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A TYMPANAL HEARING ORGAN IN SCARAB BEETLES

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Summary

We describe the paired hearing organ of the scarab beetle *Euetheola humilis*. The auditory structures of the beetle are typical of other insect ears in that they have a thinned tympanic membrane backed by a tracheal airsac with associated chordotonal sensory structures. The tympanic membranes of the beetle are part of its cervical membrane and are located behind the head, where the cervix attaches dorsally and laterally to the pronotum. Each membrane is approximately 3 μm thick. The chordotonal sensory organ, which lies within the tracheal airsac, contains 3–8 scolopidia that attach by accessory cells directly to the tympanic membrane. Neurophysiological recordings from the neck connective of the beetle revealed that the auditory system is sensitive to frequencies between 20 and 80 kHz and has a minimum threshold of approximately 58 dB at 45 kHz. The neurophysiological audiogram is identical to the behavioral audiogram for a head roll, one behavioral component of the beetle's startle response elicited by ultrasound. Blocking experiments show that the membranous structures on the cervix are indeed the hearing organs. Neurophysiologically determined thresholds increased by more than 35 dB when drops of water covered the tympanic membranes and were essentially restored to the control level when the water was later removed. At least three other genera of Dynastinae scarabs have similar tympanum-like structures located in their cervical membranes. Behavioral and neurophysiological data show that the frequency tuning of species in two of these genera, *Cyclocephala* and *Dyscinetus*, is nearly identical to that of *E. humilis*. Our discovery represents only the second group of beetles known to respond to airborne sounds. However, the hearing organs of these scarab beetles differ in structure and placement from those of the tiger beetles, and thus they represent an independent evolution of auditory organs in the Coleoptera.

Key words: *Euetheola humilis*, Scarabaeidae, hearing, tympanum, acoustic startle response, ultrasound, beetle, audiogram.

Introduction

Audition is one of the primary sensory modalities used by insects. During the reception of acoustic signals, pressure waves are transduced by tympanic membranes (Bennet-Clark, 1983), and tympanal hearing organs have evolved numerous times within the insects (Yack and Fullard, 1993). Species in at least seven orders of insects are known to hear using tympana (Hoy and Robert, 1996). Insect ears function primarily in detecting acoustic signals emitted by conspecifics or for detecting sound generated by predators or prey. The tympanic auditory structures of insects have three general features. First, they have a thinned area of the cuticle, the tympanic membrane. Second, the tympanic membrane is associated with an expansion of the tracheal system, an airsac. Finally, the transduction of the vibration of the membrane into neural signals is accomplished by bipolar sensory cells in the chordotonal sensory organ.

Previously, we described the acoustic startle response of a night-flying scarab beetle, *Euetheola humilis*, the first scarab beetle shown to hear airborne sounds (Farris, 1994; Forrest *et al.* 1995). In field experiments, beetles were trapped beneath speakers broadcasting bat-like ultrasound, presumably because they detected the ultrasound and took evasive action from a potential bat predator. In laboratory experiments, we showed that the beetles were ultrasound-sensitive and that their behavioral response had a short latency and was graded with stimulus intensity (Forrest *et al.* 1995). Interestingly, the beetles show a similar ultrasound startle response in contexts other than flight (Farris, 1994).

In the present paper, we describe the auditory organs of *Euetheola humilis*. Neural activity correlated with an acoustic stimulus indicated that these scarab beetles are sensitive to frequencies between 20 and 80 kHz, a frequency range commonly used by echolocating bats. Using the neural correlate to pulses of sound as an indicator of hearing and blocking the putative ears with water, we showed that tympanal structures located on the neck membranes of the

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beetle are responsible for its sense of hearing. The tympanal organs of these scarabs differ from those of tiger beetles (Cicindelidae), the only other beetles known to possess a tympanal hearing organ (Spangler, 1988). Finally, we examined museum specimens of other dynastine scarabs to determine the taxonomic distribution of these auditory structures in the group.

Materials and methods

Animals

All *Euetheola humilis* were collected near the University of Mississippi campus. We kept beetles at room temperature (20–24 °C) in 18 cm×15 cm×9 cm plastic boxes containing moistened paper towels, 10–30 beetles per box. Beetles were fed pieces of apple twice weekly. This housing proved adequate for maintaining the beetles for more than 6 months.

Morphological studies

On the basis of behavioral observations, we knew that the beetles must possess a pressure-sensitive tympanal hearing organ. We inspected several beetles and tried to locate an auditory organ necessary to detect airborne ultrasound. We used three criteria for determining candidate auditory structures (Hutchings and Lewis, 1983): (1) a thinned area of cuticle acting as the tympanum, (2) a large tracheal airsac associated with the tympanum, and (3) a chordotonal sensory organ associated with the tracheal airsac or tympanum. Initially, we examined the first abdominal segment in the area where the tympanic membranes of the tiger beetle are located (Spangler, 1988). There were no obvious tympanal structures, and ablation of the area did not decrease the behavioral response of the beetle to ultrasound.

The first two criteria were met by an area on the cervical membrane where it attaches dorsally and laterally to the pronotum. The thin clear cuticle was backed by a tracheal airsac. To determine whether a scolopidial sensory organ was linked to the tympanal organ or the tracheal airsac, we prepared histological sections of the prothorax of the beetles. To avoid tissue disruption near the tympanal organ during sectioning, we removed most of the thick cuticle of anesthetized beetles by sanding it away using a Dremel® Moto-Tool®. The beetles were then injected with alcoholic Bouin's fixative. The legs and abdomen, and in some cases the heads, of the beetles were removed and the thoraces were immersion-fixed for 24 h at room temperature. After fixation, the thoraces were transferred to 70 % ethanol saturated with Li_2CO_3, dehydrated in ethanol and embedded in JB-4® plastic. We sectioned transversely or horizontally at 5 µm and stained the sections with a 0.5 % solution of Toluidine Blue in 0.5 % sodium borate.

Stimulus generation

To measure neurophysiological thresholds to sound, we digitally generated 10 ms duration pulsed sinusoids (5–80 kHz) using custom-written software. A 1 ms raised-cosine ramp was applied to the onset and the offset of all pulses. The pulses were played from a 16-bit digital-to-analog (D/A) converter (TDT DD1) at a sampling frequency of 200 kHz. The output of the D/A converter was amplified using a Harman/Kardon HK 6150 integrating amplifier, and the stimuli were broadcast from an ESS AMT-1 tweeter located 30 cm from the insect preparation. At the site of the insect preparation, the amplitudes of the test frequencies were more than 30 dB (typically more than 40 dB) above all other frequency components in the broadcast spectrum. Stimulus level was adjusted using a TDT PA-4 programmable attenuator. Sound pressure level at the preparation was calibrated using a Bruel and Kjaer (B&K) model 4138 microphone (90 ° angle of incidence) at the location of the beetle's head (insect removed). The microphone was connected to a model 2608 B&K measuring amplifier (fast, linear weighting). We standardized the calibration systems using a B&K model 4220 pistonphone calibrator. We measured all sound pressure levels (SPL) with a linear weighting, and we report these levels in dB referenced to 20 µPa.

Neurophysiological tuning curve

Cold-anesthetized beetles were mounted ventral side up on a small platform using low-melting-temperature wax. The platform was 12 cm above the floor of a 130 cm×70 cm×60 cm, foam-lined Faraday cage that reduced acoustic reflections and electrical interference. We removed all of the legs from the anesthetized beetle at the coxal joint, and the head was waxed in a position to expose the tympanic membranes. A ventral dissection through the prothoracic area surrounding the legs exposed the prothoracic ganglion and neck connectives. We used a sharpened tungsten hook electrode to record extracellular neural activity from one neck connective. An indifferent electrode was inserted into the beetle's abdomen. The activity of the electrode was amplified using an amplifier (AM Systems model 1700) whose output was recorded along with the stimulus envelope on separate channels of a Vetter PCM recorder for later analysis. We measured the neurophysiological tuning by presenting the beetles with five 10 ms pulses at a rate of $1\ s^{-1}$. The output of the electrode was monitored visually on an oscilloscope or aurally on an audio monitor of the amplifier output. For different carrier frequencies, we determined the minimum sound pressure level (to the nearest 1 dB) required to elicit neural activity correlated with three of the five stimulus presentations.

Auditory blockage studies

Preliminary blocking and ablation studies suggested that the thinned areas on the neck membranes were the tympanal organ. First, ablation of the structures increased behavioral thresholds in one beetle by 6–10 dB and decreased the probability of a response by more than 50 % in two other beetles. We used a mixture of Vaseline and oil to coat the cervical membranes of another individual that had previously responded to 10 presentations of ultrasound. After coating the membranes, the beetle responded to only 5 of 10 further presentations. When the mixture was removed from the membranes, the beetle

responded to 10 of 10 stimulus presentations. In addition, beetles rarely responded to high-intensity ultrasound (>80 dB SPL) when their head was tucked into the socket formed by the pronotum. The head is always slightly extended from the socket during flight and while walking.

To show conclusively that these membranes were responsible for sound reception, we used a reversible blocking technique while recording extracellular responses to sound from the neck connective (as above). For each individual (N=6), we determined the neurophysiological threshold for 10 ms pulses having a frequency of 30 kHz. The tympanic membranes were then loaded with small drops of water carefully applied with a syringe so that only the cervical membranes were covered. The threshold was determined as before. Finally, the water was wicked away with a small paper towel and the threshold again determined. In addition, we measured thresholds for two other beetles before and after the entire body, except the neck membranes, had been covered with wax or submerged in water.

Taxonomic distribution of scarab ears

The auditory structures of these scarabs are easily visible under a dissecting microscope. To assess the occurrence of these ears in other scarabs, we examined specimens from the Entomology Museum collection at Cornell University. The following taxonomic groupings are based on Endrödi (1985). We obtained specimens from the following tribes within the subfamily Dynastinae: Cyclocephalini (*Cyclocephala* and *Dyscinetus*), Pentodontini (*Euetheola*, *Oxygrylius*, *Bothynus* and *Aphonus*), Dynastini (*Dynastes*) and Phileurini (*Phileurus*). We also obtained specimens from two other scarab subfamilies, Cetoniinae (*Cotinus*) and Rutelinae (*Pelidnota* and *Popillia*). The Cetoniinae are considered to be closely related to the Dynastinae (Ratcliffe, 1991).

All specimens were relaxed in a closed container with moist paper towels and an anti-fungal agent. Once the beetles were relaxed, we carefully removed the head of each specimen and examined it under a dissecting scope for the presence of auditory structures resembling those of *Euetheola humilis*.

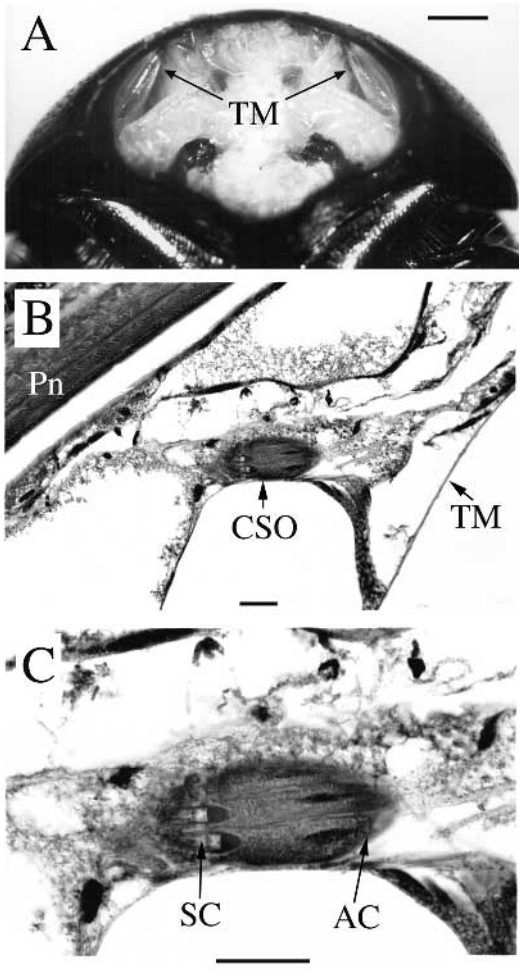

Fig. 1. Auditory organ of the scarab beetle *Euetheola humilis*. (A) Frontal view of the beetle with its head removed showing the location of the paired tympanic membranes (TM) situated dorsally and laterally where the cervical membrane attaches to the pronotum. Scale bar, 1 mm. (B) Transverse sections through the prothorax (Pn, pronotum) showing the chordotonal sensory organ (CSO) associated with the right tympanic membrane (TM). Two scolopidia in the organ are clearly visible. Scale bar, 20 µm. (C) Magnification of B showing the scolopidial cells (SC) and the attachment cells (AC) that connect the scolopidia directly to the tympanic membrane. Scale bar, 20 µm.

Results

Morphological studies

The paired tympanic membranes (TM, Fig. 1A) of *Euetheola humilis* are located in the cervical membrane where it attaches dorsally and laterally to the pronotum. The tympanic membranes are 2–3 µm thick and are backed by a tracheal airsac (Fig. 1B). The thickness of other parts of the cervical membrane is typically more than 5 µm. Histological sections in this area revealed a chordotonal sensory organ (CSO, Fig. 1B) within the tracheal airsac. The sensory organ contains at least three (but fewer than eight) scolopidia (SC, Fig. 1C) that attach directly to the tympanic membrane by accessory cells (AC, Fig. 1C). The attachment of the sensory organ to the tympanic membrane is near the dorsal medial apex of the membrane.

Neurophysiological tuning curve

Extracellular recordings from neck connectives showed a neural correlate with our broadcasts of acoustic stimuli (Fig. 2A). On the basis of recordings of the neural response, the beetles are sensitive to ultrasound and their average thresholds are below 70 dB SPL for frequencies between 20 and 80 kHz (Fig. 2B). The lowest thresholds averaged 58 dB at 45 kHz. For four of the six individuals tested, no neural response was detected for 5 kHz pulses at the highest sound pressure level our system could produce (104 dB SPL). The neurophysiological tuning curve (circles, Fig. 2B) was almost

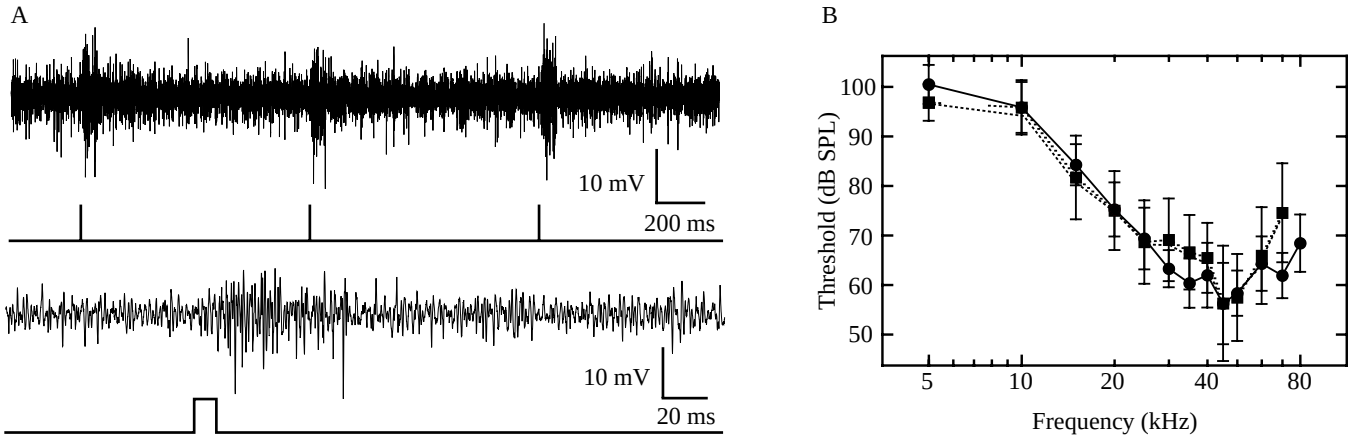

Fig. 2. (A) Correlated response of extracellular recording from neck connective of *Euetheola humilis*. The stimulus is a 10 ms duration 40 kHz pulse presented every 1 s (indicated by the stimulus envelope below each neurophysiological trace). The top trace shows the neural response to three successive pulses at 25 dB above the beetle's threshold. The second trace is the second stimulus presentation in the top trace on an expanded time scale. (B) Neurophysiological (circles and solid lines) and behavioral (squares and dotted lines) audiograms for the scarab beetle *Euetheola humilis*. Behavioral thresholds are the mean (±1 S.D.) minimum sound pressure level (SPL) (dB re 20 μPa) required to elicit an observable head roll (data from Forrest *et al.* 1995) (*N*=11). Stimuli used in the neurophysiological recordings were 10 ms pulses having 1 ms raised-cosine ramps at the onset and offset. Pulses were presented at a repetition rate of 1 s^{-1}. Thresholds are the mean (±1 S.D.) SPL (dB re 20 μPa) required to produce a correlated neural response recorded from the beetle's neck connective in three of five stimulus presentations (*N*=6 individuals).

identical to the behavioral audiogram for these beetles (squares, Fig. 2B from Forrest *et al.* 1995).

Auditory blockage studies

The neurophysiological thresholds before and after the ears had been covered with water were measured. The average thresholds for the beetles prior to treatments to the tympanic membranes were 66±6 dB SPL (mean ± S.D., *N*=6) for 10 ms pulses having a 30 kHz carrier frequency. After loading the tympanic membranes with several drops of water, the thresholds increased by more than 35 dB to 102±4 dB. Removing the water covering the membranes restored thresholds to within 3 dB of pre-treatment threshold (69±5 dB). Pre- and post-treatment thresholds remained within 3 dB for two individuals whose entire body except the neck membranes had been covered with a soft wax. Thus, unimpeded tympanic membranes were necessary and sufficient to provide the neural response.

Taxonomic occurrences of scarab ears

Of the 11 genera for which we examined specimens for tympanal organs (Table 1), we found evidence of tympana in four: *Euetheola*, *Cyclocephala*, *Dyscinetus* and *Oxygrylius*. We tested one of these night-flying scarabs with tympana-like structures for responses to ultrasound. Like *Euetheola humilis*, *Dyscinetus morator* responded behaviorally to presentations of ultrasound. The acoustic startle behavior of *D. morator* was less robust than the stereotypical head roll exhibited by *E. humilis*. Instead, the startle of *D. morator* was a rapid tuck of the head into the neck socket. *Dyscinetus morator* was sensitive to ultrasound and had a behavioral tuning curve similar to that of *E. humilis* (Fig. 3). In addition, in field experiments like those described in Forrest *et al.* (1995), we caught 13 *Cyclocephala lurida* in a trap beneath speakers broadcasting high-intensity ultrasound (40 kHz pulses) with a

Table 1. *Taxonomic distribution of tympanal organs within the dynastine Scarabaeidae*

SUBFAMILY Tribe Genus	Tympanic membranes
DYNASTINAE	
Cyclocephalini	
Cyclocephala	Yes
Dyscinetus	Yes
Pentodontini	
Euetheola	Yes
Oxygrylius	Yes
Bothynus	No
Aphonus	No
Dynastini	
Dynastes	No
Phileurini	
Phileurus	No
Oryctoderini	?
Oryctini	?
Agaocelphalini	?
CETONIINAE	
Cotinus	No
RUTELINAE	
Pelidnota	No
Popillia	No

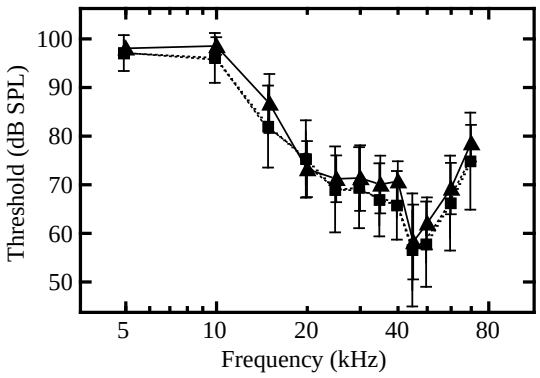

Fig. 3. Behavioral audiogram of the scarab beetles *Dyscinetus morator* (triangles and solid lines) and *Euetheola humilis* (squares and dotted lines from Forrest *et al.* 1995) . The stimulus was a 300–500 ms train of 5 ms duration pulses each with 1 ms linear ramps at the onset and offset. Pulses were presented at a repetition rate of $50\,s^{-1}$. Thresholds are the mean (±1 S.D.) sound pressure level (dB re 20 μPa) required to elicit a noticeable movement of the head in three of five stimulus presentations (N=6 individuals). The behavior of *D. morator* elicited by the ultrasound differs from that of *E. humilis* and is not a head roll. Rather, the beetles tuck their head into the socket formed by the pronotum when presented with an ultrasound stimulus.

low-frequency sound but we caught none in a trap broadcasting only the low-frequency sound. We have also measured neural responses correlated to ultrasound pulses in both *Cyclocephala lurida* and *Dyscinetus morator*.

Discussion

We had previously shown that the scarab beetle *Euetheola humilis* responds to ultrasound in free flight and when tethered (Forrest *et al.* 1995). We have now demonstrated that the auditory structures are paired tympanic membranes typical of insect ears. These structures are necessary and sufficient for a correlated neural response to ultrasound stimuli to be measured.

Frequency tuning

The extracellular neural response of *Euetheola humilis* is multi-unit and complex. For other insects showing an acoustic startle, measurements of neural thresholds at the primary afferent or ascending interneurons are typically 10–20 dB below thresholds measured behaviorally. However, the neural and behavioral audiograms we measured for *E. humilis* are nearly identical and, thus, we suspect that the measured neural response from the neck connective is likely to be a descending response that drives the motor output. We know that there are a small number of auditory afferents, but at present have no data on the numbers of auditory interneurons or their thresholds to acoustic stimuli.

The auditory organs

Euetheola humilis ears are located behind the head in the cervical membranes. In contrast, the ears of tiger beetles are located on the tergum of the first abdominal segment (Spangler, 1988), and thus they represent an independent evolution of the auditory organs.

The sensory cells associated with the tympanic membranes of *E. humilis* contain only 3–8 scolopidia. The small number of cells suggests minimal peripheral processing by the beetles, and the system probably functions as a predator detector that generates an acoustic startle for predator avoidance (Forrest *et al.* 1995). The auditory systems of many insects showing pronounced acoustic startle to ultrasound usually have only a few afferent cells sensitive to high frequencies. For instance, bat detection by notodontid moths is mediated by a single auditory cell, the A cell, in each metathoracic ear (Surlykke, 1984). Noctuid moths have a similar hearing organ with only two A cells, one functions at low intensity and the other at high intensity (Roeder, 1967). Field crickets (*Teleogryllus oceanicus*) have approximately 70 sensory cells in the auditory organ located in each foretibia. Of these 70 receptor cells, only a few, possibly 2–4, are ultrasound-sensitive (Pollack, 1994). In the locusts approximately 10 % of a total of 80 sensory cells are associated with the pyriform vesicle (Gray, 1960), the area of the Müller's organ involved in high-frequency hearing (Michelsen, 1971). The mantis *Tenodera aridifolia* has only 13–16 scolopidia attached to the tympanum (Yager and Hoy, 1986), whereas a much larger mantid species, *Hierodula membranacea*, has 35–40 scolopidia associated with the tympanic membrane (Yager, 1996). The tiger beetle *Cicindela marutha* has between 4 and 20 auditory afferents. In contrast, green lacewings *Chrysopa carnea* have a relatively large number of sensory units, 28–33, associated with the tympanal organ on the forewing (Miller, 1970).

Taxonomic occurrences of scarab ears

The ears of scarabs clearly differ morphologically from those of tiger beetles (Spangler, 1988; Yager and Spangler, 1995), and the two groups are separated taxonomically into different suborders within the Coleoptera. There can be no doubt that these ears represent separate and independent evolutionary events. Tympanal hearing organs are highly specialized structures that have evolved a number of times within the insects (Hoy and Robert, 1996). Therefore, insect auditory structures should be considered to be highly derived characters that may be extremely useful in understanding evolutionary relationships between closely related taxa. It appears that the paired auditory structures of scarabs are found only on species in the Cyclocephalini and Pentodontini, two tribes within the Dynastinae. Interestingly, we also found that some genera within the Pentodontini do not possess these ears (Table 1), suggesting a possible loss of ears or that the group may be polyphyletic.

It is surprising that tympanal hearing has only been found in two distantly related groups of beetles, Scarabaeidae and Cicindelidae. Because many beetles are nocturnal fliers and are thus exposed to an intense risk of predation from echolocating bats, we believe that other ultrasound-sensitive beetles are likely to be discovered in the near future.

We wish to thank the Hoy laboratory for helpful comments and criticism during this work. We thank Paul Lago at the University of Mississippi for identifying the beetles and for shipping a number of beetles to our laboratory. Brett Ratcliffe was very generous with information about the current systematics of dynastine scarab beetles. We also thank Rick Hoebeke for help in providing specimens from the Entomology Museum at Cornell University. Voucher specimens of scarab beetles examined for ears are deposited under lot 1226 at the Cornell Entomology Museum. Funding for this work came from NIH grant RO1 DC00103 to R.R.H. H.E.F. was also supported from USDA-ARS cooperative agreement grant 58-6408-104 to the National Center for Physical Acoustics.

References

BENNET-CLARK, H. C. (1983). Insect hearing: acoustics and transduction. In *Insect Communication* (ed. T. Lewis), pp. 49–82. London: Academic Press.

ENDRÖDI, S. (1985). *The Dynastinae of the World*. Dordrecht: Dr W. Junk Publishers.

FARRIS, H. E. (1994). Risk assessment by insects: behavioral response to acoustic stimuli. MS thesis. University of Mississippi, MS, USA.

FORREST, T. G., FARRIS, H. E. AND HOY, R. R. (1995). Ultrasound acoustic startle response in scarab beetles. *J. exp. Biol.* **198**, 2593–2598.

GRAY, E. G. (1960). The fine structure of the insect ear. *Phil. Trans. R. Soc. B* **243**, 75–94.

HOY, R. R. AND ROBERT, D. (1996). Tympanal hearing in insects. *A. Rev. Ent.* **41**, 433–450.

HUTCHINGS, M. AND LEWIS, B. (1983). Insect sound and vibration receptors. In *Bioacoustics: A Comparative Approach* (ed. B. Lewis), pp. 181–205. London: Academic Press.

MICHELSEN, A. (1971). The physiology of the locust ear. I. Frequency sensitivity of single cells in the isolated ear. *Z. vergl. Physiol.* **71**, 49–62.

MILLER, L. A. (1970). Structure of the green lacewing tympanal organ (*Chrysopa carnea*, Neuroptera). *J. Morph.* **131**, 359–382.

POLLACK, G. S. (1994). Synaptic inputs to the omega neuron of the cricket *Teleogryllus oceanicus*: differences in EPSP waveforms evoke by low and high sound frequencies. *J. comp. Physiol.* A **174**, 83–89.

RATCLIFFE, B. C. (1991). The scarab beetles of Nebraska. *Bull. Univ. Nebraska State Mus.* **12**, 1–333.

ROEDER, K. D. (1967). *Nerve Cells and Insect Behavior*. Cambridge: Harvard University.

SPANGLER, H. G. (1988). Hearing in tiger beetles (Cicindelidae). *Physiol. Ent.* **13**, 447–452.

SURLYKKE, A. (1984). Hearing in notodontid moths: a tympanic organ with a single auditory neurone. *J. exp. Biol.* **113**, 323–335.

YACK, J. E. AND FULLARD, J. H. (1993). What is an insect ear? *Ann. ent. Soc. Am.* **86**, 677–682.

YAGER, D. D. (1996). Nymphal development of the auditory system in the praying mantis *Hierodula membranacea* Burmeister (Dictyoptera, Mantidae). *J. comp. Neurol.* **364**, 199–210.

YAGER, D. D. AND HOY, R. R. (1986). The cyclopean ear: a new sense for the praying mantis. *Science* **231**, 727–729.

YAGER, D. D. AND SPANGLER, H. G. (1995). Characterization of auditory afferents in the tiger beetle, *Cicindela marutha* Dow. *J. comp. Physiol.* A **176**, 587–599.