

## BEHAVIORAL RESPONSE TO ULTRASOUND BY THE TIGER BEETLE *CICINDELA MARUTHA* DOW COMBINES AERODYNAMIC CHANGES AND SOUND PRODUCTION

DAVID D. YAGER<sup>1,\*</sup> AND HAYWARD G. SPANGLER<sup>2</sup>

<sup>1</sup>*Department of Psychology, University of Maryland, College Park, MD 20742-4411, USA and*  
<sup>2</sup>*US Department of Agriculture, Agriculture Research Service, Carl Hayden Bee Research Center,*  
*2000 East Allen Road, Tucson, AZ 85719, USA*

*Accepted 15 November 1996*

### Summary

Tethered flying tiger beetles, *Cicindela marutha*, respond to trains of bat-like ultrasonic pulses with a short-latency, multi-component behavior. The head rolls to one side, the metathoracic legs kick to the opposite side, the elytra swing backwards towards the hindwings and pronate, the hindwings increase their stroke excursion and frequency, and the plane of the hindwing motion tilts forward. In addition, the beetles produce trains of ultrasonic clicks typically containing 100–200 clicks in response to a 1 s stimulus. The clicks average 85–90 dB SPL at 2 cm. The latencies for hindwing changes and elytra swing in response to stimuli more than 10 dB over threshold are 90–110 ms; the latency to clicking is 120–150 ms. Neither the head roll nor the leg kick appears to be directional

relative to the sound source. The behavioral response is broadly tuned with greatest sensitivity at 30–60 kHz and mean behavioral thresholds of 75–80 dB SPL. Physiological audiograms from the auditory afferents show substantially greater sensitivity and sharper tuning than the behavioral response, which suggests that tiger beetles may use their hearing in other contexts as well as during flight. The combination of aerodynamic components and arctiid-moth-like clicking may provide these insects with a powerful defense against attack by echolocating bats.

Key words: insect hearing, Cicindelidae, tiger beetle, *Cicindela marutha*, bat evasion, ultrasound, startle response, defensive behavior.

### Introduction

The broad range of insects with ultrasonic hearing in the range 30–60 kHz attests to the ecological pressure exerted on them by echolocating, insectivorous bats (reviewed by Hoy *et al.* 1989). While some of these insects also use their hearing for intraspecific communication, its use in bat evasion has been substantiated in many species by establishing that bat-like trains of ultrasonic pulses trigger specific behaviors in flying animals that are unique to that behavioral context. In two cases (moths and mantises), behavioral observations in the field have demonstrated the efficacy of the insects' ultrasound-triggered responses in evading capture by hunting bats (Roeder, 1967; Yager *et al.* 1990).

The ultrasound-triggered behaviors vary both in complexity and in type, although most involve wingbeat pattern or rate changes. Green lacewings *Chrysopa carnea* (Miller and Olesen, 1979) and tettigoniids *Neoconocephalus ensiger* (Libersat and Hoy, 1991) respond by closing their wings and dropping, locusts *Locusta migratoria* (Robert, 1989) and crickets *Teleogryllus oceanicus* (Moiseff *et al.* 1978) employ specific steering motions, and mantises *Parasphendale*

*agrionina* extend their forelegs and dorsiflex the abdomen (Yager and May, 1990). Among the moths, the noctuids vary their response according to stimulus intensity (=bat range), turning away from weak signals and diving erratically from more imminent danger (Roeder, 1967). The arctiids answer the threat with a unique strategy: flight path changes are much less common than in other moths, but they consistently produce trains of ultrasonic clicks that are very effective in averting the bat's attack (Dunning and Roeder, 1965; Dunning *et al.* 1992; Acharya and Fenton, 1992). Startle, disruption of the bat's echolocation system and advertisement of the moth's distastefulness are mechanisms that singly, or in combination, may explain how the clicks protect the moths (Fullard *et al.* 1994).

Behavioral and physiological experiments have recently established that some tiger beetles (Cicindelidae) hear using two ears located on the dorsal surface of the first abdominal segment (Spangler, 1988a; Yager and Spangler, 1995). Afferent nerve recordings show that the auditory system has a sensitivity of 50–60 dB SPL in its most sensitive frequency

\*e-mail: dy5@umail.umd.edu.

range of 25–35 kHz. The function of hearing in these beetles is not yet known. Since they produce sounds with a peak frequency of 30–35 kHz in non-defensive contexts, intraspecific communication is a possibility (Freitag and Lee, 1972; Pearson, 1988). However, an alternative hypothesis is also viable: tiger beetles fly at night, as shown by frequent black-light captures of many species (Larochelle, 1977; Pearson, 1988) and coexist with many species of insectivorous bats (Hoffmeister, 1986), so their auditory system may help them avoid capture by bats. These two hypotheses are not mutually exclusive.

The present study lends support to the hypothesis of ultrasound-mediated bat evasion by tiger beetles by demonstrating a complex behavioral response to pulsed ultrasound that occurs only during flight. Furthermore, the behavioral response is unique in that it combines both wing motion changes (presumably altering the flight path) and the production of loud ultrasonic clicks.

## Materials and methods

### *Animals*

We collected adult male and female *Cicindela marutha* Dow (Coleoptera: Cicindelidae) near Willcox Playa, approximately 137 km east of Tucson, AZ, USA, during late July and August. We conducted experiments both in Tucson and in College Park. Some beetles were held in the laboratory at 24–28 °C for up to 4 weeks in sand-filled aquaria containing puddles of standing water; day length was 12 h. They were fed small crickets, flies and insect parts several times a week.

### *Stimulation procedures*

Experiments were carried out either in an acoustic isolation chamber (Industrial Acoustics Corp.) or at the volume center of a 1 m×1 m×2 m box lined with acoustic foam to minimize echoes. We suspended each beetle from a thin wire affixed to its pronotum with a small drop of wax. For experiments in the anechoic box, the position of the beetle was standardized at 0.7 m from speakers (Technics EAS10TH400B) at either end of the box. In the acoustic chamber, stimuli were broadcast from Realistic 40-1375 leaf tweeters 0.4–0.5 m away from the beetle or from a custom-built bat simulator (40 kHz, 10 ms pulses at 10 s<sup>-1</sup>) 0.15 m away from the beetle, depending on the experiment.

Stimuli were trains of 10 ms trapezoidal sound pulses (0.5–3.0 ms rise/fall times for different experiments; 10 or 20 ms interpulse interval) produced by standard electronics. The rise/fall times were adequate to eliminate onset and offset transients from the speaker in all cases. Sound pressure level (SPL) was calibrated from 1 to 100 kHz using a Brüel & Kjaer 2230 SPL meter and 4135, 6.25 mm microphone (grid off). Sound pulses used for calibration were longer than 100 ms to allow adequate meter response time. The calibration system frequency response was flat to within 1 dB from 1 to 70 kHz; appropriate corrections were included in the calibration procedure to compensate for a roll-off above 70 kHz. Stimulus

SPLs are reported here as dB (RMS) re 20 µPa (dB SPL). Our calibration confirmed attenuator accuracy over the range used and speaker linearity up to 95 dB for frequencies ≤80 kHz. Harmonics with the greatest energy (usually the second) were more than 35 dB (most were more than 45 dB) below the fundamental at all frequencies (Stanford Research Systems SR760 FFT analyzer).

### *Data collection*

Tethered beetles initiated flight either spontaneously or in response to puffs of wind. Sustained flight did not require continuous wind, and we did not use a fan during data collection. The data are from more than 50 beetles. Temperatures during testing were 22–26 °C.

We monitored the sound produced during normal flight and after an ultrasonic stimulus using a Brüel & Kjaer 4135 6.25 mm microphone with the Brüel & Kjaer 2230 SPL meter as an amplifier. Unless otherwise noted, the microphone pointed at the wings from 2 cm behind the animal and 30–45 ° above the horizontal. A consistent pressure wave is produced during each cycle of wing motion, so we also used this arrangement to measure wingbeat frequency (we confirmed these measurements using the shadow cast on a photocell by the hindwing as it broke a laser beam). For spectral analysis, the signal was stored on a Racal IV-D tape recorder running at 38 cm s<sup>-1</sup> (recording system frequency response was flat to within 2 dB up to 100 kHz). For experiments not requiring acoustic analysis (presence or absence of clicks, measurement of wingbeat frequency, etc.), we stored the data on either a Marantz PM-455 cassette recorder (±2 dB to 16 kHz) or a BioLogic digital instrumentation tape recorder (±1 dB to 20 kHz).

For click SPL measurements, we recorded 30 kHz reference tones of known SPLs between 65 and 95 dB SPL on the tape (Racal recorder) before recording ultrasound-evoked clicks on the same tape at identical gain. Click SPLs were then derived by comparison of click *versus* reference tone output voltages on an oscilloscope.

We used photographic techniques to assess changes in wing position and movement pattern after ultrasonic stimulation (tripod-mounted Nikon N90 camera with Nikkor 105 mm macro lens; Kodak TMAX 100 film). By choosing an appropriate shutter speed, we could either freeze motion (shutter speed faster than 0.01 s) or obtain an 'average' position over approximately 25 wingbeat cycles (shutter speed 0.5 s). Four different photographic series optimized viewing angle for measurement of specific aspects of elytra or hindwing change. Stimuli were 500 ms trains of 10 pulses at 30 kHz and 15 dB over threshold. Data were collected and analyzed in a paired design using photographs immediately before and after each stimulus.

We established the latency from stimulus onset to first elytral motion by first aiming a 0.5 mm laser beam from above so that it struck the extreme leading edge of the opaque elytrum about half-way along its length when the beetle was in stable flight. As the elytrum moved backwards after stimulation, it

exposed a photocell positioned below the animal to the laser beam. Voltage output of the photocell and a stimulus monitor were stored on separate channels of the digital tape recorder.

Stimuli used to determine the tuning for components of the ultrasound-triggered response were 300 ms pulse trains (10 ms pulses; 10 ms interpulse interval) with intertrain intervals of greater than 5 s to minimize habituation. Frequencies were presented in a semi-random order so that adjacent frequencies did not occur in series. Threshold was defined as the lowest SPL eliciting the response in at least two out of three trials.

#### Analyses

We used a Kay Elemetrics DSP Sona-Graph 5500 in its 32 kHz range for analysis of the beetle-produced clicks. The signals on the data tapes were reproduced at one-quarter of the recording speed on the Racal tape recorder, allowing analysis to cover the entire frequency range of the recording.

Analyses of temporal patterns and latency utilized data acquisition and analysis software, SuperScope II (GW Instruments, Somerville, MA, USA), running on a Power Macintosh computer (Apple). Analog-to-digital conversion (MacAdios II board; GW Instruments) used sampling periods of 10  $\mu$ s or above, depending on the analysis.

We obtained angular measurements of the elytra and hindwing movements from the four series of photographs by first scanning the contact sheets of each roll of film into the Power Macintosh computer (La Cie Silverscan scanner controlled by ColorIt! 3.0 software). Image enhancement and analysis tools were provided by NIH Image software. In some cases, measurements were made on images projected from negatives by a photographic enlarger. We estimate that the overall resolution of the analysis was  $\pm 1^\circ$ . We define  $0^\circ$  in the horizontal plane as directly in front and  $0^\circ$  in the vertical plane as directly above the animal. We used circular statistics for computations dealing with angular measurements and a non-parametric test for angular comparisons (sign test) to avoid assumptions about the underlying data distribution (Batschelet, 1981).

For determining mean tuning curves, data in decibels were converted to a linear scale (pressure) prior to statistical manipulation and the results were then converted back to decibels for reporting. This explains the unequal lengths of the standard deviation bars above and below data points on the figures.

Averages are expressed in the text as mean  $\pm$  standard error (S.E.M.) for linear data and mean  $\pm$  angular standard deviation (S.D.) for angle data unless otherwise noted. The significance level for all statistical tests is 0.05.

## Results

### Normal flight

*C. marutha* flies strongly on a tether without any wind stream, and many individuals only showed obvious signs of fatigue after 30–45 min of continuous flight. Under our conditions, females flew more reliably than males, but we

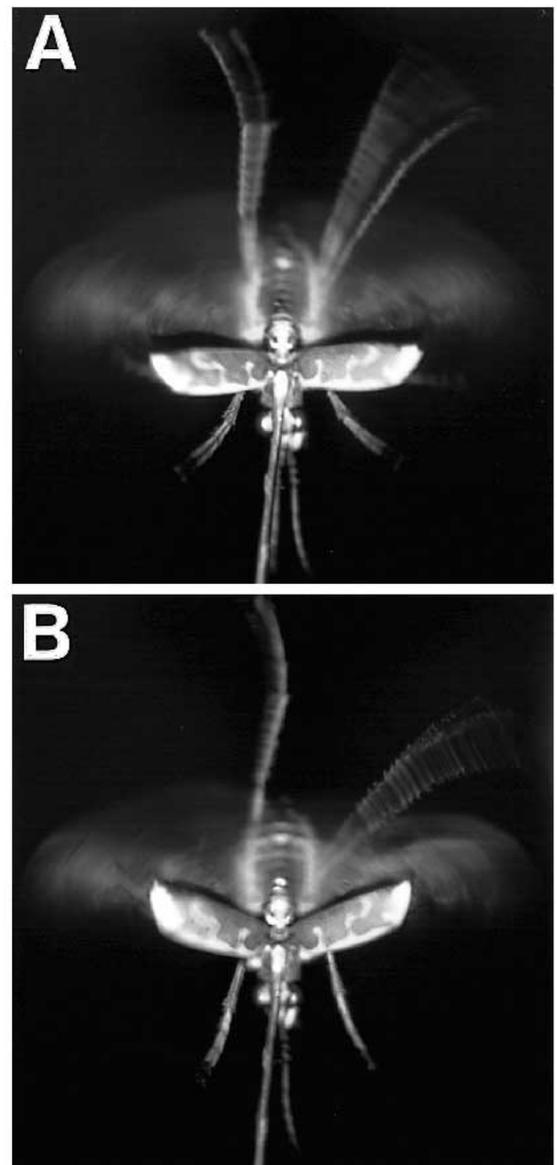


Fig. 1. Control-test pair of photographs viewing the flying tiger beetle from directly above. Shutter speed was 0.5 s. (A) Normal, stable flight. The elytra are held at approximately  $90^\circ$  to the long axis of the body, the hindlegs trail straight behind, the forelegs are partly extended ahead of the animal. The tether enters the picture from the bottom center and is attached to the pronotum. Body length is approximately 12 mm. (B) The same beetle immediately after a stimulus presentation from its right. This frame was taken less than 5 s after A. The elytra have swung backwards, there is a moderate leg kick away from the ultrasound source and a slight head roll. The abdomen appears broader and shorter because of its dorsiflexion and forward compression. The change in hindwing stroke plane can be seen by comparing the position of the top edge of the hindwings relative to the tip of the abdomen in the two photographs.

detected no other behavioral differences between the sexes in our tests. We noted a consistent time-of-day effect: the beetles flew most readily in the morning and evening and were reluctant to fly during mid-afternoon.

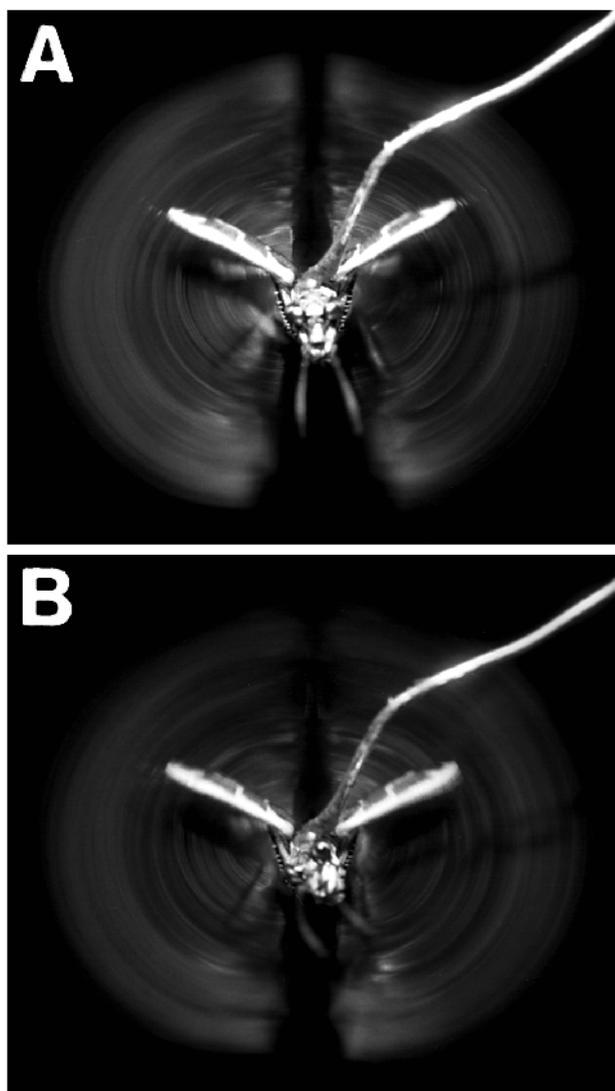


Fig. 2. Control-test pair of photographs viewing the flying tiger beetle from directly in front. Shutter speed was 0.5 s. (A) Before the stimulus, the head is straight. The hindwing excursion extends between  $90^\circ$  at the top of the stroke and approximately  $160^\circ$  at the bottom. The mesothoracic tarsi are faintly visible in their normal position protruding laterally from under the tips of the elytra. (B) The same beetle immediately after a stimulus presentation from its right. This frame was taken less than 5 s after A. The top of the head is rolled towards the stimulus. Hindwing excursion has increased at both the top and bottom of the stroke. No movement of the elytra is evident in this trial. Note that there is significantly less light reflected from the hindwings than in the control. This consistent effect is due to the change in stroke plane angle of the hindwings.

Tiger beetles do not flap their elytra. The only movement of these thickened forewings is a slight vibration arising from the action of the hindwings transmitted through the thorax. In normal, stable flight, the elytra are held symmetrically to the sides at  $97 \pm 7^\circ$  (with  $0^\circ$  to the front of the animal; 254 measurements on 10 beetles) relative to the long axis of the body (e.g. Fig. 1A). They are raised above the horizontal by

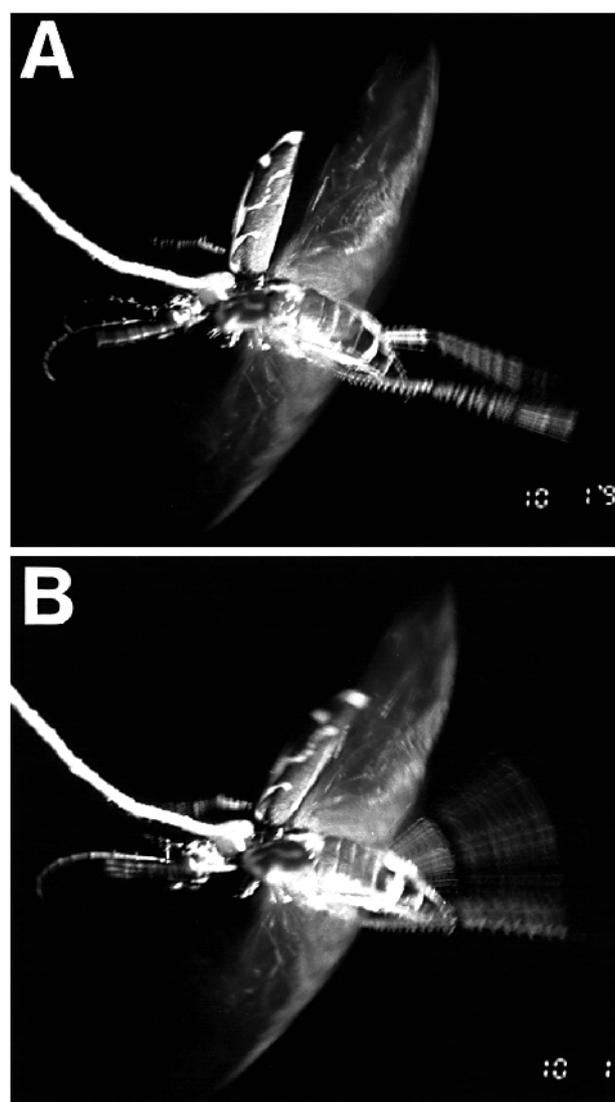


Fig. 3. Control-test pair of photographs viewing the flying tiger beetle from its left side (also slightly above and behind). Shutter speed was 0.25 s. (A) Before stimulation. Note the gap between the elytra and the hindwings. Both antennae are visible pointing straight ahead of the animal. (B) The same beetle immediately after a stimulus presentation from its left. This frame was taken less than 5 s after A. The elytra have swung back so that no gap is visible between them and the hindwings. There is both a head roll and a leg kick. The abdomen and the plane of the hindwings are tilted slightly forward. The dorsiflexion of the abdomen can be seen by comparing the apparent width of the rostral abdominal segments in the two photographs.

$21 \pm 6^\circ$  (226 measurements on 10 beetles), forming a dihedral (a shallow 'V' shape; Fig. 2A).

The hindwings flap at a mean of  $45.9 \pm 0.4$  strokes  $s^{-1}$  (Hz; 156 measurements from five beetles) in stable, tethered flight at  $22^\circ\text{C}$ . Eleven beetles flying under the same conditions, but at a higher temperature ( $26^\circ\text{C}$ ) had an average wingbeat frequency (WBF) of  $52.8 \pm 0.9$  Hz. Among all beetles, the range of WBFs was from 34 Hz to a maximum of 57 Hz. The WBF

for an individual beetle during a testing period was not constant, but varied by 2–3 Hz around the mean.

The hindwing excursion in stable, tethered flight is  $167 \pm 9^\circ$  (see Fig. 2; 226 measurements on 10 beetles). The two wings almost touch at the top of the stroke ( $90^\circ$  to the horizontal); the bottom of the stroke is  $77^\circ$  below the horizontal. The hindwings do not beat vertically (see Fig. 3). The average stroke plane angle shown by the 0.5 s photographic exposures of beetles viewed from the side is  $118 \pm 8^\circ$  (53 measurements on five beetles) relative to a line through the head and thorax; the top of the stroke is further caudal than the bottom. These photographs do not allow distinction between the angles of the downstroke and the upstroke.

During stable flight, the tiger beetle's head is not tilted to either side (Fig. 2). The abdomen droops somewhat below the line defined by the head and thorax (Fig. 3). The prothoracic legs are partly extended to the front, and the metathoracic legs trail straight behind the animal (Figs 1, 3). The mesothoracic legs are also fully extended and kept tight against the lower surface of the elytra (possibly held in a groove); the tarsi extend past the elytral tips (Fig. 1).

#### Non-acoustic response to ultrasound

##### Basic description

Flying *C. marutha* react to ultrasound with a multiple-component behavior (Figs 1–3). Within 150 ms, the beetle (1) rolls its head to the side, (2) deflects one or both metathoracic legs to the side, (3) swings the elytra backwards and pronates them, and (4) increases hindwing stroke frequency and excursion, and changes the stroke plane angle. It often also contracts the dorsal abdominal muscles, causing the abdomen to compress forward slightly. In some cases, the prothoracic legs also swing to the side. The response is context-specific: it occurs only during flight. In fact, we did not observe any reaction to ultrasound by beetles walking or standing on the substratum in more than 10 trials.

##### Elytra movement

During stable flight, the beetles hold the elytra approximately at right angles to the body ( $97^\circ$ ). The rapid posterior swing of the elytra triggered by ultrasound (Figs 1, 3) moves them to an average angle of  $103 \pm 7^\circ$ , a statistically significant change (sign test; 10 animals). The maximum swing we observed was  $23^\circ$ , and swings of  $10$ – $20^\circ$  were frequent. It is not uncommon, especially soon after flight onset, for the beetles to swing the elytra all the way back, closing them and halting flight. The beetles also often lower the elytra slightly so that the dihedral viewed from the front becomes shallower (Fig. 2). The mean dihedral angular change, however, is small (from  $21 \pm 6^\circ$  to  $17 \pm 7^\circ$ ) and is not statistically significant. The shallower dihedral may actually reflect pronation in some cases rather than, or in addition to, a change in elytra position. In many trials, ultrasound triggered a clearly visible tilting of the elytra so that the leading edge dropped towards the horizontal. While the pronation appeared to be symmetrical, our photographs do

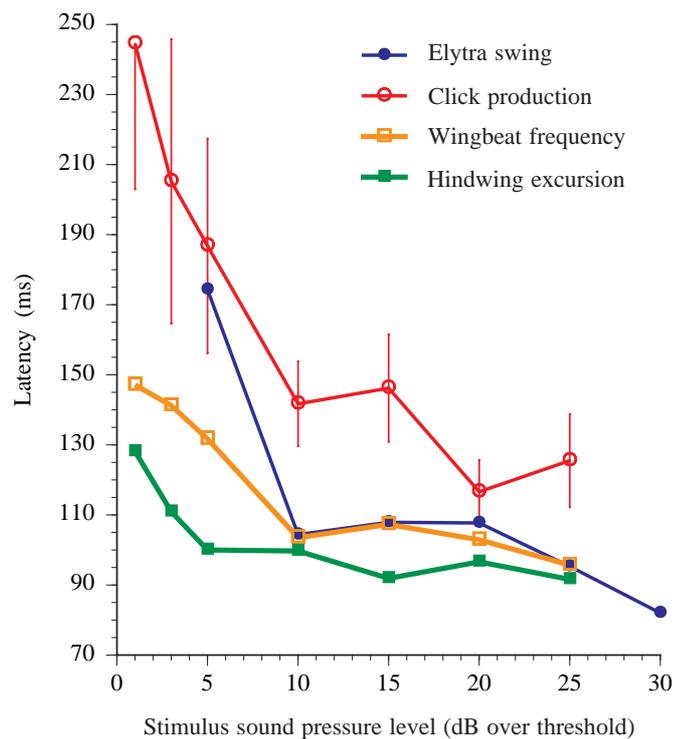


Fig. 4. Change in latency with increasing stimulus sound pressure level (SPL) for four components of the tiger beetle's ultrasound-triggered response. Standard error bars are shown for click production; the variability is comparable for the other curves. Each point is the mean from six animals (5–12 trials at each SPL) for elytra swing and five animals (6–11 trials at each SPL) for the other behaviors.

not allow us to make the angular measurements necessary to confirm this.

As shown in Fig. 4, latencies to the first movement of the elytra are less than 110 ms for SPLs of 10 dB over threshold or higher. The shortest mean latency we measured in any individual beetle was 78.7 ms at 25 dB over threshold. The latency rises very steeply at lower SPLs; the one measurement obtained at 2 dB over threshold (data not shown) was longer than 300 ms.

The elytra swing component of the behavior is broadly tuned (Fig. 5). Greatest sensitivity is at 30 kHz, but the response is only slightly less sensitive at 60 kHz. The lowest individual threshold value we recorded was 68 dB SPL at 30–35 kHz. The sensitivity drops off very sharply below 20 kHz.

##### Hindwing changes

The hindwing component of the ultrasound-triggered behavior is itself complex. Wingbeat frequency increases in all cases, and the duration of the increase usually exceeds that of the stimulus (e.g. Fig. 6C), although the response duration can be shorter than the stimulus duration for SPLs just above threshold. The magnitude of the WBF increase depends on the WBF just before the stimulus so that the beetle always responds to an ultrasonic stimulus with a maximum WBF in

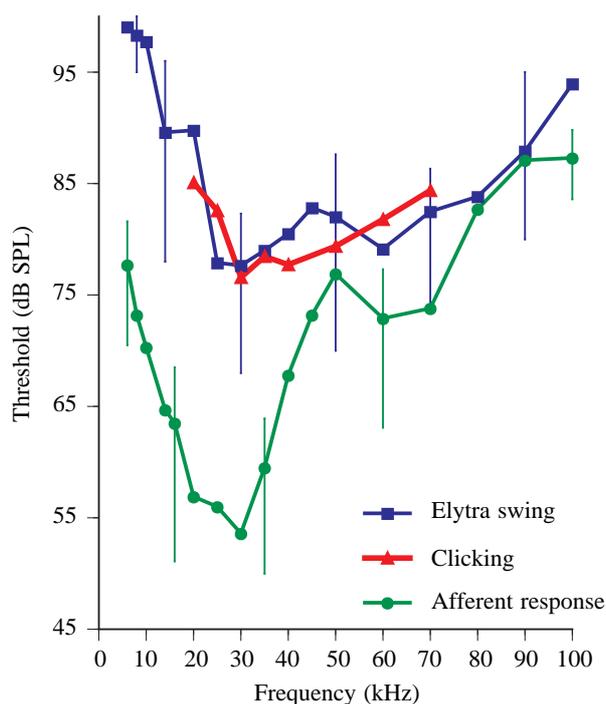


Fig. 5. Mean tuning curves for the elytra swing ( $N=9$ ) and click production ( $N=6$ ) compared with the corresponding data recorded from the auditory afferents of *Cicindela marutha* ( $N=13$ ; from Yager and Spangler, 1995). Standard deviation bars are shown for the afferent response and elytra swing; those for click production are comparable.

the range 50–60 Hz (Fig. 7). For most of our beetles, the increase was 6–10% (3–5 Hz). Considering only pre-test WBFs of 43 Hz or greater, the slope of the linear regression is significantly different from zero ( $F$ -test; d.f.=47) for both the percentage increase (1.44% decrease per Hz increase in pre-test WBF;  $r^2=0.294$ ) and the maximum WBF (0.46 Hz increase in maximum WBF per Hz increase in start WBF;  $r^2=0.172$ ). We found no relationship between the SPL of the stimulus and the magnitude of the WBF change. In some cases, the increase in WBF was preceded by a decrease lasting for 2–3 cycles.

Hindwing stroke excursion also increases in response to ultrasound (Fig. 2). The control angle of  $167\pm 9^\circ$  measured from the front-view photographs changes to  $172\pm 8^\circ$  after stimulation, a statistically significant difference (sign test; 10 animals). As for WBF, the magnitude of the change depends on the pre-test condition: beetles with smaller initial excursion angles showed greater increases so that the response angle was always close to  $180^\circ$ . Even though the hindwings normally reach  $90^\circ$  to the horizontal at the top of the stroke, we saw the increased excursion reflected there as well. Observations using a stroboscopic light suggested that the wingtips may actually touch at the top of the stroke following a stimulus. The hindwing excursion increase substantially outlasts the stimulus (Fig. 6A).

The stroke plane angle of the hindwing relative to the body after stimulation is difficult to measure with reasonable

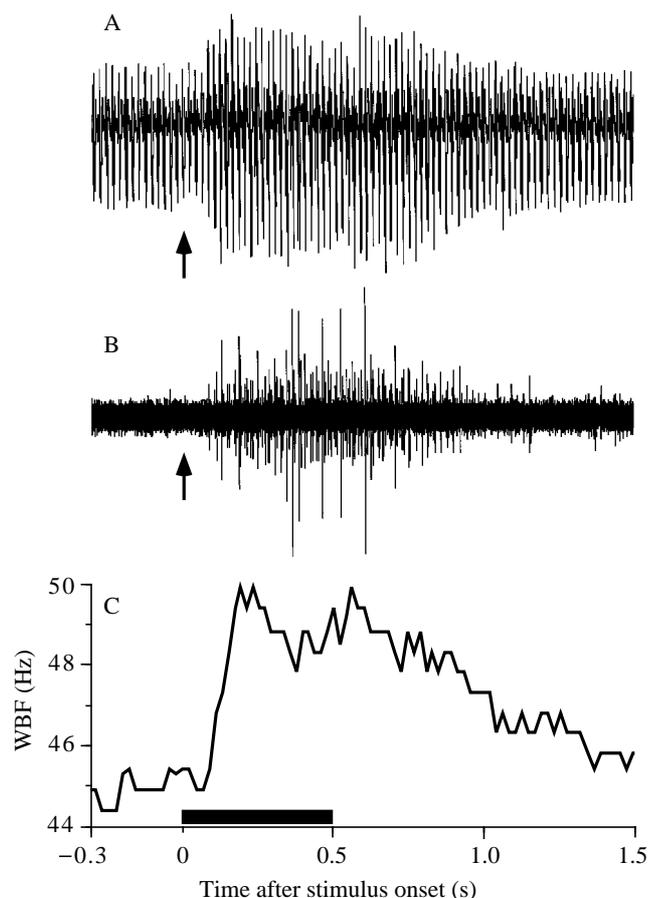


Fig. 6. Three components of the behavioral response to the same 0.5 s stimulus train (15 dB above threshold) shown aligned with the time axis of C. A microphone 2 cm behind and slightly above the flying beetle recorded the sound produced during flight. The arrows in A and B indicate the stimulus onset, and the bar in C shows the stimulus time. (A) The sound recording was low-pass filtered below 5 kHz to show changes in the pressure wave caused by hindwing movement. The increased amplitude following the stimulus onset reflects increased excursion and possibly also changes in stroke plane angle. (B) The recording was high-pass filtered above 10 kHz to show click production. The clicks are substantially attenuated because the frequency response of the recorder did not extend much above 20 kHz. (C) Wingbeat frequency (WBF) calculated for each cycle. The increase following the stimulus onset in this example is approximately 12%.

precision in our 0.5 s exposure, side-view photographs because the anterior wing borders become blurred compared with the pre-test exposure. Observations from the photographs suggest that the tops of the wings are further forward after stimulation (see Fig. 3). However, the actual angular difference measured is less than  $2^\circ$ , which is not a statistically significant change (sign test; five animals). We argue, nevertheless, that a change in the hindwing path is a real component of the overall behavior on the basis of two observations: (1) our front-view photographs reinforce the suggestion of a change in the stroke plane of the hindwings (the overall reflection of light from the hindwings differs before and after the stimulus) (Fig. 2); (2) in

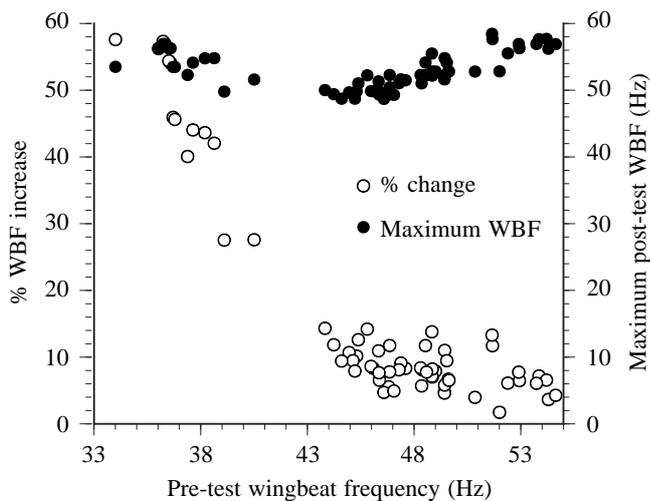


Fig. 7. The magnitude of the increase in wingbeat frequency (WBF) after an ultrasonic stimulus depends on the WBF immediately before the stimulus, whereas the maximum WBF after the stimulus does not. Pre-test WBF is the average of the 20 cycles just before the stimulus. There are 65 data points from five animals in each series. Stimuli were more than 15 dB over threshold.

strongly responding animals, the entire posterior half of the animal appears to tilt forwards in response to an ultrasound stimulus, possibly the combined visual effect created by a change in the stroke plane angle of the hindwings and contraction of the dorsal abdominal musculature (Fig. 3).

The pattern of latency change with increasing SPL is very similar for the changes in WBF and hindwing excursion after stimulation (Fig. 4). Minimum mean latencies for individual beetles were 75 and 79 ms for excursion and WBF, respectively. In contrast with the elytra swing, however, hindwing changes show relatively smaller latency increases as SPL decreases towards threshold.

#### Directionality

We find no convincing evidence that the ultrasound-triggered response is consistently oriented relative to the sound source. We tested 10 beetles by presenting 30 kHz stimuli at 90 dB SPL alternately from 90° to the left and right. Each of the 20 trials for each beetle was followed by a disruptive stimulus (wind puff or light flash) from straight ahead that returned the animal to a symmetrical flight posture. Only three of the animals showed a statistically significant bias (binomial probability) in the direction of the head roll; the top of the head turned towards the stimulus more often than away from it. Pooling the data from the 10 beetles yields a small, but significant ( $\chi^2$ ), difference: towards the stimulus in 115 trials and away in 86. Five of the animals showed a significant bias (binomial probability) in the direction of the leg kick, but two kicked mostly towards the stimulus and three away from the stimulus. The pooled data indicate no preferred direction ( $\chi^2$ ) for the leg kick.

While not consistently directional relative to the sound

source, the response is clearly lateralized. For instance, the legs almost always (171 out of 180 trials) kick in the direction opposite to the head roll. It is especially easy to appreciate the lateralization when observing reactions to longer (0.3–2 s) ultrasonic stimuli at 95–105 dB: after an initial strong response, many beetles ‘waggle’ – the head, legs and wings appear to swing from side to side in a coordinated manner.

#### Acoustic response to ultrasound

The behavioral response of flying *C. marutha* to ultrasonic pulses also includes a prominent acoustic component. The beetles produce trains of loud, ultrasonic clicks (Fig. 8A).

The individual clicks produced by the beetles are very short (Fig. 8C). The major portion of the click is over within 150  $\mu$ s; a low-amplitude tail lasts for an additional 100–150  $\mu$ s. The spectral energy is distributed over 10–70 kHz, with a broad peak at 30–40 kHz (Fig. 8B). There is sufficient energy below 15 kHz for the clicks to be faintly audible to the human ear. Normal wingbeat sounds have virtually all of their energy below 8 kHz and do not include click-like components.

The average maximum SPL of the clicks from five beetles (9–16 trials each) was 89.6 dB SPL (peak) (+4.3 dB, –8.4 dB, s.d.) with a range of 81–95 dB SPL; the microphone was pointed at the animal from 2 cm behind and approximately 30° above the horizontal. The wingbeat sound measured just before each stimulus averaged 69.4 dB SPL (peak) (+2.3 dB, –3.2 dB, s.d.). In four beetles, we compared the maximum SPL of the clicks recorded from behind and from a symmetrical position ahead of the animal. In all cases, the clicks were louder from the front (*t*-tests; d.f.=19–49). The average difference was 5.2 dB.

Using 1 s trains of 10 ms pulses (20 ms period) at 30 kHz and 90 dB SPL, we assessed click production in seven beetles. Each stimulus train elicited an average of  $78.9 \pm 8.6$  clicks ( $N=52$ ). Weakly responding beetles produced fewer than 20 clicks per stimulus, whereas strong responders routinely produced 150–190. Clicking greatly outlasted the stimulus in all cases (Figs 6B, 8A).

Both the latency to the first click and the number of clicks produced varies with SPL. As for the elytra swing, the latency decreases very rapidly as SPL increases above threshold (Fig. 4). The shortest mean latency we measured for an individual beetle was 98.1 ms at 20 dB over threshold. The number of clicks per 500 ms stimulus train follows a similar (but inverse) pattern (Fig. 9). The clear plateau suggests a dynamic range of approximately 20 dB.

The behavioral tuning curve for click production matches the curve for elytra swing very closely (Fig. 5). The lowest individual threshold value we observed was 65 dB SPL at 30 kHz.

Click production is clearly linked to the wingbeat cycle. As Fig. 10 shows, the clicks occur at the wingbeat frequency. The primary, high-amplitude click occurs just before the wingbeat waveform in every cycle. (The wingbeat waveform is a pressure wave lasting 5 ms or less that was recorded by the microphone once each wingbeat cycle whenever the wings are

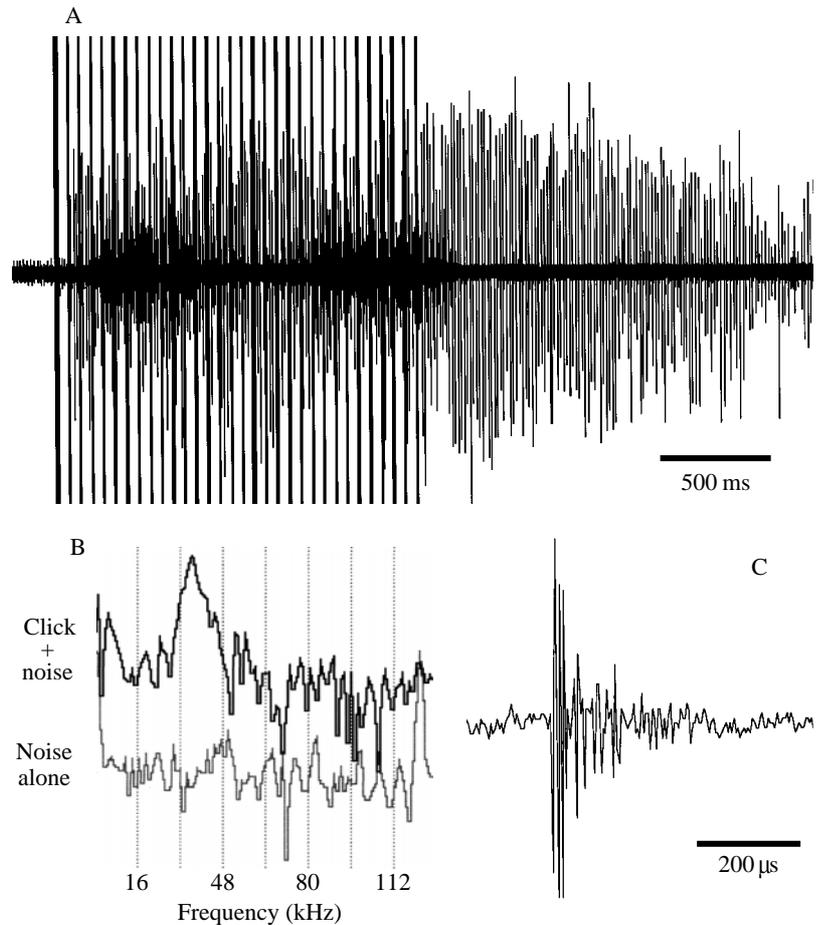


Fig. 8. (A) A train of 10ms, 40kHz stimulus pulses (thick vertical bars; clipped in this oscillogram) triggers a prolonged burst of ultrasonic clicking by a flying tiger beetle. The loudest clicks are approximately 90 dB SPL measured at 2 cm. (B) Power spectrum of a single tiger beetle click. The lower trace is the spectrum of 200 μs of noise immediately before the click. The upper trace is the spectrum of 200 μs of noise including the click plotted on the same scale. (C) Oscillogram of a single tiger beetle click.

flapping. We do not know its exact relationship to wing position.) When a smaller secondary click is present (as in three cycles in Fig. 10), it also has a constant temporal relationship to the wingbeat waveform. The second click typically occurred 4–6 ms after the first. For 2301 cycles, seven

beetles produced an average of 1.78 clicks per cycle. While the majority of cycles had one or two clicks, 21.2% of the cycles had three or more clicks (maximum of six; generally low in amplitude compared with the primary click). High click densities were limited to strongly responsive animals and to the portion of the click train during the stimulus in moderate responders. The ‘extra’ clicks were not scattered randomly throughout the wingbeat cycle, but clustered with the primary and secondary clicks.

Tiger beetles did not produce clicks in response to tactile stimuli, even when flying.

**Discussion**

Trains of ultrasonic pulses elicit a complex behavioral response in flying *C. marutha*. Changes in wingbeat pattern

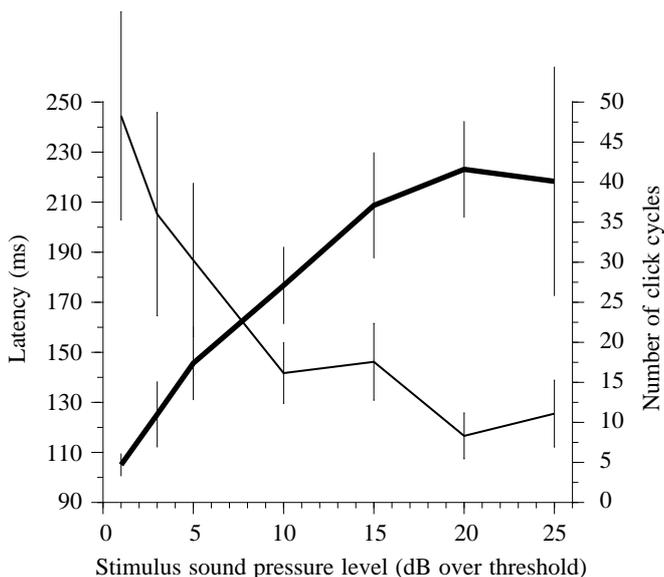


Fig. 9. The number of clicks produced changes in concert with a decrease in latency to click production as stimulus sound pressure level increases (thick line). Stimuli were 500 ms trains of 10 ms pulses (20 ms period). The right-hand ordinate is the number of wingbeat cycles during which any clicks occurred (thin line); the actual numbers of clicks are approximately twice the values shown on this scale. Standard error bars are shown. Data are from approximately 300 trials from five beetles for each curve.

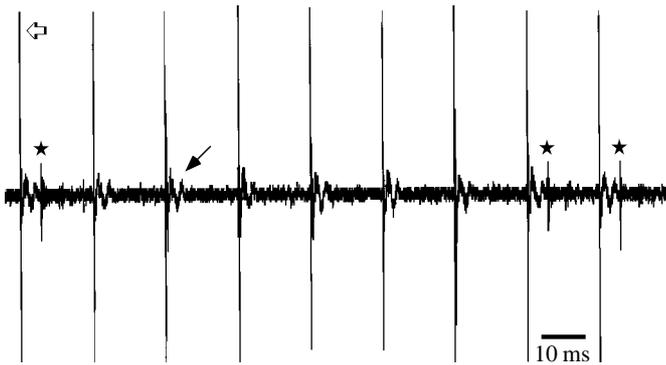


Fig. 10. Oscillogram showing the relationship of clicks to the wingbeat cycle. The high-amplitude primary clicks (open arrow) occur at the wingbeat frequency and in a constant temporal relationship to the wingbeat waveform (filled arrow; see text). Smaller, secondary clicks (stars) occur in three cycles in this trace, also in a fixed temporal relationship to the wingbeat cycle.

and movements of the head, legs and elytra are coupled with production of loud ultrasonic clicks. This combination of behavioral components is unique among insects responding to ultrasound. While both the phylogeny and the distinctive structure of the tiger beetle auditory system ensure that this behavioral suite evolved independently, some of the components show striking similarities to acoustic behaviors in other insects.

#### Comparisons

Ultrasound triggers defensive behaviors in green lacewings *Chrysopa carnea* (Miller, 1975; Miller and Olesen, 1979), tettigoniids *Neoconocephalus ensiger* (Libersat and Hoy, 1991), moths (reviewed by Roeder, 1967; Spangler, 1988b), crickets *Teleogryllus oceanicus* (Moiseff *et al.* 1978; Nolen and Hoy, 1986), locusts *Locusta migratoria* (Robert, 1989) and mantises *Parasphendale agrionina* (Yager and May, 1990) as well as in tiger beetles. In all cases, context is crucial in determining the nature of the response and, in fact, most insects do not respond at all unless they are flying.

With the exception of the elytra swing, the non-acoustic components of the tiger beetle's response are also found in various combinations in the other ultrasound-responsive insects. For example, crickets, locusts and tiger beetles share a directional leg kick. In crickets and locusts, there is a directional abdominal bending, but in mantises and tiger beetles there is a symmetrical contraction of the dorsal muscles. WBF changes are common to all, but mantises decrease the WBF while lacewings and tettigoniids stop flapping altogether. The head roll is absent in tettigoniids and possibly in lacewings.

These insects also share important functional characteristics of their ultrasound-triggered responses, presumably reflecting convergence driven by the shared problem of bat predation. In every case, the behavioral tuning curves are broad, spanning the range 30–60 kHz. Minimum thresholds range from

40–50 dB SPL (locusts, moths) to 70–80 dB SPL (tiger beetles). Latencies are uniformly short: less than 150 ms even for complex behaviors and as low as 25–35 ms for electromyographic events in muscles controlling specific components of the response (crickets, tettigoniids). The duration of the response substantially exceeds that of the stimulus in each case.

Arctiid moths and tiger beetles are the only insects known to click in response to ultrasound in a defensive context. In both cases, the clicks are very short (50–200  $\mu$ s) and have a broad (30–80 kHz) frequency spectrum (Fullard and Fenton, 1977). Click SPL is also closely comparable: Krasnoff and Yager (1988) and Surlykke and Miller (1985) measured peak SPLs at 2 cm of 79–90 dB SPL for three arctiid species. Whereas *C. marutha* clicks at an average rate of 100–120 clicks  $s^{-1}$ , click rates vary among arctiid species from less than 20 to greater than 1500 clicks  $s^{-1}$  depending largely on the structure of the tymbals that produce the clicks (Fullard and Fenton, 1977; Surlykke and Miller, 1985). The arctiid response to ultrasound includes some behavioral components other than clicking (WBF changes and flight cessation; Dunning and Roeder, 1965; Fullard, 1979), but in recent field studies (Dunning *et al.* 1992; Acharya and Fenton, 1992) nine species of arctiids known to click copiously survived bat attacks without performing any obvious evasive maneuvers in more than 80% of observations (in control experiments, deafened and/or muted moths were captured in more than 60% of the trials). In contrast to tiger beetles, arctiids will click in response to a range of threatening stimuli, whether in flight or not.

Some of the insects discussed here do not predictably orient their response relative to the ultrasound source (mantises, lacewings, tettigoniids), while others show strong directionality (moths, crickets, locusts). Tiger beetles appear to fall in the former category. Yager and Spangler (1995) showed that directional information is available to the central nervous system in the afferent responses, but also reported considerable variability.

Recently, Forrest *et al.* (1995) reported an ultrasound-triggered response in a scarab beetle. The comparison with cicindelids is especially interesting since these two coleopteran auditory systems must have evolved independently (T. G. Forrest, personal communication). Their study focused on the head roll shown by walking scarabs in response to ultrasonic pulses. The tuning was broad (20–70 kHz) at levels of 60–70 dB SPL. Latency to response in the neck muscles measured electromyographically was 30–40 ms. Of particular note is the lack of context-specificity: the scarabs responded both when walking and when flying. The in-flight response, however, has not yet been characterized.

#### Function(s) of hearing in tiger beetles

The results presented in the present study may help to resolve the question raised by Yager and Spangler (1995) regarding how tiger beetles use their hearing. Two theories dominate: intraspecific communication and predator avoidance. Prey localization is unlikely since most of these

insects are diurnal, visual hunters (Pearson, 1988). Yager and Spangler (1995) argued that both theories are possible, but that bat evasion was best supported by their physiological data.

The behavioral data presented here strongly support the hypothesis that hearing in *C. marutha* functions as part of a system for evading capture by hunting, echolocating bats. The context-specificity of the response clearly dictates a function during flight, and light-trap captures demonstrate that some tiger beetles (including *C. marutha*) fly at night in substantial numbers (nocturnal activities are primarily dispersal and, for some species, moving to oviposition sites; Laroche, 1977; Pearson, 1988; D. L. Pearson, personal communication). Aerodynamically, the increases in hindwing excursion and WBF predict higher power output, and the elytra pronation, tilting forward of the stroke plane of the hindwings and forward compression of the abdomen would all contribute to a dive. The asymmetries in the head and leg movements suggest that the dive will be to the side, but non-static aerodynamic testing will be required to confirm this. The tuning of the behavioral response matches the power spectrum of the most common bat echolocation calls, as well as the audiograms of other insects known to use hearing to evade bats (reviewed by Yager and Hoy, 1989; Yager *et al.* 1990). Calculations using the behavioral threshold data for tiger beetles to determine the response time available to the insect when approached by a hunting bat (assuming beetle flight speed of  $3 \text{ m s}^{-1}$ , bat flight speed of  $6 \text{ m s}^{-1}$  and echolocation cry SPL of 100 dB SPL at 10 cm; Yager *et al.* 1990) yield response times of 530 ms for a bat approaching from behind and 170 ms for a bat approaching from the front. Even in the worst circumstance, the behavioral latencies of less than 150 ms give the beetle adequate time to initiate evasive maneuvers before it can be captured.

The arctiid-like clicking of tiger beetles potentially adds another dimension to their defense against bats. It is well-established that arctiid moth clicks very effectively deter and/or disrupt bat attacks (Dunning and Roeder, 1965; Dunning *et al.* 1992; Acharya and Fenton, 1992). The exact mechanism is not clear and may vary depending on the circumstances. On the basis of the similarity of their power spectrum and SPL, tiger beetle clicks, like those of arctiids, will be readily audible to an insectivorous bat and could startle it (Bates and Fenton, 1990) or possibly interfere with its echolocation (Fullard *et al.* 1994). Also like arctiid moths, tiger beetles produce noxious defensive secretions (Pearson *et al.* 1988), so the clicks might deter attack by warning an experienced bat (aposematism). An intriguing possibility is that tiger beetles may be acoustic mimics (Batesian or Müllerian) of the distasteful, clicking moths.

The behavioral data also support the hypothesis that hearing in *C. marutha* is used for intraspecific communication. While the behavioral tuning curves for tiger beetle defensive behavior match closely those of other bat-evading insects, they do not match well the audiogram obtained physiologically from the tympanal nerve (Fig. 5; Yager and Spangler, 1995). The auditory afferent response is much more sharply tuned (to 30 kHz) and is more than 20 dB more sensitive. This implies a

second function for hearing in tiger beetles. Acoustic signals produced by tiger beetles when on the ground are species-specific click trains with very different temporal patterns from defensive clicking (Freitag and Lee, 1972; H. G. Spangler, unpublished observations), reinforcing the idea that a second function may be intraspecific communication. This would be analogous to the situation in some crickets, for example, with broad ultrasonic tuning for evasion and low-threshold, sharp tuning, at a lower frequency, for mate attraction (Nolen and Hoy, 1986). The nature and function of intraspecific communication in *C. marutha* remain unknown.

#### *Click production mechanism*

Flying insects can produce ultrasonic clicks using a diversity of mechanisms. Arctiid moths use tymbals located on the sides of the thorax (Fullard and Heller, 1990). Agaristine moths have specialized 'castanets' on the forewings that strike each other at the top of the upstroke (Bailey, 1978), and some noctuid moths produce weak clicks using the same method, but without wing specializations (Waters and Jones, 1994). A nymphalid butterfly makes loud clicks when the tegula near the wing base hits a 'costal clicker' (a modified wing cell) on the forewing (Møhl and Miller, 1976). Motion of a projection on the forewing of pyralid moths deforms a modified tegula to produce clicks (Spangler and Takessian, 1986). A hard exoskeleton such as that of the tiger beetle provides innumerable opportunities for moving structures to strike or scrape together to yield clicks.

While we do not as yet know how tiger beetles produce their ultrasonic clicks, our data do provide some clues. Most suggestive is the tight temporal locking of the clicks to the wingbeat cycle. A mechanism not linked to the wings, such as the tymbals of arctiids, is therefore unlikely. The elytra do not flap, so any mechanism requiring rhythmic forewing movement can also be excluded. The power spectrum of the clicks shows a prominent peak at 30–40 kHz. This matches the power spectrum of 'quacks', acoustic signals of unknown function produced by *C. marutha* and many other tiger beetles (Freitag and Lee, 1972), and may mean that the same resonating/radiating system, possibly on the hindwings, is utilized in both contexts.

The most distinctive component of the tiger beetle's ultrasound-triggered behavior is the swing of the elytra backwards towards the hindwings. Our latency data show that the elytra swing always occurs before clicking begins. We have preliminary data from ablation experiments and stroboscopic photographs that suggest (a) that the elytra swing is necessary for click production and (b) that the hindwings may strike the elytra during a response, sometimes hard enough to deform the hindwings. As our current primary hypothesis for the click production mechanism, elytral impact with the hindwings has the added appeal of an elegantly crude economy: the single, simple motion of the elytra could alter the aerodynamics of hindwing motion (as happens when the cricket's leg hits its hindwing; May and Hoy, 1990) and at the same time produce loud ultrasonic clicks.

We thank Dr David Pearson for identifying the beetles. Amy Harron assisted with data collection and analysis. Dr Pearson and Dr Winston Bailey made very helpful suggestions on the manuscript. Dr Kevin O'Grady provided statistical advice. This research was supported by research grant 5-R29-DC01382 from the National Institute on Deafness and other Communication Disorders, National Institutes of Health to D.D.Y.

### References

- ACHARYA, L. AND FENTON, M. B. (1992). Echolocation behavior of vespertilionid bats (*Lasiurus cinereus* and *Lasiurus borealis*) attacking airborne targets including arctiid moths. *Can. J. Zool.* **70**, 1292–1298.
- BAILEY, W. J. (1978). Resonant wing systems in the Australian whistling moth *Hecatesia* (Agarosidae, Lepidoptera). *Nature* **272**, 444–446.
- BATES, D. L. AND FENTON, M. B. (1990). Aposematism or startle? Predators learn their responses to the defenses of prey. *Can. J. Zool.* **68**, 49–52.
- BATSCHULET, E. (1981). *Circular Statistics in Biology*. New York: Academic Press.
- DUNNING, D. C., ACHARYA, L., MERRIMAN, C. B. AND DAL FERRO, L. (1992). Interactions between bats and arctiid moths. *Can. J. Zool.* **70**, 2218–2223.
- DUNNING, D. C. AND ROEDER, K. D. (1965). Moth sounds and the insect-catching behavior of bats. *Science* **147**, 173–174.
- FORREST, T. G., FARRIS, H. E. AND HOY, R. R. (1995). Ultrasound startle responses in scarab beetles. *J. exp. Biol.* **198**, 2593–2598.
- FREITAG, R. AND LEE, S. K. (1972). Sound producing structures in adult *Cincindela tranquebarica* (Coleoptera: Cincindelidae) including a list of tiger beetles and ground beetles with flight wing files. *Can. Ent.* **104**, 851–857.
- FULLARD, J. H. (1979). Behavioral analyses of auditory sensitivity in *Cyncia tenera* Hübner (Lepidoptera: Arctiidae). *J. comp. Physiol.* **129**, 79–83.
- FULLARD, J. H. AND FENTON, M. B. (1977). Acoustic and behavioural analyses of the sounds produced by some species of Nearctic Arctiidae (Lepidoptera). *Can. J. Zool.* **55**, 1213–1224.
- FULLARD, J. H. AND HELLER, B. (1990). Functional organization of the arctiid moth tymbal (Insecta, Lepidoptera). *J. Morph.* **204**, 57–65.
- FULLARD, J. H., SIMMONS, J. A. AND SAILLANT, P. A. (1994). Jamming bat echolocation: the dogbane tiger moth *Cyncia tenera* times its clicks to the terminal attack calls of the big brown bat *Eptesicus fuscus*. *J. exp. Biol.* **194**, 285–298.
- HOFFMEISTER, D. F. (1986). *Mammals of Arizona*. Tucson: University of Arizona Press.
- HOY, R. R., NOLEN, T. G. AND BRODFUEHRER, P. D. (1989). The neurotheology of acoustic startle and escape in flying insects. *J. exp. Biol.* **146**, 287–306.
- KRASNOFF, S. B. AND YAGER, D. D. (1988). Acoustic response to a pheromonal cue in the arctiid moth *Pyrrharctia isabella*. *Physiol. Ent.* **13**, 433–440.
- LAROCHELLE, A. (1977). Cicindelidae caught at lights. *Cincindela* **9**, 50–60.
- LIBERSAT, F. AND HOY, R. R. (1991). Ultrasonic startle behavior in bushcrickets (Orthoptera: Tettigoniidae). *J. comp. Physiol. A* **169**, 507–514.
- MAY, M. L. AND HOY, R. R. (1990). Leg-induced steering in flying crickets. *J. exp. Biol.* **151**, 485–488.
- MILLER, L. A. (1975). The behaviour of flying green lacewings, *Chrysopa carnea*, in the presence of ultrasound. *J. Insect Physiol.* **21**, 205–219.
- MILLER, L. A. AND OLESEN, J. (1979). Avoidance behavior in green lacewings. I. Behavior of free flying green lacewings to hunting bats and ultrasound. *J. comp. Physiol.* **131**, 113–120.
- MØHL, B. AND MILLER, L. A. (1976). Ultrasonic clicks produced by the peacock butterfly: a possible bat-repellent mechanism. *J. exp. Biol.* **64**, 639–644.
- MOISEFF, A., POLLOCK, G. S. AND HOY, R. R. (1978). Steering responses of flying crickets to sound and ultrasound: Mate attraction and predator avoidance. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4052–4056.
- NOLEN, T. G. AND HOY, R. R. (1986). Phonotaxis in flying crickets. I. Attraction to the calling song and avoidance of bat-like ultrasound are discrete behaviors. *J. comp. Physiol. A* **159**, 423–439.
- PEARSON, D. L. (1988). Biology of tiger beetles. *A. Rev. Ent.* **33**, 123–147.
- PEARSON, D. L., BLUM, M. S., JONES, T. H., FALES, H. M., GONDA, E. AND WITTE, B. R. (1988). Historical perspective and the interpretation of ecological patterns: Defensive compounds of tiger beetles (Coleoptera: Cicindelidae). *Am. Nat.* **132**, 404–416.
- ROBERT, D. (1989). The auditory behavior of flying locusts. *J. exp. Biol.* **147**, 279–310.
- ROEDER, K. D. (1967). *Nerve Cells and Insect Behavior*. Cambridge, MA: Harvard University Press.
- SPANGLER, H. G. (1988a). Hearing in tiger beetles (Cicindelidae). *Physiol. Ent.* **13**, 447–452.
- SPANGLER, H. G. (1988b). Moth hearing, defense and communication. *A. Rev. Ent.* **33**, 59–81.
- SPANGLER, H. G. AND TAKESSIAN, A. (1986). Further observations on sound production by the lesser wax moth, *Achroia grisella* (F.) (Lepidoptera: Pyralidae). *J. Kansas Ent. Soc.* **59**, 555–557.
- SURLYKKE, A. AND MILLER, L. A. (1985). The influence of arctiid moth clicks on bat echolocation; jamming or warning? *J. comp. Physiol. A* **156**, 831–843.
- WATERS, D. A. AND JONES, G. (1994). Wingbeat-generated ultrasound in noctuid moths increases the discharge rate of the bat-detecting A1 cell. *Proc. R. Soc. Lond. B* **258**, 41–46.
- YAGER, D. D. AND HOY, R. R. (1989). Audition in the praying mantis, *Mantis religiosa* L.: Identification of an interneuron mediating ultrasonic hearing. *J. comp. Physiol. A* **165**, 471–493.
- YAGER, D. D. AND MAY, M. L. (1990). Ultrasound-triggered, flight-gated evasive maneuvers in the praying mantis, *Parasphendale agrionina*. II. Tethered flight. *J. exp. Biol.* **152**, 41–58.
- YAGER, D. D., MAY, M. L. AND FENTON, M. B. (1990). Ultrasound-triggered, flight-gated evasive maneuvers in the praying mantis, *Parasphendale agrionina*. I. Free flight. *J. exp. Biol.* **152**, 17–39.
- YAGER, D. D. AND SPANGLER, H. G. (1995). Characterization of auditory afferents in the tiger beetle, *Cincindela marutha* Dow. *J. comp. Physiol. A* **176**, 587–600.