase_{PTM}$) was nearly 0 deg across the entire sound spectrum tested (Fig. 8A). At the resonant frequency, the phase difference was 2.96 deg (±1.15, N=9). This suggests that either the membranes move in the same direction or they move in opposite directions. From Fig. 6B,C it is observed that both membranes vibrate with similar phase (positive deflection occurs in both membranes simultaneously), but because the membranes are in reversed position, both membranes move in opposite directions. Hence, the ATM and PTM displacements in response to a four-cycle pure tone of 3.6 kHz were also in phase with the incident stimulus (Fig. 8B).

DISCUSSION

Weta hear over a narrow range of frequencies (Field et al., 1980). In effect, the conversion of acoustic energy into mechanical energy at the auditory periphery reveals that excellent sensitivity exists in the narrow frequency range of the conspecific call. Interestingly, there is no notable difference in the mechanical response between males and females, suggesting that hearing is equally important to both sexes (Greig and Greenfield, 2004). The mechanical response in the ultrasonic range is very weak (<50 µm s Pa^{-1} between 20 and 40 kHz), indicating that they are unable to hear the echolocation calls of predatory bats. Tree weta are preyed upon by one endemic species of bat, the lesser short-tailed bat (*Mystacina tuberculata*) (Arkins, 1996). This bat is specialised to forage on the ground and along tree branches. When hunting on substrate, it continues to emit ultrasound as well as incidental sound of movement through leaf litter (Jones et al., 2003; Parsons et al., 2010). This incidental sound is audible to weta (Lomas, 2007). From this evidence, it is suggested that there has been no selective pressure for weta to evolve sensitivity to echolocation-related ultrasonic frequencies.

The tympana of *H. thoracica* are remarkable in several respects. First, they are the thickest so far described in insects. Large tympana are characteristic of the Anostostomatidae. The largest recorded tympana (4.0×2.1 mm) is from the giant weta *Deinacrida hetercantha*, measured from a juvenile female ~100 mm long (live body weight >70 g) (Ball and Field, 1981). The tympana of other ensiferan families are much smaller. For example, the cricket *Macrogryllus ephippium* has the same body size (length 41 mm, adult male) as *H. crassidens* and *H. thoracica*, yet its tympanal surface area is only a quarter of that of the weta (*ca.* 4 mm^2) (Ball and Field, 1981). The tympana of *H. thoracica* are much thicker than those of most other insects, ranging from 19 to 185 µm. The locust ear (*Locusta migratoria*) is also large, with a total area of ~3 mm^2. However the tympanum is 8–10 µm at its thickest point (Michelsen, 1971) and only ~250 nm at its thinnest (N. Mhatre, T. Scott and D.R., unpublished data).

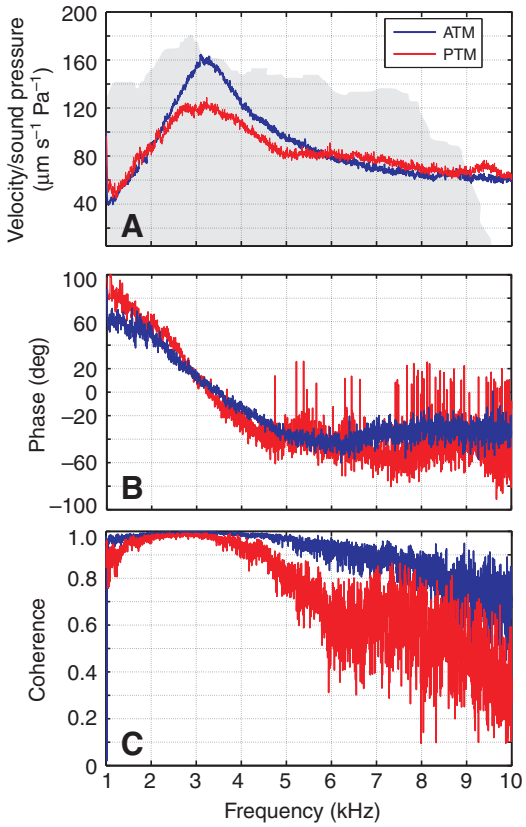

Fig. 5. Mechanical response of the tympanal membranes of *H. thoracica*. (A) Mean response across the ATM (blue trace) and PTM (red trace) in one female specimen. The shaded area corresponds to the spectral content of the calling song. The frequency of the maximal response is 3.33±0.09 kHz (mean±s.d.) for the ATM and 3.36±0.09 kHz for the PTM. (B) Phase spectrum of the response of each eardrum. (C) Coherence function between the vibration velocity and microphone signal averaged across the entire membrane. Note that coherence is high at the resonant frequency.

As the tympana of *H. thoracica* are thick and large, they may be expected to have significant mass, material density, as well as low compliance and lower responsiveness to incident sound pressure (Beranek, 1954). Yet our results show that the tympana are highly mechanically sensitive and within the hearing range of other insects (e.g. Windmill et al., 2005). In *Hemideina*, this mechanical sensitivity enables a neural threshold of 39 dB SPL (Field et al., 1980) (present study). The quality factor (Q_{-3dB}) of the PTM and ATM, a measure of energy dissipation in a system, within this frequency range is ~2. Whilst this is similar to the tuning reported in other cricket species with much thinner tympana (Larsen et al., 1989), it is very different from the lack of mechanical tuning reported in the tree cricket *Oecanthus henryi*, a species also sensitive to frequencies of ~3 kHz (Mhatre et al., 2009).

The tympanum of *H. thoracica* behaves as a simple mechanical oscillator that responds maximally to acoustic forcing at 3.3–3.4 kHz. Maximum deflection occurs in the thin region of the membrane, which vibrates at resonance with simple drum-like deflection profiles. The second region of the tympanum, the thickened region, does not display such motion. It oscillates as a stiff plate driven by the thinner surrounding regions. This region behaves like a hinge on a door, giving support to the proposed 'swing door model' of Bangert et al. (Bangert et al., 1998). The heavy thickened region moves with the thinned region at its medial edge with little movement along the rim of the tympanum, giving the impression that the thickened region is 'hinged' along that edge. This may be a mechanical adaptation contributing to filtering out frequencies, especially higher frequencies, that are not relevant to the acoustic communication of *H. thoracica*. The thickened region may play an important part in this filter by absorbing high frequencies and acting as a damper, allowing only the relevant low frequencies to be transmitted to auditory receptors. The thinnest region of the tympanum, the area of maximum deflection, lies directly adjacent to the enlarged tympanal vesicles, which in turn are in close proximity to the CA (Fig. 2, see also supplementary material Movie 1).

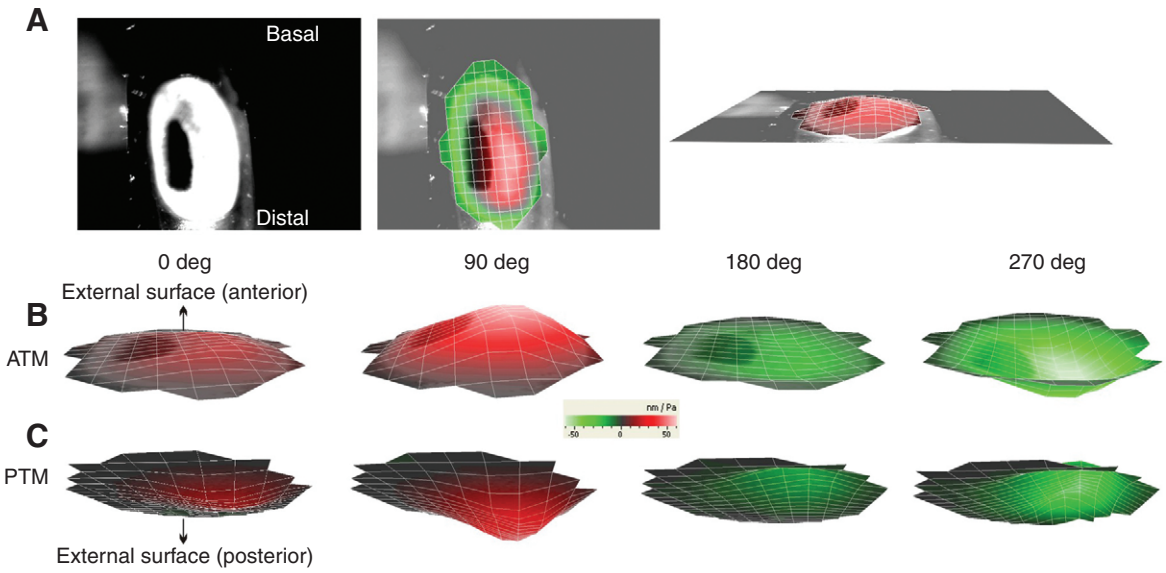

Fig. 6. Deflection shapes and displacement of the tympanal membranes of *H. thoracica*. (A) To establish orientation, left and middle panels show the tympanal area and the laser scanning lattice, respectively. The right panel shows the perspective under which deflections are presented in B and C. (B) Tympanal deflection shape of the ATM. (C) Deflection of the PTM. Red, outward tympanal deflections (towards speaker); green, inward tympanal deflections. Deflections (nm Pa^{-1}) are shown for five phase angles through a single oscillation cycle at 3 kHz.

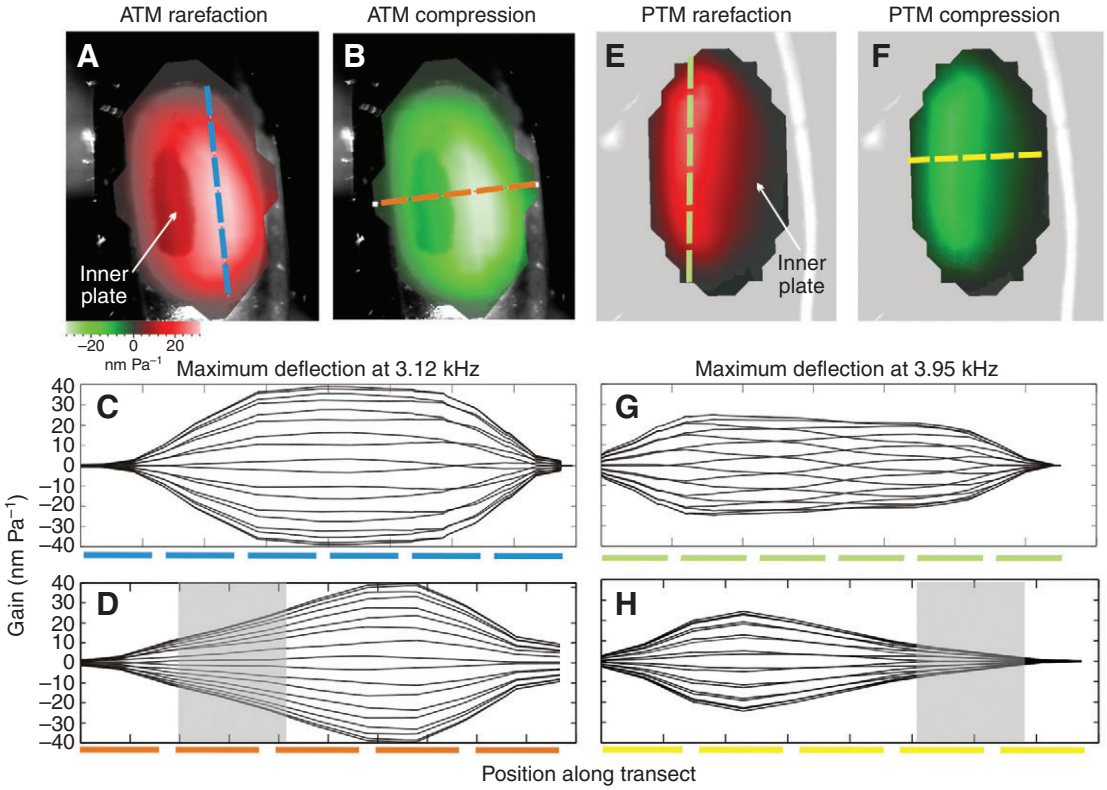

Fig. 7. Deflection envelopes of the ATM and PTM (right leg) of *H. thoracica* measured along transect lines in response to broad-band acoustic stimulation. (A,B,E,F) Displacement maps of the ATM and PTM, respectively. Red, maximum outward deflection (towards speaker); green, maximum inward deflection. Deflections are shown at respective resonances for this specimen (3.12 kHz for the ATM and 3.95 kHz for the PTM). Blue, orange and yellow lines show the transect lines associated with following panels. (C,D,G,H) Deflection envelopes of membrane displacement (across the defined transects) are shown every 20 deg of phase along the stimulus cycle. The shaded areas in D and H show the position of the inner plate.

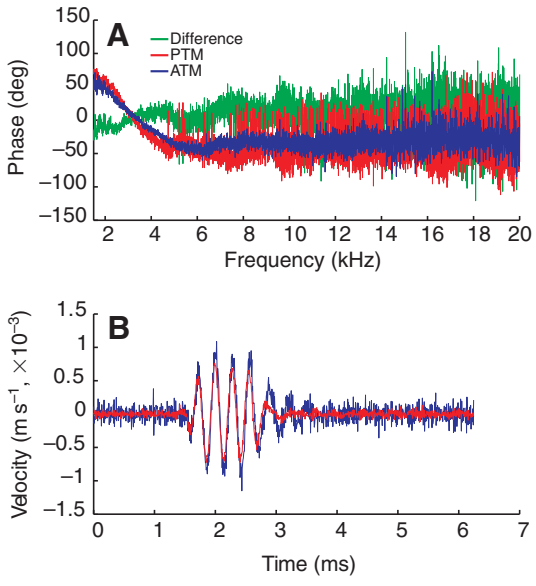

Fig. 8. Phase and time-resolved response of the ATM and PTM of *H. thoracica* to incident sound. (A) The phase response of membrane vibrations shown as transfer functions between the vibration velocity and the reference acoustic stimulus (periodic chirps 1–20 kHz; N=9). The green trace is the mathematical difference between the two responses. (B) Time response of the ATM (blue trace) and the PTM (red trace) at the point of maximal velocity for each membrane.

The mechanical properties of the weta tympanal membranes reveal a peripheral frequency filtering mechanism, a first important step in the chain of hearing. Acting prior to neuronal processing, the tympanal membranes of *H. thoracica* bandpass-filter frequencies in a narrow range relevant to intraspecific communication. This property is different from the mechanical behaviour of the tympana in other insects that might show broad frequency responses and/or multiple resonant modes (Michelsen and Larsen, 1978; Michelsen et al., 1994; Bangert et al., 1998; Windmill et al., 2005; Sueur et al., 2006; Sueur et al., 2008; Windmill et al., 2008; Mhatre et al., 2009).

Such narrow mechanical filtering is uncommon in Orthoptera. For example, the tympanic membranes of the tree cricket *O. henryi* display a flat, non-resonant mechanical response across a broad range of frequencies (Mhatre et al., 2009). The mechanical response of some bushcrickets studied to date is also relatively broad-band, (Bangert et al., 1998; Nowotny et al., 2010); however, in these bushcricket species the response is not as flat as that seen in *O. henryi*. In *O. henryi*, frequency specificity might then be expected to emerge through the characteristics of the mechanoreceptors, for instance interneuronal filtering. Whilst the tympanal membranes of the field cricket species studied so far (using similar approaches as those described here) exhibit resonance in the range of 3–10 kHz (Michelsen et al., 1994) (F.M.-Z and D.R., unpublished measurements), the neural activity relating to external acoustic stimuli has a much narrower response at ~4 kHz (Nocke, 1972; Hill, 1974; Kostarakos et al., 2009). In Tettigoniidae, individual auditory

receptors are arranged tonotopically along the CA and the removal of the tympanum does not affect the tuning of these receptors (Oldfield, 1982). Therefore, the mechanisms of auditory processing in Orthoptera are very diverse and the mechanical processing, through tympanal response and neural processing, ought to be considered as part of an integrated chain of events leading to the perception of sound. The mechanical tuning seen in *H. thoracica*, which implies filtering prior to neural processing, could be plesiomorphic to the more elaborate sound receptor found in many Tettigoniidae and Gryllidae.

Alternatively, species of the families Anostostomatidae, Haglidae and, to a lesser extent, Tettigoniidae also have thickened pigmented inner plates on their tympanic membranes (Schumacher, 1975; Ball and Field, 1981; Sickmann et al., 1997; Mason et al., 1999; Nowotny et al., 2010). It is also interesting to note here that such pigmentation and the associated plate-like anatomy tend to occur in tympana that are visible, as opposed to those that are protected by cuticular flaps in many Tettigoniidae species [e.g. *Tettigonia viridissima* (Bangert et al., 1998)]. Further research is required to establish the exact function of tympanal anisotropy, its relation to the complex tracheal structures of the inner ear and the transformation of acoustic energy into mechanical forces acting on the CA and its mechanosensory cells.

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