

Beware of bats, beware of birds: the auditory responses of eared moths to bat and bird predation

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The allotonic frequency hypothesis (AFH) proposes that the preponderance of moths in the diets of some bats (e.g., Rhinolophidae) is the result of these bats echolocating at allotonic frequencies, that is, outside of the typical hearing range of most moths (ca., 20–60 kHz). The broader hearing range of African moths (5–110 kHz) suggests that their ears may function at frequencies usually considered allotonic. We investigated 1) whether moth ears were functionally audible to the Cape horseshoe bat, *Rhinolophus capensis* (Rhinolophidae), which forages in dense vegetation and echolocates at 84 kHz, and 2) whether moth auditory sensitivity below 10 kHz allows them to detect the rustling noises made by bird predators as they pursued moths through vegetation. The calls of *R. capensis* were audible to moths albeit over shorter distances relative to syntonic bats. Shorter detection distances combined with the constrained spaces in the cluttered habitat in which rhinolophids forage give moths both less time and less space within which to react to an attacking bat. Thus, the AFH in combination with habitat offers a better explanation for the preponderance of moths in the diets of rhinolophids than either of them on their own. Moths also responded both neurologically and behaviorally to the rustling sounds made by birds (Cape Bulbul, *Pycnonotus capensis*) as they pursued moths. We suggest that the high sensitivity of moths to frequencies from 5 to 10 kHz allows them to avoid these avian attacks by using responses that have traditionally been considered solely anti-bat behavior. *Key words*: allotonic frequency hypothesis, audition, bats, birds, moths, predation. [*Behav Ecol* 19:1333–1342 (2008)]

Coevolution, in the strictest sense of the word, has occurred when a trait in one species has evolved in response to a trait in another and the trait in the second species also evolved in response to the trait in the first (Janzen 1980; Futuyma 1986). This results in reciprocal selection pressure being exerted on all partners in the interaction. Coevolution is likely to occur in systems where the same cue is used by the predator to detect prey and by the prey to detect that predator, for example, the use of echolocation calls by bats to detect moths through reflected echoes and the use of the bats' calls by moths to detect bats. The interaction between bats and tympanate moths is often cited as an example of a coevolutionary arms race between predator and prey (Fenton and Fullard 1979; Fullard 1988; Rydell et al. 1995; Miller and Surlykke 2001). With many species of tympanate moths and many species of bats putatively involved in this arms race, it may be more accurately described as diffuse coevolution (Rothstein 1990; Rydell et al. 1995).

There is much evidence to support the conclusion that ears exist in moths primarily for the purpose of detecting bats (Fullard 1988). For example, ears have evolved in moth species that make no sounds of their own (i.e., they cannot fulfill a communication role—Fullard 1987; Waters 2003), and tympanate moths respond to bat echolocation calls by taking evasive action (Roeder 1967). At local spatial scales, the sensitivities of moth ears reflect the echolocation frequencies of sympatric bats (Fenton and Fullard 1979; Fullard 1982,

1987). African moths, for example, are sensitive to a wider range of frequencies (5–110 kHz) than North American moths of the same family (20–60 kHz) as a result of being exposed to bat assemblages of higher diversity and thus wider ranges of echolocation frequency (Fullard 1982, 1987). However, sensitivity does decrease at frequencies above 65 kHz, and most moths are functionally deaf at frequencies higher than 100 kHz (Fenton and Fullard 1979) supporting Novick's (1977) idea that higher than normal frequencies permit bats to increase their hunting success on eared moths. Such frequencies are called allotonic (other tone) because they fall outside of the hearing range of most moths (Fullard 1988). The allotonic frequency hypothesis (AFH) takes this relationship one step further and proposes that the evolution of tympanate organs has exerted selection pressure on bats resulting in the evolution of strategies that counter the auditory defenses of moths, completing the reciprocity required by coevolution.

The AFH provides a potential explanation for the preponderance of moths in the diets of high-frequency echolocating bats such as the Rhinolophidae and Hipposideridae (Jones 1992). It has received neurophysiological (Fullard and Thomas 1981), species-specific (Fullard and Dawson 1999), and community-level support (Jacobs 2000; Schoeman and Jacobs 2003; Pavey et al. 2006). However, if African moths have greater hearing sensitivities above the syntonic range of their North American counterparts, as a result of predation by bats that use higher frequency calls (Fullard 1982, 1987), it suggests that allotonicity is geographically dependent and African moths may be able to respond to the ostensibly allotonic frequencies of rhinolophid bats with enough time to avoid capture. Ears with hearing sensitivities at higher frequencies would favor moths that have them, increasing their proportions in

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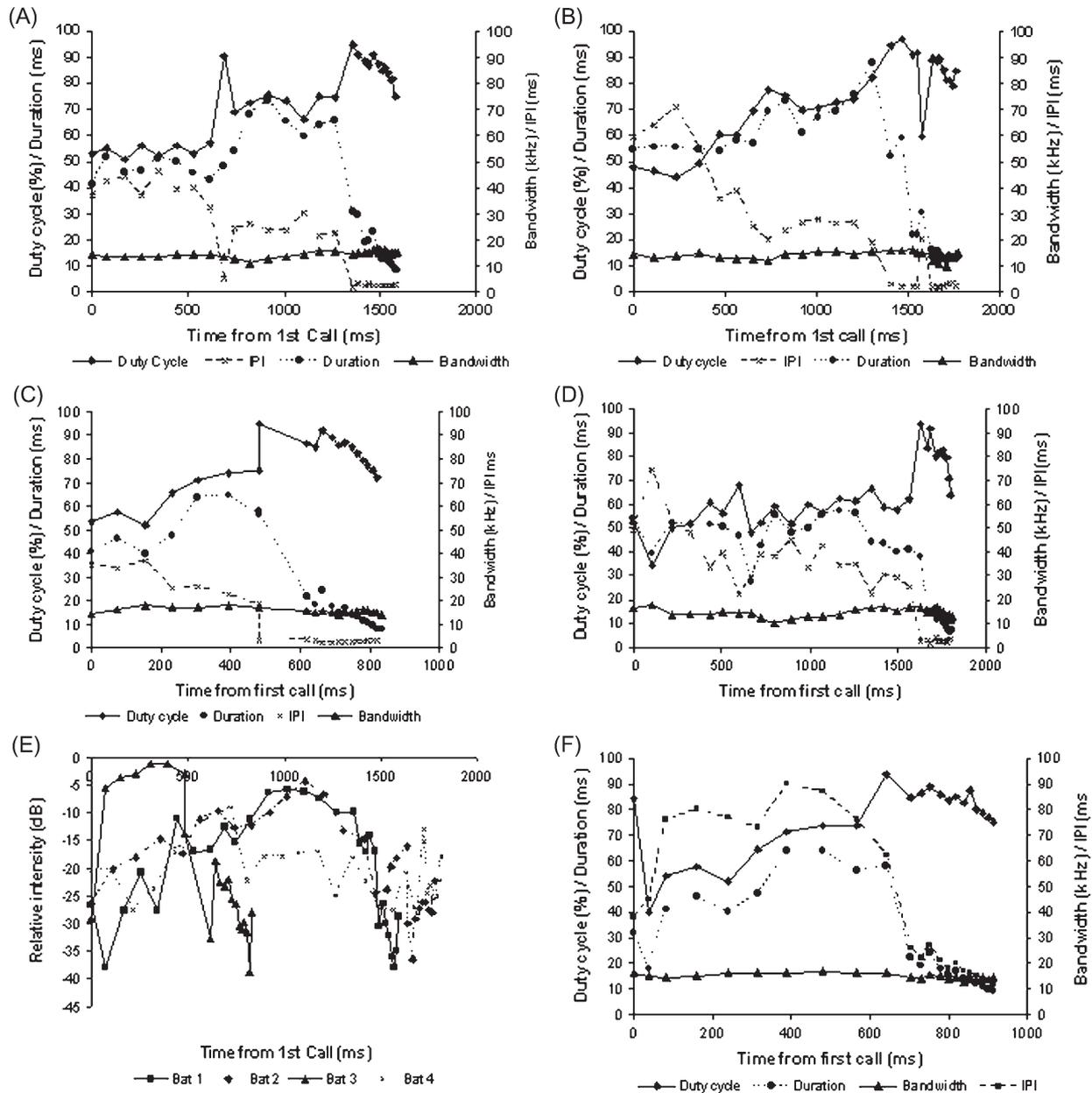


Figure 1 (A–D) Changes in the echolocation parameters of 4 *Rhinolophus capensis*; (E) changes in relative echolocation intensity for the same 4 bats during their attacks on tethered moths and (F) an attack sequence recorded in the field.

the population. Thus, some bats using high-frequency echolocation calls may be only partially allotonic to such moths, that is, the high-frequency calls are still audible to moths but at reduced distances. If so, the AFH alone may not explain the preponderance of moths in the diets of bats like the Rhinolophidae.

Unlike many bats, horseshoe bats (family Rhinolophidae) are clutter tolerant, that is, they detect and capture their insect prey within dense vegetation (Heller and von Helversen 1989; Jacobs et al. 2007). Whereas most bat species worldwide determine target distance by separating call and returning echoes in time, rhinolophids—which are found only in the Old World—do so using slight differences between emitted call frequencies and the frequency of the returning echoes (Doppler-shift compensation) (Schnitzler 1973). As a result, rhinolophid bats are able to produce calls more than 50% of

the time (see Figure 1) and are not susceptible to the backward masking that other bats may experience when call and returning echoes overlap in cluttered environments (Schnitzler and Kalko 2001). Furthermore, most rhinolophid species use call frequencies higher than typical of bats in general (Jones 1999; Jacobs et al. 2007) and may thus circumvent the auditory defenses of insects with bat-detecting ears tuned to lower frequencies representing the average echolocation call range of sympatric insectivorous bats.

To date, the sensory neural responses of moth ears to the echolocation calls of an aerial hawking attack have only been demonstrated using playbacks of the common North American big brown bat, *Eptesicus fuscus*, a species which typically produces calls less than 10% of the time (a low-duty cycle bat) and does so using calls well within the peak auditory sensitivity of both North American and African moth species (Fullard

et al. 2003). Here we investigate the AFH as an explanation for the preponderance of moths in the diets of rhinolophids using *Rhinolophus capensis*, a high-duty cycle bat (45–90% duty cycle) calling at 84 kHz (Jacobs et al. 2007), a frequency expected to be at the periphery of sympatric moths' peak hearing sensitivity (Fullard and Thomas 1981). Attack sequences from a rhinolophid, where the bat's position and distance relative to the microphone were known, did not exist until now. We use these attack sequences to determine the auditory responses of 2 moth species, the noctuid, *Helicoverpa armigera*, and the notodontid, *Desmeocraera griseiviridis*, to a bat which uses what we expected were allotonic frequencies.

The hearing sensitivity of certain African moths to frequencies below 20 kHz raises the possibility that bats may not be solely responsible for the hearing sensitivities of moths at these low frequencies. Diurnal moths may be exposed to bird predation, and moth audition may have responded to sounds (e.g., rustling of vegetation) that bird predators make as they hunt for moths. If so, these sounds should fall within the 5–20 kHz frequency range (Bell 1982; Fullard 1988). The influence of non-bat predators on moth hearing at these low frequencies would be hidden in environments where sympatric bats also echolocate at these low frequencies but may be evident in bat-free environments. For example, in the bat-free oceanic islands of French Polynesia, the ears of endemic moths show auditory degeneration by being less sensitive to high-frequency sounds (>30 kHz) than adventive (recently arrived) species but retain high sensitivity at the lower frequency range (<20 kHz; Fullard 1982; Fullard et al. 2004; Fullard et al. 2007). Similarly, although the ears of endemic noctuid moths on the island of Kaua'i in the Hawaiian archipelago are sensitive to the peak echolocation frequency (27.8 kHz) of the single bat species that occurs there, the endemic *Lasiurus cinereus semotus*, the ears of these noctuids are most sensitive at 15 kHz (Fullard 1982, 1987). Fullard (1982, 1987) ascribes this to these moths listening to the social calls of *L. cinereus semotus* (9.6 kHz), but it could instead be a defense against predation from the rich avian fauna on the Hawaiian Islands, a function for which it was not originally adapted or selected (an "exaptation" sensu Gould and Vrba 1982). Thus, moth ears that arose in the context of bat predation may now also function against bird predation. We thus also investigated the potential benefits incurred by moth hearing for the detection of gleaning, diurnal insectivorous birds.

MATERIALS AND METHODS

This study was done in De Hoop Nature Reserve (34°26'S/20°25'E) near Bredasdorp, Western Cape, South Africa, in November 2006. Bats were captured in mist nets placed about 5–10 m from the entrance to Hothole cave, a sink hole within which bats roosted. The dominant vegetation in the reserve surrounding the cave is coastal fynbos (low growing, sclerophyllous vegetation) dominated by restios (*Chondropetalum* spp.) with a maximum height of approximately 2 m. The dominant flowering plant was *Agathosma collina* (Rutaceae), which grew to a height of about 1.5 m and covered large tracts of the fynbos.

Echolocation

We flew 4 adult female *R. capensis* in a flight room 10 × 3 × 2 m to record their echolocation calls as they attacked tethered moths; mostly earless monkey moths, *Phyllalia* spp. (Euperotidae) but also eared *H. armigera* (Noctuidae). *H. armigera* refused to fly in the presence of echolocating rhinolophids, and rhinolophids are unable to detect nonfluttering prey (Trappe and Schnitzler 1982; Bell and Fenton 1984). These moths thus

had to be deafened by destroying the tympanic membrane with a fine needle, a procedure that does not involve damaging musculature or result in loss of hemolymph.

We recorded echolocation calls from bats as they attacked tethered moths in the flight room using the Avisoft UltraSoundGate 416 (Avisoft Bioacoustics, Berlin, Germany) with the microphone placed 970 mm behind the tether. Calls were recorded onto an hp Compaq nx7010 notebook computer with Avisoft SASLab Pro software at a sampling rate of 500 kHz with 16-bit resolution. We analyzed calls using BatSound Pro software (Version 3.20, Pettersson Elektronik AB, Upsala, Sweden). We measured one sequence from each bat chosen by the following criteria: 1) the bat was flying head-on toward the microphone, 2) the bat took the moth and feeding buzzes were recorded as it did so, and 3) the sequence had good signal-to-noise ratio (9–28 dB). We also recorded the attack sequences of free-flying *R. capensis* as they hunted around their cave roost using the same recording equipment placed on the ground in the bats' flight path with the microphone facing slightly upward.

For each call in the sequence, we measured the peak frequency (PF) from the power spectrum (4096 Fast Fourier Transform [FFT]) and the intensity at PF, call duration, interpulse interval (IPI), and interonset interval (time from the onset of one call to the onset of the next call in the sequence) from the oscillogram and minimum frequency of the frequency-modulated (FM) tail from the spectrogram (4096-point FFT). Call duration was measured from the initiation of the constant frequency (CF) component of the call to the terminus of the downward sweeping FM component of the call. IPI was measured from the end of one call to the initiation of the next call. Duty cycle was calculated by dividing pulse duration by the interonset interval. Bandwidth was calculated by subtracting the minimum frequency of the FM component from the frequency of the CF component.

Bird predation

We focused our observations on the Cape Bulbul, *Pycnonotus capensis*, a bird that was very common and active where the moths were active. Because it was difficult to follow unmarked birds individually, we recorded all predation events on *H. armigera* during a 2-h period on one day between 0800 and 1000 h at Koppie Alleen, a site in the reserve 6 km to the east of Hothole. Birds were observed with a Nikon 9 × 25 CF III binoculars (Nikon Vision Co., Ltd, Tokyo, Japan), but predation events could also be seen readily with the naked eye. We compared the frequency of the observed predation modes with expected frequencies using a Chi-square test and compared the success rates of the 2 most successful predation modes using a Fisher's Exact test.

Moth auditory analyses

We used the eared moths *H. armigera* (Noctuidae) and *D. griseiviridis* (Notodontidae) in all playback experiments. *Helicoverpa armigera* were collected during the day as they fed on the flowering bush, *A. collina*, and both *H. armigera* and *D. griseiviridis* were collected at night from ultraviolet lights.

The action potentials of the auditory receptors in the moths' tympanic nerves (IIN1b; Nüesch 1957) were recorded with a stainless steel hook electrode referenced to a second in the moths' abdomen (Fullard et al. 2003). Neural responses were amplified (Grass Instruments P-15 Pre-amplifier, Astro-Med, West Warwick, RI) and observed either online or stored in a laptop PC using digital acquisition boards (ADC 212/3 [sampling rate = 3 MHz]; Pico Technology, Cambridgeshire, UK, or UltraSoundGate 416–200 [sampling rate = 250 kHz];

Avisoft) and oscilloscope-emulating software (PicoScope 5.10.7 or Recorder 2.9, respectively). Spike records were later analyzed with a customized MATLAB (Version R2006b, The MathWorks, Natick, MA) application. In keeping with previous studies (Roeder 1964; Fullard et al. 2003; Nabatiyan et al. 2003; Marsat and Pollack 2006, 2007), we report instantaneous spike periods (time from the peak of one spike to the peak of the next) rather than averaged rates (e.g., spikes per second) as a direct measure of the auditory receptors' activity and their likelihood to activate postsynaptic neural components (Hedwig 2006).

Acoustic stimulation

Moth auditory preparations were exposed to pulsed synthetic sounds generated by a broadcast from a speaker (Technics EAS-10TH400B, Panasonic, Mississauga, Canada) mounted 30 cm from the moths. Intensities were recorded as voltages delivered to the speaker and then converted to peak equivalent sound pressure levels (decibels peSPL) (root mean square [RMS] re 20 μ Pa) (Stapells et al. 1982) from equal-amplitude continual tones as measured with a Brüel and Kjaer (B&K, Nærum, Denmark) 4135 microphone and 2610 measuring amplifier. The entire system was calibrated before and after the study with a B&K 4228 pistonphone. Auditory threshold curves (audiograms) were derived using 20 ms sound pulses and 0.1 ms rise/fall times from 5 to 100 kHz delivered 2 s^{-1} at 5 kHz increments of random sequence with A1 cell threshold determined as the stimulus intensity that evoked 2 receptor spikes per stimulus pulse.

This setup was used to play back prerecorded attack sequences of *R. capensis*, the local bat and those from an attack sequence of *E. fuscus*, a North American representative of a typical aerially foraging, insectivorous vespertilionid. We used an attack sequence from a North American bat because we had a good attack sequence for it, and it was representative of bats that used syntonic frequencies similar to those used by African species (e.g., *Myotis tricolor*, *Neoromicia capensis*, and *Miniopterus natalensis*). It also allows direct comparison with Fullard et al. (2003).

For the *R. capensis* sequence, we adjusted the amplitude of each call independently based on the distance the bat was from the microphone as it produced each call such that it would represent the relative intensity of the call at the moth's ears (decibels peSPL), rather than the microphone (1 m from the moth). The bat's distance from both moth and microphone was estimated for each call using the synchronized audio-video recordings and a number of morphometric measurements of the bat (e.g., forearm length, distance between ears, skull breadth, etc.). Such correction was not necessary for the *E. fuscus* attack sequence (Fullard et al. 2003). Because the intensities of calls during the attack phase are unknown for most bats, we used a range of intensities from 70 to 100 dB in 6-dB steps for our playbacks. For each attack sequence, intensities were set by matching the voltage of the highest amplitude call (indicated by asterisks in Figures 2 and 3) to that of continual 25 kHz (for *E. fuscus*) or 85 kHz (for *R. capensis*) tones of known intensities as generated using the setup described above. Frequency spectra (FFT size 1024, Hanning window) of the calls were derived using BatSound Pro (Version 3.31) (Pettersson Electronic).

Moth auditory preparations were also exposed to the rustling sounds of the bush, *A. collina*, from whose flowers *H. armigera* fed during the day when it was attacked by birds. We simulated the sounds that a foraging bird would make as it strikes the bush by rustling a branch of the bush at 20, 50, and 100 cm from the moths while their tympanic nerves were being monitored. We tested whether these simulated sounds

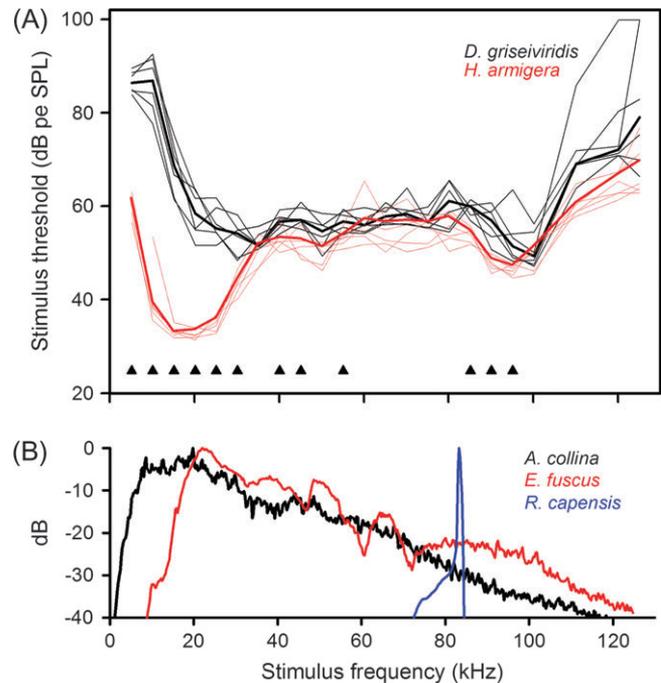


Figure 2

(A) Auditory threshold curves of the A1 receptor in the noctuid, *Helicoverpa armigera*, and the notodontid, *Desmeocraera griseiviridis*. Curves represent the median (heavy lines) of individual moths (thin lines), $n = 6$ for both species; significant differences (Mann–Whitney T -tests) indicated by triangles. (B) Frequency spectra of the sounds used as stimuli to the moth ears.

reflect the sounds of rustling birds by recording the sounds that birds made as they landed on an *A. collina* bush in the flight room. We recorded the sound of 4 Bulbuls as they landed (7 landings in total) in a bush using a B&K $\frac{1}{2}$ " microphone (model 4190, frequency response 1 Hz to 20 kHz \pm 0.2 dB) connected to a Sound Devices 722 solid-state recorder (sampling at 96 kHz with 24-bit precision). The microphone was 25 cm from the birds and on axis as they landed. Sound intensities were referenced against a B&K pistonphone (model 4231) producing 94 dB SPL at 1 kHz. Calls were high-pass filtered (cut off 1 kHz) to remove wind noise.

Moth avoidance of bird predation

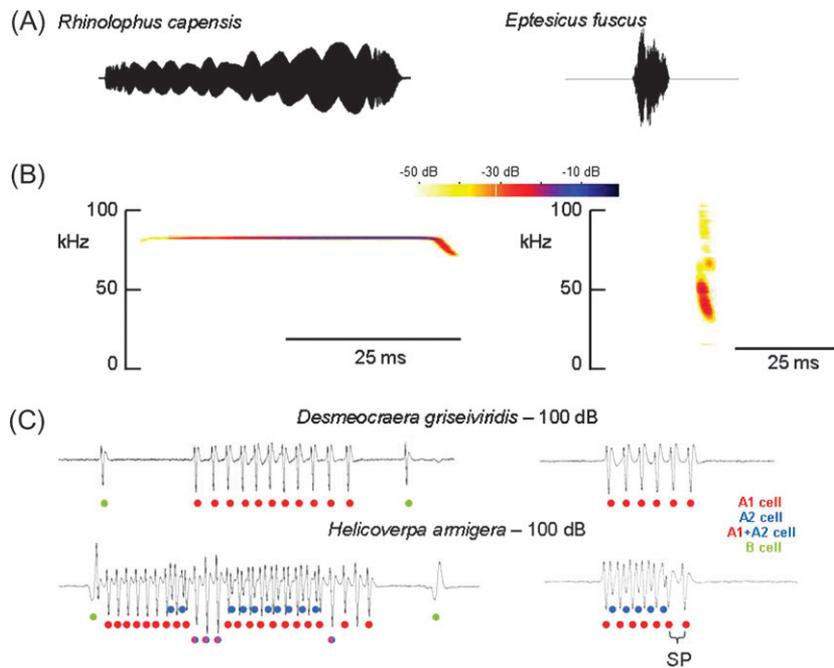
We did a series of tests to determine whether *H. armigera* responded to visual or auditory cues. We tossed stones into *A. collina* bushes on which *H. armigera* were feeding and recorded whether they reacted to the stone while it was in the air or when it rustled through the bush. We also made audible hissing sounds by placing our tongues against our clenched teeth and blowing air through our teeth.

Statistical analyses were done using Statistica (Version 8.0, StatSoft, Inc., Tulsa, OK) and JMP (Version 7.0 SAS Institute, Cary, NC).

RESULTS

Moth activity

Desmeocraera griseiviridis was not obviously active during the day, and we only caught them at light traps at night. *Helicoverpa armigera*, on the other hand, were active both day and night.

**Figure 3**

Auditory responses of *Desmeocraera griseiviridis* and *Helicoverpa armigera* to the prerecordings of single echolocation calls of *Rhinolophus capensis* and *Eptesicus fuscus*. (A) Oscillograms of the 2 species. (B) Spectrograms illustrating the frequency structure of the calls. (C) Representative traces of the tympanic nerve of the 2 moth species in response to the calls in (A) at the highest intensity tested. Individual A1 and A2 spikes as well as simultaneous firing cells are indicated by the symbols, as well as the (B) (nonauditory cell). Spike period is indicated (SP) for measurements in subsequent analyses.

Bat echolocation behavior

Rhinolophus capensis, a high-duty cycle bat, readily took earless monkey moths from the tether. In many low-duty cycle aerial hawking bats, an increase and then decrease in duty cycle has been reported over the course of an attack (e.g., Surlykke and Moss 2000). For these bats, the echolocation call attack sequence has for many years been delineated into search (pre-detection), approach (postdetection), and terminal phases (Griffin et al. 1960). However, this terminology may not accurately describe what *R. capensis* is doing over the course of its attack, and, with the exception of the terminal phase calls (those calls beginning at 90% duty cycles and steeply dropping off thereafter and ending at the capture of the moth), we hesitate to use them here. We do, however, note that in 2 of the 4 echolocation sequences (Figure 1A,B), 2 changes in duty cycle were obvious and, with the exception of the differences in duty cycle that define low- and high-duty cycle bats, qualitatively similar to the changes seen in low-duty cycle species (Griffin et al. 1960; Surlykke and Moss 2000). Duty cycle started low at about 50% and after about 750 ms into the sequence, it increased to about 75% and finally at about 1400 ms into the sequence it increased further to about 90%. Within the terminal phase, there is a rapid decrease in duty cycle back to approach phase values (Figure 1). In the remaining 2 sequences (Figure 1C,D), these changes are also clear; however, the time elapsed over the course of the first duty cycle increase is brief, and these sequences are shorter than the first 2. The change in duty cycle in *R. capensis* is caused mostly by changes in call duration but also, to a minor degree, by changes in IPI (Figure 1). The terminal phase is defined by a decrease in peak duty cycle that is due to a decrease in call duration, IPI remaining unchanged (Figure 1A–D). There was a general increase in intensity as the bat approached the microphone (placed directly behind the tethered moth) but a decrease as the bat entered the terminal phase of its echolocation sequence (Figure 1E). Bandwidth remained more or less constant throughout the attack sequence of all 4 bats (Figure 1A–D). Similar patterns of echolocation behavior were evident in the attack sequences of free-flying *R. capensis* (Figure 1F), suggesting that bats in our flight room were behaving normally.

Moth auditory responses

Figure 2A describes the auditory sensitivity of 6 *H. armigera* and *D. griseiviridis* collected in the same habitat as *R. capensis*. Both species reveal the typically high sensitivity of African moths sympatric with diverse bat communities (Fullard 1982), but they differ from each other at frequencies below 35 kHz where *H. armigera* reveals significantly lower thresholds than *D. griseiviridis*. The audiograms for *H. armigera* resemble those of other southern African noctuids (Fullard and Thomas 1981) in their maximum tuning at 15–30 kHz but reveal a high sensitivity relative to North American moths (Fullard 1982), at 80–100 kHz, a characteristic also exhibited by *D. griseiviridis*. Figure 2B illustrates the frequency spectra of the entire echolocation attack sequence of both bats and illustrates the broad bandwidth (20–70 kHz) of the FM calls of *E. fuscus* compared with the narrower bandwidth (69–85 kHz) of the CF calls of *R. capensis*. Figure 2B also includes the spectrum of the sounds produced by rustling a branch of the flowering bush, *A. collina*, revealing a broad spectral bandwidth from 1 to 60 kHz.

Moth auditory responses to single echolocation calls emitted early in the attack sequence of *R. capensis* and *E. fuscus* are illustrated in Figure 3. The long (mean \pm standard deviation [SD] = 47.8 \pm 4.0 ms, n = 10 search phase calls), CF (83.4 \pm 0.0 kHz, n = 10 search phase calls) call of *R. capensis* evokes many more A1 spikes in both moths than the shorter call of *E. fuscus*. The A2 cell is not present in notodontids (Eggers 1919; Surlykke 1984) but fires vigorously in *H. armigera* at the 100-dB intensity. Both moths also exhibit B cell activity, but this cell is not acoustically activated (Roeder and Treat 1957) and shows no change in activity to the attack calls of bats (Fullard et al. 2003).

Figures 4 and 5 show typical moth auditory responses to the entire attack sequences (*E. fuscus*: 604 ms; *R. capensis*: 2239 ms) of the 2 bats at the lowest and highest playback intensities used. The spike periods are plotted as a function of the total number of echolocation calls emitted by each bat which although similar (37 in *R. capensis* vs. 39 in *E. fuscus*) results in a total amount of time that is longer in *R. capensis* than *E. fuscus*. All the spike periods are compared with that of 2.6 ms. This is the conservative end of Roeder's (1964) estimate of the

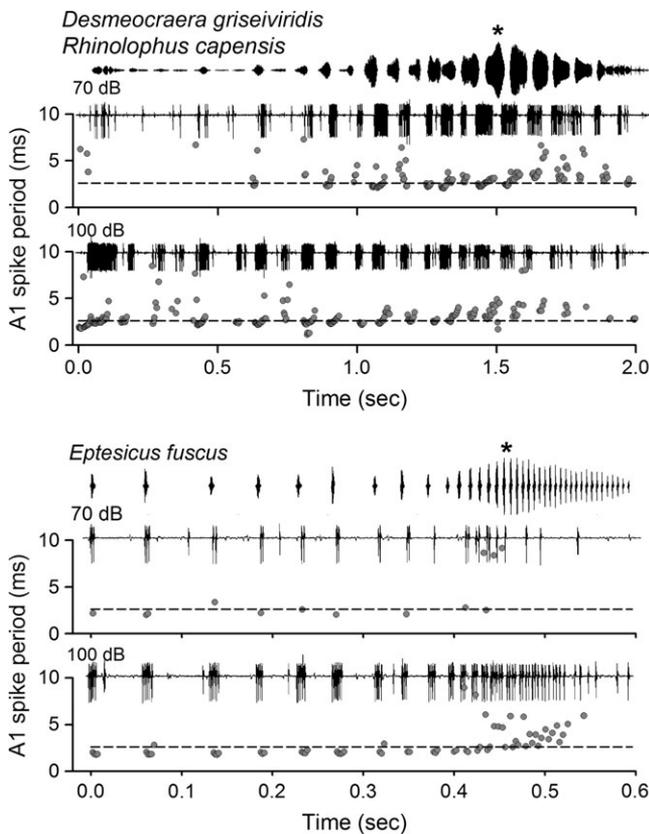


Figure 4

Representative oscillogram of the auditory response of *Desmeocraera griseiviridis* to the prerecorded echolocation attack sequences of *Rhinolophus capensis* and *Eptesicus fuscus*. For each bat, the entire echolocation sequence is the top trace (asterisk marks the call that was used to set sequence intensities [see text for further explanation]). The trace below is of the moth's tympanic nerve at the lowest and highest intensities tested (70 and 100 dB peSPL). The bottom trace plots the A1 auditory receptor spike period for the duration of the entire attack sequence and the dashed line indicates 2.6 ms, the value identified by Roeder (1964) as necessary for evoking defensive flight responses in noctuid moths.

A1 spike period required to activate diving responses in North American noctuids because we do not know what if any differences exist in African versus American moths. This estimate is hereafter called the critical spike period (CSP). Against *R. capensis* calls at the 70-dB playback intensity, the auditory response of both moths reveal a similar trend exhibiting few A1 spikes, most with periods greater than the CSP for the first half of the attack sequence. After this, A1 spikes appear in both moths, but for *D. griseiviridis*, most periods remain in excess of the CSP for the rest of the attack. The stronger response of *H. armigera* to the calls of *R. capensis* is more apparent at the 100-dB playback intensity with this moth readily firing A1 spikes with most periods below the CSP. In contrast, the calls of *E. fuscus* evoke strong A1 responses at low and high intensities in both moths with spike periods consistently below the CSP for most of the attack sequence. The final 200 ms of *E. fuscus*' attack shows a similar A1 dropout as previously reported in North American moths (Fullard et al. 2003), but this is less apparent for *H. armigera* than for *D. griseiviridis*.

We ran four 2-way repeated measures analysis of variances to investigate potential within-subject effects of bat species, play-

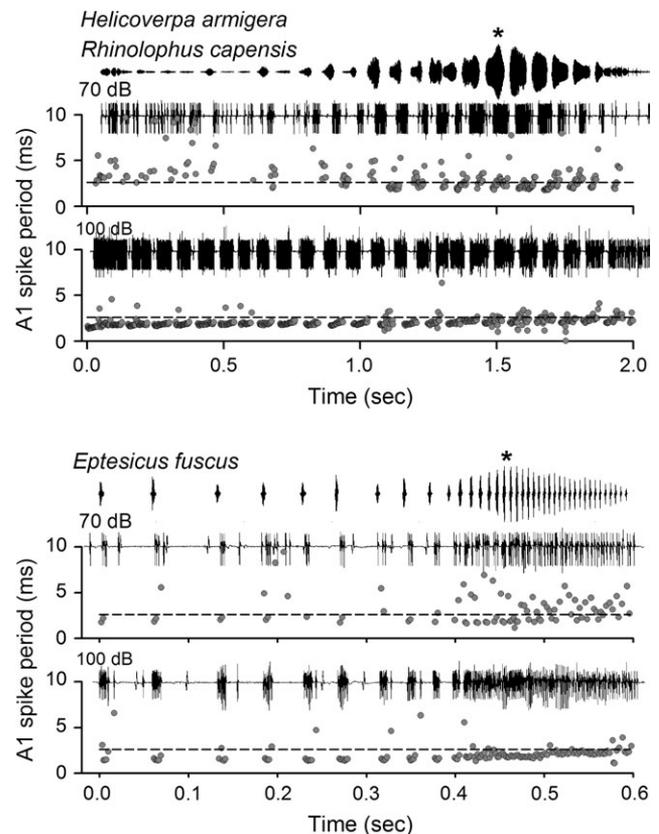


Figure 5

Response of the auditory response of the noctuid, *Helicoverpa armigera*, to the echolocation attack sequences of *Rhinolophus capensis* and *Eptesicus fuscus* (legend as for Figure 3).

back intensity, and species–intensity interactions. For *D. griseiviridis*, total A1 spike number was 1) significantly higher in response to the *R. capensis* attack sequence ($F = 110.76$, $P < 0.0001$), 2) increased significantly in response to increasing playback intensities ($F = 12.72$, $P = 0.0144$), and 3) the species–intensity interaction was also significant ($F = 6.48$, $P = 0.0471$) (Figure 6A). For this same moth species, percentage of A1 spikes below CSP 1) did not differ significantly between *R. capensis* and *E. fuscus* attack sequences ($F = 2.063$, $P = 0.1888$), 2) increased significantly in response to increasing playback intensities ($F = 36.655$, $P = 0.002$), and 3) the species–intensity interaction was not significant ($F = 6.371$, $P = 0.0492$) (Figure 6B). For *H. armigera*, total A1 spike number was 1) significantly higher in response to the *R. capensis* attack sequence ($F = 1081.88$, $P < 0.0001$), 2) increased significantly in response to increasing playback intensities ($F = 185.26$, $P < 0.0001$), and 3) the species–intensity interaction was also significant ($F = 133.07$, $P = 0.0002$) (Figure 6C). For *H. armigera*, percentage of A1 spikes below CSP was 1) significantly lower in response to the *R. capensis* attack sequence ($F = 35.76$, $P = 0.0003$), 2) did not increase significantly in response to increasing playback intensities ($F = 2.922$, $P = 0.1605$), and 3) the species–intensity interaction was not significant ($F = 1.628$, $P = 0.3287$) (Figure 6D).

Figure 7A illustrates representative auditory responses of both moths to the sounds of the bush, *A. collina*, when rustled at 3 distances. Figure 7B summarizes the averaged results from *D. griseiviridis* ($n = 4$) and *H. armigera* ($n = 5$) moths to the sounds and indicates that compared with *D. griseiviridis*,

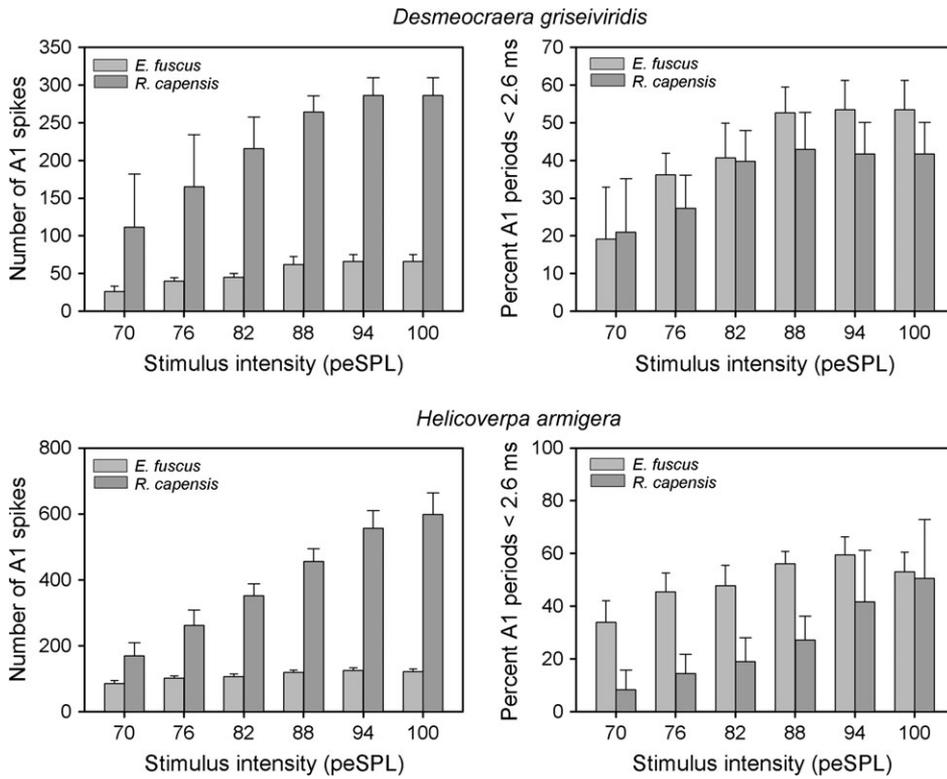


Figure 6 Statistical comparisons of the auditory responses in the 2 species of moths ($n = 5$ for both) to the calls of *Rhinolophus capensis* and *Eptesicus fuscus*.

H. armigera is overall more sensitive to these sounds responding with vigorous A1 firing, many of which have spike periods less than 2.6 ms. The rustling sounds that captive birds made when landing on a bush had a mean (RMS) \pm SD intensity of

60.5 \pm 7.8 dB at 25 cm with most energy between 1.5 and 20 kHz. This frequency range was thus similar to those from a shaken bush (Figure 2B), and the intensity was well above the threshold intensity for *H. armigera* at 5–20 kHz (Figure 2A).

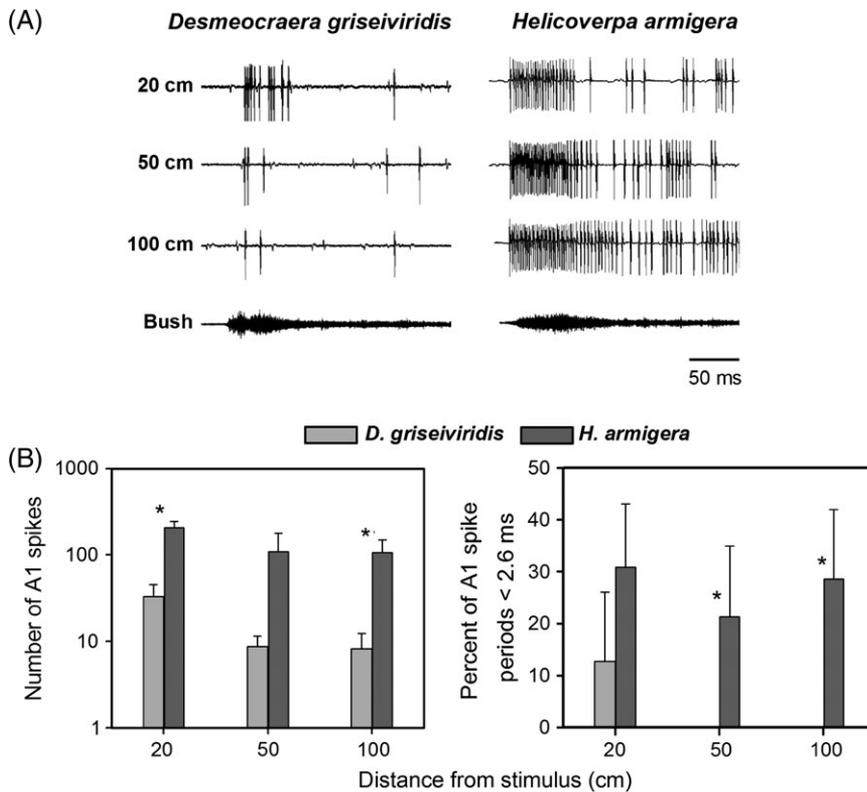


Figure 7 (A) Oscillograms of the auditory response in *Desmeocraera griseiviridis* and *Helicoverpa armigera* to the sounds created by rustling the bush *Agathosma collina* at 3 distances from the auditory preparation. (B) Averaged A1 spiking responses of the 2 moths (*D. griseiviridis*, $n = 4$; *H. armigera*, $n = 5$) to the bush sounds at 3 distances (statistical analyses involved 2 sets of three 2-sample t -tests with sequential Bonferroni correction: $\alpha_1 = 0.017$, $\alpha_2 = 0.025$, $\alpha_3 = 0.05$; * = significant corrected P values).

Bird predation

Helicoverpa armigera were active throughout the day and could be easily observed as they probed the flowers for nectar. Inflorescences of *A. collina* were on the top layer of the plant. We observed a total of 24 predation events on moths by the Cape Bulbul, 13 (54%) of which were successful. The birds captured the moths in several ways all of which involved scanning the habitat from a perch and then taking off after a moth. First, they would land on a bush, scan into it, and then dive into the bush after a moth either eating it on the ground or flying back to the top of a perch and consuming it there (9 of 24 predation events). We called this "ground gleaning," which included the bird chasing a moth that was flying just above the vegetation into the bush before catching and consuming it (3 of the 8 successful ground gleaning attempts). Second, birds would skim over the vegetation and glean moths from bushes as the moths probed the flowers (11 of 24 predation events). We called this "aerial gleaning." In 3 of the 5 successful aerial gleaning attempts, the moth managed to escape from the bird's beak. The third method was fly catching in which the bird flew after a moth flying just above the vegetation and attempted to catch the moth on the wing (4 of 24 predation events). Birds were unsuccessful in all the 4 attempts made to catch moths in this way and their failure was due to the fast and erratic flight used by moths. This was probably the reason why birds seldom chased after the moths that took to the air when the bird landed on a bush (2 out of 11 bird landings that did not include predation). In the 2 instances in which they did, birds were unsuccessful at catching the moth. There was no difference in the frequency with which birds used the different predation modes (observed vs. expected frequency Chi square = 3.2, degrees of freedom [df] = 2; $P > 0.1$). There was also no difference in the success rate between ground and aerial gleaning (Fisher's Exact test [2 tailed] = 2.1, df = 1, $P > 0.2$).

Moth avoidance of bird predation

Moths did not appear to actively avoid birds that aerial gleaned them from inflorescences and appeared to be oblivious to them. However, 3 of the 5 moths captured in this way were able to escape from the bird's beak, suggesting that the moths scales were slippery and/or that when aerial gleaning the bird does not get a good enough grip on the moth. We observed birds landing on a bush containing moths on 13 occasions. In 11 of these, at least 10 moths took flight when a bird landed on the bush. These flights were short, and the moths almost immediately flew down into bushes a short distance from the bush from which they were flushed by the alighting bird. The bird did not take off after the moths but instead scanned either the habitat or the bush on which it was sitting occasionally diving in after a moth that had not taken flight. On 2 of the 13 occasions when a bird landed on a bush, only 1 and 2 moths, respectively, took flight out of the bush. In both cases, the bird chased after them but was only successful in catching the single moth that had taken off, following it down into another bush and capturing it there. When it chased after the 2 moths, that continued flying together, it missed both of them as they disappeared into a bush.

Moths did not respond to tossed stones while it was in the air ($n = 10$). However, they always took off when a stone rustled through a bush and before it hit the ground ($n = 10$). Moths on bushes over which the stones were thrown did not react ($n = 10$). Similarly, during aerial gleaning, the other moths in the bush, from which a bird took a moth, did not react ($n = 11$). Moths also took off when we rustled nearby bushes that were not in contact with the bush on which the moths were

feeding ($n = 10$). Lastly, moths took flight from the bushes they were sitting on when we made audible hissing sounds by placing our tongues against our clenched teeth and blowing air through our teeth ($n = 10$).

DISCUSSION

The nocturnal and diurnal flight activity of the noctuid moth, *H. armigera*, during the day and at night exposes it to both bat and bird predation. We suggest that this is reflected in its auditory sensitivity which, although primarily evolved to detect the bat echolocation assemblage to which it is exposed at night, has secondarily rendered it sensitive to the rustling sounds that certain birds make as they pounce on the vegetation in pursuit of moths during the day. The hearing sensitivity of the notodontid, *D. griseiviridis*, on the other hand, appears to be adapted only to the bat assemblage. Both species of moths displayed auditory sensitivity to frequencies higher than that of North American moths from the same family.

Birds and moths

The observation that *H. armigera*, when resting or foraging on the flowers of *A. collina*, exhibit escape flight when exposed to the sounds of the bush caused by attacking birds suggests a novel function for the ears of this and perhaps other diurnally active moths. We are confident that this escape behavior of the moths was acoustically evoked because nonvisual stimuli (e.g., hissing) also caused moths to fly from their perches, whereas visual stimuli (e.g., rocks thrown over a bush and birds aerial gleaning) did not evoke a response. We suggest that the high sensitivity of *H. armigera*'s ears enables it to detect the relatively low-intensity sounds generated by a bird as it lands on a bush when attacking moths. To our knowledge, this is the first report of acoustically mediated escape behavior from bird predation in moths. It suggests that moth ears that originally arose in the context of bat predation may now be effective against bird predation. Once exposed to bird predation selection pressure from birds might then have driven the evolution of increased sensitivity in the ears of *H. armigera* to frequencies between 5 and 10 kHz because bats hunting at the level of vegetation do not echolocate at frequencies below 20 kHz. This is supported by the fact that *D. griseiviridis* does not show this sensitivity and is apparently not active during the day and thus not exposed to bird predation. At least in North America, Notodontidae are the least diurnal clade measured in a study on moth activity (Fullard and Napoleone 2001). However, the hearing sensitivity of *D. griseiviridis* matches that of *H. armigera* at frequencies above 35 kHz, including that of the 80–100 kHz range. This frequency range matches the range in echolocation frequency used by bats at De Hoop (*N. capensis* 42 kHz, *M. tricolor* 52 kHz, *R. capensis* 84 kHz, and *R. clivosus* 92 kHz; Schoeman and Jacobs 2003; Stoffberg and Jacobs 2004; Jacobs et al. 2007). Furthermore, the lowest frequency echolocation calls a moth is likely to encounter at De Hoop Nature Reserve is 23 kHz (*Tadarida aegyptiaca*; Schoeman and Jacobs 2003) and elsewhere in Africa 10 kHz (*Otomops martiensseni*; Fenton et al. 2004). Both molossid species likely confined to hunting in open space (Norberg and Rayner 1987).

Bats and moths

Given the absence of social uses for hearing in most moths (Scoble 1992; Conner 1999; Yager 1999), moth ears are physiologically designed to detect the frequencies produced by the most abundant acoustic predators that they encounter.

In moth assemblages thus far tested, these frequencies are contained within the echolocation calls of the aerially foraging bat communities that form acoustic assemblages peaked at 20–60 kHz (Fullard 1982), the range to which most moths are maximally sensitive (Fullard 1987). Frequencies outside this range are traditionally considered to be allotonic (Fullard 1988). However, the occurrence of highest sensitivity in *D. griseiviridis* at a frequency range of 80–100 kHz suggests that syntonicity, and therefore its converse allotonicity, is specific to the predator assemblage and cannot be regarded as having a set range of frequencies across all moth species at all locations. Instead, syntonicity is determined by the assemblage of predators a moth is exposed to over its evolutionary history and its natural history. Thus, for *D. griseiviridis*, allotonic frequencies would be frequencies above 100 kHz rather than outside the normal range of 20–60 kHz as for most moths. Similarly, for *H. armigera*, the range of sensitivity at 80–100 kHz means that these frequencies are at best only partially allotonic. Sensitivity in this range could only have evolved if it conferred some advantage to individual moths. This range coincides with the PF of *R. capensis* and other rhinolophid species that occur in Africa (e.g., *R. clivosus* 92 kHz; Jacobs et al. 2007) and which are active at De Hoop all year round, unlike other migratory species, for example, *M. tricolor* and *M. natalensis* (McDonald et al. 1990). The fact that the echolocation calls of *R. capensis* are only partially allotonic suggests that the AFH may not be the sole explanation for the high preponderance of moths in the diets of rhinolophid bats (Jones 1992; Jacobs et al. 2007) at least in the Cape Floral Kingdom where the proportion by volume may range from 2% to 69% for *R. clivosus* (PF 92 kHz) and from 7% to 97% for *R. capensis* (Jacobs et al. 2007).

Roeder (1964) concluded from observing the ears and anti-bat behavior of various North American noctuids that an A1 spike period of 1.5–2.6 ms is required to activate the last-ditch avoidance response of diving to the ground. This suggests that a moth exposed to the calls of *R. capensis* will not activate the erratic flight maneuvers normally seen during the attack stage of the more typical aerial foraging bat (e.g., *E. fuscus*) used in our study, at least at intensities <95 dB. However, this differential is more pronounced at low playback intensities and appears to have effectively disappeared at >95 dB. Thus, despite the zone of sensitivity at 80–100 kHz, this means that calls of *R. capensis* may be detected, and evoke a response, by moths like *H. armigera* over relatively shorter distances than those calls of bats echolocating at frequencies between 20 and 60 kHz. This shorter detection distance combined with the cluttered habitat in which rhinolophids forage give moths both less time and less space within which to react to an attacking bat. This would make moths more vulnerable to bat predation even if it is from a relatively audible bat. In a test of the AFH at De Hoop Nature Reserve and Algeria Forestry Station, both sites of similar habitat in the Cape Floral Kingdom, support was found for the AFH in the form of a correlation between peak echolocation frequency and the proportion of moths in the diets of bats (Schoeman and Jacobs 2003; Schoeman 2006). However, bats like *R. capensis*, *R. clivosus*, and *Nycteris thebaica* that consumed a high proportion of moths also hunted in dense vegetation. The higher proportion of moths in the diet of the smaller *R. capensis* at De Hoop relative to that of the larger *R. clivosus*, which echolocates at a higher frequency (92 kHz; Jacobs et al. 2007), may thus be due to the better maneuverability of the smaller bat in dense vegetation. Bats that hunted in the open (*T. aegyptiaca* and *Sauromys petrophilus*) or that foraged at the margins of vegetation rather than within it (*M. tricolor*, *N. capensis*) took relatively fewer moths. Thus, the AFH may not be the only explanation for the high preponderance of moths in the diets

of aerial hawking bats, and factors such as prey availability and habitat can play a major role.

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