

Nocturnal activity positively correlated with auditory sensitivity in noctuid moths

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We investigated the relationship between predator detection threshold and antipredator behaviour in noctuid moths. Moths with ears sensitive to the echolocation calls of insectivorous bats use avoidance manoeuvres in flight to evade these predators. Earless moths generally fly less than eared species as a primary defence against predation by bats. For eared moths, however, there is interspecific variation in auditory sensitivity. At the species level, and when controlling for shared evolutionary history, nocturnal flight time and auditory sensitivity were positively correlated in moths, a relationship that most likely reflects selection pressure from aerial-hawking bats. We suggest that species-specific differences in the detection of predator cues are important but often overlooked factors in the evolution and maintenance of antipredator behaviour.

Keywords: antipredator behaviour; primary and secondary defences; sensory ecology

1. INTRODUCTION

Edmunds (1974) categorized antipredator behaviours into primary defences (preventing predator encounters) and secondary defences (surviving encounters). Most animals have defences that encompass both categories and they switch to secondary defences when primary defences fail (Endler 1991), but there is variation in the dependence of different species on either strategy. Studies have found that structurally (Galatowitsch & Mumme 2004) or visually (Brodie 1989) defended prey are more likely to exhibit escape behaviours than those relying on crypsis. Rarely studied, predator detection threshold may be equally important in explaining variation in defence strategy (e.g. Jabłoński & Strausfeld 2001; Matheson *et al.* 2004); to switch from primary to secondary behavioural defences, an animal must first detect the predator. Although there is substantial literature on behavioural responses of animals to predator cues (Kavaliers & Choleris 2001), these data are not directly applicable to detection threshold; animals may not demonstrate a perceptible response at the time of detection, and whether an animal responds

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depends on various factors (Broom & Ruxton 2005). Identifying thresholds is also difficult because most animals use several sensory systems for predator detection. Neural activity, however, provides a more objective measure of detection threshold.

Moths are ideal for investigating the influence of sensitivity to predator cues on defence strategy. Many moth lineages have evolved ears sensitive to the echolocation calls of insectivorous bats (Yack *et al.* 1999). Hearing is the only modality by which moths are known to detect bats, and, with few exceptions (Conner 1999), detecting bats is the sole purpose of moths' ears. The noctuid moth ear is a simple structure with one or two sensory cells depending on the family (Yack *et al.* 1999). Neural activity in response to pulsed sound is easily recorded from the auditory nerve in live preparations. When moths fly at night, they are susceptible to predation by bats (Roeder 1998). Upon hearing bat echolocation calls, eared moths perform avoidance manoeuvres (secondary defences) such as flying away from a quiet, distant bat or diving to the ground in response to a loud, close bat (Roeder 1998). Earless moths compensate for their inability to detect bats using primary defences; they fly less, closer to vegetation and more erratically than eared moths (reviewed in Fullard 1998). Within eared moths, there is a range of sensitivity to ultrasound, which could translate into variation in the reliance on primary or secondary defences. We predicted that the amount of time eared moths fly at night was correlated with their threshold for detecting the echolocation calls of bats.

2. MATERIAL AND METHODS

(a) Auditory neurophysiology

We collected moths and conducted our experiments from June to August 2006 and 2007 at the Queen's University Biological Station (QUBS), Chaffey's Lock, Ontario, Canada. Moths were decapitated (as in Fullard & Dawson 1999 and Surlykke *et al.* 1999), which renders the moth quiescent and removes the possibility of a shift in tympanal response due to acoustic stimulation (Windmill *et al.* 2006), and fixed ventral side up to modelling clay using metal struts with the wings spread to reveal the ears. Following thoracic dissection, a stainless steel electrode was hooked onto the exposed auditory nerve and a reference electrode inserted into the abdomen. Neural activity was amplified (Grass P15 AC amplifier) and displayed online using a data acquisition board and oscilloscope emulating software (PicoSCOPE v. 5.10.7, Pico Technology). Sound pulses were generated by a MATLAB application and delivered to the moth preparation via a high-speed data acquisition board (National Instruments, BNC 2110), amplifier (Avisoft Bioacoustics, model 70101) and speaker (Technics leaf tweeter, EAS 10TH400B), positioned 30 cm from the moth's ear. Pulses were 10 ms in duration (plus a 1 ms rise/fall time) and produced every 500 ms at a sampling rate of 500 kHz.

We generated audiograms (threshold–response curves) by broadcasting randomized frequencies from 5 to 100 kHz at 5 kHz increments and increasing the amplitude for each frequency until pulses consistently elicited one or two action potentials from the auditory nerve. The voltage produced by the amplifier at this intensity was converted to sound intensity (dB peak equivalent SPL) by calibrating the speaker to continual tones with a Brüel and Kjaer 6.35 mm condenser microphone (type 4135) and measuring amplifier (type 2610). We took two measurements from each audiogram: (i) best threshold (lowest intensity in dB to elicit a neural response) and (ii) overall sensitivity (area between the horizontal line set at 90 dB and the response curve (pascals×kHz), see Fullard 1982 for details; figure 1). Table 1 provides a list of species included in this study with data values and the sources of data. Data were the means for each species. Log-transformed data for overall sensitivity explained a significant amount of variation in best threshold ($r^2=0.49$, $F_{1,15}=14.46$, $p<0.002$). We feel the former is a more ecologically relevant indicator of sensitivity to the

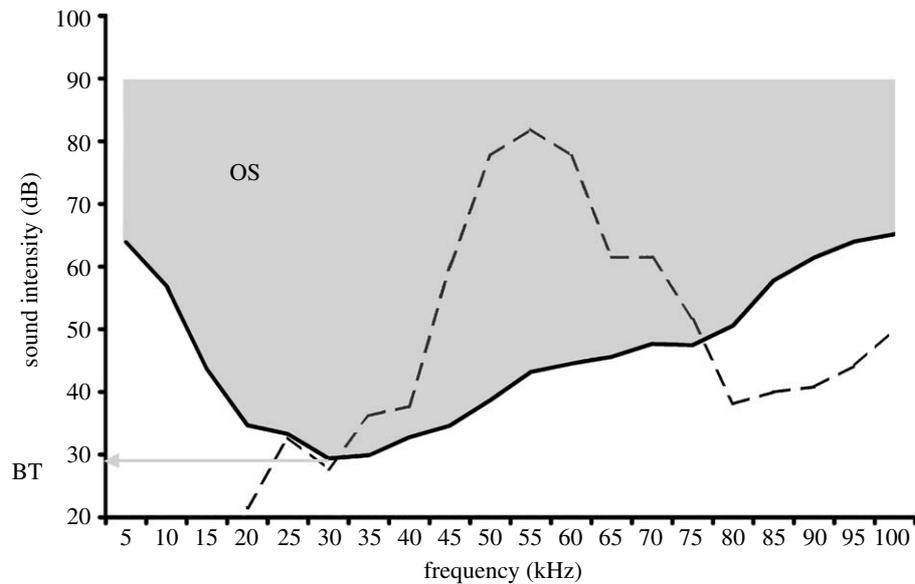


Figure 1. Audiogram of *Caenurgina erechtea* (solid line, absolute dB peSPL) and power spectrum of a search phase echolocation call from the bat, *Myotis lucifugus* (dashed line, relative dB, J. M. Ratcliffe 2001, unpublished data). OS, overall sensitivity (shaded area); BT, best threshold (arrow).

Table 1. Mean values per moth species for measures of auditory sensitivity, size and nocturnal activity. (BT, best threshold; OS, overall sensitivity; BF, best frequency; SA, surface area; NFT, nocturnal flight time; Pa, pascals.)

species	BT (dB)	OS (Pa*kHz)	BF (kHz)	<i>n</i>	source	SA (mm ²)	NFT (%)
Arctiidae							
<i>Ciseps fulvicollis</i>	33.9	60.5	30	5	this study	191.1	41.0
<i>Ctenucha virginica</i>	34.6	50.4	30	5	Fullard & Dawson (1999)	413.6	10.8
<i>Cyenia tenera</i>	52.0	53.6	50	5	Fullard & Dawson (1999)	357.5	7.4
<i>Hypercompe scribonia</i>	39.1	59.6	25	5	this study	795.3	33.2
<i>Phragmatobia fuliginosa</i>	40.1	57.4	50	2	this study	275.8	8.3
Lymantriidae							
<i>Lymantria dispar</i>	45.9	54.7	50	5	Fullard & Dawson (1999)	551.1	10.7
Noctuidae							
<i>Acrionicta americana</i>	33.0	59.3	35	10	Surlykke et al. (1999)	894.1	24.0
<i>Amphipyra pyramidoides</i>	30.0	59.4	25	10	Surlykke et al. (1999)	648.9	18.5
<i>Anagrapha falcifera</i>	28.3	59.1	20	3	this study	358.2	28.4
<i>Caenurgina erechtea</i>	27.7	59.9	30	10	Surlykke et al. (1999)	514.5	26.0
<i>Catocala cerogama</i>	27.7	60.2	30	10	Surlykke et al. (1999)	1727.6	24.8
<i>Feltia jaculifera</i>	33.8	58.7	30	5	this study and Surlykke et al. (1999)	368.1	14.9
<i>Panthea furcilla</i>	42.0	52.5	30	10	Surlykke et al. (1999)	394.8	8.9
Notodontidae							
<i>Clostera albosigma</i>	46.6	53.6	40	5	this study	328.2	3.1
<i>Datana contracta</i>	49.2	53.4	50	5	this study	512.5	7.7
<i>Nadata gibbosa</i>	45.4	54.7	35	5	this study	547.3	8.2
<i>Peridea angulosa</i>	47.0	54.2	35	5	this study	523.6	10.3

echolocation calls of sympatric bats because it incorporates data on thresholds for all frequencies (Fullard 1982). Therefore, we consider only overall sensitivity here.

(b) Nocturnal flight time

We calculated nocturnal flight time (percentage of the night each species was active) using original data for actograms published by Fullard & Napoleone (2001). Briefly, five moths per species (three for *Clostera albosigma*) were videotaped under ambient light conditions in individual cages for 24 hours. Moths were scored each minute as either in-flight or stationary; per cent activity equals in-flight minutes divided by the total number of minutes of night.

(c) Potential confound: surface area

Surlykke et al. (1999) showed that noctuid moths of larger surface area have lower best thresholds; to control for potential effects in

our sample, we measured the surface area for five individuals of each species or used the values of Surlykke et al. (1999). Scanned images of spread moths were imported into IMAGE J (v. 1.38) and the surface area (mm²) was calculated for each individual and averaged for each species.

(d) Comparative analyses

For all analyses, we used log-transformed data (to create linear relationships) of three measurements: overall sensitivity; surface area; and per cent nocturnal flight time. To control for the possible effects of surface area on overall sensitivity for specific species, we also took standardized residuals of overall sensitivity versus surface area. For comparisons at the species level and using independent contrasts (Felsenstein 1985) as implemented by the Crunch procedure in CAIC v. 2.6.9 (Purvis & Rambaut 1995), we ran linear regressions of (i) flight time versus surface area, (ii) flight

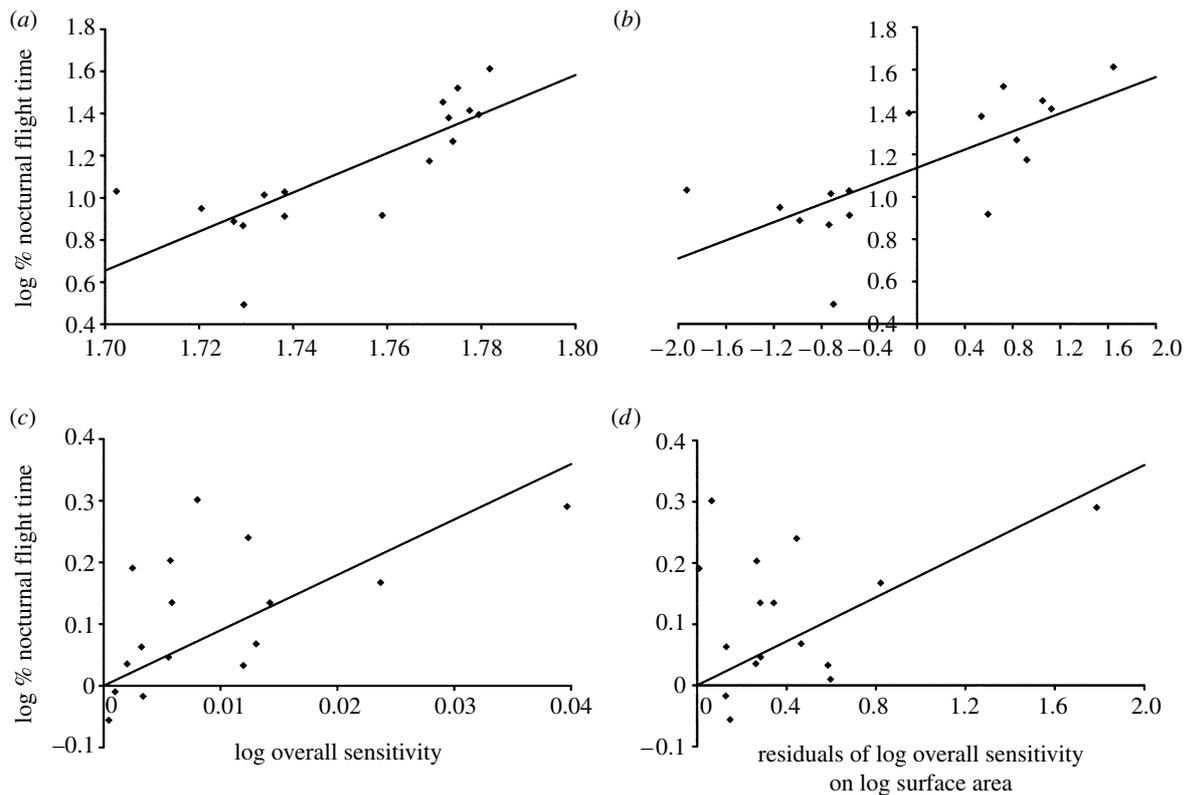


Figure 2. Per cent nocturnal flight time in relation to overall sensitivity. (a,b) Species data ($r^2=0.62$, $p<0.0002$; $r^2=0.52$, $p<0.002$) and (c,d) PICs ($r^2=0.62$, $p<0.0002$; $r^2=0.46$, $p<0.003$). Analyses displayed in (b,d) used standardized residuals to control for potential effect of size on sensitivity.

time versus overall sensitivity, and (iii) flight time versus overall sensitivity residuals (i.e. values independent of the surface area). For independent contrast analyses carried out using CAIC, we used a composite phylogeny (see the electronic supplementary material) based on the molecular phylogeny of Mitchell *et al.* (2006) for inter-familial relationships and those of Weller *et al.* (1994), Jacobson & Weller (2002) and DaCosta & Weller (2005) for species-level relationships not resolved by Mitchell *et al.* (2006). Owing to differences in the methods used to construct these phylogenies, branch lengths were set as equal.

3. RESULTS

Both species-level and independent contrast-level analyses produced similar results (figure 2). Per cent nocturnal flight time was not significantly related to surface area (species level (SL): $r^2=0.07$, $F_{1,15}=1.08$, $p=0.315$; phylogenetically independent contrasts (PICs): $r^2=0.05$, $F_{1,15}=0.87$, $p=0.367$). Per cent nocturnal flight time was significantly and positively related to overall sensitivity (SL: $r^2=0.62$, $F_{1,15}=24.88$, $p<0.0002$ (figure 2a); PIC: $r^2=0.62$, $F_{1,15}=24.24$, $p<0.0002$ (figure 2c)). This positive relationship between flight time and sensitivity held after controlling for surface area (SL: $r^2=0.52$, $F_{1,15}=16.06$, $p<0.002$ (figure 2b); PIC: $r^2=0.46$, $F_{1,15}=12.62$, $p=0.003$ (figure 2d)). One of the independent contrasts is an outlier (0.3, 0.04 (figure 2c) 0.3, 1.8 (figure 2d)), but the regressions remain significant with this contrast removed ($r^2=0.54$, $F_{1,14}=16.57$, $p<0.002$ (figure 2c); $r^2=0.32$, $F_{1,14}=6.47$, $p=0.02$ (figure 2d)).

4. DISCUSSION

Our results demonstrate that moths' echolocation call detection threshold, before and after controlling for

both the surface area and putative evolutionary relationships between species, is positively correlated with nocturnal flight time. We suggest that this relationship reflects predation risks associated with flying in search of mates while insectivorous bats are foraging. Although an increase in predation pressure should select for greater sensitivity to predator cues, moths face morphological constraints on auditory sensitivity (Surlykke *et al.* 1999), which may instead select for greater reliance on primary behavioural defences. While our study does not explicitly test for causal relationships, it does highlight the relationship between detection thresholds and primary and secondary behavioural defences in prey. Moths with less sensitive ears appear to rely more on primary behavioural defences such as those used by earless species (e.g. reduced flight time: Fullard 1998), whereas those with more sensitive ears appear to rely more on their ability to initiate effective anti-bat flight behaviours.

Comparative studies specifically relating predator detection threshold to associated antipredator behaviour are rare due to difficulties in measuring neural thresholds and controlling for alternate sensory modalities of detection; those that have, however, report results similar to our own. For example, Jabłoński & Strausfeld (2001) demonstrated that in response to a visual stimulus representing an approaching predator, fly species with shorter and greater diameter giant descending neurons (GDN) initiate escape responses earlier than those with longer and narrower GDNs. Matheson *et al.* (2004) found that the descending contralateral movement detector, an interneuron that responds to visual stimuli, habituated faster in solitary

phase desert locusts (*Schistocerca gregaria*), which are cryptic and tend to move less, than in gregarious phase locusts. Over evolutionary time, differences in predator detection thresholds could evolve in concert with, or drive/be driven by, differential primary anti-predator defensive behaviours.

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