Hawkmoths produce anti-bat ultrasound

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Bats and moths have been engaged in aerial warfare for nearly 65 Myr. This arms race has produced a suite of counter-adaptations in moths, including bat-detecting ears. One set of defensive strategies involves the active production of sound; tiger moths’ ultrasonic replies to bat attack have been shown to startle bats, warn the predators of bad taste and jam their biosonar. Here, we report that hawkmoths in the Choerocampina produce entirely ultrasonic sounds in response to tactile stimulation and the playback of biosonar attack sequences. Males do so by grating modified scraper scales on the outer surface of the genital valves against the inner margin of the last abdominal tergum. Preliminary data indicate that females also produce ultrasound to touch and playback of echolocation attack, but they do so with an entirely different mechanism. The anti-bat function of these sounds is unknown but might include startling, cross-family acoustic mimicry, warning of unprofitability or physical defence and/or jamming of echolocation. Hawkmoths present a novel and tractable system to study both the function and evolution of anti-bat defences.

1. Introduction

Aerial warfare between echolocating bats and their insect prey has escalated for nearly 65 Myr [1]. Ultrasonic ‘bat-detecting’ ears have independently evolved a minimum of 19 times in five insect orders [1]. Most insect ears are connected directly to neuronal circuits that steer the animals away from bats at low biosonar call intensities and trigger aerobatic evasive behaviours (loops, spirals and dives) at high call intensities [2]. Tiger moths (Erebidae: Arctiinae) have accelerated this arms race with an additional set of acoustic strategies. These moths have evolved paired metathoracic tymbals to create ultrasonic clicks to answer attacking bats. Empirical work supports three non-mutually exclusive functions for these acoustic signals. Sounds can startle naive bats [3], warn of unpalatability [4,5] and jam biosonar [6].

Roeder et al. [7] demonstrated almost a half century ago that choerocampine hawkmoths (Sphingidae) have bat-detecting ears. Hawkmoths are in a different superfamilly from tiger moths, and the two groups are distantly related [8]. However, sphingids are also reported to produce sounds in the audible spectrum [9–12], though the function of sound production in sphingids is unknown. Here, we provide the first evidence that some hawkmoths produce ultrasound to playback of echolocation attack. Males of at least three choerocampine species produce entirely ultrasonic defensive sounds by stridulating modified scales on the genital valves against the inner margin of the last abdominal tergum. Preliminary evidence from two of the same hawkmoth species indicates that females also produce ultrasound, but with an entirely different mechanism. Both males and females produce ultrasound in response to both tactile stimulation and to playback of bat attacks.

2. Material and methods

(a) Data collection

We sampled hawkmoths with 175 W mercury vapour lights in Malaysia (peninsular and Borneo) during March 2012 and focused on three choerocampine species, Cechenena lineosa, Theretra boisduvalii and Theretra nessus. We tethered moths in free flight at the end

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3. Results and discussion

Three closely related choerocampine species \[18\] produce entirely ultrasonic signals when touched and when stimulated with bat echolocation playback (table 1). This discovery brings

| (b) Analysis |
| We measured moth signals using Avisoft SASLab Pro and defined each cycle of the stridulatory apparatus as a modulation cycle. All signal parameters were computed from three modulation cycles per individual. We used responses from tactile trials to characterize signals to prevent corruption by overlapping bat sounds in the echolocation playback trials. To determine whether moth sounds were spectrally or temporally different when produced during tactile or playback trials, we examined several modulation cycles, and found no differences. We measured temporal parameters from the oscillogram and spectral and intensity values from power spectra (FFT 1024, 50% overlap). We converted relative intensity to peak equivalent SPL (dB peSPL re. \(2 \times 10^{-5} \mu Pa\)) using a reference tone of a known intensity \[16\] and adjusted for the frequency response of the microphone. To calculate duty cycle of the moth sounds, we divided this value by 100. We used this approach to allow a direct comparison with tiger moth acoustic analyses \[6,17\].

| Table 1. Response characteristics of three choerocampine hawkmoth species to tactile stimulation and bat echolocation playback. Time of onset to bat attack, referenced to the last biosonar cry of the playback, is included for three echolocation sequences. We measured temporal response from the end of the recording, due to higher signal-to-noise rates. Sample size \(n\) listed for onset timing note subsets of moths out of the total number that responded to one or more playbacks. Values are mean \(\pm S.D.\) indicated data collected during a separate expedition. |
| species | no. and percentage (in brackets) of response to tactile stimulation | no. and percentage (in brackets) of response to playback of bat attack | onset from end of bat attack 1, ms (n) | onset from end of bat attack 2, ms (n) | onset from end of bat attack 3, ms (n) |
| Ceratopa lineosa | 26 (100%) | 24 (92%) | 2.6 (15) | 3.3 (18) | 3.3 (18) |
| Theretra boisduvalii | 26 (100%) | 24 (92%) | 2.6 (15) | 3.3 (18) | 3.3 (18) |
| Theretra nessus | 26 (100%) | 24 (92%) | 2.6 (15) | 3.3 (18) | 3.3 (18) |

of a 5 mm diameter hollow plastic rod; a monofilament line was tied between the thorax and abdomen, threaded through the rod and held securely while the flying moth was queried for acoustic response to tactile stimulation and playback of bat attacks. We examined acoustic responses in several tethering scenarios and elected to use the above method, as it did not interfere with sound production. To record moth sounds, we used Avisoft UltraSoundGate 116 Hn hardware (sampling at 375 kHz onto a laptop computer running Avisoft Recorder software) and a CM16 condenser microphone \((\pm 3 \text{ dB}, 20–140 \text{ kHz})\) positioned 10 cm from the posterior end of the moth’s abdomen (location of the sound-producing structures). Using the same computer and software, we presented moths with three echolocation attack sequences played back via an Avisoft UltraSoundGate Player BL Pro Speaker/Amplifier \((\pm 6 \text{ dB}, 20–110 \text{ kHz})\), playback sampling rate 250 kHz) placed 10 cm from the moth’s head (location of ultrasound-sensitive ears); in order of playback, \(Lasiurus borealis\), \(Eptesicus fuscus\), and a synthetic bat attack designed to approximate the echolocation behaviour of several bat species (see the electronic supplementary material, figure S1, for details). We obtained recordings of \(Eptesicus\) and \(Lasiurus\) biosonar attacks in a flight room as bats attacked mealworms tethered 10 cm from the same microphone as above. While these bat genera are not found in Malaysia, the family (Vespertilionidae) is well represented at our sample sites. The final second of biosonar attacks is similar across bats \[12\] and as hawkmoths cannot discriminate frequency \[13,14\], the temporal and amplitude dimensions of the attack are the relevant parameters. On a subsequent expedition (April 2013), we queried three \(C.\ lineosa\) moths with a ‘Malaysian synthetic attack sequence’ created from the echolocation attack of \(Kerivoula papillosa\) (characteristics taken from fig. 1 in \[15\]) to further address issues of the ecological relevance of our playback stimuli. Each attack was approximately 700–900 ms in duration and had a peak equivalent sound pressure level (SPL) of 116 \(\pm 2 \text{ dB}\), as measured by a B&K \(\dagger\) microphone (grid off) at 10 cm \((\pm 0.5 \text{ dB}, 1–100 \text{ kHz})\). Four seconds of silence separated the playback of each attack. Voucher specimens are stored in the collection of the FLMNH, University of Florida; acoustic files are archived at the Cornell Laboratory of Ornithology Macaulay Library (accession no. ACC3006).
The specific function of anti-bat ultrasound production in hawkmoths remains unknown, but it might play a similar role as in tiger moths—to startle, warn of chemical defense or jam biosonar. It is unlikely that sphingids are warning bats of bad taste, as they do not appear to sequester host plant toxins as adults [25,26]. However, larvae of some species can sequester defensive chemicals and regurgitate gut contents containing chemical compounds in the direction of predators [27], and this might be why some sphingid caterpillars are aposematically coloured [26]. Hawkmoth adults might benefit through Batesian mimicry, as has been shown in tiger moths [5]. Sphingids, which include species that can fly up to 5 ms⁻¹ [28], could also be signalling unprofitable characteristics such as flight speed and difficulty of capture, or their pronounced tibial spines [29]. Jamming is also a viable function for hawkmoth sounds. Duty cycle, or sound production per unit time, is theoretically and empirically related to jamming efficacy. Two tiger moth species have been pitted against naive bats to experimentally test jamming and only one, Bertha
dina trigona, has been shown to jam biosonar; the duty cycle of its signal is approximately 44 per cent [6]. Euchaetes egle did not successfully avoid naive bats and has a duty cycle of approximately 3 per cent [4]. The three hawkmoth species that we tested are intermediate between these two with a duty cycle of
approximately 20 per cent. Clearly, experiments are needed to further elucidate the function of hawkmoth ultrasound.

There are anecdotal reports of audible sounds from only a few genera of hawkmoths, but these genera are widely distributed across all three subfamilies of the Sphingidae [9–12,18], arguing that sound production is deeply rooted within the history of the group. To fully understand the evolution of hawkmoth anti-bat sounds, many moth species must be tested for response to bat echolocation attack and the data correlated with phylogeny.

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