

Behavioural context regulates dual function of ultrasonic hearing in lesser waxmoths: bat avoidance and pair formation

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Abstract. Ultrasonic hearing in moths evolved to detect bat echolocation cries and has been co-opted into the mating system of some species. Some moths evade or approach ultrasonic stimuli that overlap in their spectral and temporal features. The present study addressed the evaluation of ultrasonic stimuli by lesser waxmoths, a species in which ultrasonic hearing functions in bat evasion and pair formation. Playback experiments show that the role of different stimulus features varies with behavioural context. Flying moths have a single evasive response, which is performed mainly on the basis of pulse length and stimulus power: they dive upon hearing loud pulses ≥ 1 ms in length, and respond more strongly to high-power stimuli above that pulse length. Female moths walking on the ground respond with positive phonotaxis mainly on the basis of pulse rate by orienting toward pulses delivered at rates ≥ 30 pulses s^{-1} . Consequently, moths on the ground approach stimuli that elicit evasion when detected in flight. However, the different responses given to stimulus features by flying and walking moths match the distinguishing features of bat cries and conspecific males in both contexts. Stimulus evaluation, modified by behavioural context, gives lesser waxmoths the flexibility to show appropriate evasion or approach responses to stimuli that overlap in their features.

Key words. *Achroia grisella*, acoustic signalling, female mate preferences, Lepidoptera, Pyralidae.

Introduction

Adaptive animal behaviour often requires that different responses be given to stimuli with overlapping features. A striking example of this discrimination occurs in moths. Most moth species are sensitive to ultrasound, can detect the echolocation cries of bats and evade them (Roeder, 1962, 1964, 1967; Spangler, 1988; Scoble, 1992; Miller & Surlykke, 2001). Some moths also use ultrasonic hearing in sexual communication (Spangler, 1988; Conner, 1999). Spe-

cies that orientate away from and towards ultrasound occur among noctuid, arctiid and pyralid moths (Conner, 1999). Negative and positive responses are performed although the stimuli overlap in their spectral and temporal features. The broad tuning of moth ears is not likely to allow discrimination of these stimuli based on their frequency (Spangler & Takessian, 1983; Surlykke & Fullard, 1989; Jones and Waters, 2000; Skals & Surlykke, 2000; Miller & Surlykke, 2001; Fullard *et al.*, 2003). It has therefore been suggested that the discrimination of stimuli associated with bat predators and conspecifics is based on temporal features (Surlykke & Fullard, 1989; Greenfield & Weber, 2000; Skals & Surlykke, 2000; Jones *et al.*, 2002) and on cues provided by the behavioural context of the moth (Conner, 1999).

The present study addressed the dual function of ultrasonic hearing in lesser waxmoths, *Achroia grisella*

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F. (Lepidoptera: Pyralidae). Lesser waxmoths are widespread honeybee symbionts in which ultrasonic hearing functions in sexual communication and bat evasion. Males produce ultrasonic advertisement calls to which females orientate by walking (Greenfield & Coffelt, 1983; Spangler *et al.*, 1984; Jang & Greenfield, 1996, 1998). Males fan their wings and cause tymbals at the base of the forewings to generate pairs of short (approximately 100 μ s) pulses of broadband ultrasound (70–130 kHz). Females prefer high intensity calls delivered at fast pulse rates. Pair formation usually takes place on a substrate such as the outside of beehives or nearby vegetation.

Ultrasonic hearing in lesser waxmoths also functions in bat evasion: when stimulated with some types of pulsed ultrasound, walking moths and calling males stop movement, and flying moths dive towards the ground (Spangler, 1984b; Greenfield & Weber, 2000; Greenfield & Baker, 2003). Lesser waxmoths may be exposed to predation by bats that hunt insect prey in flight (Brandt, 2003; these bats are known as aerial-hawking bats), by bats that glean prey from foliage (gleaning bats), and by bats that engage in both hunting strategies (Faure & Barclay, 1994; Schnitzler & Kalko, 2001; Ratcliffe & Dawson, 2003).

Overlap exists between the features of stimuli associated with these predators and with conspecific males. Nonetheless, there may exist cues that allow moths to respond appropriately in the presence of bats using different hunting strategies and in the presence of male moths. The cries of aerial-hawking bats vary in pulse rate (from approximately 2–200 pulses s^{-1}) along the hunting phases of search, approach and capture, but their pulses remain long (= 1 ms), at least before the final capture phase, compared to the pulses of male lesser waxmoths (Griffin *et al.*, 1960; Roeder, 1967; Simmons *et al.*, 1979; Fenton & Fullard, 1981; Miller & Degn, 1981; Fenton, 1982; Miller, 1983; Griffin, 1986; Rydell, 1990; Kalko, 1995; Schnitzler & Kalko, 2001). The pulse rate of gleaning bats does not increase above approximately 30 pulses s^{-1} and, instead, the bats strongly reduce the amplitude of their cries or become silent as they approach their prey (Fenton, 1990; Faure & Barclay, 1994; Arlettaz *et al.*, 2001; Miller & Surlykke, 2001). The pulse rate of calling male lesser waxmoths varies between 60 and 140 pulses s^{-1} and their pulses are very short (0.1 ms) (Jang & Greenfield, 1996).

Previous studies with lesser waxmoths have shown that calling, walking and flying are inhibited by long pulses of ultrasound delivered at slow rates (Greenfield & Weber, 2000; Greenfield & Baker, 2003). Responsiveness to bat cries is not context-dependent, as simulated bat cries cause lesser waxmoths both to dive when in flight or to stop moving when on the ground (Greenfield & Weber, 2000). The present study expands these considerations to test whether stimulus evaluation is context-dependent. The influence of behavioural context (flight vs. walking) on the evaluation of the temporal features of ultrasonic stimuli (pulse length and pulse rate) and in eliciting evasion and approach responses is examined.

Materials and methods

The moths used in the present study came from a captive colony established with over 200 individuals collected on abandoned beeswax in Lawrence, Kansas, in 1997 and 1999. The colony was maintained with a breeding protocol that minimized inbreeding and prevented assortative mating. Moths were reared on a diet of flour, glycerol, brewer's yeast, beeswax, honey and water (modified from Dutky *et al.*, 1962), and kept at 25 °C at LD 12 : 12 h. Pupae were isolated in individual plastic cups to ensure that eclosing adult females were unmated and likely to respond to male calls. Experiments were performed during the first 5 h of the scotophase with 1–2-day-old virgin females. Testing took place in a 3.5 × 3.5 × 2.5-m semianechoic room at 25 °C under dim red illumination, except for experiments involving high-speed filmography and free-flying moths (see below), which were conducted in the laboratory under fluorescent-light illumination at a room temperature of approximately 24 °C.

Signal generation and playback

Digitally-modified recordings of male calls and digitally-generated simulations of bat echolocation cries were used. A recording of a calling male moth with features close to the mean of the population was used to generate male call playbacks. The recording was made with a condenser microphone (ACO Pacifica Inc., Belmont, California; frequency response \pm 6 dB from 10–160 kHz) fitted to a preamplifier (ACO model 4012). The signal was high-pass filtered (Krohn-Hite 3202, Avon, Massachusetts) at 20 kHz, transferred to a 486 computer (66 MHz cpu) via a soundcard (SoundFX Engineering Version, Silicon Soft, San José, California), and sampled at 298 kHz. Custom-designed software was utilized to vary the temporal parameters of this recording of playback. Playbacks were broadcast with an Ultra Sound Advice S56 loudspeaker (frequency response \pm 6 dB from 10–200 kHz), amplified by an Ultra Sound Advice S55 amplifier (frequency response \pm 3 dB from 18–300 kHz). Recordings of the playbacks, made as for the calling male, showed that the playbacks had 0.1-ms pulses and a concentration of energy between 70 and 87 kHz (frequency cutoffs 6 dB below maximum energy; fast Fourier Transform performed with custom-designed software), thus approximating the mean values of these traits in the population (Jang & Greenfield, 1996).

In constructing simulated bat echolocation cries, it was recognized that lesser waxmoths have been preyed upon by various species of Old World bats for most of their evolutionary history. The two known *Achroia* species are symbionts of honeybees (*A. grisella* on *Apis mellifera* and *Achroia innotata* on several *Apis* species in South-east Asia; Corbet & Tams, 1943; Whalley, 1964). *Apis* is native to the Old World, and *A. mellifera* probably arose in Central Asia (Engel, 1997, 1999; Michener, 2000). Searching-phase echolocation cries representative of bats that hunt in

open spaces between tree canopies in the Old World were therefore simulated. These cries have approximate values of 40 kHz for frequency and 13 ms for pulse length (Neuweiler, 1989). Pulse lengths of 0.1 ms (male moth calls) and 1.0, 3.0 and 6.5 ms were also assayed to explore the range between bat cries and male moth calls. Pure 40-kHz tones were produced with a B&K Precision 3010 function generator (B&K Precision Corporation, Yorba Linda, California). The output of the function generator was transferred to the computer described above, and the file was edited to produce playbacks with the desired temporal features.

Playbacks fully resembling male moth calls and bat echolocation cries were used to perform a realistic comparison of the moths' responses to the different stimuli. Playbacks of male moth calls and bat cries differed in frequency (70–87 kHz and 40 kHz, respectively) and in the presence of single or double pulses. It is highly unlikely that the difference in frequency influenced the behaviour of the moths, since the tuning of their ears is broad (Spangler & Takessian, 1983) and previous experiments indicate that their behavioural responses are not influenced by the frequency of ultrasonic stimuli (Greenfield & Weber, 2000). It has been suggested that greater waxmoths (*Galleria mellonella*, Pyralidae) may distinguish frequencies of 25–32 kHz from frequencies of 35–250 kHz (Spangler, 1984a, 1988). However, this differential responsiveness between frequency ranges probably reflects the lower end of a broad spectrum of sensitivity to ultrasound, rather than frequency discrimination. The other difference between playbacks of male moth calls and bat cries was the presence of a double-pulse in the male calls. There is no indication that lesser waxmoths use this feature to distinguish male moths from bats: they respond just as well to single- or double-pulse stimuli if other features are appropriate (Greenfield & Weber, 2000).

Experimental design

The experiments reported here had three purposes: (i) to evaluate the role of different stimulus temporal features in eliciting evasion in flight; (ii) to evaluate their role in eliciting approach on the ground; and (iii) to evaluate the role of behavioural context in mediating these responses.

The role of stimulus temporal features in eliciting evasive responses in flight. The responses of moths were evaluated in tethered flight. Female moths were cooled to 5 °C for at least 15 min. They were then attached by the thorax to an entomological pin with hot glue and allowed approximately 5 min to adjust to room temperature. They were then raised above the substrate and exposed to a weak air current (mean \pm SE air velocity = 0.40 ± 0.03 ms⁻¹) to induce and maintain wing beating.

Moths in tethered flight were presented with stimuli having pulse lengths of 0.1, 1.0, 3.0, 6.5 and 13.0 ms, delivered at pulse rates of 10 and 90 pulses s⁻¹, and at amplitudes of

75 and 86 dB peSPL (0 dB = 20 μ Pa). Playback amplitude was calibrated with the peak equivalent method (Jang & Greenfield, 1996) and monitored with an oscilloscope (Tektronix 7313 dual channel oscilloscope, Beaverton, OR). Because it was impossible to have 13-ms pulses delivered at 90 pulses s⁻¹ because of a duty cycle higher than 100%, 10-ms pulses were used instead of 13-ms pulses. The stimuli using 0.1-ms pulses were not tested at rates lower than 90 pulses s⁻¹. These stimuli encompassed the full range of moth and bat signals. An amplitude of 86 dB peSPL corresponds to bat echolocation cries of intermediate intensity (75–100 dB peSPL measured 10 cm from the bat) detected at a distance of 10–50 cm, or to higher-intensity cries (≥ 110 dB peSPL) detected at approximately 1.6 m (Fenton & Bell, 1981; Fenton & Fullard, 1981; Griffin, 1986; Schnitzler & Kalko, 2001).

Stimuli were broadcast with the Ultra Sound Advice loudspeaker and amplifier described above. The loudspeaker was placed 60 cm away from the moths. Stimulus presentation lasted approximately 1 s. The behaviour of the tethered female moths was recorded with a MotionScope high-speed video camera (Redlake Imaging Corporation, San Diego, CA) at 250 frames s⁻¹. Broadcast stimuli were monitored with the microphone and amplifier described above. These were connected to a Hameg HM 203-7 20-MHz oscilloscope (Frankfurt, Germany), and the oscilloscope was placed behind the tethered moths such that it was hidden from them but visible to the camera. Thus, there was a synchronous film record of their behaviour and the onset of playback. In frame-by-frame analysis of the video recordings, the percentage of moths that responded with evasive manoeuvres was noted, along with response latency (delay between the onset of the playback and the beginning of the response) and response duration.

To aid the interpretation of the tethered flight tests, responses were also studied in free-flight. Female moths were released in the laboratory, approximately 1.5 m above the ground and 30 cm from the loudspeaker. After approximately 1 s of flight, the moths were exposed to 13-ms pulses delivered at 10 pulses s⁻¹, broadcast at 114 dB peSPL (as measured at the release point of the moths, 30 cm away from the loudspeaker). This amplitude simulated bat cries of intermediate to high intensity close to the moth.

The role of stimulus temporal features in eliciting positive responses on the ground. The lowest pulse rate that elicited positive phonotaxis (pulse-rate threshold) was measured in female moths presented with two stimuli having extreme pulse lengths: 0.1 ms (male calls) and 13 ms (bat cries). One-day-old virgin females were placed at the centre of a screen arena (80 cm radius), 60 cm from a loudspeaker directly outside the arena. Stimuli with pulse rates of 30–130 pulses s⁻¹ were presented in increments of 10 pulses s⁻¹. For the rate of 90 pulses s⁻¹, 10-ms pulses were used instead of 13-ms pulses. The stimuli were presented in random order until the phonotaxis threshold was identified for the female. A randomly chosen pulse rate higher

than the threshold was then presented to check that it elicited phonotaxis, thus ensuring that the moths had not habituated. If a female approached a stimulus delivered at 30 pulses s^{-1} , lower rates were presented in decrements of 10 pulses s^{-1} until her threshold was obtained. Time between presentation of each stimulus to a female was > 30 min. Playback amplitude was adjusted to 86 dB peSPL at the position of the female in the arena before stimulus presentation. This value is the mean amplitude of calling males in the laboratory population, measured 8–10 cm from the male (range = 73–92 dB peSPL, $n = 25$). In each stimulus presentation, females were allowed 1 min to begin walking and an additional 1 min to complete phonotaxis. All females that responded started to walk within 50 s, some as soon as within 1 s of stimulus presentation. Positive phonotaxis was scored when a female walked towards the loudspeaker and was within 10 cm of it; females often wing-fanned as they walked or when they came close to the speaker, a behaviour that indicates sexual receptivity (Greenfield & Coffelt, 1983).

Thresholds were first obtained to stimuli with 0.1-ms pulses (male calls), and the next day thresholds of the same individual females to stimuli with 13-ms pulses (bat cries) were obtained. Each female was exposed to the sequence of stimuli only once for each pulse length. The pulse-rate thresholds of phonotaxis to the two pulse lengths were then compared.

The role of behavioural context. This section considered whether individual female moths in flight show evasive responses to stimulus features that are related to their approach responses to the same stimulus features on the ground. Individual female moths were therefore subjected to evasion assays in flight and to approach assays on the ground. Pulse-rate thresholds of response were measured in each context. Evasion thresholds are likely to be critical for lesser waxmoths: early evasion is probably crucial for these slow, clumsy fliers, and the onset of their non-graded evasive response (see Results) can be adequately quantified with a threshold measure. Thresholds of approach indicate which male pulse rates are acceptable to a female, and show substantial genetic variance (Rodríguez & Greenfield, 2003).

Thresholds were obtained by the method of random playback order outlined above. Thresholds of phonotaxis to 0.1-ms pulses were first obtained. The next day, evasion thresholds to the pulse rate of simulated bat cries were obtained for the same individual female moths in tethered flight. Pulses of 6.5 and 13 ms were assayed as simulated bat cries, using separate sets of moths for each pulse length. Pulse rates varying from 4 to 90 pulses s^{-1} were presented in increments of 20 pulses s^{-1} , except for the two lowest pulse rates, which were 4 and 10 pulses s^{-1} . For the rate of 90 pulses s^{-1} , 10-ms pulses were used instead of 13-ms pulses. Stimuli were broadcast at 86 dB peSPL.

Statistical analysis

The data showed homogeneity of variance, so tests were performed on untransformed data except for the proportional

data that were arcsine-transformed (Sokal & Rohlf, 1995). Pearson's Product-Moment correlations (r), paired t -tests and one-way analyses of variance (ANOVAS) were calculated. When a test involved two- or three-way comparisons with categories with different sample sizes, ANOVAS were performed with the General Linear Model, which allows for an unbalanced design. Statistical power ($1 - \beta$) was calculated according to Sokal & Rohlf (1995).

Results

Lesser waxmoths responded with evasion or approach on the basis of different stimulus features in different behavioural contexts. Pulse length and stimulus power were most influential in determining evasion in flight, whereas pulse rate was most influential on the ground.

Females in free flight responded to simulated bat echolocation cries with dives of 10–30 cm towards the ground, followed by flight resumption: 58% of 24 female moths assayed responded in this manner; the other moths continued in undisturbed flight. Two of the moths that responded also changed the direction of their flight as they dived, and turned away from the loudspeaker.

Females in tethered flight responded to bat echolocation cries with brief modifications of their flight pattern. Slow-motion observation of the video recordings revealed that, in undisturbed tethered flight, the moths beat their wings at 37 ± 1 cycles s^{-1} (mean \pm SE, $n = 29$). Following stimulation with simulated bat cries, they interrupted this movement and held their wings at the top position of their arc of motion for 177 ± 40 ms ($n = 42$), or seven wing cycles. This movement is referred to as a flutter. Overall, flutter latency (delay between stimulus onset and the beginning of flutters) was 106 ± 28 ms ($n = 56$).

Tethered moths often stopped flying whether a playback had been presented or not (47% of 190 tethered females stopped flying before the stimulus sequence was completed). Flight cessation sometimes occurred shortly after the onset of the playback, but was most often not associated with playbacks and occurred before their onset or up to 1 s after. Flight cessation was thus not a reliable assay of evasion. The moths also moved the tips of their abdomens continuously and erratically, even in undisturbed flight.

Some females that did not flutter in response to playbacks performed half-wing beats instead: wing beating continued but the wings did not descend beyond the horizontal in their arc of motion. Half-wing beats encompassed 5 ± 2 wing cycles (range = 1–16, $n = 11$), or 237 ± 81 ms ($n = 6$). Half-wing beat latency (delay between stimulus onset and the beginning of half-wing beats) was 156 ± 57 ms ($n = 12$), which was not significantly different from flutter latency ($F_{1,66} = 0.60$, $P = 0.44$). Half-wing beats occurred infrequently, representing 12 of the 83 observed responses in tethered flight. Five of these 12 half-wing beats were elicited by 0.1-ms pulses; one moth presented with 3-ms pulses (delivered at 10 pulses s^{-1} and at 75 dB peSPL) first

performed 11 half-wing beats over 372 ms and then performed a flutter.

Thus, dives in free flight, and flutters and half-wing beats in tethered flight, were the only unequivocal responses seen. Dives and flutters occurred in response to the same stimuli. Flutters therefore appear to indicate that evasive dives would have occurred had the moths been in free flight.

The role of stimulus temporal features in eliciting evasive responses in flight

The percentage of female moths in tethered flight that responded with flutters was significantly influenced by the amplitude ($F_{1,8} = 27.3$, $P = 0.001$), pulse length ($F_{3,8} = 13.7$, $P = 0.002$), and pulse rate ($F_{1,8} = 5.72$, $P = 0.04$) of simulated bat echolocation cries (Fig. 1; this test conservatively excluded 0.1-ms pulses because no female moth responded to this stimulus by fluttering). Thus, stimuli with more overall acoustical power elicited a higher percentage of responses. However, very short pulses did not elicit flutter responses regardless of power. For example, 1-ms pulses delivered at 10 pulses s^{-1} and at 86 dB peSPL elicited flutters in 44% of the moths assayed (Fig. 1), but 0.1-ms pulses never elicited flutters, even when delivered at 90 pulses s^{-1} (or even at 130 pulses s^{-1} in additional trials), although these stimuli had comparable power.

The latency of the flutter response was also affected by pulse rate ($F_{1,50} = 10.97$, $P = 0.002$) and pulse length ($F_{2,50} = 4.42$, $P = 0.02$; Fig. 2). Here, stimuli with more acoustic power elicited shorter latencies. The effects of the

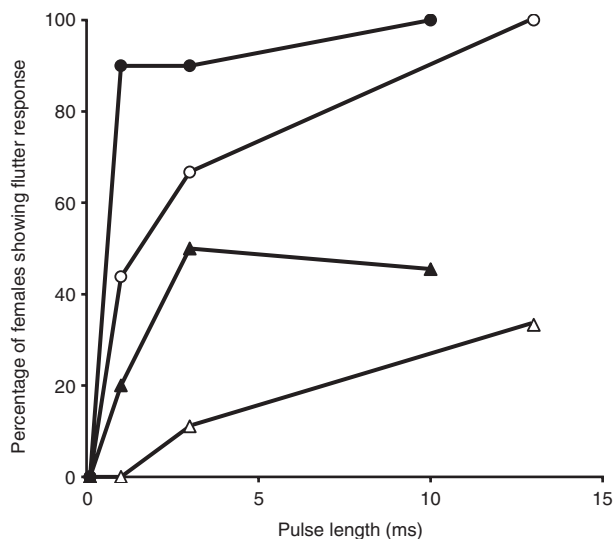


Fig. 1. Incidence of evasive response to simulated bat echolocation cries. Percentage of tethered females that fluttered in response to 40-kHz ultrasound delivered at four pulse lengths, two pulse rates and two amplitudes. Filled symbols: pulse rate = 90 pulses s^{-1} ; open symbols: pulse rate = 10 pulses s^{-1} ; circles: amplitude = 86 dB peSPL; triangles: amplitude = 75 dB peSPL. At least 10 female moths were assayed per stimulus combination.

two variables depended on each other (interaction component: $F_{2,50} = 4.32$, $P = 0.002$): shorter pulses had to be delivered at higher rates to elicit the short latencies that long pulses generated regardless of pulse rate (Fig. 2). Consequently, moths responded with flutters after hearing different numbers of pulses for different stimuli. With the longest pulses, the moths responded after hearing a single pulse, with 3-ms pulses the moths responded after 1–2 pulses even when the pulse rate was low and, with 1-ms pulses, the response occurred after four pulses at low pulse rates. Too few moths responded with flutters to stimuli presented at 75 dB peSPL to assay the influence of amplitude on flutter latency in the above ANOVA. Overall, flutter latency at 75 dB peSPL was 193 ± 72 ms ($n = 16$), which was not significantly different from the latency at 86 dB peSPL ($F_{1,70} = 1.85$, $P = 0.18$).

The role of stimulus temporal features in eliciting approach responses on the ground

Overall, positive phonotaxis to male calls (with 0.1-ms pulses, delivered at 86 dB peSPL) occurred at a threshold pulse rate of 84 ± 2 pulses s^{-1} (range = 40–130 pulses s^{-1} , $n = 174$; for this overall description, data were pooled from this section and the next). To check for an effect of playback order, a correlation between the pulse rate of the first playback presented to a female and her threshold was tested. With habituation, there should be a positive correlation; with sensitization, a negative one. No correlation was

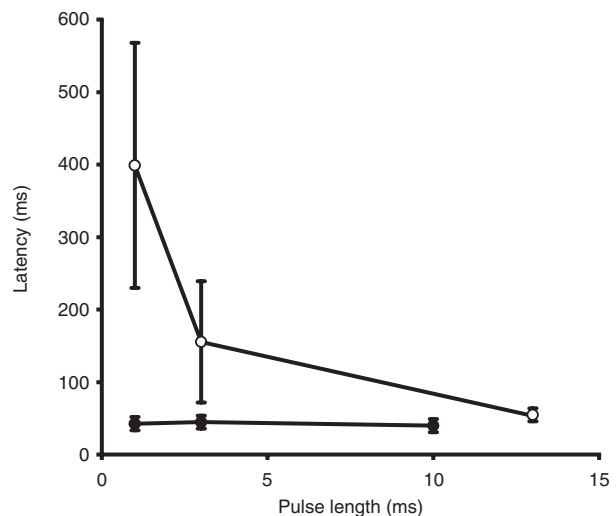


Fig. 2. Latency of evasive response of tethered females to simulated bat echolocation cries. Mean \pm SE latency are shown for flutter responses to pulses of 40-kHz ultrasound delivered at 86 dB peSPL, with four different pulse lengths and two pulse rates. Filled circles: pulse rate = 90 pulses s^{-1} ; open circles: pulse rate = 10 pulses s^{-1} . At least seven female moths were assayed per stimulus combination.

found ($r = 0.02$, $P = 0.77$, $n = 148$), indicating that the phonotaxis thresholds were not influenced by playback order.

Individual females had significantly lower pulse-rate thresholds of approach for stimuli with 13-ms pulses than for stimuli with 0.1-ms pulses (paired t -test for 23 females presented with both stimuli: $t = 2.30$, $P = 0.03$; Fig. 3). However, this difference of 17 pulses s^{-1} in mean pulse-rate thresholds between the two stimuli was the product of a difference of two orders of magnitude in pulse length (0.1 vs. 13 ms). For 13-ms pulses, the distribution of pulse-rate thresholds was truncated at the lower end (Fig. 3), suggesting an absolute threshold that longer pulses would not shift further down. It should be noted that female moths on the ground approached stimuli that, when presented in flight,

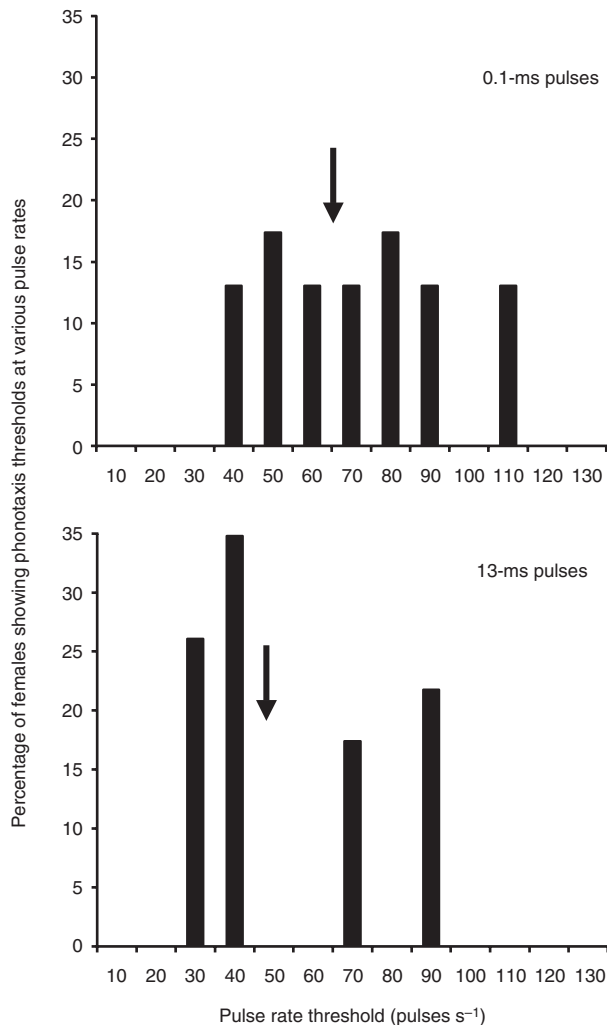


Fig. 3. Positive phonotaxis of female moths on the ground. Pulse-rate thresholds of 23 female moths, each presented with short pulses (0.1 ms, above) and long pulses (13 ms, below; note positive phonotaxis to stimuli that elicit evasion in flight). Arrows indicate the mean for each set of thresholds. The figure shows thresholds of response; female moths approaching a given pulse rate therefore also approach higher rates.

elicited evasive responses. Female moths that did not show positive phonotaxis in these trials remained stationary at the centre of the arena.

The role of behavioural context

This section considered results for the set of female moths that were each presented with male calls on the ground and bat cries in flight. Pulse-rate thresholds were very different for evasion in flight and approach on the ground. Thresholds for evasion of bat echolocation cries ranged only between 4 and 10 pulses s^{-1} , the two lowest rates assayed. By contrast, thresholds for approach of male calls occurred at higher rates and over broader range (Fig. 4). Results did not differ between assays with bat echolocation cries having 6.5- or 13-ms pulses: there were no differences in the percentage of females that responded with evasion, nor in their thresholds of evasion or approach (all tests: $P \leq 0.15$). Results were thus pooled from both assays and the pooled data are shown in Fig. 4. An effect of playback order on evasion thresholds was checked for by testing for a correlation between the pulse rate of the first playback presented to a female and her threshold. Habituation should result in a positive correlation and sensitization in a negative one. No correlation was found ($r = 0.05$, $P = 0.70$, $n = 60$) and it was concluded that thresholds were not influenced by presentation order.

To compare the responses of individual female moths in the contexts of evasion in flight and approach on the ground, moths were first categorized according to their evasion thresholds in flight, and then a difference in the approach thresholds on the ground was tested between these categories. Females with evasion thresholds in flight of 4 pulses s^{-1} did not have significantly different approach thresholds on the ground from females with evasion thresholds in flight of 10 pulses s^{-1} (Fig. 4) ($F_{1,42} = 0.73$, $P = 0.40$; this test had power to detect differences ≥ 20 pulses s^{-1}). Thus, there was no association between responses to the pulse rate of bat echolocation cries in flight and responses to the pulse rate of male calls on the ground.

Discussion

In lesser waxmoths, evasion and approach responses to ultrasonic stimuli are mediated by context-specific stimulus evaluation. In flight, there is a threshold pulse length ($0.1 < \text{threshold} \leq 1.0$ ms) below which lesser waxmoths do not respond with evasion regardless of power. Above this pulse length, more moths respond with evasion to higher-power stimuli, and response latencies are shorter. Studies with greater waxmoths and other pyralid and noctuid moths also show that evasion in flight and on substrates is influenced by stimulus power (Acharya & McNeil, 1998; Skals & Surlykke, 2000; and see Skals and Surlykke's discussion on other experiments that varied the pulse rate of ultrasonic stimuli). The shorter response latencies

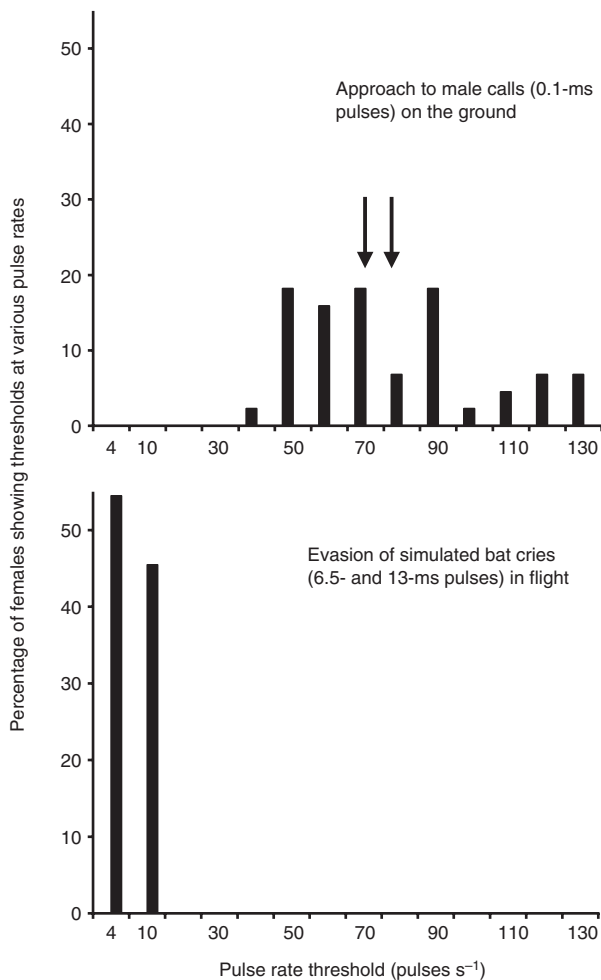


Fig. 4. Evasive and approach thresholds in lesser waxmoths. The distribution of pulse-rate thresholds is shown for positive phonotaxis on the ground (above) and for evasion in flight (below). Arrows indicate mean positive phonotaxis thresholds of female moths whose evasion threshold was either 4 pulses s⁻¹ (left, $n = 24$) or 10 pulses s⁻¹ (right, $n = 20$). The figure shows thresholds of response. Therefore, female moths approaching a given pulse rate also approach higher rates, and female moths showing evasion at a given rate also evade higher rates.

found in the present study (Fig. 2) are comparable to those found for greater waxmoths (mean = 72 ms; Skals & Surlykke, 2000), but latencies found in the present study for lower-power stimuli are much longer (Fig. 2) than those reported for greater waxmoths. This difference may occur because the present study includes lower-power stimuli (longer latencies occur in response to stimuli with 1.0-ms pulses delivered at 10 pulses s⁻¹) than the study by Skals & Surlykke (2000).

Where evasion in flight is mostly elicited by high-power stimuli above a minimum pulse length, positive phonotaxis on the ground is regulated by pulse rate. Increasing stimulus power by lengthening the pulses reduces the pulse-rate threshold of positive phonotaxis; walking female moths

even approach stimuli that elicit evasion in flight. However, great changes in pulse length achieve only small changes in pulse-rate threshold, and there is a threshold pulse rate (30 pulses s⁻¹) below which moths do not respond with positive phonotaxis regardless of pulse length or power. An absolute threshold of approximately 30 pulses s⁻¹ is also arrived at in separate trials where the amplitude of male calls is varied experimentally (Greig & Greenfield, unpublished data). Previous experiments with playbacks of male moth calls also show a stronger effect of pulse rate on positive phonotaxis on the ground, although increases in pulse length (and consequently in power) also increase attractiveness (Jang & Greenfield, 1996). Interestingly, inhibition of the activity of lesser waxmoths on the ground is mostly dependent on slow pulse rates (Greenfield & Weber, 2000; Greenfield & Baker, 2003), although there is also a threshold pulse length ($0.6 < \text{threshold} \leq 3.0$ ms) below which inhibition does not occur (Greenfield & Weber, 2000). At pulse rates above the minimum, female lesser waxmoths prefer calls having faster pulse rates, longer pulses and higher amplitudes (Jang & Greenfield, 1996, 1998), and overall stimulus power influences positive phonotaxis (Greig & Greenfield, unpublished data).

Thus, ultrasonic stimulus processing in lesser waxmoths does not proceed by straightforward integration, where the level of the response would be linearly related to stimulus power integrated over time. For evasion flight, there appears to be straightforward integration above a minimum pulse length. For evasion on the ground, lower pulse rates and longer pulses are crucial. For positive phonotaxis on the ground, there appears to be straightforward integration above a minimum pulse rate. Because of the relevance of different stimulus parameters in different contexts, what little variation there is in responses to the pulse rate of bat cries in flight is unrelated to the broad and important variation in the responses to the pulse rate of male moth calls on the ground. Behavioural context therefore mediates stimulus evaluation for evasion and approach responses.

Context-dependent stimulus evaluation can facilitate appropriate responses to bat echolocation cries and male moth calls. Reliance on pulse length may best distinguish bat cries from male moth calls for flying lesser waxmoths: the pulse rate of the cries of aerial-hawking bats varies from low to high (2–200 pulses s⁻¹) along the phases of searching, approach and capture (Griffin *et al.*, 1960; Roeder, 1967; Simmons *et al.*, 1979; Fenton & Fullard, 1981; Miller & Degn, 1981; Fenton, 1982; Miller, 1983; Griffin, 1986; Rydell, 1990; Kalko, 1995; Schnitzler & Kalko, 2001). However, their pulses remain long (≥ 1 ms, at least before the final capture phase) compared to those of male moth calls (0.1 ms). Furthermore, evaluating pulse length only requires detecting a single pulse and can be performed more quickly than evaluating pulse rate, potentially facilitating faster evasion responses. Above the minimum pulse length, higher-power bat cries may reflect predation risk to a certain point. Noctuid and pyralid moths (including greater waxmoths) show stronger evasive responses on the basis of predation risk reflected by stimulus power

(Acharya & McNeil, 1998; Jones *et al.*, 2002). A recent study showed that stimulation of the ear of a moth diminishes during the final capture-buzz phase of the attack of an aerial-hawking bat due to a reduction in pulse length and amplitude (Fullard *et al.*, 2003). The span of a bat attack during which long pulses and increasing stimulus power indicate predation risk may thus be limited to the searching and approach phases of aerial-hawking bats; lack of neural coding for low-power but high-risk capture phase bat cries may be due to low selection pressure on the moths during phases when it already is too late to evade the predator, or to selection on the bats to overcome the predator evasion strategies of moths (Fullard *et al.*, 2003).

On the ground, reliance on pulse rate may best distinguish the cries of gleaning bats from male moth calls for lesser waxmoths: the pulse rate of gleaning bats does not increase above approximately 30 pulses s^{-1} , and instead they may become silent as they approach the prey (Fenton, 1990; Arlettaz *et al.*, 2001; Miller & Surlykke, 2001), whereas the pulse rate of male moth calls ranges between 60 and 140 pulses s^{-1} (Jang & Greenfield, 1996). Additionally, moths on the ground may have more time to process ultrasonic stimuli associated with gleaning bats because their capture phase lasts longer than the capture phase of aerial-hawking bats (Faure & Barclay, 1994; Arlettaz *et al.*, 2001). A separate study that measures lower pulse-rate thresholds of approach and upper pulse-rate thresholds for evasion for individual female moths on the ground (Greig & Greenfield, unpublished data) shows a positive correlation between avoidance and approach thresholds on the ground (i.e. females with lower avoidance thresholds also have lower approach thresholds), and also shows that an individual female's upper and lower thresholds are very close in value. These findings suggest that female lesser waxmoths on the ground perceive pulse rate categorically (Green & Marler, 1979), as either bat cries (slower pulse rates) or male moths (faster rates). Similarly, the findings of a previous study (Greenfield & Weber, 2000), together with those of the present study, suggest that flying lesser waxmoths may perceive pulse length categorically, as either bat cries (long pulses) or male moths (short pulses).

It is not to be inferred that pulse rate evaluation by lesser waxmoths on the ground has the sole function of distinguishing predators from male moths. Above threshold pulse rates, female lesser waxmoths prefer faster pulse rates, longer pulses and higher-amplitude calls, and these mate preferences may yield mate-choice benefits in the form of offspring of higher viability and attractiveness (Jang & Greenfield, 1996, 1998; Collins *et al.*, 1999). Instead, it is proposed that the features of the echolocation cries of aerial-hawking and gleaning bats, and their behaviour, may have selected for context-specific stimulus evaluation that influenced the evolution of ultrasonic sexual communication in lesser waxmoths (Endler, 1992). The positive correlation between the pulse-rate thresholds of individual moths for the inhibition of movement and for approach on the ground (Greig & Greenfield, unpublished) indicates that

female moths that are cautious regarding bats are also more discriminating regarding males. The benefits of predator evasion and mate choice may be synergistic.

A suggestion of serial processing of acoustic stimuli in flying lesser waxmoths is emphasized. Some moths perform half-wing beats (14% of observed responses in tethered flight). This behaviour does not appear to be evasive; instead, it may allow further stimulus evaluation. One moth that performed half-wing beats also subsequently performed flutters, both in response to 3-ms pulses delivered at a low amplitude. Are half-wing beats followed by dives if what is heard subsequently may indicate the presence of a predator? Keeping the wings above the horizontal during half-wing beats may improve sensitivity for sounds coming from below the moth, present a reduced echo profile (Roeder, 1967), or diminish lift (Dickinson *et al.*, 1999; Dickinson, 2001).

In conclusion, lesser waxmoths possess the behavioural flexibility to perform appropriate predator evasion or pair formation in response to ultrasonic stimuli with overlapping features. This flexibility is achieved by varying the features used for stimulus evaluation in different behavioural contexts. Context-specific stimulus evaluation thus allows relevant responses to the same stimulus.

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