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Auditory Relationships to Size in Noctuid Moths: Bigger Is Better

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Since large targets reflect stronger echoes than small ones, bats should first detect moths with large wing and body surface areas (SAs) at greater distances than moths with small SAs.

We hypothesized that since the increased conspicuousness of large moths reduces their safety margin over bats, their ears would compensate by being more sensitive. The sensitivity and frequency tuning of ears of noctuid moths sampled from Canada and Denmark depend significantly

on SA, with larger moths possessing lower thresholds and lower best frequencies. The echo intensities from artificial echolocation pulses reflected from moths predict that, although large moths will be detected by bats at greater distances, their greater auditory sensitivity ensures them a sufficient safety margin. The increased sensitivity of large moths is not simply a by-product of their greater ear size since tympanal radius increases less with SA than that of a nonauditory body part. This suggests that some auditory constraint (e.g., frequency tuning) limits the maximum size to which ears (and perhaps moths) can reach.

Noctuid moths use a pair of tympanal ears to detect the echolocation calls of hunting bats, encoding their intensities as a cue to the closeness of the bat (Roeder 1967). These ears are serviced by only two receptor cells, and simple electrophysiological techniques make it possible to measure

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this insect's total auditory defense. Since moths are slower fliers than bats, their ears must sufficiently alert them to an approaching bat (by detecting its echolocation pulses) before the bat is aware of the moth (by detecting its echo) to allow the moth a detection distance safety margin (Roeder 1966). Bats use detectable sonar echoes as a cue to the presence of a potential target (Møhl 1988), and moths with large surface areas (SAs) should therefore be detected at greater distances (Forrest et al. 1995), thus reducing their safety margin. We tested the hypothesis that large moths compensate for their increased detectability by possessing ears that are more sensitive than those of small moths. To test whether this relationship exists in geographically independent communities we conducted separate studies in two disparate temperate locations, Canada and Denmark. The following noctuids [sample size, mean surface area (mm²)] were collected from wild populations at ultraviolet lights in Canada at the Queen's University Biological Station in eastern Ontario: *Idia aemula* (6, 151.7), *Lithocodia carneola* (1, 158.3), *Lacinipolia renigera* (4, 198.3), *Ochropleura plecta* (9, 253.4), *Chytonix palliatricula* (8, 253.8), *Leuconycta diphteroides* (1, 285.9), *Raphia frater* (10, 353.4), *Acronicta inclara* (10, 360.6), *Feltia jaculifera* (2, 368.1), *Panthea furcilla* (10, 394.8), *Charadra deridens* (1, 418.0), *Colocasia propinquila* (8, 476.1), *Acronicta laetifica* (3, 484.6), *Feltia subgothica* (4, 487.2), *Acronicta innotata* (8, 488.1), *Caenurgina erechtea* (10, 514.5), *Panopoda rufimargo* (5, 555.4), *Amphipyra pyramidoides* (10, 648.9), *Panopoda carneicosta* (6, 650.9), *Acronicta americana* (10, 894.1), *Catocala coccinata* (1, 1211.5), *Catocala concumbens* (1, 1726.6), *Catocala cerogama* (10, 1727.6), *Catocala palaeogama* (1, 1845.4), *Catocala ilia* (10, 2082.3), and *Catocala cara* (1, 2085.5). The following were collected in Denmark on the island of Fyn near Odense University: *Oligia versicolor* (1, 219.3), *O. strigilis* (1, 219.3), *Hadena compta* (5, 290.3), *Axylia putris* (5, 333.2), *Caradrina morpheus* (8, 369.3), *Euxoa tritici* (4, 396.0), *Noctua janthe* (6, 497.3), *Xestia c-nigrum* (4, 515.3), *Noctua orbona*

(3, 645.8), *Polia bombycina* (5, 710.5), *Noctua comes* (7, 716.0), *Apamea monoglypha* (4, 749.8), *Amphipyra pyramidea* (5, 917.0), *Noctua pronuba* (9, 969.6), *Noctua fimbriata* (6, 1038.8), *Catocala nupta* (1, 1824.0), and *Catocala fraxini* (2, 3030.0). Various subfamilies were sampled to increase the phylogenetic diversity of our data base.

Moths were decapitated and placed ventrum up while their auditory nerves were exposed, rinsed with saline, and hooked onto stainless steel electrodes. Receptor responses were amplified with Grass P15 preamplifiers (Canada) or custom-built amplifiers (Denmark). Stimuli were generated with a WaveTek Function Generator, shaped into 10-ms pulses at 2 s, amplified, and broadcast from a Technics EAS-10TH400B leaf tweeter (Canada) or with a custom-built computer system to generate 8-ms sound pulses (0.5 ms rise/fall) which were delivered into custom-built power amplifiers and 60 mm electrostatic loudspeakers (Denmark) 30 cm from the preparation. Moth surface areas were measured by first scanning them after they had been spread as for voucher specimens and then analyzing the images with morphometric software [Canada: HP PicturePlace (Hewlett-Packard), SigmaScan (Jandel Scientific); Denmark: VISION-plus Overlay Frame Grabber (Imaging Technology), GIPS software (Image House)]. Tympanal areas were measured on 22 of the 26 Canadian moths included in this study (sample sizes from one to eight specimens) by importing video-grabbed stereomicroscope images of the ears (SNAPSHOT, Cardinal Technology) into the above software.

Figure 1a illustrates the most sensitive species audiograms of the Canadian and Danish noctuid moths tested. The audiograms are similar to those reported from moths in other locales (Surlykke and Gogala 1986; Fullard 1988; Fullard et al. 1997) in their absolute sensitivities (as indicated by their best thresholds, BTs). To test the relationship between SA and auditory sensitivity we applied linear regressions of mean BTs onto the mean log-transformed SAs of each species sampled. BTs ranged

from 26.0 to 51.3 dB SPL (Canada) and 23.4 to 37.8 dB SPL (Denmark) and were significantly inversely related to SAs in Both locations (Fig. 1b). We expanded our estimation of auditory sensitivity to include broader regions of the audiogram (e.g., bandwidths at BT plus 5, 10, and 20 dB), and in all cases regressions were highly significant. We also examined the relationship of frequency tuning and SA by regressing species mean best frequencies (BFs) onto SAs. BFs ranged from 24.0 to 51.3 kHz (Canada) and 18.3 to 35.0 kHz (Denmark) and also revealed significant regressions on SAs (Fig. 1c). Nonparametric analyses (Spearman's rank correlations) were also performed on the data and in all cases there were significant correlations of mean BTs and BFs with log-transformed SAs.

Our results demonstrate that large moths possess ears that are both more sensitive and tuned to lower frequencies than those of small species and is the first report of the influence of body size on auditory receptor sensitivity in an insect. The first question that arises from our results is whether the greater auditory sensitivity of large moths is sufficient to offset their increased conspicuousness. To test this, we first estimated the effects of SA on detectability by measuring the echo strengths of artificial echolocation pulses at 10, 30, and 100 kHz reflected from the dorsal surfaces of freshly killed moths. Echo strengths were measured on all the Danish moths included in this study (sample sizes from two to eight specimens) The wings were spread as for voucher specimens and the moth was placed with its dorsal surface 22 cm from a loudspeaker (>20 kHz: custom built electrostatic; ≤20 kHz: Vifa D26NC). The position of the moths was adjusted carefully to reflect the maximum echo corresponding to the "glint" from a flying insect when its wings are perpendicular to the sound path (Schnitzler et al. 1983). Echoes were recorded with a 0.25 in. Brüel and Kjær (B&K) microphone (type 4135) 22 cm from the moth in the same orientation as the loudspeaker, preamplified (B&K type 2633) and amplified (B&K type 5935). The

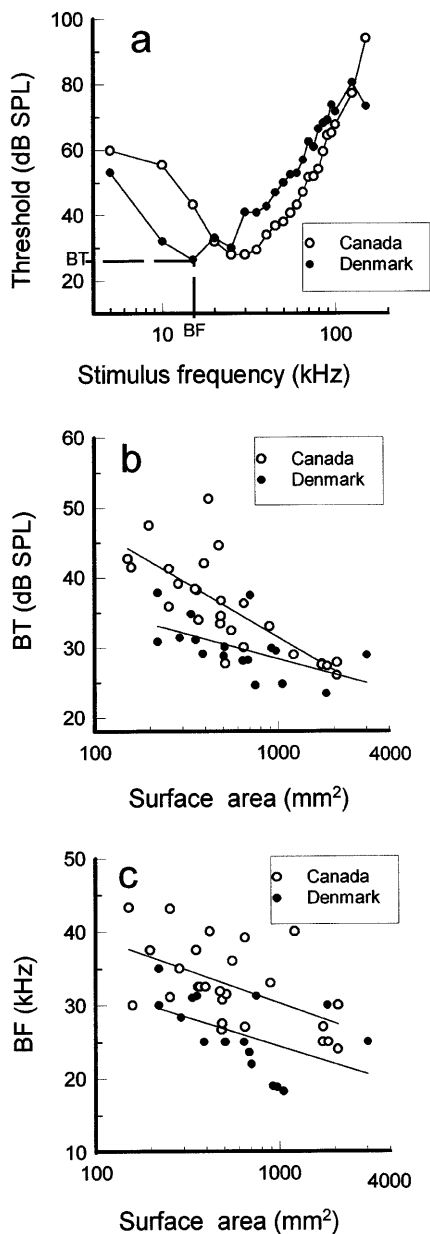


Fig. 1. a) Mean audiograms of the most sensitive species of Canadian (*Catocala ilia*) and Danish (*Noctua fimbriata*) noctuid moths. Thresholds were determined as sound intensities (dB SPL) that elicited three receptor action potentials per stimulus pulse for four consecutive pulses. *BT*, Best threshold (lowest stimulus intensity required to elicit threshold); *BF*, best frequency (the stimulus frequency eliciting BT). b) Linear regressions of species best threshold means onto log transformed (to achieve normality) species SA means. Canada: $BT = 87.3 - (18.4 \times \log SA)$, $R^2 = 0.61$, $P < 0.001$; Denmark: $BT = 49.2 - (6.9 \times \log SA)$, $R^2 = 0.31$, $P < 0.05$. c) Linear regressions of species best frequency means onto log transformed species SA means. Canada: $BF = 57.8 - (9.2 \times \log SA)$, $r^2 = 0.30$, $P < 0.01$; Denmark: $BF = 75.9 - (18.5 \times \log SA)$, $R^2 = 0.67$, $P < 0.001$

Next we used the target strengths to estimate the bat's detection distances (DDs, the distances at which the bat first detects the moth's echo) to moths of various SAs by iterating the distances that would produce echoes of 20 dB (the assumed detection threshold, DT, including noise, detectable by the bat). DDs for the bat were calculated using a source level (SL) of 110 dB SPL (the intensity of the echolocation pulse at 10 cm in some common bat species), the target (echo) strength (TS) from the values in Fig. 2a, an atmospheric attenuation factor (atm. att.) at 25 kHz of 0.492 dB/m (70% RH; (Evans and Bass 1972; Surlykke 1988) and the formula:

$$20 \text{ dB DT} = \text{SL} + \text{TS} - 2(\text{TL}) - \text{noise}$$

(noise set to 0, included in DT),

where TL is the transmission loss due to spherical spreading and atmospheric attenuation. Since we used dorsal (maximum) areas for size and echo measurements, we were likely to obtain maximum estimates of the detection distances. Figure 2b illustrates that the estimated DD of the bat to the Danish moths in our study was between 4.6 m and 9.5 m, which is higher than the values observed for bats in the field (Kalko and Schnitzler 1989; Surlykke et al. 1993) but within those reported for laboratory-tested bats (Kick 1982) and those predicted for other bats (Waters et al. 1995). Finally we estimated the moths' detection distances to bats. We calcu-

lated the DDs as the distance at which the one-way transmission loss at the moth's BF is equal to the difference between the bat's source level (110 dB SPL) and the moth's BT. Figure 2b shows that Danish moth DDs range from 26.7 to 92.7 m, values also higher than those reported from field studies (Roeder and Treat 1961; Roeder 1966). The discrepancy between predicted and field-observed distances may arise from differences between the obstacle-free sound path assumed for theoretical calculations compared to the cluttered environment of natural bats and moths. The regression line of the differences between moth and bat DDs is included in Fig. 2b and represents the safety margin that a moth has over the bat in detection distance. Although the absolute DDs are probably overestimates, it is likely that the relative differences between DDs for large and small moths are fairly realistic since DDs are biased in the same direction for moths of all sizes. As expected, the safety margin remains in favor of the moth regardless of its SA, but, unexpectedly, the safety margin is not constant, indicating that large moths have greater absolute distances to escape bats. One possible explanation is that large moths may be less maneuverable. Besides, at longer absolute distances where larger moths are detected the sound beam emitted by the bat is wider. Therefore large moths may have to get further off from the bat's flight path to remain undetected.

The second question that arises from our results is whether the greater sensitivity of large moths is an evolved adaptation or merely an epiphenomenal by-product of their size. If the simple tympana of moths behave as microphones (Brüel & Kjaer Handbook, 1996), high SA moths would be more sensitive only because their ears are larger (although this would not necessarily rule out an adaptive advantage). To test this, we computed the radii from the measured tympanal areas of 22 of the Canadian species that were used in the auditory analyses. Figure 2c describes that tympanal radius increases with SA with a significantly lower slope than the length of a non-auditory sclerite (scu-

sound pulses (generated by the same apparatus as for the audiograms) were only 1.2 ms long to avoid pulse/echo overlap. Target strengths were calculated as the echo intensity at a standard distance of 0.1 m relative to the incident sound intensity on the target (Fig. 2a). As expected, larger moths reflect stronger echoes predicting that for a bat echolocating at 25–30 kHz (i.e., a typical temperate aerial forager), the largest moths we tested return echoes that are 10–15 dB more intense than those of the smallest species.

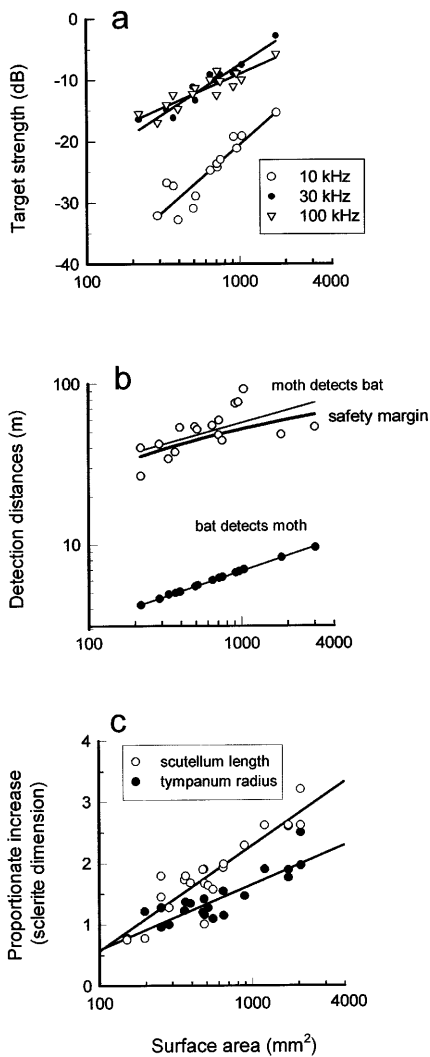


Fig. 2. a) Linear regressions of target strength (0.1 m) at various pulse frequencies onto log transformed species SA means of Danish moths at 10 kHz, 30 kHz, and 100 kHz. b) Predicted detection distances (DDs) for a bat echolocating at 25 kHz to the Danish moths used in our study [$DD = -6.8 + (4.6 \times \log SA)$, $r^2 = 0.99$, $P < 0.001$] compared to the detection distances of the moths to the bat [$DD = -29.6 + (29.5 \times \log SA)$, $R^2 = 0.3$, $P < 0.05$]. The regression of the differences between the values of the two detection distances is plotted as the safety margin the moths have over the bat. c) Linear regressions of the proportionate increase in species mean tympanal radii (TR; computed from measured areas) and a nonauditory sclerite (scutellum) lengths (SLs) onto the proportionate increase in species mean SAs of the Canadian moths used for auditory analyses. $TR = 0.9 + (0.1 \times \log SA)$, $r^2 = 0.88$, $P < 0.001$; $SL = 1.2 + (0.1 \times \log SA)$, $r^2 = 0.80$, $P < 0.001$; the slopes of the regressions differ significantly ($t = 2.2$, $P < 0.05$)

tellum) suggesting that the interspecific increase in ear size is constrained by some factor which does not influence nonauditory body parts. Since size affects tuning as well as sensitivity (Fig. 1b; Brüel & Kjaer Handbook 1996), very large ears entail potential costs in the form of maladaptive tuning if they are to increase at the same rate as nonauditory sclerites. To remain effective at detecting bats, moth ears must preserve an adequate tuning to bat-relevant frequencies (10–50 kHz; Fullard 1988) and are restricted from becoming too large, for they would otherwise lose the advantage derived by their increased sensitivity. The result of this constraint has been the evolution of variously sized moths with ears that, while sensitive enough to maintain an advantage over the detection abilities of bats, remain appropriately tuned to the echolocation assemblage of those bats.

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