TYMPANAL HEARING IN THE SARCOPHAGID PARASITOID FLY *EMBLEMASOMA* SP.: THE BIOMECHANICS OF DIRECTIONAL HEARING

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Summary

In Diptera, tympanal hearing has evolved at least twice in flies that belong to two different families, the tachinids and the sarcophagids. Common to these flies is their parasitoid reproductive strategy, both relying on the acoustic detection and localization of their hosts, singing insects, by means of tympanal hearing organs. In the present study, the external anatomy of the unusual hearing organs of the sarcophagid fly *Emblemasoma* sp. is described. The sarcophagid ears bear numerous anatomical similarities with those of ormiine tachinids: they are located on the ventral prosternum and possess a pair of scolopidial mechanoreceptive sense organs. A striking difference, however, resides in the lack of a well-defined presternum in the sarcophagid tympanal system. Instead, a deep longitudinal fold, the tympanal fold, spans both hemilateral tympanal membranes across the midline of the animal. Measured using laser Doppler vibrometry, the tympanal mechanical response in the sound field reveals asymmetrical deflection shapes that differ from those of tachinids. Lacking a central fulcrum, the sarcophagid tympanal complex presents different vibrational modes that also result in interaural coupling. The evolutionarily convergent, yet distinct, solutions used by these two small auditory systems to extract directional cues from the sound field and the role of tympanal coupling in this process are discussed.

Key words: Diptera, tympanum, auditory mechanics, acoustic parasitism, directional hearing, convergent evolution, *Emblemasoma* sp.

Introduction

In insects of the order Diptera, the sense of hearing takes two fundamentally different forms and functions. Nematoceran flies such as mosquitoes (Johnston, 1855; Mayer, 1874), midges and, among higher flies, some Brachycerate species such as Drosophilidae (Bennet-Clark and Ewing, 1967) or Tephritidae (Webb et al., 1983) are capable of detecting the oscillations of the air molecules that accompany the propagation of sound waves. Often called 'near-field' detection because such oscillations of air molecules predominate near the sound source, this particular sensory capability is mediated by specialized appendages that typically involve the antennae, or sometimes also hairs borne on the body wall for other insects (Markl, 1973). Such sound detectors are most spectacularly exemplified by the plumose antennae of male mosquitoes and their rich sensory apparatus, the Johnston organ (Boo and Richards, 1975). This sensory modality mediates the detection of intraspecific communication signals (Bennet-Clark, 1984; Michelsen and Larsen, 1985).

Tympanal hearing organs constitute the other and rarer type of acoustic detector found in Diptera (Hoy and Robert, 1996; Hoy, 1998). Tympanal hearing has been suspected to occur for quite some time from observations of the parasitic life history of some Diptera, in particular in the family Tachinidae (Sabrosky, 1953; Léonide, 1969). More recently, direct evidence gained from phonotactic experiments (Cade, 1975; Mangold, 1978; Walker, 1988) and anatomical, physiological and biomechanical investigations (Lakes-Harlan and Heller, 1992; Robert et al., 1992; Robert and Hoy, 1998) established the presence of a tympanal auditory system in Diptera.

Parasitoids are obligate parasites at the larval stage, which means that the gravid female must find a host to provide her larvae with a food source. For some tachinid flies, the hosts of choice are diverse orthopteran species, such as Tettigoniidae for the Old World fly *Therobia leonidei* (Léonide, 1969; Lakes-Harlan and Heller, 1992) or different *Gryllus* species for the north American fly *Ormia ochracea* (Cade, 1975; Walker and Wineriter, 1990). The endoparasitic lifestyle imposes, among other things, one particular evolutionary constraint upon these phonotactic flies: they can only grow to a fraction of the body size of their hosts. As a consequence of their small body size, auditory organs can only be separated by a short interaural distance, a limitation that, in turn, generates severe

constraints for the implementation of directional sound detection in the range of frequencies produced by their hosts. The general constraints and the solutions applied by different animal species to the particular problem of directional hearing have long been recognized, investigated and abundantly discussed in the literature (Autrum, 1940; Bennet-Clark, 1984; Knudsen, 1980; Michelsen and Larsen, 1985; Michelsen, 1994). For the fly *Ormia ochracea*, it has recently been shown, on the basis of anatomical, neurophysiological and biomechanical investigations, that directional hearing relies on the mechanical coupling of the two hemilateral tympanal membranes (Miles et al., 1995; Robert et al., 1996b, 1998).

Acoustic parasitism is, however, not only found in flies of the family Tachinidae. Quite remarkably, some species of another large family of parasitoids, the Sarcophagidae, have been reported to detect and locate their singing host acoustically, in this case a cicada (Soper et al., 1976). Interestingly, the genus *Emblemasoma* has been taxonomically characterized by the presence of an inflated prosternum (Shewell, 1987), the same character that provides the key to the tribe Ormiini of the tachinids (Wood, 1987). Because size constraints also apply to the sarcophagid flies, the question of the directional sensitivity of their tympanal organs, and the mechanical basis for it, is germane and constitutes the main object of the present study. First, the external anatomy of the sarcophagid prosternal region is described and compared with that of tachinid flies. To examine the acoustic inputs to this small auditory system, an analysis of sound diffraction around the fly's body is presented. On the basis of laser Doppler vibrometry, the present biomechanical analysis provides evidence for asymmetrical tympanal vibrations in the sound field. The analysis of the deflection shapes establishes that these tympanal vibrations differ from those reported earlier in tachinid flies and that this is due to a type of interaction between the tympanal membranes that has not been reported previously.

Materials and methods
Animals

The flies used in the present study were captured in the field, at the University of Mississippi Biological Field Station, Lafayette County, Mississippi, USA. Female parasitoid flies *Emblemasoma* sp. (Shewell, 1987) were captured at sound traps broadcasting the song of the local cicada, *Tibicen pruinosa* (H. Farris, in preparation). Captures were made in the afternoon, the period during which the cicadas are acoustically active. Upon capture, each fly was placed individually into a 20 g vial and supplied with a cotton ear-tab wetted with 3–5 % sugar water. Within a day or two after capture, the specimens were sent either to Ithaca or Binghamton (New York) or to Zürich (Switzerland) for laboratory investigations.

For the sarcophagid flies of the genus *Emblemasoma*, taxonomic identification is still uncertain. Like tachinid flies of the genus *Ormia* (Walker, 1988), the genus *Emblemasoma* may encompass different sympatric and closely related species that

are difficult to distinguish morphologically. Therefore, it cannot be excluded that the present technique of acoustic trapping attracts more than one species at a time (H. Farris, personal communication). Such sarcophagid specimens are quite rare and, despite a recent and valuable taxonomic revision at the species level (Pape, 1990), identification remains problematic. Mindful of the characters described by Pape (1990), our examination did not reveal morphological differences that hinted at the presence of several different species in our sample.

Scanning electron microscopy and light scanning microscopy

The tympanal ears of female *Emblemasoma* sp. flies were examined using scanning electron microscopy and light scanning microphotography. For scanning electron microscopy, the decapitated flies were air-dried and sputter-coated with gold. Specimens were examined and photographed on an AMR-1000A scanning electron microscope. Although providing less optical resolution than scanning electron microscopy, light scanning microphotography was used to gain information about the transparency and colour (material distribution) of the tympanal structures.

Experimental apparatus and procedures
Positioning of the preparation and measurement of the mechanical response

The fly specimen was tethered on a small positioning stage that allowed it to be positioned accurately relative to the sound field and relative to the laser vibrometer. The specimens had to be decapitated to allow the laser beam to be positioned precisely on different parts of the tympanal complex. Fine spatial adjustments of the fly preparation were important since they permitted the reflection of the laser beam from cuticular structures to be optimized. The positioning system was also designed so that the orientation of the specimen relative to the laser beam could be altered without changing its position in the sound field and its azimuthal angle relative to the acoustic stimulus. Control measurements indicated that the removal of the head did not influence the tympanal mechanical response.

The mechanical response of the fly's tympanal hearing organs to an incident sound pressure was measured using laser Doppler vibrometry. The laser vibrometer used in these experiments (Polytec OFV 2100 control electronics and OFV 302 optical sensor head) could detect vibration velocities as low as $0.5\,\mu m\,s^{-1}$ over a frequency range from $0.1\,Hz$ to $500\,kHz$. The laser beam could be focused to a dot $5\,\mu m$ in diameter and positioned with a precision of $2–3\,\mu m$, both features permitting the measurement of the mechanical response of selected locations on the hearing organs. The reflectivity of the tympanal membranes or other cuticular structures and the sensitivity of the vibrometer were sufficient to obtain highly coherent data without using reflective particles. The measurements were thus made under non-loading conditions. Further details about the methods employed to measure the mechanical response of the fly's tympanal structures are reported by Miles et al. (1995) and

Robert et al. (1996b). Experiments were conducted at ambient temperatures ranging from 22 to 25 °C.

Sound stimulation

The sound stimulus was usually a 10 ms burst of random noise at an overall sound pressure level of 104 dB re 20 μPa. The stimulus was digitally synthesized, bandpass-filtered from 1 to 30 kHz, amplified [Brüel and Kjær (B&K) type 2706 power amplifier] and broadcast from a tweeter (ESS-AMT1) at a repetition rate of approximately 1 Hz. This loudspeaker could deliver signals over the frequency range used with minimal distortions (Fig. 1A). In addition, to investigate the temporal response of the hearing organs, sound bursts were used that resembled the calling song of the cicada host, *Tibicen pruinosa*. These stimuli were 15 ms long pulses of 3.7 kHz carrier frequency, with rise and fall times of 1.3 ms (Fig. 1B). As measured with the reference microphone at the specimen, the first harmonic was 43.2 dB lower than the fundamental. During the vibrometric experiments, all stimuli could be delivered to the specimen at angles of incidence ranging from $-90°$ to $+90°$ azimuth and $0°$ elevation.

Mechanical transfer functions

The mechanical response of the tympanal ears was expressed as frequency transfer functions. The transfer functions were computed as the cross-power spectrum between the laser vibrometer and the reference microphone (B&K type 4138) signal divided by the auto-power spectrum of the microphone signal. For each location measured on the tympanal system, the cross and autospectra used to calculate the transfer functions were averaged using the results of 10 consecutive stimulus presentations.

Response linearity and noise level

The linearity of the data and the level of signal contamination by unrelated noise were estimated by means of a coherence function (Miles et al., 1995; Robert et al., 1996b). Magnitude-squared coherence was computed as the squared absolute value of the cross-power spectra between the laser and microphone signals divided by the product of the autopower spectra of the laser and microphone signals (Kates, 1992). In the frequency range from 1 to 30 kHz, coherence values were typically above 0.95 (range 0–1). This indicates that unrelated noise accounts for less than 5 % of the data. Also, during laser measurements, coherence between the electrical signal fed to the loudspeaker and the reference microphone signal was very close to 1, indicating that the loudspeaker caused minimal distortions (Fig. 1A).

Acoustic conditions and estimate of diffraction

To estimate the sound field incident on the tympanal system, acoustic measurements were made to assess the conditions of sound diffraction around the specimen. The B&K (type 4138) condenser microphone (3.2 mm diameter) was positioned beside the fly's thorax, as shown in Fig. 1C, and the sound source was positioned at different angles (from $-180°$ to
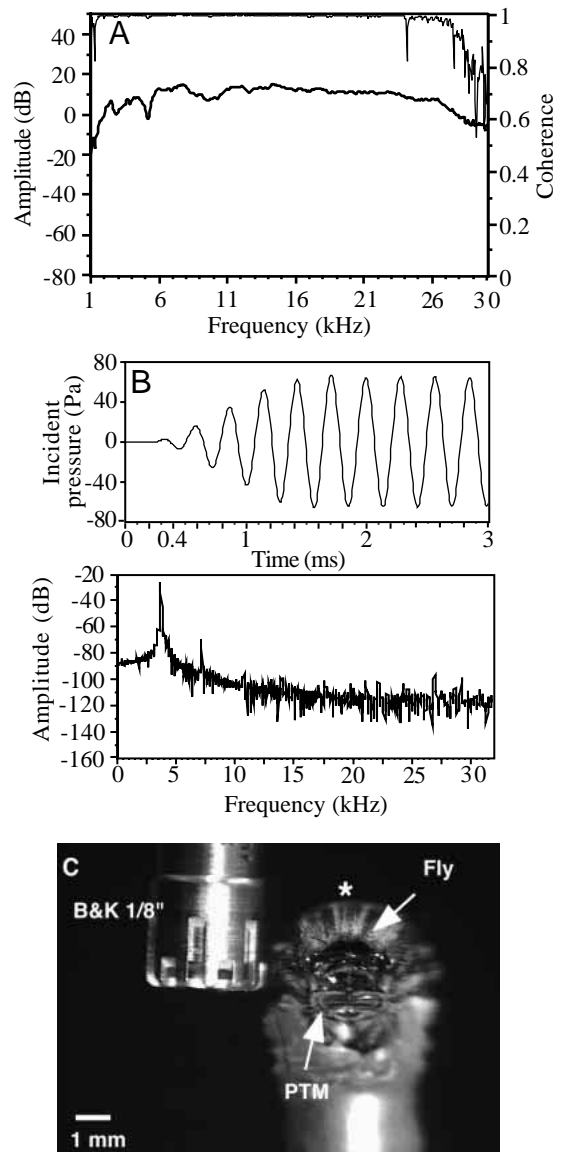

Fig. 1. Acoustic stimuli and position of the specimen in the experimental arrangement. (A) Amplitude frequency spectrum of the band-limited noise stimulus (lower line) and coherence between the electrical signal fed to the loudspeaker and the sound measured by the reference microphone at the location of the fly (upper line). (B) Oscillogram of the tone stimulus simulating the cicada host *Tibicen pruinosa*. The first 3 ms of the 15 ms long stimulus is shown to illustrate the ramping (upper panel). The lower panel shows the frequency spectrum of the tone stimulus. (C) Position of the reference microphone (B&K 4138) relative to the fly and the prosternal tympanal membranes (PTM). During estimates of diffraction, the microphone was positioned beside the fly; during vibrometric measurements, it was placed directly above the specimen at the location indicated by an asterisk.

$+180°$) around the specimen. The band-limited random noise (20 Hz to 30 kHz) broadcast by the loudspeaker was measured by the fixed microphone, digitized, and stored as a frequency spectrum (Fast Fourier Transform at 1024 lines) for various

angles of incidence. To estimate the sound diffraction caused by the fly's body, the frequency spectra of complementary angles of incidence (i.e. +20° and −20°) were subtracted from each other. In an acoustic free field, these difference spectra approach a value of zero only under non-diffractive conditions. However, in an experimental arrangement such as that used here, free-field conditions cannot be met because the measuring apparatus, the optical table and the tethering system interfere with the propagation of sound waves. Thus, the difference spectra may indicate not only diffraction around the specimen but also heterogeneities in the sound field (such as standing waves due to various echoes) that are unrelated to diffractive effects from the fly itself. Although inconvenient, this experimental shortcoming can be taken into account by also estimating the magnitude of the heterogeneities in the sound field in the absence of the specimen, thus establishing a baseline against which to compare the difference spectra measured across the fly's body. To do this, each difference spectrum is subtracted from its corresponding 'free-field' difference spectrum. In practice, the 'free-field' difference spectrum is computed from the spectra measured for two complementary angles of incidence (i.e. +20° and −20°) without the specimen in the sound field. The frequency spectrum thus obtained constitutes a better estimate of the diffraction solely caused by the specimen. These diffraction spectra show that no significant diffraction takes place at the frequencies of behavioural relevance (from 3 to 5 kHz) (Fig. 2A–C). In this frequency range, the difference spectra take mean values of −0.03±0.51 dB (mean ± S.D.) for an angle of incidence of ±30°, 0.15±0.49 dB for an angle of incidence of ±90° and −0.30±0.31 dB for an angle of incidence of ±130° (N=10). For particular frequencies, however, diffraction can reach higher values, such as 4.45 dB at 14.75 kHz for an angle of incidence of 90° or 4.97 dB at 21.75 kHz for an angle of incidence of 130°. When averaged over the entire frequency range (1–30 kHz) and displayed as a function of the angle of incidence, diffraction values are not significantly different from zero, but show the expected tendency to increase for angles orthogonal (e.g. 90°) to the longitudinal axis of the specimen (Fig. 2D). Thus, while the small body size of the specimen does not preclude diffractive effects from occurring at some frequencies, the measurements indicate that no consistent and significant diffraction takes place at frequencies below 7–8 kHz (Fig. 2A–C).

Additional measurements were made with probe microphones to quantify more accurately the sound pressures and the time of arrival of the incident sound at the hearing organs (Robert et al., 1996b). These measurements were made subsequently using dried specimens that had been rehydrated overnight for this purpose. The probe microphones were customized Knowles miniature microphones (type 3068) fitted with concentric steel tubes flaring into a thin tip. The tip of the probe had a minimal internal diameter of 100 μm. To measure incident pressure, the two cross-calibrated probe microphones were each positioned directly in front of one tympanum. The interaural difference in the time of arrival of the incident sound pressure (a pure tone at 3.7 kHz) was 10.3±0.9 μs (mean ± S.D.; N=10). In these conditions, no significant interaural difference in sound pressure level could be measured (0.20±0.22 dB; N=10). Thus, in terms of interaural time difference and interaural intensity difference, the acoustic input to the auditory system of *Emblemasoma* sp. provides only very small cues for directional sound detection.

Results

The external auditory anatomy

The hearing organs of the parasitoid sarcophagid fly *Emblemasoma* sp. are located on the ventral prothorax, between the prothoracic coxae and the base of the neck
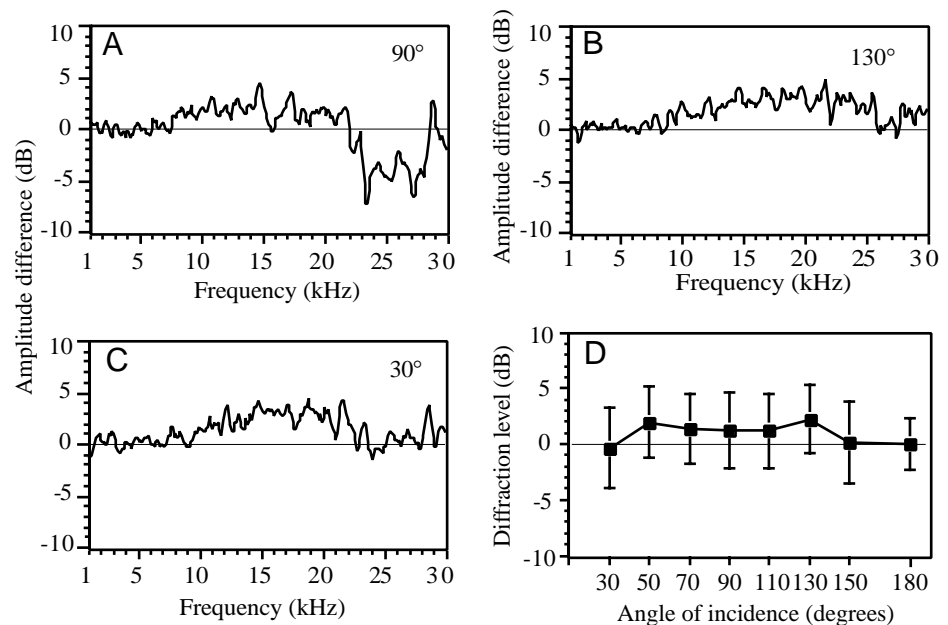

Fig. 2. Assessment of the diffractive conditions in the sound field surrounding the specimen. (A–C) Difference frequency spectra indicating diffraction for different angles of incidence (see Materials and methods). A value of zero indicates the absence of diffraction caused by the fly's body. (D) Level of diffraction, expressed in dB (± S.D.), as a function of the stimulus azimuthal angle of incidence. The level is calculated as the average of the 233 frequency values for each angle of incidence (as in A–C). Positive values indicate higher sound pressure levels for the side ipsilateral to the sound source.

(Fig. 3A,B). As seen in the light scanning micrograph of Fig. 3A, the tympanal hearing organs occupy the entire width of the ventral prosternal region. Conspicuously, the probasisternum, a sclerite that in other sarcophagid flies is deeply recessed between the prothoracic coxae (e.g. in *Neobelleria bullata*; Edgecomb et al., 1995) protrudes anteriorly to form a prosternal inflation. In fact, this inflation constitutes an important taxonomic character since it is the key character common to the sister genera *Emblemasoma* and *Colcondamyia*, both parasitoid sarcophagids (Shewell, 1987).

Each hearing organ is externally identifiable by an area of thin, membranous cuticle; the prosternal tympanal membrane (Fig. 3A,B). The tympanal membrane is made of the apposition of thin exocuticle with the prosternal tracheal air sac; it is approximately 1–2 μm thick over most of its surface. Previous histological investigations showed that a pair of
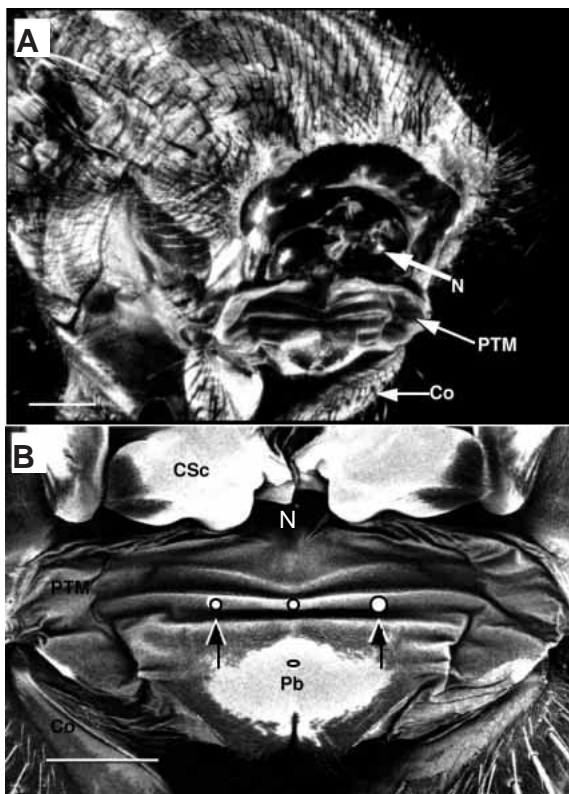

Fig. 3. The tympanal auditory organs of the parasitoid sarcophagid fly *Emblemasoma* sp. (A) Light scanning micrograph of the anterior prothoracic region in an oblique frontal view. The ears are situated between the prothoracic coxae (Co) and the neck insertion (N) to the thorax. The arrow labelled PTM indicates the fly's left prothoracic tympanal membrane. The fly's head has been removed to make the auditory organs visible. (B) Scanning electron micrograph of the tympanal hearing organs in frontal view. The white dots to the left and right of the midline and on the probasisternum (median dot) indicate the locations of laser vibrometric measurements. The arrows indicate the insertion of the cuticular apodemes linking the mechanoreceptive organs to the tympanal system. Pb, probasisternum; PTM, prothoracic tympanal membrane; CSc, cervical sclerite; Co, prothoracic coxa.; N, neck. Scale bars, 500 μm.

scolopidial sense organs are attached to the tympanal membranes *via* stiff cuticular apodemes (D. Robert, M. P. Read and R. R. Hoy, unpublished observations). Dissections of fresh specimens also confirm that both scolopidial mechanoreceptive organs are situated in the unpartitioned tracheal sac that backs the prosternal tympanal membranes. This basic plan conforms to the conventional design of an insect tympanal ear and is very similar to that reported previously for the acoustic parasitoid fly *Ormia ochracea* (Robert et al., 1994, 1996a).

There are, however, some striking peculiarities of the external anatomy of the sarcophagid ears. First, the membranous tympanal regions are smooth rather than possessing the fine radial corrugations found in several different species of the genus *Ormia* (Robert et al., 1996a). Second, and unlike *Ormia* spp., the tympanal system does not possess a well-defined presternum. The boundary between the presternum and the tympanal membranes is neither clearly delimited nor easily recognizable as a clear change in structure, thickness or colour. Although the presternum is readily identifiable in atympanate sarcophagids (Edgecomb et al., 1995), its exact morphological delineation remains unclear in *Emblemasoma* sp.

Another special feature of the sarcophagid ears is the thickened horizontal folds (creases) that join the tympana. The cuticle that forms these folds is slightly thicker than that of the prosternal tympanal membranes. The scolopidial sensory organs make mechanical contact with the tympanal system at the level of the fold that is immediately dorsal to the probasisternum (Fig. 3B, arrows). The movements of the coxal joint are not transmitted to the adjacent prosternal tympanal membranes, as in *Ormia* spp. Such mechanical isolation between the coxal and the prosternal membranes is due to the ventrolateral extensions of the probasisternum and is absent in atympanate sarcophagids (D. Robert, unpublished observation).

The mechanical response of the tympanal system to an incident sound wave

The mechanical response of the cuticular structures involved in the reception of sound waves was measured by pointing the beam of the laser vibrometer at selected locations. First, the vibrations of the ipsilateral and contralateral contact points between the chordotonal organs and the tympana were measured and compared; they were then also compared with the response of the thicker cuticle on the probasisternum (Fig. 3B, black-circled white dots). When the sound source is at an angle of incidence of 90° in azimuth (0° elevation), the side ipsilateral to the sound source vibrates more than the contralateral side (Fig. 4A). The mean ipsi/contralateral difference for frequencies between 3 and 9 kHz is 8.2±3.2 dB (mean ± S.D.; N=97 frequency points, eight animals). In comparison, the probasisternum remains relatively immobile; its level of vibration in the same frequency range being on average 13.8±2.1 dB (mean ± S.D.; N=97 frequency points, eight animals) lower than that of the contralateral side (Fig. 4A).
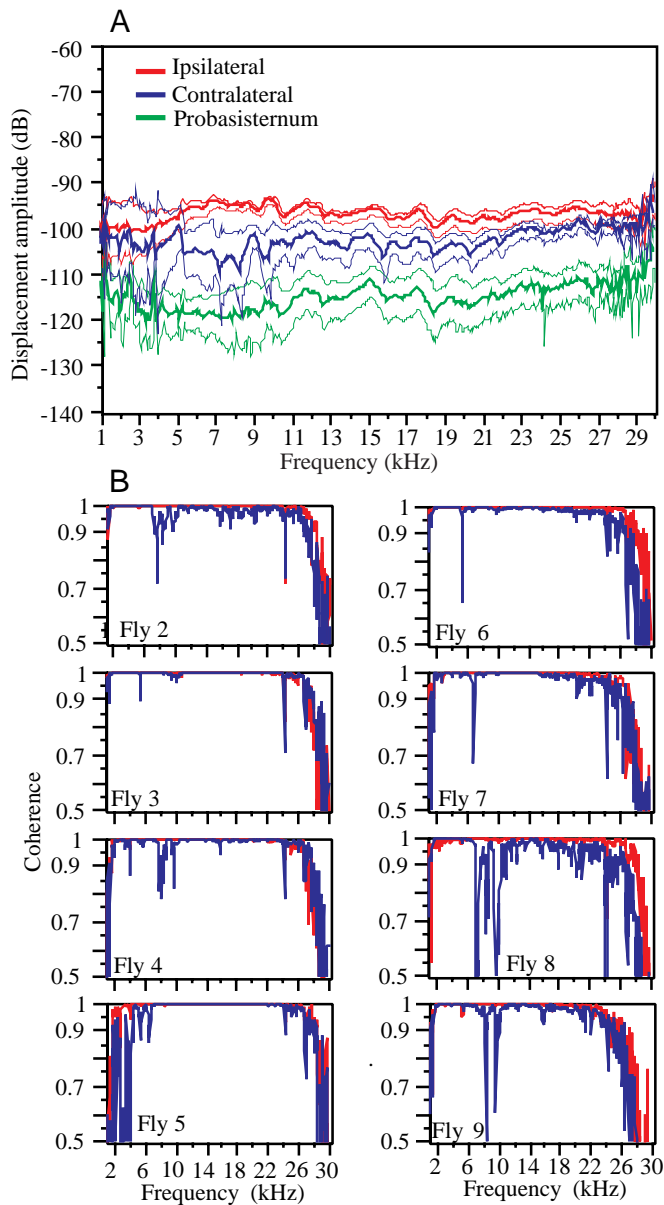
A

B

Fig. 4. Amplitude frequency spectra of the mechanical response of the tympanal system. (A) Mechanical displacement of the ipsilateral and contralateral points of insertion of the sensory organs and of the probasisternum (location of measurement shown in Fig. 3B). Thick lines show mean values from eight different animals. Thin lines show ± 1 S.D. around the mean. (B) Coherence functions between mechanical displacement and sound input to the system for each of the eight animals. Red lines, ipsilateral coherence functions. Blue lines, contralateral coherence functions. Note that only the upper 50 % of the coherence data are shown. The sound stimulus was delivered at 90 ° azimuthal angle of incidence and 0 ° elevation.

This result demonstrates that an incident sound can induce vibrations of the cuticular structures anatomically identified as tympanal membranes. It also demonstrates that, because the probasisternum vibrates much less than the prosternal tympanal membranes, these measurements cannot result from an artefactual situation in which the entire specimen would
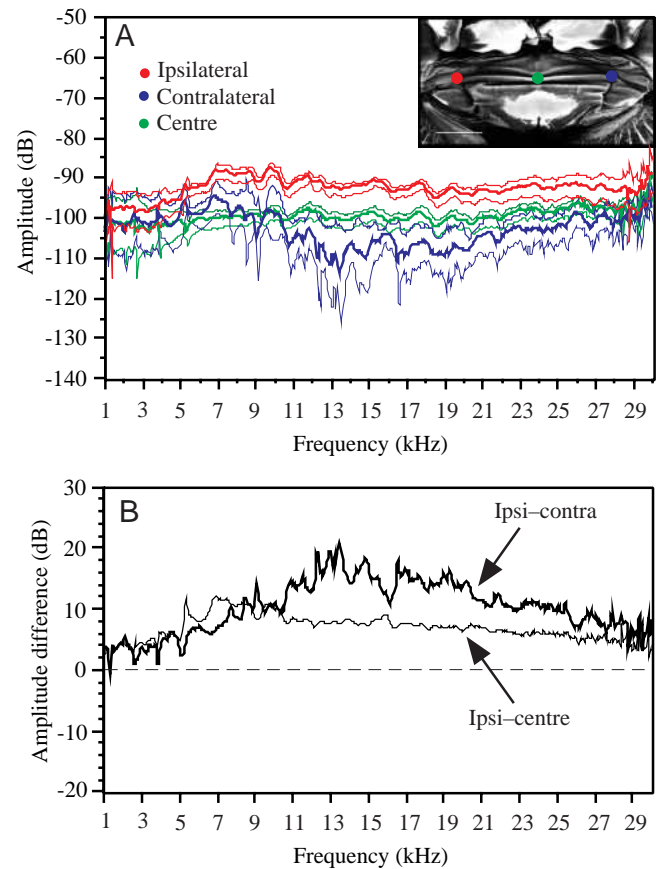
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Fig. 5. Amplitude frequency spectra of the mechanical displacement of the tympanal membranes and of the median presternum. (A) Inset, locations of vibrometric measurement. Red dot, ipsilateral membrane. Blue dot, contralateral membrane. Green dot, median presternum. Scale bar, 500 μm. Thick lines show mean values from eight animals; thin lines show ± 1 S.D. The sound stimulus was delivered at 90 ° azimuthal angle of incidence and 0 ° elevation. Amplitude is given in dB re 1 mm Pa^{-1}. (B) Difference spectra of the mechanical response of the ipsilateral and contralateral (Ipsi–contra) tympanal membranes and the ipsilateral tympanal membranes and the median presternum (Ipsi–centre), as shown in A.

be set into vibration by the sound stimulus. To investigate further the possible presence of artefacts due to unrelated noise input to the system, the linear relationship between the input sound signal and the mechanical responses was examined in terms of their coherence functions. As shown in Fig. 4B, coherence is relatively high for frequencies below 26 kHz for both ipsi- and contralateral responses. Although occasional local reductions in coherence can be observed for the contralateral side, the high levels of coherence confirm the absence of experimental artefacts in this data set.

The vibratory behaviour of the ipsi- and contralateral tympanal membranes and the centre of the median fold was also investigated (Fig. 5A). Consistent with the measurements shown in Fig. 4A, the tympanal membrane ipsilateral to the sound source (90 ° azimuth, 0 ° elevation) vibrates more than the contralateral one for all frequencies measured. Between 3 and 9 kHz, the mean difference between

the ipsi- and contralateral tympanal displacement is 6.4±2.5 dB (mean ± S.D.; N=97 frequency points, eight animals). Interestingly, in the same frequency range, the level of vibration of the median presternum (green dot on the animal's midline) is slightly lower than that of the contralateral membrane (by 1.9±1.9 dB; mean ± S.D.; N=97, eight animals). Above 10–11 kHz, however, the mechanical displacement of the median presternum is intermediate between that of the ipsi- and the contralateral tympanal membranes (Fig. 5A). It is also worth noting that, at frequencies below 4–5 kHz, the difference in the vibration levels between these three points of measurement is smaller than at higher frequencies. This point is illustrated in Fig. 5B, in which the differences between the mechanical response of the ipsilateral and contralateral tympanal membranes and the ipsilateral tympanum and the centre point are displayed as difference frequency spectra. At low frequencies, there is little difference in displacement between the lateral and central cuticular elements of the tympanal system. As frequency increases, the difference between the ipsilateral and central displacement (red and green dots in Fig. 5A) becomes smaller than that between the ipsi- and contralateral sides (red–blue). These data indicate that the relative displacements of these three anatomical locations differ. The exact nature of these displacements, in terms of tympanal deflection shapes, will be addressed below.

Directional sensitivity

The directional sensitivity of this tympanal system was also measured by varying the azimuthal angle of incidence of the sound stimulus from 0° to 90° around the specimen. For each angle, the difference in the response amplitude between the ipsilateral and contralateral tympana was measured and averaged for eight animals over the frequency range from 3 to 5 kHz (Fig. 6). As expected, the interaural difference in amplitude of the mechanical response is larger when sound waves are orthogonally incident to the longitudinal axis of the animal (90°).

The temporal organization of the mechanical response

The mechanical displacement of the tympanal system was also measured in response to a sound stimulus simulating the calling song of the cicada host (Fig. 1B). The mechanical response was measured from the ipsilateral and contralateral points of insertion of the sensory organs, the median presternum and the median probasisternum (centre; as shown in Figs 3, 5). From the oscillographic traces of the tympanal vibrations, shown as displacement velocity, it can be seen that the response amplitude of the ipsilateral ear is larger than that of the median presternum and contralateral tympanum (Fig. 7). To control for a possible artefact in which the whole preparation vibrates in the sound field, the vibrations of the thick cuticle of the probasisternum were also monitored. This structure moves relatively little compared with the tympanal structures (Fig. 7). These measurements are consistent with those obtained with a band-limited random noise stimulus and indicate that the structures responsible for the conversion of acoustic energy into mechanical energy, the tympanal membranes, undergo vibrations in the sound field that are significantly larger than those of associated supporting cuticular elements, or even the rest of the animal.

The oscillographic traces of the mechanical response also reveal that both tympanal membranes oscillate with a period of 270 μs, thus following the acoustic pressure variation of the 3.7 kHz stimulus (Fig. 7). In this example, the time delay between the ipsi- and contralateral oscillations amounts to approximately 42 μs. The temporal relationship between the vibration of the ipsilateral and contralateral sides was also investigated on a larger sample by cross-correlation analysis. For eight animals stimulated in identical conditions, the mean time delay obtained for best correlation (typical r>0.97) between the ipsilateral and contralateral response signals is 42.9±31.0 μs (mean ± S.D.; range 22–110 μs). This mechanical time delay is considerably larger than the difference in the time of arrival of the incident sound wave at the tympana (the acoustic interaural time difference) measured to be 10.3±0.9 μs (mean ± S.D.; N=10) with the probe microphones.
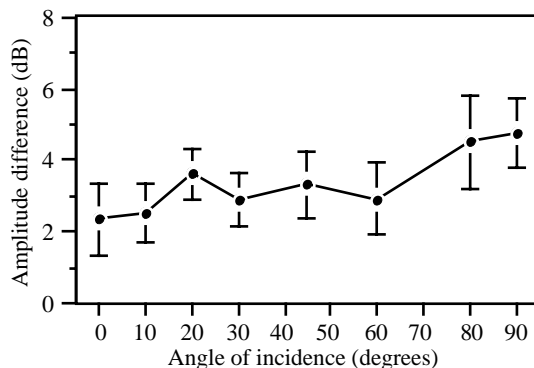

Fig. 6. Mechanical response as a function of the stimulus azimuthal angle of incidence. Response is given as the amplitude difference between the ipsilateral and contralateral points of insertion of the sensory organs (as shown in Fig. 3). Values are means ± S.D. for the frequency range from 3 to 5 kHz; N=8 animals.
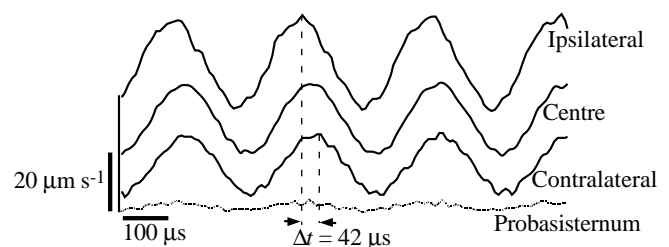

Fig. 7. Mechanical response of the tympanal system to the simulated song of the cicada host delivered at an azimuthal angle of incidence of 90° (0° elevation). The traces show the displacement velocity of the ipsilateral and contralateral points of insertion of the sensory organ to the tympanal membranes, of the median region of the presternum (centre) and of the probasisternum (for measurement locations, see Figs 3, 5). Δt, time delay between ipsilateral and contralateral vibrations.

Tympanal dynamics

To investigate further tympanal dynamics in *Emblemasoma* sp., vibrations were monitored at a higher spatial resolution by measuring the mechanical response to random noise at nine different locations across both ears (Fig. 8). The actual physical displacement velocity of the tympanal membranes at a frequency of 3.7 kHz is shown graphically as a deflection shape (Fig. 8A,B). Deflection shapes were reconstructed from the real and complex transfer functions by plotting the instantaneous velocity (the amplitude information) of all nine measured locations at successive times (the phase information) within one period of oscillation at a particular frequency. At 3.7 kHz, the frequency of the cicada host, the ipsilateral and contralateral tympanal membranes (red and blue dots, respectively) experience inward and outward deflections that are similar in shape but slightly different in amplitude (Fig. 8A). In this example, the interaural difference in deflection (measured at the insertion of the sensory organs to the tympanal organs) is 1.8 dB at 3.7 kHz (Fig. 8), while for eight animals the mean difference is 3.9 dB (Fig. 5). The median position (green dot) moves along with the entire tympanal system, although slightly less than the two hemilateral tympana. This can be seen as the saddle-shaped deflection at maximal deflection (Fig. 8A). The difference in maximal deflection amplitude between the ipsilateral tympanum and the median presternum is 2.8 dB in this example.

Ipsilateral and contralateral tympanal deflections reach their maxima at different times (relative phase) within the stimulus cycle (as seen in Fig. 7). This fact is best illustrated by a two-dimensional contour plot in which the amplitude of deflection is colour-coded (Fig. 8B). The time difference between ipsi- and contralateral maximal deflections (the blue peaks) is 38 μs, closely corroborating the time delays obtained by cross-correlation analysis.

In Fig. 9, the dynamic response of the tympanal membranes is shown, as contour plots, for four different stimulus frequencies. At low frequency (2 kHz), the amplitude
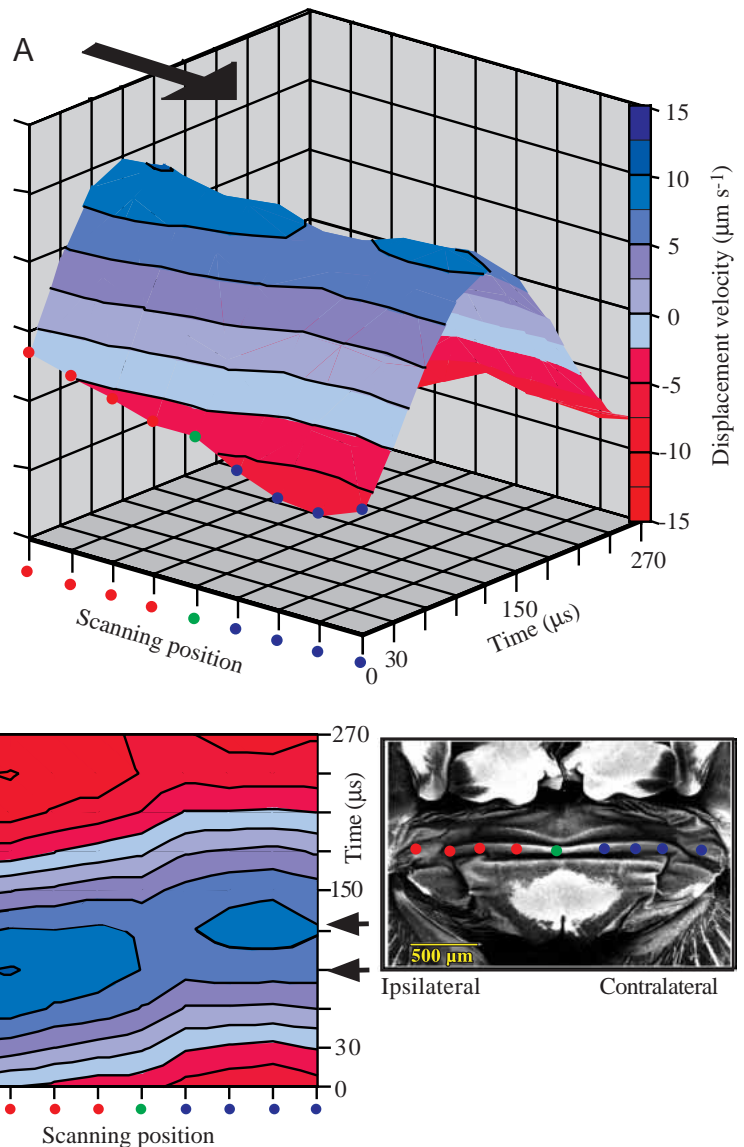

Fig. 8. Deflection shapes of the tympanal ears as a function of time at 3.7 kHz. The scanning electron micrograph shows the anatomical location of the vibrometric measurement; red is ipsilateral to the stimulus, green is medial and blue is contralateral. Angle of incidence of stimulus, 90° (large arrow). (A) The 'scanning position' axis (*x*-axis) indicates the measurement locations, while the time axis (*y*-axis) shows one period of the 3.7 kHz sound stimulus (270 μs). Deflection velocities of both tympanal membranes are shown on the vertical *z*-axis. Outward deflections are indicated by positive values (blue hue), while inward deflections take negative values (red hue). (B) Contour plot of the deflection shapes at 3.7 kHz. Colour convention as in A. Note that synchronous deflections would result in straight and parallel contour lines. The time difference between ipsi- and contralateral maximal deflections is shown by the arrows on the time axis.
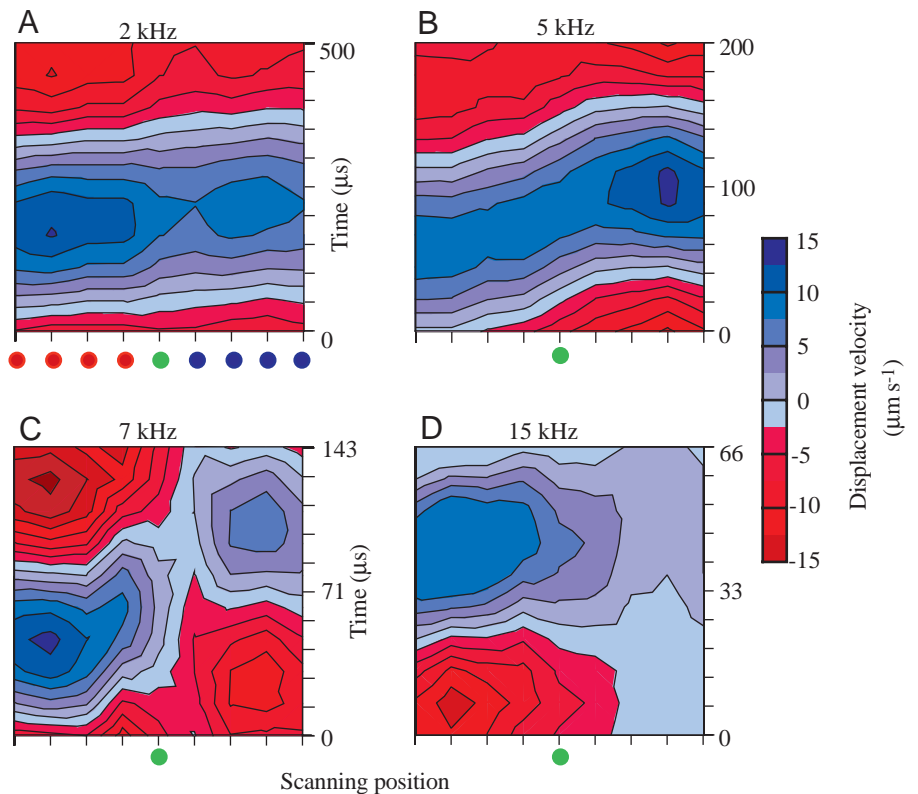
Fig. 9. Contour plots of the tympanal deflection shapes as a function of time for different stimulus frequencies. Conventions as in Fig. 8.

difference between the ipsi- and contralateral maximal deflections is 4.9 dB ($N=8$) (Fig. 9A, see also Fig. 5). Ipsilateral and contralateral deflections of maximal amplitude (blue peaks) occur with a time delay of 49 μs. For a stimulus frequency of 5 kHz, the ipsilateral and contralateral deflection shapes are very similar, while the difference in deflection amplitude is 3.4 dB and the interaural delay reaches 43 μs (Fig. 9B). Deflection shapes, however, become quite different at 7 kHz (Fig. 9C). The outward displacement of the ipsilateral side (blue peak) is accompanied by an inward displacement of the contralateral side (red valley). The ipsilateral and contralateral tympanal membranes oscillate with a maximal amplitude difference of 6.9 dB. Also, maximal contralateral and ipsilateral outward displacements are delayed by approximately 58 μs at 7 kHz. Interestingly, at this frequency, the deflection amplitude of the central point (green dot) becomes significantly lower than that of both tympanal membranes (ipsi–median difference 11.3 dB, Fig. 9C). This indicates that the tympanal membranes are rocking about the relatively immobile central point. At higher frequency (15 kHz), the ipsilateral tympanum oscillates in the sound field, while the contralateral side vibrates relatively little (amplitude difference 17.7 dB, for eight animals, 17.2 dB) (Fig. 9D). In this case, the central point experiences vibrations intermediate in amplitude (7.8 dB lower than the ipsilateral side). No time delay can be estimated in this case because the time of maximal deflection on the contralateral side cannot be measured reliably.

Taken together, these results demonstrate the strong frequency-dependence of interaural amplitude differences over a range from 2 to 18 dB and time differences of the order of 38–58 μs. In the frequency range around 3.7 kHz, amplitude differences remain modest but not negligible (2–6 dB). The present data therefore show that, in response to an incident sound arriving from one side of the animal, the peripheral auditory system of the fly *Emblemasoma* sp. oscillates asymmetrically. Such a pattern of tympanal deflections is quite unlike that expected from two independent tympanal systems set only 1 mm apart.

In effect, these asymmetrical deflections provide, at the level of tympanal mechanics, interaural time and amplitude differences that are much larger than those available in the sound field.

Discussion

Diffractive effects and the inputs to the auditory system

Sound diffraction around the body, or the head, depending on the animal species considered, generates an important acoustic cue used by the auditory system for the localization of a sound source (Michelsen, 1994; Middlebrooks and Green, 1991). Here, diffractive effects were estimated by measuring difference spectra across the fly's body (Fig. 1C) and normalizing them to the inherent heterogeneities of the sound field. Diffraction spectra thus represent the amount by which sound pressure differs from one side of the body to the other, with reference to the sound pressure at this particular location in the absence of the animal. According to sound diffraction theory (Morse and Ingard, 1968; Beranek, 1988), objects smaller than approximately one-tenth of the sound wavelength

cause little diffraction in the sound field. For *Emblemasoma* sp., the ratio between body size (3.8 mm) and wavelength (93.3 mm) at the frequency of the host's calling song (3.7 kHz) is 0.04. Accordingly, diffractive effects should be very small at the carrier frequency of the host's calling song. Although somewhat variable, the present diffraction spectra fail to indicate any systematic deviation from zero in the frequency range tested (Fig. 2A–C). For this particular fly species, a body size to wavelength ratio of 10 is reached at approximately 9 kHz. As indicated in Fig. 2, it is above this frequency that diffractive effects begin to take place, thus corroborating theoretical predictions. These diffraction experiments demonstrate that, in the absence of significant sound diffraction around the fly's body, the directional acoustic cues to the tympanal ears, in terms of acoustic interaural intensity difference (IID) and interaural time difference (ITD), are very small. By themselves, an IID of 0.20±0.22 dB (mean ± S.D.; N=10) and/or an ITD of 10.3±0.9 μs (mean ± S.D.; N=10) cannot account for the large interaural differences observed in the mechanical responses of tympanal membranes. As reported previously for tachinid flies, the key to directional sensitivity is to be found in the particular tympanal mechanics of the peripheral auditory system.

Asymmetrical deflection shapes and modes of vibrations

The mechanism underlying directional hearing in the ormiine tachinid fly *Ormia ochracea* has been described previously in some detail (Miles et al., 1995; Robert et al.,1996b). In the ormiine system, the asymmetrical tympanal vibrations observed in response to an incident sound wave are brought about by the mechanical coupling of the two tympanal ears. A crucial anatomical element contributing to this process is an unpaired and median sclerite (anatomically the presternum, functionally the intertympanal bridge), which behaves much like a flexible see-saw in the sound field (Miles et al., 1995; Robert et al., 1996b). The mechanical nature of this intertympanal coupling has also been investigated in experiments applying direct mechanical tympanal stimulation through the controlled vibrations of a pin (Robert et al., 1998). In these experiments, coupling through the intertympanal bridge occurs during the mechanical stimulation of only one tympanum, i.e. in the absence of any acoustic input to the system, thus demonstrating the pure mechanical nature of coupling (as opposed to acoustic coupling) *via* the intertympanal bridge (Robert et al., 1998). It has also been proposed that this system represents a third and distinct kind of receiver principle for directional hearing (Robert and Hoy, 1998), complementing the other two well-documented uncoupled pressure receiver system found in most mammals (Rayleigh, 1907; Middlebrooks and Green, 1991) and the acoustically coupled pressure-difference receiver system of some birds (Hill et al., 1980; Knudsen, 1980), some small mammals (Coles et al., 1982), frogs (Narins et al., 1988) and some insects (Michelsen, 1994).

Whether the asymmetrical tympanal vibrations of the sarcophagid peripheral auditory system can be explained in terms analogous to those proposed for tachinid flies will be addressed here on the basis of comparative morphological and biomechanical evidence.

The anatomical hallmark of dipteran auditory organs

The sarcophagid ears bear numerous anatomical similarities with those of the ormiine tachinids; they feature a series of modifications that also constitute the hallmark of tachinid tympanal ears (Robert et al., 1996a). Located on the ventral prosternum, the sarcophagid auditory organs feature (1) an inflated probasisternum that provides a rigid frame mechanically isolating the tympanal membranes from the nearby coxal membranes; (2) an increase in the surface area, and a thinning, of the prosternal membranes; (3) a modified prosternal tracheal system featuring an enlarged and unpartitioned prosternal air sac that backs the tympanal membranes (the presence of other modifications of the tracheal system, such as larger mesothoracic spiracles and the subpartitioning of their spiracular atrium, as found in tachinid flies, Robert et al., 1994, remains to be investigated); (4) the presence of two mechanoreceptive chordotonal organs in the prosternal air sac; (5) stiff cuticular apodemes linking the chordotonal organs to the tympanal membranes.

Although sarcophagid ears possess the essential features of dipteran tympanal ears, one of their striking features resides in the absence of a well-defined presternum. In the tachinid *O. ochracea*, the presternum is a forked and unpaired sclerite that is easily distinguishable from the tympanal membranes because it is made of thicker cuticle and therefore is less transparent. In *Emblemasoma* sp., the delineation of the presternum is not as straightforward as in *Ormia*, even raising the question of its presence on the tympanal complex. Since the prosternal chordotonal mechanoreceptive organs attach to the presternum in all higher Diptera, tympanate and atympanate, investigated so far (Edgecomb et al., 1995), one may surmise, on the basis of homology, that the presternum is present in tympanate sarcophagids. Thus, in *Emblemasoma* sp., the segment of cuticle situated between the points of insertion of the chordotonal organs to the tympanal membranes (Fig. 3, arrows) may be the presternum. Strikingly, unlike in *Ormia* spp., the presternum and the prosternal membranes together form a deep longitudinal fold, the tympanal fold, that runs across the tympanal system. The evidence presented below suggests that this folding is responsible for the mechanical coupling observed between the tympanal membranes.

The mechanical response of the sarcophagid ears

Phenomenologically, the mechanical response of the sarcophagid peripheral auditory system is distinct from that of the tachinid fly. Both systems have come to solve the problem of directional hearing in two ways that are unique to flies, but that are also quite different from each other. As shown in Figs 8 and 9, the cuticular structures at the midline of the animal (green dots) undergo significant displacement at most frequencies tested. This is a clear indication of the lack of a fulcrum such as that found in tachinids, for which at all
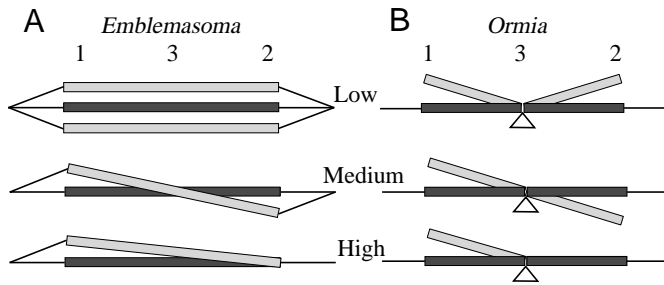
Fig. 10. Deflection modes of the tympanal systems in the sarcophagid fly *Emblemasoma* sp. (A) and in the tachinid fly *Ormia ochracea* (B). The filled bars represent the horizontal tympanal fold in A and the intertympanal bridge in B. The open triangles, in B only, show the stable fulcrum point. For both species, the lateral thin lines indicate the prosternal tympanal membranes. Also indicated are the insertions of the sensory organs (1, 2) and the midline of the presternum (3). Low, Medium and High indicate low (2–4 kHz), medium (6–8 kHz) and high (>15 kHz) frequencies, respectively. See Discussion for a detailed explanation.

frequencies the presternum oscillates about a relatively immobile fulcrum (Miles et al., 1995). This structural difference has been documented histologically (data not shown) and also experienced by gently pushing on the midline of the tympanal system and observing that the whole tympanal complex is very compliant. In *Emblemasoma* sp., the median part of the presternum is not supported by the apical aspect of the probasisternum and therefore moves along with the rest of the tympanal system (Fig. 8).

To help explain the different ways in which these two auditory systems achieve asymmetrical tympanal deflections, Fig. 10 schematically presents the major anatomical differences between the sarcophagid and tachinid systems as well as the deflection shapes at different frequencies. The tachinid system consists of two distinct beams, the two arms of the presternum, that join medially at the fulcrum (pivot) (Fig. 10B, label 3). At low frequencies, the presternum deflects in such a way that bending occurs (Miles et al., 1995; Robert et al., 1996b). Experimental evidence shows that insertion points 1 and 2 experience displacements of similar amplitude at frequencies below approximately 4 kHz. Interestingly, at such frequencies, the sarcophagid system, consisting of a single beam without support in the middle, deflects inwards and outwards with only little bending (Figs 8, 9A, 10A). As a result, points 1, 2 and 3 move together with only slightly different displacement amplitudes, and the bending mode described for tachinid flies finds its equivalent in the translational mode observed for low frequencies in sarcophagids. At intermediate frequencies (e.g. at 7 kHz), both tympanal systems oscillate in a similar manner; maximal outward displacements of point 1 are accompanied by maximal inward displacements of point 2 (Figs 9C, 10A,B). In this rocking mode, both tympanal systems swing about their midline. Note that, in the sarcophagid fly, this occurs in the absence of an anchored fulcrum. As seen in the Results, the difference in the amplitude of displacement between one side

of the tympanal system and the other also becomes larger as frequency increases (Fig. 5). The data show that the point of lowest displacements, the node, is displaced towards the side of the tympanal system contralateral to the sound source. At 15 kHz, for instance, the point of lowest displacement shifts contralaterally to the insertion point of the chordotonal organ, and the single beam (the tympanal fold) sways about point 2 (Figs 9D, 10A). In tachinids, the same result was shown to occur through the combination of both bending and rocking modes so that contralateral vibrations are minimized (Fig. 10B) (Miles et al., 1995; Robert et al., 1996b).

The experimental evidence presented here highlights a notable finding. Both tachinid and sarcophagid auditory systems achieve asymmetrical tympanal deflections despite interaural distances of the order of 1 mm. Their common operating principle relies on mechanical coupling between the two hemilateral tympanal membranes. However, the anatomical means of achieving mechanical coupling is embodied in two different morphologies that elicit two distinct biomechanical responses in a sound field. The tachinid solution is a flexible see-saw anchored at its centre that couples the two hemilateral tympana; the sarcophagid solution relies on the longitudinal tympanal folds that provide the structural rigidity, the mechanical anisotropy in stiffness, necessary for intertympanal coupling. These two solutions converge at the functional level and make use of distinct and divergent anatomical constructions. Thus, through functionally convergent but anatomically divergent evolutionary innovations, these two fly families have independently solved the problem of the directional detection of low-frequency sound by tympanal membranes separated by approximately 1 mm.

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