The Journal of Experimental Biology 214, 2569-2578

doi:10.1242/jeb.057612

RESEARCH ARTICLE

Matching sender and receiver: poikilothermy and frequency tuning in a tree cricket

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Accepted 9 May 2011

SUMMARY

Animals communicate in non-ideal and noisy conditions. The primary method they use to improve communication efficiency is sender–receiver matching: the receiver's sensory mechanism filters the impinging signal based on the expected signal. In the context of acoustic communication in crickets, such a match is made in the frequency domain. The males broadcast a mate attraction signal, the calling song, in a narrow frequency band centred on the carrier frequency (CF), and the females are most sensitive to sound close to this frequency. In tree crickets, however, the CF changes with temperature. The mechanisms used by female tree crickets to accommodate this change in CF were investigated at the behavioural and biomechanical level. At the behavioural level, female tree crickets were broadly tuned and responded equally to CFs produced within the naturally occurring range of temperatures (18 to 27°C). To allow such a broad response, however, the transduction mechanisms that convert sound into mechanical and then neural signals must also have a broad response. The tympana of the female tree crickets exhibited a frequency response that was even broader than suggested by the behaviour. Their tympana vibrate with equal amplitude to frequencies spanning nearly an order of magnitude. Such a flat frequency response is unusual in biological systems and cannot be modelled as a simple mechanical system. This feature of the tree cricket auditory system not only has interesting implications for mate choice and species isolation but may also prove exciting for bio-mimetic applications such as the design of miniature low frequency microphones.

Key words: tree crickets, Oecanthinae, frequency tuning, hearing.

INTRODUCTION

Crickets are nocturnal insects that communicate acoustically in the context of long-distance mate attraction (Alexander, 1967). Typically, the senders are adult males who produce loud calling songs that the receivers, conspecific females and males, use to locate them (Alexander, 1967). The song of each species has a unique set of temporal and spectral features as well as amplitude profiles (Otte, 1992), which are important to the females who use them to distinguish conspecific from heterospecific males (reviewed in Gerhardt and Huber, 2002). The songs also play a role in mate choice, providing cues for discrimination between conspecific males and, most functionally, locating the males in darkness (reviewed in Gerhardt and Huber, 2002; Kostarakos and Römer, 2010).

Communication systems have to deal with acoustic interference in noisy real-world conditions (Forrest, 1994). The primary way they deal with such interference is to match the response of the receiver's sensory system to the sender's signals. Hence, a suitable match between sender and receiver has to be made in both the spectral and temporal domains of calling song. In the spectral domain, which is the focus of the present study, cricket calling song is nearly tonal and most of the energy in the song is centred on a single frequency, called the carrier frequency (CF) (Bennet-Clark, 1999). In field crickets, the female receiver matches the sender's song spectrum and responds more to frequencies near the CF (Moiseff et al., 1978; Thorson et al., 1982; Stout et al., 1983). At the peripheral level, the mechanical system that transduces sound, the tympanum, is resonant at frequencies close to the CF (Paton et al., 1977; Larsen and Michelsen, 1978). At the next level, the auditory receptors are tuned to different frequencies, with a large number tuned to frequencies close to the CF, each with a much higher selectivity than the tympanal membrane alone (Oldfield et al., 1986; Ball et al., 1989; Larsen et al., 1989; Imaizumi and Pollack, 1999). The tuning of these two systems effectively acts as a filter set, reducing the representation of non-CF frequencies in the auditory system, making the receiver less likely to perceive heterospecific songs. This manifests itself in cricket behaviour as a greater degree of orientation towards CF-like frequencies (Moiseff et al., 1978; Thorson et al., 1982; Stout et al., 1983; Kostarakos et al., 2008).

In addition to this, the field cricket directionality system is also frequency dependent (Larsen et al., 1984; Michelsen and Löhe, 1995; Michelsen, 1998), and calls at non-CF frequencies are thought to prevent females from finding males (Ulagaraj and Walker, 1975). However, the two frequency filters in the field cricket system are only sometimes matched with each other (Kostarakos et al., 2008; Kostarakos et al., 2009).

In the temporal domain, the tympanum represents a range of temporal patterns faithfully (Poulet and Hedwig, 2001). Females, however, orient preferentially towards conspecific temporal patterns (Poulet and Hedwig, 2005), and this behaviour appears to be driven by processing at a higher neuronal level (Schildberger, 1984; Nabatiyan et al., 2003; Poulet and Hedwig, 2005).

Male calling songs can, however, change. In poikilotherms, body temperature and hence neuromuscular function are affected by ambient temperature. As a result, the temporal patterns of male

advertisement calls change with temperature (Walker, 1962; Pires and Hoy, 1992a; Souroukis et al., 1992; Martin et al., 2000). In the Oecanthinae, or tree crickets, even the CF changes markedly with temperature (Walker, 1962; Metrani and Balakrishnan, 2005). To maintain a sender–receiver match under these conditions, acoustic communication systems can adopt one of two possible strategies: the receiver can either be narrowly tuned to signal properties and shift tuning concomitantly with the changing signal [called temperature coupling (Gerhardt and Huber, 2002)], or the receiver can be broadly tuned and therefore receptive to the entire natural range of the signal. The second strategy imposes a trade-off on the communication, and the broader the tuning, the greater the probability of masking of the signal by noise. Yet another possibility is that a mismatch may be tolerated. This would depend on ecological context; for example, a mismatch may not pose significant difficulty for the female if the conspecific CF is quite different from the sympatric acoustic community. Such mismatches in frequency tuning both slight and extreme have indeed been reported in insects (Bailey and Römer, 1991; Mason et al., 1999; Kostarakos et al., 2008; Kostarakos et al., 2009) and anurans (Gerhardt and Mudry, 1980).

Much of what we know about sender–receiver matching in acoustic communication is from the temporal domain of song. Most of the insects and anurans investigated so far use the temperature coupling strategy (Gerhardt, 1978; Gerhardt and Doherty, 1988; Pires and Hoy, 1992b; Gerhardt and Huber, 2002), with the notable exception of the grasshopper *Chorthippus biguttulus*, which uses a 'broad-tuning' strategy (von Helversen and von Helversen, 1981).

At the level of spectral tuning, much less is known, especially about the behaviour of the tympanum in response to different temperatures. In anurans, female preference for call spectral characters is temperature dependent, as is the tuning of auditory receptors (Stiebler and Narins, 1990; Smotherman and Narins, 1998; Gerhardt and Huber, 2002), even though the spectral characters of their calls are not (Gerhardt and Mudry, 1980). In tree crickets, where such spectral change is observed, little is known about the temperature dependence of either the behaviour, the neurobiology of the female preference or the biophysics of the tympanal membrane (but see Mhatre et al., 2009).

At least four species of tree crickets belonging to the genus *Oecanthus* are found in southern India (Metrani and Balakrishnan, 2005). The syllable repetition rate and CF of all four species increase in a linear fashion with temperature (Metrani and Balakrishnan, 2005). One of the species, *Oecanthus henryi*, is acoustically active in the wild over a large temperature range from 18 to 28°C and its CF changes by nearly 1 kHz (median CF: 2.8 kHz, range: 2.4–3.3 kHz) (Metrani and Balakrishnan, 2005). *Oecanthus henryi* thus provides us with a suitable system to investigate frequency tuning in response to temperature at the behavioural and physiological levels. More generally, it allows us insight into the strategies used by poikilothermic signalling systems to deal with changing environments.

In this study, we investigate the behavioural response of *O. henryi* females to songs with different CFs, using a no-choice phonotaxis paradigm. We also investigate the mechanical frequency response of the *O. henryi* tympanum in an attempt to elucidate the basis of the behavioural response to song CF.

MATERIALS AND METHODS

Behaviour

Animals

Experiments were performed with adult virgin *Oecanthus henryi* Chopard 1936 females. *Oecanthus henryi* nymphs were captured in fields in Bangalore and Ullodu, Karnataka, India, where they are found associated with a fragrant weed (*Hyptis suaveolens*) throughout their life cycle. Female nymphs were maintained individually and reared to adulthood, ensuring virginity. Experiments were conducted 2 to 3 weeks after the final moult. Fresh apple pieces and water were provided *ad libitum*. The animals were maintained on a 12 h:12 h light:dark cycle at room temperature, which varied between 18 and 24°C, within the natural range of temperature variation in the field.

Experimental setup

Indoor phonotaxis experiments were carried out in a testing arena consisting of two loudspeakers (Creative SBS 240, Creative Technology Ltd., Singapore) placed 120 cm apart and approximately 60 cm from the ground (Fig. 1). A stem of the plant *H. suaveolens*, approximately 60 cm long and stripped of all branches, was placed vertically in between the two loudspeakers. Stripped branches of the same plant were placed at the top of the stem and at right angles to it to form a T-junction extending towards the two speakers (Fig. 1). The ground, the surrounding walls and the mount on which the loudspeakers were placed were covered with acoustic foam (Monarch Tapes and Foams Ltd., Bangalore, India) to minimize echoes. The stems extending from the T-junction ended on the acoustic foam in front of each speaker and were thus not in contact with either the speakers or the platform on which they were placed.

Animals were released singly at the base of the vertical stem during each trial. Their movements were recorded using an infrared-sensitive video camera (Sony DCR-TRV 17E, Tokyo, Japan).

Experiments were conducted with only one loudspeaker active, and females had a choice of either walking towards the active or the mute loudspeaker. The playback of the song model to be tested was begun before releasing the female on every trial. An animal reaching the playback loudspeaker was scored as a responder and the time it took to reach the playback loudspeaker, i.e. the latency, was also recorded. A silent control was carried out before each experiment, in order to measure the probability of a female 'responding' in silence, i.e. reaching either of the two mute loudspeakers. Females were observed for at least 120 s after release on the plant and their movements were recorded. The frequencies of responders in the silent and playback trials were compared to evaluate whether more females reached the loudspeaker that played sound stimuli.

Experiments were carried out at three temperatures (18, 22 and 27°C) and each animal was tested at all three temperatures, one each on three consecutive days. The experimental room was either cooled with an air conditioner or heated using a room heater in order

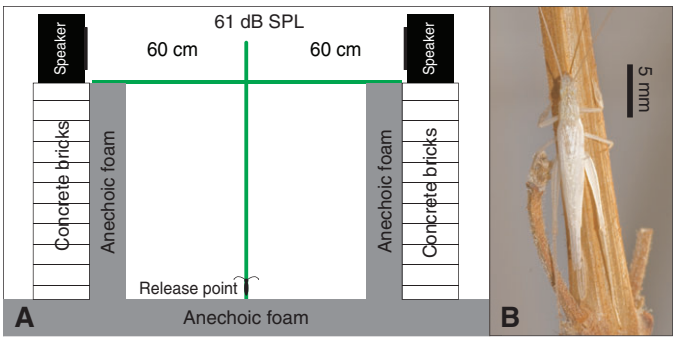

Fig. 1. (A) Schematic representation of the experimental setup used for the phonotaxis experiments. The figure is not to scale. (B) Photograph of an *Oecanthus henryi* female for scale comparison.

to maintain the desired temperature. The temperature of the room was maintained with an accuracy of ±1°C and was continuously monitored using a Testo 110 precision thermometer (Testo Ltd., Alton, Hampshire, UK) during the experiment. Each animal was acclimatized to the testing temperature for 1 h before conducting the experiments. The experiments were conducted in darkness between 19:00 and 21:00 h (the peak calling time of the test species).

Acoustic stimuli

To determine whether temperature affected the response to song CF, individual female responses to different song models were tested at three different temperatures. For each of the three temperatures, a set of eight calls was synthesized, each set having a fixed temporal pattern appropriate for that temperature (syllable repetition rate of 43.3, 51.3 and 66.7 Hz at 18, 22 and 27°C, respectively). The CF of the songs within each set was varied from 1.5 to 8.5 kHz in steps of 1 kHz. The stimuli were designed by multiplying envelopes with a sine wave of the desired CF in MATLAB (version 6.5, The MathWorks Inc., Natick, MA, USA). The envelope for a single syllable was extracted from a natural song and then the syllable was repeated at the appropriate syllable repetition rate to make a chirp, which was in turn repeated at the appropriate chirp rate. The temporal features of the stimuli were obtained from *O. henryi* calling songs recorded in the field at different ambient temperatures (Metrani and Balakrishnan, 2005). A natural advertisement call was not included in the set of stimuli as previous experiments had shown that the animals responded equally well to natural and synthetic songs, which had the same features as natural song (Appendix).

Songs were broadcast at 61 dB SPL (r.m.s. re. 2×10^{-5} N m^{-2}), based on field measurements of the SPL of male *O. henryi* calling song at comparable distances (R. Deb and R.B., unpublished data). The SPL broadcast from each speaker was measured before each trial using a Brüel and Kjær microphone (Type 4133) and Integrating Sound Level Meter (Type 2231) (Brüel & Kjær Sound & Vibration Measurement A/S, Nærum, Denmark) using the fast integration, r.m.s. and flat response settings. The measurement was made at the T-junction of the *H. suaveolens* branches.

Experimental design

Each female was tested with all eight song models at all temperatures. The order in which the animals were exposed to the three temperatures was cycled so that every possible combination was used.

At any given temperature, the first two stimuli were the extremes of the range of CFs being tested (i.e. 1.5 and 8.5 kHz); if the animal was exposed to 1.5 kHz first, then it was exposed to 8.5 kHz next and *vice versa*. After this, the animal was exposed to frequencies in descending order, i.e. 7.5, 6.5, 5.5, 4.5, 3.5 and 2.5 kHz. The stimulus CFs 3.5 and 2.5 kHz are closest to the natural song frequencies and are expected to elicit a response. A female having been exposed to song-like CFs may not respond to the other higher CFs because of a loss of motivation if these were presented after the optimal frequencies. Hence, 3.5 and 2.5 kHz were the last CFs to be presented to the females.

Successive stimuli broadcast to an animal were alternated between the loudspeakers in order to control for direction of presentation. At a population level, it was thus ensured that every stimulus was played from both loudspeakers an equal number of times.

Statistical analysis

Two response variables were tested statistically: the frequency of positive responders, negative responders and non-responders, and the latency of positive responses. For an overall analysis, responses were analyzed using a linear mixed model fitted by maximum likelihood (R version 2.10.1, R Foundation for Statistical Computing, Vienna, Austria), where the temperature of testing and the CFs were treated as fixed factors and the identity of the animals was used as a random factor.

Next, in order to examine whether responses to the various stimulus frequencies were significantly different from chance, the response to each CF was compared with the response to the silent trial. A McNemar chi-square test was used to analyse the proportion of responders and a Wilcoxon matched pairs test was used to analyse response latency. This analysis was carried out for all CFs at each of the three temperatures. Thereafter, the same test was used to compare responses between pairs of stimulus CFs.

Vibrometry

Animals

Laser vibrometry experiments were carried out with adult *O. henryi* females. Wild-caught adult females were maintained at 26°C under a 12 h:12 h light:dark cycle with *ad libitum* access to food and water. Females were immobilised and mounted for laser vibrometry experiments as described in Mhatre et al. (Mhatre et al., 2009). In order to increase stability, the mounting procedure was slightly modified for some experiments. Instead of gluing their forelegs to a separate balsa platform, the knee joint was immobilised on Blu-Tack® (Bostik Ltd, Stafford, Staffordshire, UK) attached to the lateral edges of the brass plate on which the animal was mounted. Thus the animal was immobilised on a single adjustable holder allowing for easy manipulation and orientation. The results from the two methods of mounting were compared and found to be identical (N.M., unpublished results).

Experimental setup

Vibrometry experiments were carried out on a vibration isolation table (TMC 784-443-12R; Technical Manufacturing Corp., Peabody, MA, USA). The vibration isolation table and the experimental setup on it were placed in an acoustic isolation booth (IAC series 1204A; 4.50×2.25×1.98 m; Industrial Acoustics, Bronx, NY, USA). Vibration velocities were measured using a microscanning laser Doppler vibrometer (Polytec PSV-300-F; Waldbronn, Germany) with an OFV-056 scanning head fitted with a close-up attachment, and digitised using Polytec Scanning Vibrometer software (version 7.4) through a data acquisition board (National Instruments PCI-4451; Austin, TX, USA). The experiments were carried out at three ambient temperatures (18, 22 and 27°C) and the experimental chamber was maintained at the desired temperature using a wall-mounted air conditioner and room heater. The temperature was monitored using a Testo 110 precision thermometer at the beginning and end of the measurement and the measurement was deemed acceptable only if the temperature remained with ±2°C of the desired temperature. Vibrometric measurements were made on one of the two anterior tympanal membranes (ATMs). The laser beam was perpendicular to the ATM being measured and scanned its entire surface.

Acoustic stimuli

Ipsilateral sound stimulation was used in all of the experiments. Acoustic stimuli were produced using Polytec Scanning Vibrometer software, amplified (Sony Amplifier Model TA-E570) and passed to a loudspeaker (Maplin L65AW, Maplin Electronics, Rotherham, UK, for the smaller bandwidth and ESS AMT-1; ESS Laboratory, Inc., Sacramento, CA, USA, for the larger bandwidth). Sounds were

constantly and simultaneously measured and recorded during the experiment using a calibrated 1/8 inch precision pressure microphone (Brüel and Kjær 4138) and preamplifier (Brüel and Kjær 2633). The microphone has a flat response in the measured frequency range and was positioned approximately 2 mm directly above the thorax of the animal. Periodic chirps were presented in two frequency ranges: 0.5 to 10 kHz at 22.5 mPa (61 dB SPL re. 2×10^{-5} N m^{-2}; to be comparable with behavioural data) and 2 to 20 kHz at 40 mPa [66 dB SPL re. 2×10^{-5} N m^{-2}; to be comparable with data in Mhatre et al. (Mhatre et al., 2009)]. The SPL of the signals was kept constant (±2 dB) over the entire frequency range.

Measurements of tympanal vibration

Analyses of tympanal membrane velocity and SPL were carried out using Polytec Scanning Vibrometer software (versions 7.4 and 8.5). The velocity of vibration of the membrane was sampled simultaneously with the acoustic signal at the appropriate minimum sampling rate set by the programme. Averages of 20 responses were made at each point in the scanning lattice, which was placed over the entire tympanal membrane. Using a fast Fourier transform with a rectangular window, a frequency spectrum was calculated (resolution of 12.5 Hz for the smaller bandwidth signal and 25 Hz for the larger bandwidth). The data were smoothed using a moving average window of 20 points (equivalent to 250 and 500 Hz, respectively). The laser and microphone signals were then used to calculate the coherence, gain and phase of the responses. The transfer function (nm Pa^{-1}) of the membrane displacement (nm) with respect to the reference sound level of the acoustic stimulus (Pa) and the coherence between the vibrometer and the microphone signals were calculated as in Windmill et al. (Windmill et al., 2005). Data were considered reliable only when the coherence of the transfer function was above 0.8. The membrane showed a deflection pattern as described in Mhatre et al. (Mhatre et al., 2009), and the data shown here are for the point of maximal deflection on the membrane. Two sets of seven females each were tested at the two frequency ranges.

RESULTS

Behavioural response of *O. henryi* females to song frequency at different temperatures

An overall analysis of female response across the three temperatures and for all the stimuli revealed that CF was the best predictor of a positive response and temperature was not; and the response was also not individual dependent (linear mixed-effects model: frequency, t=–5.7, $P\ll0.001$; temperature, t=1.5, P=0.125; Fig. 2).

Pairwise comparisons of female responses were made between all CFs at each of the three temperatures in order to determine the specific CFs at which females responded. At 18°C, as there were no responders in the silent trial, the responses were compared with the response to CF 1.5 kHz. The response to CF 1.5 kHz has been found to be equivalent to the response to a silent trial (M.B. and R.B., unpublished data), a result also obtained for the other two temperatures tested in the present study.

At 18°C, when compared with the response to 1.5 kHz, a significant increase in response was found only to stimuli with CFs of 2.5 kHz (χ^2=13.07, $P<<0.001$), 3.5 kHz (χ^2=7.11, P=0.007) and 4.5 kHz (χ^2=7.69, P=0.005). For all other CFs, female response frequencies were not significantly different from chance (P>0.05 for responses to 5.5, 6.5, 7.5 and 8.5 kHz; Fig. 2A).

At 22°C, five females responded in the silent trial. When this response was compared with the response to the other stimuli, only the responses to CFs of 3.5 kHz (χ^2=7.56, P=0.006) and 4.5 kHz (χ^2=10.56, P=0.001) were found to be significantly different (Fig. 2B).

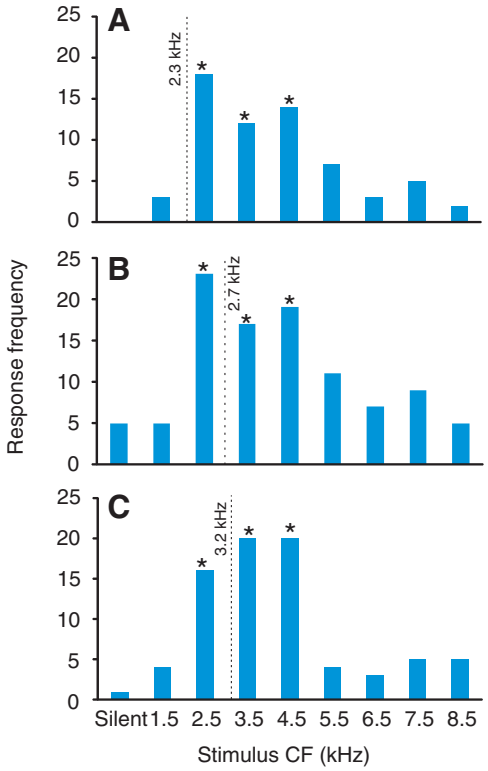

Fig. 2. Behavioural response of *O. henryi* females to stimuli with different carrier frequencies (CFs) at (A) 18°C, (B) 22°C and (C) 27°C. A total of 23 animals were tested at all CFs and all temperatures. The dashed lines represent the mean CFs of natural male song at each temperature. Asterisks indicate response frequencies that were significantly different from silent trials.

The McNemar test does not allow comparison against frequencies of zero. Because all 23 females responded to 2.5 kHz, the frequency of the negative response was zero, and a comparison of these responses to those of the silent trial was not possible. All 23 animals, however, responded positively to this frequency and hence response to a CF of 2.5 kHz can be considered significantly greater than chance. Female responses to all the other CFs, including the one to 1.5 kHz, were not significantly different from silent controls.

At 27°C, a similar trend was observed: females responded significantly more than chance only to songs with CFs of 2.5 kHz (χ^2=13.07, $P\ll0.001$), 3.5 kHz (χ^2=17.05, $P\ll0.001$) and 4.5 kHz (χ^2=17.05, $P\ll0.001$; Fig. 2C).

Comparing the number of females showing a positive response to 2.5, 3.5 and 4.5 kHz across the three temperatures did not reveal any significant differences (P>0.05 for all comparisons between temperatures for CFs of 2.5, 3.5 and 4.5 kHz). Thus, the responses to these three CFs are significantly greater than chance, but not significantly different from each other.

An overall analysis of the latencies of the positive responders across the three temperatures and for all the stimuli revealed that temperature was the best predictor of latency. CF did not have a significant effect (linear mixed-effects model: temperature, t=–2.8, P=0.005; frequency, t=0.3, P=0.762; Fig. 3). The latencies were not individual dependent.

However, Wilcoxon matched pairs tests of the latencies for the CFs of 2.5, 3.5 and 4.5 kHz between the three temperatures did not reveal any significant differences (P>0.05 for all comparisons between

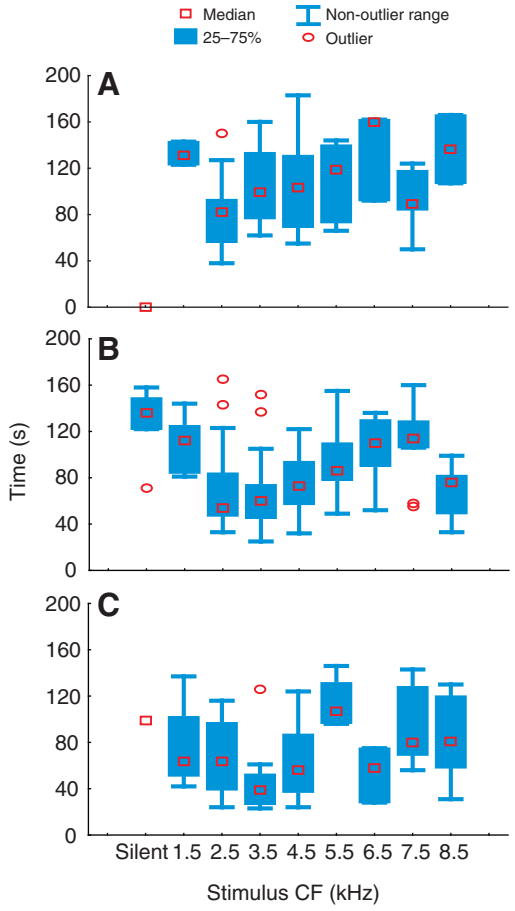

Fig. 3. Box and whisker plots of the latencies of response shown by *O. henryi* females to stimuli with different CFs at (A) 18°C, (B) 22°C and (C) 27°C. A total of 23 animals were tested at all CFs and all temperatures; only data from positive responders are considered here.

temperatures for the CFs of 2.5, 3.5 and 4.5 kHz). Thus, the difference in the latencies detected in the overall analysis may be caused by the differences in female responses at the CFs 1.5, 5.5, 6.5, 7.5 and 8.5 kHz. The response to these song frequencies, however, was not significantly different from chance. Hence, we conclude that the latency of the female response to the song frequencies of 2.5, 3.5 and 4.5 kHz does not differ significantly over the three temperatures.

Mechanical response of the tree cricket ATM

The CF of the calling song of *O. henryi* males varies from 2.4 to 3.3 kHz between 18 and 28°C and the female behavioural response shows a peak between 2.5 and 4.5 kHz (Figs 2, 3). If the basis of the behaviour lies in the mechanics of the tympanal membrane, one would expect a similar peak in the frequency response of the tympanal membranes of *O. henryi* females. The displacements of the ATMs of individual females did not, however, show a distinct peak at any of the three temperatures tested (Fig. 4A,D,G and Fig. 5A,D,G). However, the mean of the responses showed a minor peak between 3 and 4.5 kHz at all three temperatures only in the narrow band stimulation (Fig. 4A,D,G). We calculated the height of this peak in relation to the baseline response of the ATM. To calculate the baseline of the mean response, we considered the mean of the mean displacement from 1.75 to 3 kHz and from 4.5 to 10 kHz. Both the baseline and the maximum displacement are marked by dashed lines in Fig. 4A,D,G. The peaks are 36.51, 39.30 and 36.80% higher than the baseline at 18, 22 and 27°C, respectively. However, when the individual traces were examined, they did not all show peaks in displacement between 3 and 4.5 kHz and often had peaks of similar or greater amplitudes at other higher frequencies (Fig. 4A,D,G).

In order to examine individual variation, we squared the displacement transfer function of each individual and then calculated the sum of the area under the peak and the proportion this forms of the area under the trace from 1.75 to 10 kHz. This provides a measure of the energy transferred by the sound to the individual ATMs within the frequency band of the peak. The peak band of 3–4.5 kHz accounts for 18.18% of the considered range and, on average, the areas under the peak were 25.22±2.8, 26.80±2.8 and 25.42±1.75% (N=7) at 18, 22 and 27°C, respectively. This suggests that the increase in energy transfer in the peak region is relatively minor. In addition, a similar peak was not observed in the animals tested with the broader band signals.

The phase of the transfer function was also quite different from that previously observed in a field cricket tympanal membrane (Larsen and Michelsen, 1978). At all three temperatures, the initial phase of the membranes, at the lower frequencies, began near 55 deg (55.35±18.80 deg at 1.75 kHz at 18°C) and then fell to 0 deg near 4 kHz (–0.42±7.61 deg at 18°C) (Fig. 4B,E,H). At frequencies higher than 4 kHz, the phase tended to remain near 0 deg in both measurements until approximately 12 kHz (0.31±7.99 deg at 18°C), after which the phase value fell below 0 deg in the second measurement (Fig. 4 and Fig. 5B,E,H). This shows that the membrane follows sound input cycle by cycle within this frequency range, as described previously (Mhatre et al., 2009).

Finally, the coherence measures of the transfer functions of the tympanal membranes below 1.75 kHz were less than 0.8 at all temperatures (Fig. 4C,F,I), showing that the ATMs did not respond to sound below this frequency.

Comparison to a simple harmonic oscillator model

The ATM membrane of *O. henryi* females, when excited with sound, does not behave like a classical driven simple harmonic resonator, unlike the tympanal membranes of other crickets (Paton et al., 1977; Larsen and Michelsen, 1978). A simple harmonic oscillator (SHO) resonant system, when driven with sound, will show peak displacement at its resonant frequency ω_0 as described by the equation:

$$A(\omega) = A_0\omega_0^2 / ([(\omega_0^2-\omega^2)^2 + (2\gamma\omega_0\omega)^2]^{1/2}), \quad (1)$$

where A_0 is the amplitude of the membrane at ω=0, i.e. an angular velocity of 0. The function peaks when the input $\omega=\omega_0$ and $A(\omega)=A_0/2\gamma$. The width of the peak is determined by the damping ratio γ, which relates to the quality factor Q of the resonator by $Q=1/2\gamma$. Similarly, the phase of the displacement of the membrane φ can be described by the equation:

$$\sin\varphi = (\omega_0^2 - \omega^2) / ([(\omega_0^2 - \omega^2)^2 + (2\gamma\omega_0\omega)^2]^{1/2}). \quad (2)$$

Thus, at input $\omega=\omega_0$, φ=0 deg and at $\omega \ll \omega_0$, φ=90 deg and at $\omega \gg \omega_0$, $\varphi \rightarrow -90$ deg. Thus the resonant frequency of a simple harmonic oscillator lies at the frequency where its displacement amplitude peaks and the phase of the transfer function crosses 0 deg.

Assuming that the mean phase angle at 1.75 kHz is reliable and that the resonant frequency (f_0) is at 4 kHz ($\omega_0=2\pi f_0$), we estimate from Eqn 2 that the damping ratio γ is 0.828, which would be a near critically damped SHO (γ=1). Similarly, from Eqn 1 and with an $A(\omega_0)$ of 8 nm Pa^{-1}, we estimated A_0 to be 13.25 nm Pa^{-1}. We can use these values to plot the amplitude and phase response of an

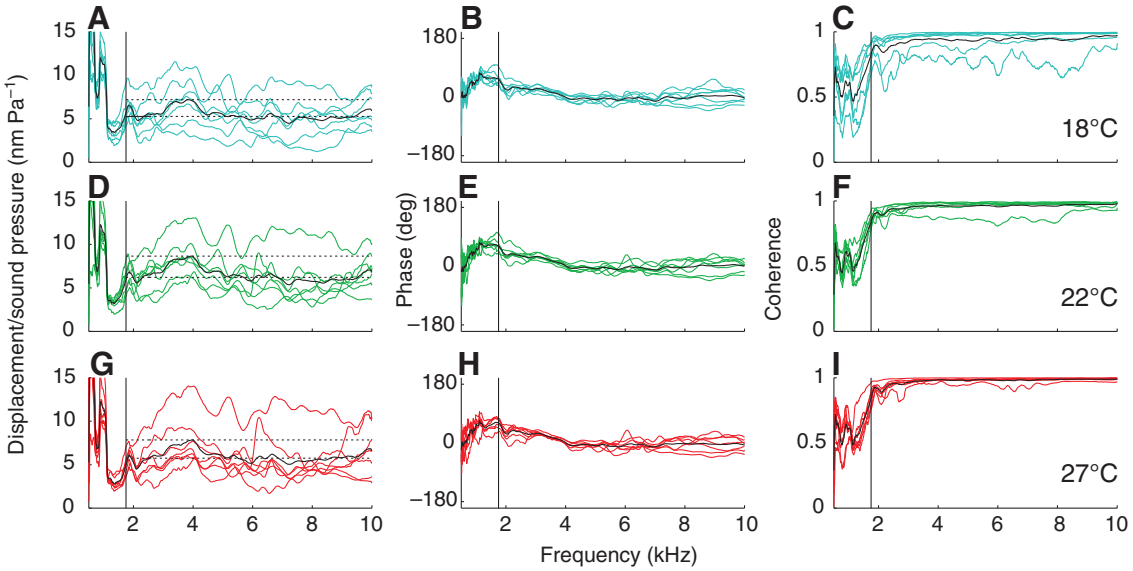

Fig. 4. The mechanical response of the anterior tympanal membrane (ATM) of *O. henryi* females at the point of maximal deflection in response to stimulation with sound at 22.5 mPa (61 dB SPL) between 0.5 and 10 kHz at different ambient temperatures. The coloured lines are data from seven females and the thick black lines are the mean responses across the females. Data shown are the amplitude (A,D,G), phase (B,E,H) and coherence (C,F,I) of the transfer function of the membrane displacement in response to sound at 18°C (blue, A–C), 22°C (green, D–F) and 27°C (red, G–I). The lower stippled line in A, D and G indicates the baseline displacement and the upper stippled line indicates the maximum displacement (see text for further explanation).

SHO model (Fig. 6). We can also vary the parameters of the model to try and achieve a better fit. As can be observed, however, the data from the *O. henryi* ATM do not match the predictions of an SHO model, neither in amplitude (Fig. 6A,B) nor in phase domain (Fig. 6C,D). A possible mechanism by which the *O. henryi* ATM may achieve a flat frequency response is the one used by microphones, i.e. placing the working frequency range below the f_0, such as an f_0 of 20 kHz (Fig. 6A). However, the phase response does not fit the expected phase response for a high f_0 (Fig. 6C). The phase response rapidly decreases from its initial value to 0 deg and then remains at this position for nearly an order of magnitude change in frequency before decreasing rapidly again (Fig. 6C,D). The only scenario in which such a phase response is possible is an SHO with a high γ where the f_0 is in the centre of the frequency range with the 0 deg phase (Fig. 6D). However, the amplitude response of a high γ SHO drops nearly two orders of magnitude around the f_0 (Fig. 6B). None of the scenarios considered here describe the frequency response of the *O. henryi* ATM. Thus variation of neither f_0 nor γ explains the difference in behaviour between an SHO and the *O. henryi* ATM. Varying the A_0 would merely shift the amplitude

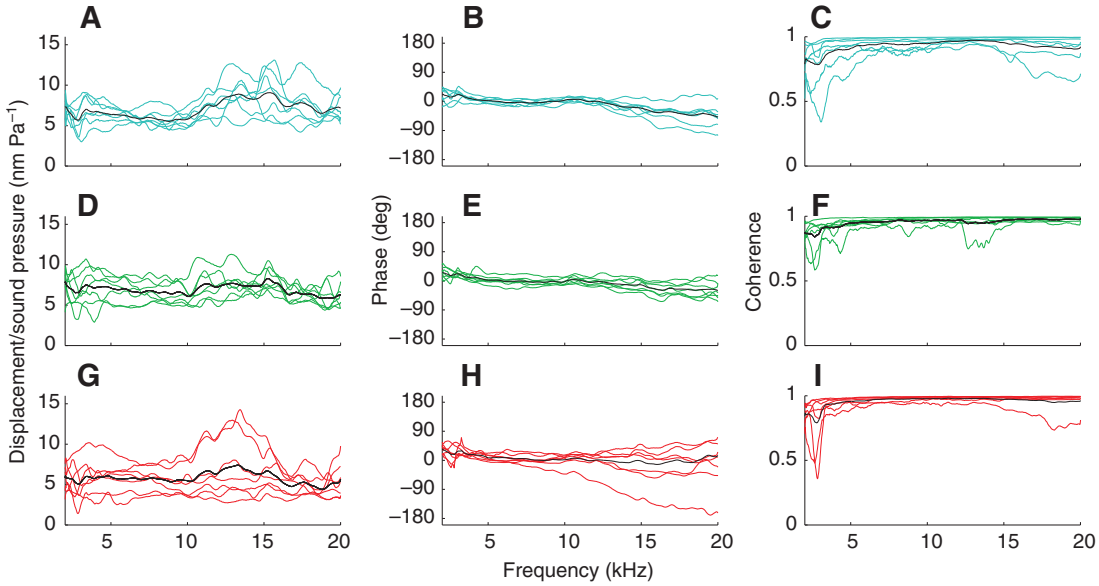

Fig. 5. The mechanical response of the ATM of *O. henryi* females at the point of maximal deflection in response to stimulation with sound at 40 mPa (66 dB SPL) between 2 and 20 kHz at different ambient temperatures. The coloured lines are data from seven females and the thick black lines are the mean responses across the females. Data shown are the amplitude (A,D,G), phase (B,E,H) and coherence (C,F,I) of the transfer function of the membrane displacement in response to sound at 18°C (blue, A–C), 22°C (green, D–F) and 27°C (red, G–I).

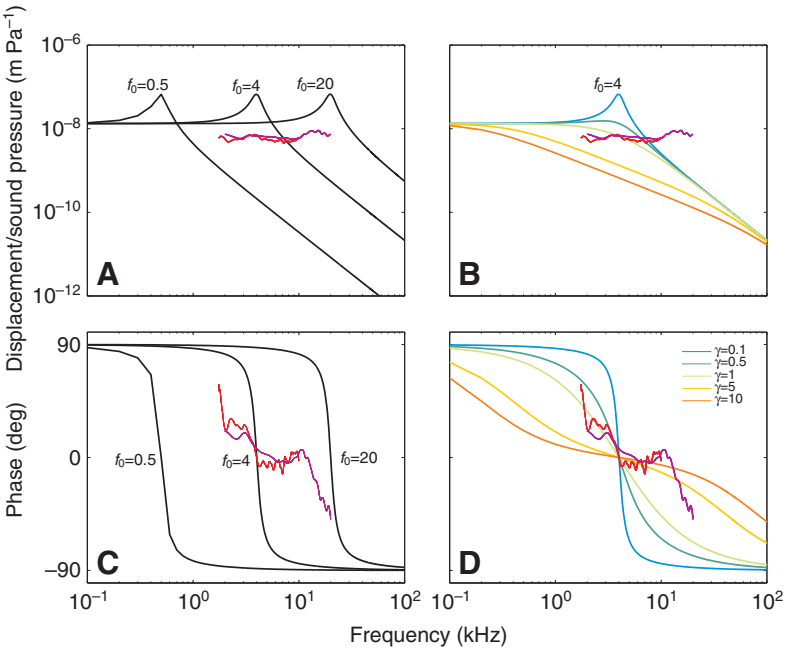

Fig. 6. The amplitude (A,B) and phase (C,D) responses of a driven simple harmonic oscillator (SHO) model when the resonant frequency (f_0) and damping ratio (γ) are varied. The mean data from Fig. 4A,B (red trace) and from Fig. 5A,B (magenta trace) are also plotted for comparison. Note that the *x*-axes are logarithmically scaled in all plots and that the *y*-axis is logarithmically scaled only in the amplitude plots (A,B). As the f_0 of the SHO increases, the amplitude and phase response shift along the logarithmically scaled frequency axis (A,C). The γ influences the shape of the amplitude response, which changes slowly with frequency close to the f_0 (B), and also the phase response, which changes slowly with frequency close to the f_0 (D). The observed *O. henryi* ATM data show a nearly constant displacement amplitude in the frequency range studied. The phase response remains near 0 deg between 4 and 12 kHz. This amplitude and phase behaviour cannot be captured by any of the single SHO models. In the region that the SHO models produce flat amplitude responses, such as before the f_0, the phase is mismatched. When the phase response of an SHO remains near 0 deg, as in an over-damped SHO, the amplitude response is mismatched.

of the resonator response (Eqn 1) and not the phase (Eqn 2), which also would not explain the ATM response. Thus, after an exploration of the parameters of an SHO model, we find that the mechanical behaviour of an *O. henryi* ATM cannot be described by simple resonant behaviour.

DISCUSSION

Behavioural response to song CF with varying temperature

The CF of *Oecanthus henryi* calling song changes with temperature (Metrani and Balakrishnan, 2005). If tree crickets were similar to field crickets who are 'tuned' at both the tympanal level (Paton et al., 1977; Larsen et al., 1984; Michelsen, 1998) and the neurophysiological level (Oldfield et al., 1986; Imaizumi and Pollack, 1999), they would perceive poorly or not at all a calling male whose CF did not match the frequency they were tuned to. Changing CF may thus present a problem for female tree crickets trying to find mates.

We investigated the tuning of female tree cricket behaviour *vis a vis* the changing CF of the male call. Previous work on nine tree cricket species, focused on the temporal domain of song, supported the temperature coupling hypothesis (Walker, 1957). The pulse rates of artificial songs were varied and it was found that conspecific pulse rate was always preferred and female preference shifted in parallel with song pattern change with changing temperature (Walker, 1957), an observation made in field crickets as well (Pires and Hoy, 1992b). An interesting detail of the experiments with tree crickets, however, was that the CF of the artificial song was not varied with temperature and females responded to the songs with mismatched CFs (Walker, 1957). A similar observation was made with *Oecanthus nigriconis* females tested at a single temperature; these crickets showed no preference for song CF within the naturally occurring CF range (Brown et al., 1996). These results thus hinted at the possibility that female response to song in tree crickets may be CF independent.

We found that, when presented with a range of CFs, the only predictor of the number of females attracted was the CF. Only naturally occurring CFs (Metrani and Balakrishnan, 2005) attracted females significantly and this pattern did not change with ambient temperature (Fig. 2). From this we can conclude that *O. henryi* females perceive, recognise as conspecific and can localise, at ecologically relevant SPLs, songs with appropriate temporal patterns and CFs between 2.5 and 4.5 kHz, without regard to ambient temperature. Our results show that *O. henryi* females solve the problem of sender–receiver matching in the spectral domain by being broadly tuned behaviourally, which fits well with the results of previous work on other *Oecanthus* species (Walker, 1957; Brown et al., 1996).

The latencies of the females who responded positively also allowed us to determine whether the attractiveness of CFs changed with temperature. It was found that the latencies were not significantly different for the frequencies of 2.5, 3.5 and 4.5 kHz across temperatures (Fig. 3). This suggests that songs with temperature-adjusted temporal patterns within the CF range 2.5 to 4.5 kHz are equally attractive across all temperatures.

A choice experiment, however, would provide clearer answers on whether particular CFs are preferred depending on temperature. Such an ability to discriminate CFs in absence of tympanal tuning, if observed, would require and hence indicate finer tuning at the level of the auditory receptors. In field and tree crickets, it has been suggested that CF is indicative of body size (Simmons, 1995; Brown et al., 1996), and lower CF songs are preferred, in a choice situation (Brown et al., 1996; Simmons and Ritchie, 1996). Such an observation is particularly interesting in the context of *Oecanthus* species, where males have the opportunity to exploit such preferences by calling from a cooler site and hence with a lower CF. Using a choice paradigm would allow us to ask whether females can discriminate between CFs, leading to greater insights into the neurophysiological mechanisms of frequency detection and discrimination.

Oecanthus henryi is also found in sympatry with three other *Oecanthus* species, one of which, *O. indicus*, shows considerable overlap in CF range, raising interesting questions regarding the mechanism of species isolation between them (Metrani and Balakrishnan, 2005).

Mechanical frequency response of the ATM

The behaviour of the mechanical transduction system, the tympanum, has to underpin the phonotactic behaviour of the

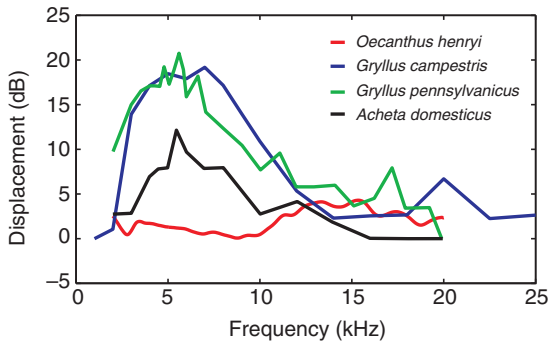

Fig. 7. A comparison of the mechanical frequency response of the *O. henryi* tympanum with those reported from three field crickets (Paton et al., 1977; Larsen and Michelsen, 1978). Velocity data were converted to displacement data and the lowest displacement in each trace was used as the reference to calculate the displacement in decibels.

females. Hence, in this study, we also investigated the frequency response of the female *O. henryi* ATM at different temperatures. The frequency response of the ATM is invariant with temperature (Figs 4, 5). The membrane responds coherently to sound only above 1.75 kHz, a strong indication of the lower limit to the ability of the female to perceive sound, and hence for the behavioural response (Figs 2, 3).

Importantly, the ATM also showed very little change in amplitude of the displacement transfer function in response to sound from 1.75 to about 12 kHz (Figs 4, 5). A slight increase (1.92, 2.44 and 2.11 nm Pa^{-1} at 18, 22 and 27°C, respectively) over the mean displacement of the membrane (5.27, 6.21 and 5.73 nm Pa^{-1} at 18, 22 and 27°C, respectively) was seen between 3 and 4.5 kHz (Fig. 4). However, this increase is small in comparison with the overall response level and is not compatible with the idea of a resonant system (Figs 4–6). Remarkably, the phase of the tympanal response also remained nearly constant near 0 deg for most of the frequency band in which the amplitude was constant (Figs 4, 5). The amplitude, phase and coherence of the displacement transfer function of this membrane are unusually flat for a biological membrane, and the displacement of the ATM encodes both the amplitude and phase of impinging sounds very faithfully over a wide range of frequencies. The tympana of two tettigoniids are the only other membranes that show a flat frequency response, albeit in membrane velocity (Bangert et al., 1998). The phase response of these membranes falls steadily over the measured frequency range (Bangert et al., 1998) and the displacement frequency response will steadily decrease with a slope of ω, as predicted for a critically damped or over-damped system (Fig. 6), because $\mathbf{v}=d\mathbf{x}/dt=\omega A\sin(\omega t+\phi)$, where $\mathbf{v}$ is the velocity of the membrane, which is the first derivative of $\mathbf{x}$, the displacement with respect to time t.

The flat frequency response observed at the tympanal level can, to a certain extent, explain the behavioural frequency response of *O. henryi* females. The lower CF limit at the behavioural level (Fig. 2) may be ascribed to the substantial drop in both coherence and amplitude of membrane displacement below 1.75 kHz (Fig. 4). The flat amplitude response also shows that the tympanum does not preferentially amplify song frequencies to any great extent (Figs 4, 5). However, the tympanal frequency response is much broader than the behavioural response and does not explain the reduction in female response above 4.5 kHz. Another level of processing, however, is available at the level of the auditory neurons (Oldfield et al., 1986), which are well known to be more sharply tuned than tympanal membranes (Gerhardt and Huber, 2002).

The mechanical basis of a flat frequency response

A simple harmonic oscillator is one of the simplest known mechanical resonant systems, one that has been used to model the response of several auditory systems (Göpfert et al., 1999; Gopfert and Robert, 2002; Göpfert and Robert, 2003; Windmill et al., 2006) and that appears to fit the response of a field cricket ear (Paton et al., 1977; Larsen and Michelsen, 1978). Membrane resonances in field crickets are centred on the conspecific song CF (Fig. 7) (Paton et al., 1977; Larsen and Michelsen, 1978) and act as a first-level filter applied to the impinging sounds, enhancing the representation of conspecific calls (Oldfield et al., 1986; Kostarakos et al., 2008; Kostarakos et al., 2009). These filter characteristics are believed to remain the same for a direct external input to the tympanum as well as for the internal tracheal input to the cricket ear (Robert, 2005), as the resonant properties of the tympanum are considered to mainly result from intrinsic material characteristics. In effect, their mechanical response serves as a filter, making heterospecific calls and other background noise less perceivable, and reduces the noise and complexity of the perceived acoustic world (Feng and Ratnam, 2000; Pollack, 2000).

However, this picture of simple resonant behaviour does not necessarily hold for all insect tympanal membranes, in particular those with heterogeneous structures. The *O. henryi* tympanal membranes are unlike the smooth, seemingly homogenous field cricket tympanal membranes and have multiple transverse folds (Mhatre et al., 2009). The response of the *O. henryi* ATM was hence compared with an SHO model to highlight its deviation from simple resonant behaviour (Fig. 6). One of the possibilities for describing the response of this membrane based on its frequency response, as well as the non-resonant time domain response, was an over-damped system. However, a comparison with the SHO model shows that although an over-damped system may explain the phase response, the flat displacement amplitude response cannot be captured (Fig. 6), suggesting that a different physical explanation may be necessary. Yet another possibility is that the f_0 of the ATM is well above song CF and the membrane response remains flat before the roll-off point (Fig. 6). Interestingly, this is a technique used to define the working frequency range of microphones. However, the measured phase response of the SHO model is incompatible with such an explanation (Fig. 6). Having explored the possibilities offered, we find that the response of the *O. henryi* ATM cannot be described by a single SHO model.

It has been observed that other insect tympanal membranes that have structural heterogeneities tend not to have simple resonant properties. Known examples include the pyriform vesicle and folded body of the locust (Stephen and Bennet-Clark, 1982; Windmill et al., 2005), the attachment sites of mechanosensory cells in moths (Windmill et al., 2006) and the inner plate structure in tettigoniids (Bangert et al., 1998; Nowotny et al., 2010). In effect, membrane modifications are likely to alter the simple resonance behaviour of the main membrane.

There are a few known ways to alter the frequency response of resonant systems. A method that is widely used in engineering applications is the coupling of oscillators, such as in the damping of bridge resonances by the use of tuned mass dampers, which oppose the motion of the main structure and reduce the amplitude of its oscillations at its resonant frequency (Den Hartog, 1947). More recently, it has been suggested that an array of masses as opposed to a single mass may be more effective in controlling such resonant

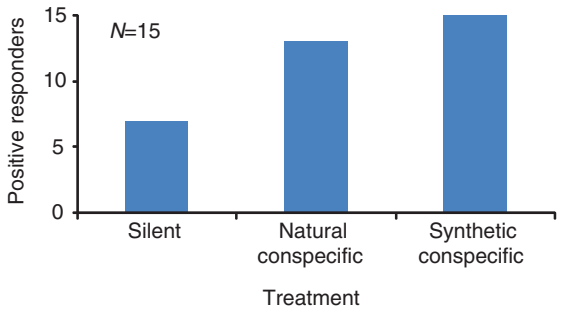

Fig. A1. Behavioural response of *Oecanthus henryi* females to natural and synthesized conspecific song. The bars represent the frequency of positive responders to the three treatments.

behaviour (Igusa, 1994). Of particular interest is a study that suggests that the amplitude and phase response of the main oscillator may be shaped as desired using an array of subordinate oscillators, making possible a flat frequency response within a small frequency range (Vignola et al., 2009). Elucidating the underlying mechanism of the surprisingly wide-range, flat frequency response of the tympanum of *O. henryi* will require further research, but opens up new and interesting avenues in the research of insect ears, as well as exciting possibilities for the design of miniature microphones.

APPENDIX

Synthetic *versus* natural song

Each *O. henryi* female was exposed to three treatments: (1) a silent trial in which the female was released at the base of the stem but no stimulus was played; (2) a natural song of an *O. henryi* male (field recording); and (3) a synthetic song with the same temporal and spectral pattern as the natural song at 26°C but synthesized using MATLAB (N=15 females). The experimental setup was similar to that reported in the Materials and methods, 'Behaviour'.

A response to the silent trial would be equivalent to the female reaching the speaker through chance alone; thus the response to both the natural and synthetic conspecific songs was compared with the response to the silent trial. In both natural conspecific (chi-square test, χ^2=9.64, P<0.01) and synthetic conspecific trials (chi-square test, χ^2=17.14, P<0.01), it was found that the response was significantly different from that for the silent trial and thus significantly different from chance (Fig. A1). A comparison between the response to the natural conspecific and synthetic conspecific songs, however, did not reveal any difference, indicating that the females respond equally well to the natural and the synthesized song (chi-square test, χ^2=2.31, P>0.01).

LIST OF ABBREVIATIONS

ATM	anterior tympanal membrane
CF	carrier frequency
SPL	sound pressure level

ACKNOWLEDGEMENTS

We would like to thank the UK India Education and Research Initiative (UKIERI) for funding this collaborative research. N.M. is supported by an IIF Marie Curie fellowship; M.B. is supported by a fellowship from CSIR, Government of India; and D.R. is supported by the Royal Society London. We would also like to thank Kathleen Lucas for her support and shared expertise throughout the experiments.

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