

Hearing diversity in moths confronting a neotropical bat assemblage

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Abstract The tympanal ear is an evolutionary acquisition which helps moths survive predation from bats. The greater diversity of bats and echolocation strategies in the Neotropics compared with temperate zones would be expected to impose different sensory requirements on the neotropical moths. However, even given some variability among moth assemblages, the frequencies of best hearing of moths from different climate zones studied to date have been roughly the same: between 20 and 60 kHz. We have analyzed the auditory characteristics of tympanate moths from Cuba, a neotropical island with high levels of bat diversity and a high incidence of echolocation frequencies above those commonly at the upper limit of moths' hearing sensitivity. Moths of the superfamilies Noctuoidea, Geometroidea and Pyraloidea were examined. Audiograms were determined by non-invasively measuring distortion-product otoacoustic emissions. We also quantified the frequency spectrum of the echolocation sounds to which this moth community is exposed. The hearing ranges of moths in our study showed best frequencies between 36 and 94 kHz. High sensitivity to frequencies above 50 kHz suggests that the auditory sensitivity of moths is suited to the sounds used by sympatric echolocating bat fauna. Biodiversity characterizes predators

and prey in the Neotropics, but the bat–moth acoustic interaction keeps spectrally matched.

Keywords Moth · Hearing · DPOAEs · Echolocation assemblage · Bat

Introduction

The tympanal ears of moths sense incoming sounds and enable the evaluation of acoustical signals from predators or mates. The evolution of moths ears took place under the selective pressure imposed by predation from sympatric bats (Roeder 1967; Fullard and Yack 1993). Moth hearing has the primary role of detecting these predators; therefore, moths commonly sense a broad range of ultrasonic frequencies with maximal sensitivity in the spectral range most used in bats' echolocation (20–60 kHz) (Roeder and Treat 1957; Fullard 1988; Nakano et al. 2015). Additionally, some species of moths have incorporated sound-emitting capabilities as a secondary acquisition (Conner 1999; Nakano et al. 2009); they produce ultrasound in mate-calling (Spangler 1988; Takanashi et al. 2010) or as predator defense (Barber and Conner 2007; Corcoran et al. 2009). Ultrasound has become a survival tool for moth species capable of detecting it or producing it.

The selective pressure imposed by bats on moth hearing is shown by studies that compare moth species that differ in their sympatric bat fauna. ter Hofstede et al. (2013) suggested that moths from the UK are more sensitive to high frequencies than moths from Canada and Denmark, possibly as a consequence of the horseshoe bats' predation on British moths. Horseshoe bats echolocate at frequencies above 80 kHz. Moths in neotropical areas are challenged by an acoustic environment

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made complex not only by a rich diversity of bat species but also by a large diversity of echolocation strategies, including low and high frequencies with several temporal designs (Fullard 1982; Kalko et al. 1996). Moths surrounded by a high diversity of bats have higher acoustic sensitivity and broader threshold curves compared with moths from sites of low bat diversity (Fenton and Fullard 1979; Fullard 1982). However, the frequencies of highest sensitivity of tropical moths still appear to be mostly under 60 kHz (Fullard and Thomas 1981; Fullard 1988; Fullard et al. 1997). The echolocation spectrum that acts as predation pressure on those moth communities includes call frequencies that are clearly above 60 kHz (Fullard and Belwood 1988). However, those frequencies do not constitute the primary selection pressure since they are produced by bats that are not primarily insectivorous or are less common species. Habitats with an assemblage of insectivorous bats employing a wide range of echolocation frequencies having similar energy over most of the spectrum may reveal a correspondingly high diversity of hearing capabilities in moths, with best frequencies covering a wide ultrasound range.

The richest bat fauna in the West Indies is found in Cuba, a subtropical island with 26 species, 70% of which are insectivorous (Silva 1979). Cuban bats illustrate the diversity of echolocation systems, and seven species echolocate at frequencies above those commonly limiting the hearing sensitivity of moths (Macías and Mora 2003; Macías et al. 2006; Mora and Macías 2011). It is unknown if these high echolocation frequencies constitute a significant predation pressure for this moth community. The echolocation assemblage most strongly affecting moths' auditory sensitivity depends not only on the echolocation frequencies, but also on certain ecological factors, such as feeding habits and population levels (Fullard and Belwood 1988). Previous bioacoustic (Mora et al. 2005; Macías et al. 2006; Mora and Torres 2008) and ecological (Silva 1979; Mancina et al. 2007) studies of Cuban bats could help to estimate the bat predation potential on the island. The distribution and population levels of insectivorous bats within this habitat, and the spectral analysis of their echolocation signals make it possible to characterize the Cuban echolocation assemblage.

The study of moth ears is essential for understanding the bat–moth interaction. Traditional studies analyzing hearing capabilities of moths have used neurophysiological recordings to evaluate tympanal nerve responses (Roeder and Treat 1957; Surlykke and Treat 1995; Fullard et al. 2003). Other studies have included behavioral criteria (Rydell et al. 1997; Dawson et al. 2004; Ratcliffe et al. 2009). An alternative method for evaluating the best hearing range in moths could be the

noninvasive recording of distortion-product otoacoustic emissions (DPOAE) (Coro and Kössl 1998; Mora et al. 2013). DPOAEs are faint sounds that appear when the ear is stimulated simultaneously with two tones; they reach their largest amplitudes at frequencies in the best hearing range of each species and reflect mechanical characteristics of sensory transduction (Kössl et al. 2008). Moth ears emit conspicuous DPOAEs, which are similar to those generated by vertebrate ears (Coro and Kössl 1998; Kössl et al. 2007). In the present work, we describe the hearing capabilities of a neotropical moth community by measuring DPOAE audiograms. We analyze the acoustic relationships between the frequencies that constitute relevant predation pressure and the auditory characteristics of sympatric moths.

Materials and methods

Animal preparation

Adult individuals of the superfamilies Noctuoidea, Geometroidea and Pyraloidea (species names in Fig. 2) were collected from fluorescent lights or from host plants in diverse localities of Havana. We also reared *Galleria mellonella* from the larval stage. These pyralid specimens were fed with a mixture of honey and wheat bran. All adult moths were fed ad libitum with a 30% sucrose solution and kept at ambient light and temperature in the Bioacoustics Lab at the Faculty of Biology, University of Havana. Each specimen was photographed and identified by comparison with specimens and vouchers previously deposited in the Institute for Ecology and Systematics and the Museum of Natural History “Felipe Poey”. All animals were tested within two days of their capture or emergence.

For auditory evaluation, specimens were tethered with modeling clay to a cork platform covered by wax. Neither dissection nor anesthetics were used. Moths with thoracic ears were positioned dorsal side up and slightly tilted to the left. Specimens with abdominal ears were placed ventral side up with the thorax slightly pulled back. To allow free coupler approach to the tympanum, wings were held to the sides and the external auditory structures were carefully displaced but not dissected. Only the right ear was examined per animal. Experiments were carried out at room temperature of 24–28 °C and relative humidity of 45–65%, inside a Faraday cage that also was acoustically isolated. The physiological state of the moths during recordings was assessed by monitoring spontaneous movements of the antennae.

Distortion-product otoacoustic emissions (DPOAEs) recording and analysis

To measure DPOAEs, a custom-built acoustic coupler was placed about 1 mm from the tympanum in an open sound system configuration. The acoustic coupler included two ½ inch microphone capsules used as loudspeakers (Microtech Gefell MK 202) for delivering tones, and a ¼ inch microphone (Microtech Gefell MK301) connected to a preamplifier (Microtech Gefell MV302) for monitoring sound pressure at the tympanum. The coupler had three channels and an overall tip diameter of 1.5 mm; it was connected to the microphone capsules through conical speculums. The acoustic signal from the microphone was amplified using a conditioning amplifier (Brüel & Kjær Nexus 2690) and digitized with a Fireface 400 sound card. A sampling rate of 192 kHz was used for stimulus generation and microphone recording. The sound system was calibrated in situ for frequencies between 10 and 96 kHz using white noise. Nonlinear distortions of the recording setup were evaluated by performing control measurements in five individuals with the coupler in front of a cuticular body surface 5 mm distant from the tympanum. Sound pressure levels used in the present study are expressed in dB SPL (dB re. 20 µPa).

The stimuli used were two simultaneous pure tones of 42.7 ms duration (1 ms rise/fall time, 0° sine phase difference between the onset of both) with a pause of 1 s. In the present study we deliberately chose short stimulus durations to avoid frequency up-tuning of the tympanum (Windmill et al. 2006; Mora et al. 2015). The frequencies and levels of these primary tones are denoted as f_1 , f_2 , L_1 and L_2 , respectively. A fixed f_2/f_1 ratio of 1.09 and a level combination of $L_1 = L_2 + 10$ dB were used since these stimulus parameters evoke large DPOAE levels in moths (Coro and Kössl 1998; Kössl and Coro 2006). For each moth, a matrix of 861 frequency-level combinations (41 frequencies \times 21 levels) was randomly presented with f_2 values from 14 to 94 kHz (2 kHz steps), and L_2 values from 30 to 70 dB SPL (2 dB steps). The microphone signal was analyzed by performing an FFT (rectangular window, 8192 data points) with a frequency resolution of 23.4 Hz. From the spectrum were measured the amplitude of $2f_1 - f_2$ DPOAE and the noise floor, calculated as the mean amplitude of 10 FFT bins at both sides and 1 kHz from the DPOAE frequency. The DPOAE audiogram was constructed by representing the levels of $2f_1 - f_2$ for each stimulus frequency-level combination in a color map. The auditory threshold was defined as the L_2 level necessary to elicit $2f_1 - f_2$ levels equivalent to 15 dB SPL. Since the spectral analysis was performed directly on the short signal recorded by the microphone without averaging, the noise floor was higher than in previous measurements where we averaged over several seconds (e.g., Kössl and

Coro 2006). Consequently, we determined the mechanical tuning curve of the moth as the 15 dB SPL iso-contour line calculated by linear interpolation in the frequency-level response matrix using the Matlab contour function. From the median species audiogram, we evaluated: (1) best frequency (BF, kHz), i.e. the f_2 frequency of maximum sensitivity, (2) minimum threshold (MTr, dB SPL), i.e., threshold at best frequency, (3) frequency range of best sensitivity delimited by the highest (F_{\max} , kHz) and lowest frequencies (F_{\min} , kHz) at +10 dB re MTr, and (4) tuning sharpness ($Q_{10\text{ dB}} = \text{BF}/(F_{\max} - F_{\min})$). Stimulation control, data acquisition, and data analysis were performed using software written in Matlab R2009 (The MathWorks, Inc., Natick, MA, USA).

Bat call analysis

Recordings of Cuban bats were obtained from the Bat Call Archive of the Research Group in Bioacoustics and Neuroethology, University of Havana. Recordings were made using an ultrasound detector (Ultrasound Advice U30; frequency response ± 3 dB, 20–120 kHz). The signals were digitized at a sampling rate of 312 kHz using a 16-bit A/D converter (Measurement Computing PC-CARD-DAS 16/330) controlled with the software Bat-Sound 2.1 (Pettersson Elektronik). Echolocation calls were resampled at 250 kHz and spectrally analyzed using Avisoft-SASLab Pro (FFT 512 points, 93.75% overlap, Hamming window).

To define the echolocation assemblage that acts as selective pressure on tympanate Cuban moths, we combined the normalized frequency spectra of the calls emitted by 15 species of insectivorous bats. Search calls from ten different individuals of each species were analyzed. To determine the assemblage spectrum, the averaged power spectrum calculated per species was weighted by a “predation factor” (Pf) that was used as a measure of their potential as predators. To calculate Pf, we took into account the distribution (D) and population abundance (PA) indexes proposed by Mancina et al. (2007) for Cuban bats. For each index these authors defined three classes to which numeric values were assigned. For species abundance they proposed: 0 common, 1 scarce and 2 rare. To categorize the geographical distribution they defined: 0 widespread, 1 moderate and 2 restricted. In the present study we propose $\text{Pf} = 5 - (\text{PA} + D)$. The cumulative maximum that the two indices can generate is 4, which indicates a rare and restricted species that, as we assume, exerts only a low predation pressure on the moths. Pf constitutes a semiquantitative assessment using the two indices that includes subtraction from 5 to avoid zero factors for the weighting of the spectral amplitudes.

Results

Moth auditory analyses

To describe the hearing of this neotropical moth community, distortion products were recorded from the tympanum of 99 individuals of 31 species. The recording of $2f_1 - f_2$ level in a matrix of frequency-level combinations allowed us to determine DPOAE audiograms for each specimen. Examples of six individual DPOAE audiograms, showing a high diversity of spectral selectivity, are illustrated in Fig. 1. Moth ears were sensitive to ultrasonic frequencies from 14 to 94 kHz. Some species showed asymmetric threshold curves (Fig. 1c, d) with steeper slopes for frequencies below the BF. However, it is noteworthy that several audiograms were limited by the highest frequency measurable by the equipment, i.e., 94 kHz (Fig. 1e, f) and, in fact, were so sensitive to highest the ultrasonic frequencies we could measure, that it was not possible to define the entire threshold curve. A similar picture emerged when assessing the frequency

range of best hearing sensitivity for all species (Fig. 2). The maximum frequency (Fmax +10 dB re MTr) was coincident with the 94 kHz limit of our recording range in 18 species (58%), suggesting a probable extension of the spectral hearing range of the moths to much higher frequencies.

The frequencies of maximum sensitivity were between 36 and 94 kHz, with a majority in the typical hearing range of most moths (ca., 20–60 kHz), although a surprising number showed best tuning to about 60 kHz (Fig. 2). Moreover, it is also noteworthy that in 14 species (45%) the BF of the median audiogram was above 60 kHz. The asymmetries of the threshold curves were also evident in this bandwidth analysis; BFs were closer to Fmin in several species (see Fig. 2). The ears were broadly tuned, Q10 dB values were generally <2 (Table 1); in only eight species, were Q10 dB values >2 recorded. In those eight species the BFs were at higher frequencies and the Fmax was cut off at 94 kHz. Minimum thresholds were found to be between 31 and 56 dB SPL (Table 1), with a median value of 48 dB SPL.

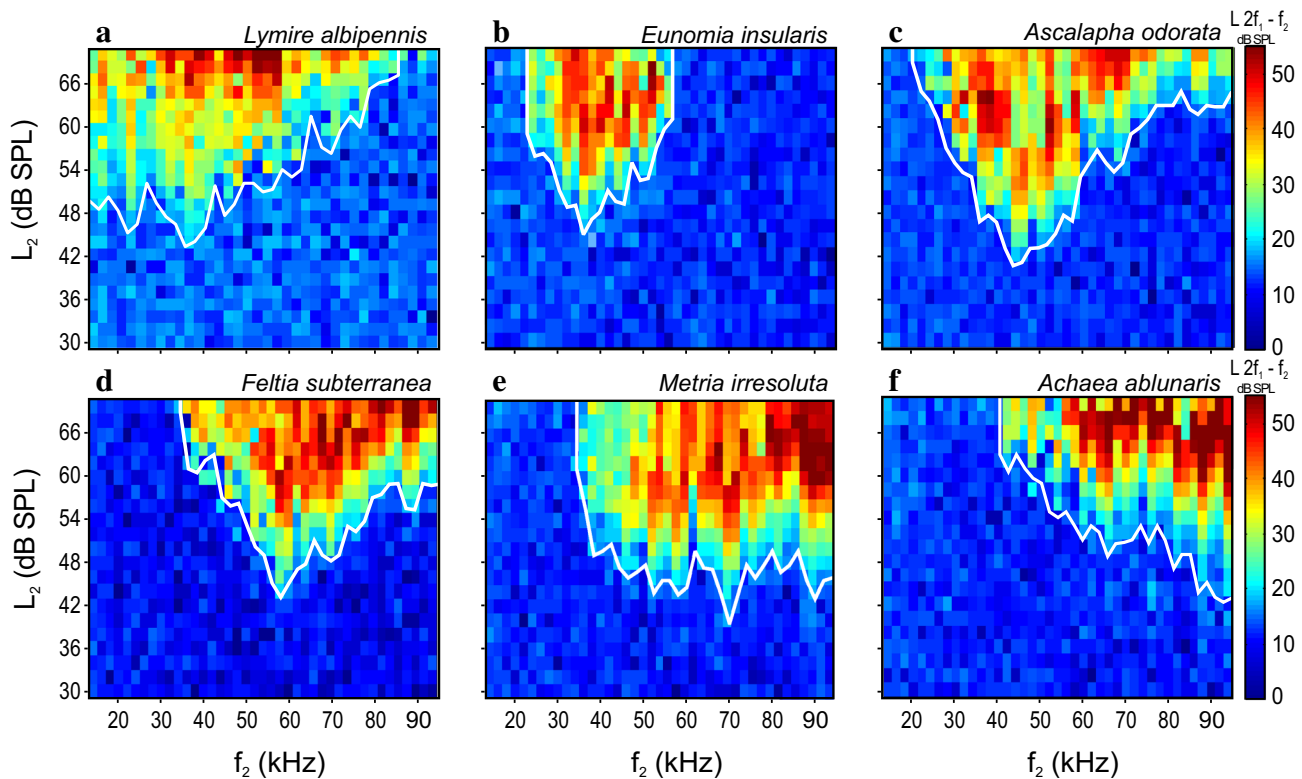
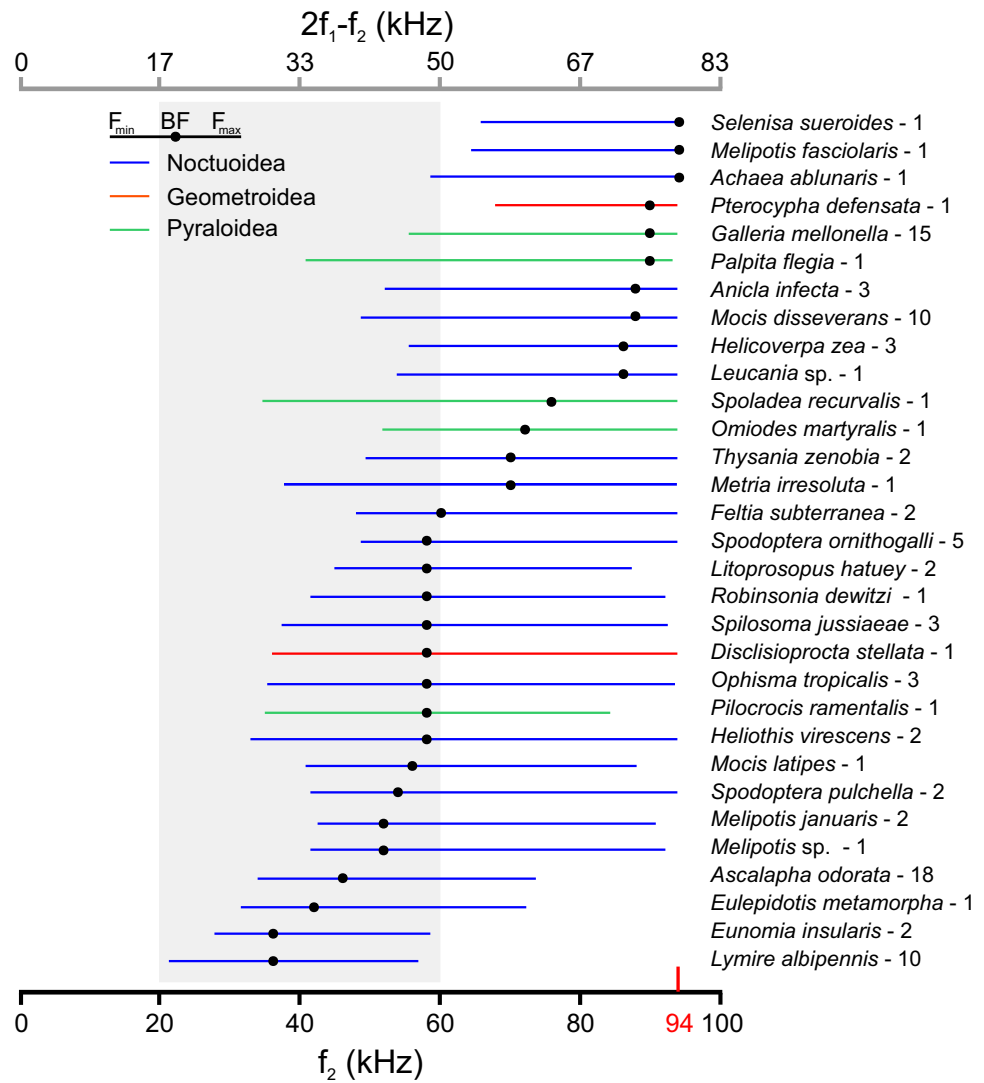


Fig. 1 Hearing diversity in neotropical moths. Examples of six individual DPOAE audiograms are represented as color maps of $2f_1 - f_2$ levels. As stimuli, two simultaneous pure tones f_1 and f_2 of 42.7 ms duration were used, at sound levels of $L_1 = L_2 + 10$ dB and at a fixed

f_2/f_1 ratio of 1.09. Auditory threshold curves (white lines) were defined by the L_2 levels necessary to elicit $2f_1 - f_2$ levels equivalent to 15 dB SPL. Species names are specified above each audiogram

Fig. 2 Spectral ranges of best hearing sensitivity. Individual or median (for species with sample size >1) frequency ranges are represented as a function of f_2 or $2f_1 - f_2$ frequencies. *Horizontal lines* depict the bandwidth, i.e. difference between the highest (F_{max}) and lowest (F_{min}) frequencies at +10 dB re minimum threshold. Best frequencies are represented by *black circles*. The spectral range of highest sensitivity previously defined for moths (Fullard 1998; Nakano et al. 2015) is delimited with a gray rectangle. Species names— N are specified to the *right*. The upper frequency limit imposed by the equipment (94 kHz) is highlighted in *red*



The echolocation assemblage

Representative echolocation calls of Cuban insectivorous bats are presented in Fig. 3a. The diversity of call designs as well as their spectral characteristics is evident. These species echolocate at frequencies from 15 to 100 kHz, but most energy is focused between 30–90 kHz, as illustrates the average spectrum (Fig. 3b, dark grey area). The echolocation assemblage that resulted from weighting by the “predation factor” (Fig. 3b, light grey area) gave a more equal distribution among frequencies with a small notch around 75 kHz. Seven bat species echolocate with frequencies above 50 kHz, four of which are mormoopids, a predominant group in Cuba (Silva 1979). Frequencies corresponding to *Pteronotus parnellii* calls, a mormoopid bat echolocating with a long (~15 ms) constant-frequency component of about 60 kHz, is highlighted as a small peak in the echolocation assemblage. With Pf

equivalent to five, mormoopids species contribute to the predominance of high frequencies in the weighted average spectrum.

Bat–moth acoustic interactions in the neotropics

The median threshold curve from all evaluated moths was estimated from the median curves for each species (Fig. 3c). This integrative analysis from ears tuned to a great diversity of frequency ranges yielded a broad curve which expands across the spectrum. Nevertheless, tuning to frequencies above 50 kHz prevails in this neotropical moth community. The spectral relation between the threshold curve of moths and the echolocation spectrum of bats (Fig. 3c) shows that Cuban insectivorous bats, as a group, echolocate with maximum energy at frequencies to which moths’ ears are most sensitive.

Table 1 Median values per moth species for measures of auditory sensitivity: best frequency (BF), minimum threshold (MTr) and tuning sharpness (Q10 dB)

Species	BF (kHz)	MTr (dB SPL)	Q _{10dB}
<i>Achaea ablunaris</i> (1)	94	43	2.66
<i>Anicla infecta</i> (3)	88	50	2.11
<i>Ascalapha odorata</i> (18)	46	42	1.16
<i>Disclisioprocta stellata</i> (1)	58	49	1.00
<i>Eulepidotis metamorpha</i> (1)	42	31	1.04
<i>Eunomia insularis</i> (2)	36	51	1.18
<i>Feltia subterranea</i> (2)	60	46	1.31
<i>Galleria mellonella</i> (15)	90	49	2.35
<i>Helicoverpa zea</i> (2)	86	49	2.25
<i>Heliothis virescens</i> (2)	58	48	0.95
<i>Leucania</i> sp. (1)	86	54	2.14
<i>Litoprosopus hatuey</i> (2)	58	40	1.37
<i>Lymire albipennis</i> (10)	36	56	1.01
<i>Melipotis fasciolaris</i> (1)	94	42	3.19
<i>Melipotis januaris</i> (2)	52	52	1.08
<i>Melipotis</i> sp. (1)	52	38	1.02
<i>Metria irresoluta</i> (1)	70	40	1.45
<i>Mocis disseverans</i> (10)	88	50	1.95
<i>Mocis latipes</i> (1)	56	48	1.18
<i>Omiodes martyralis</i> (1)	72	51	1.71
<i>Ophisma tropicalis</i> (3)	58	49	1.00
<i>Palpita flegia</i> (1)	90	47	1.69
<i>Pilocrocis ramentalis</i> (1)	58	44	1.17
<i>Pterocypha defensata</i> (1)	90	44	3.48
<i>Robinsonia dewitzi</i> (1)	58	48	1.15
<i>Selenisa sueroides</i> (1)	94	39	3.34
<i>Spilosoma jussiaeae</i> (3)	58	51	1.05
<i>Spodoptera ornithogalli</i> (5)	58	50	1.28
<i>Spodoptera pulchella</i> (2)	54	52	1.03
<i>Spoladea recurvalis</i> (1)	76	52	1.28
<i>Thysania zenobia</i> (2)	70	46	1.57

Discussion

The simple ears of moths, with their small number of auditory neurons shares an essential feature with the vertebrate ears: nonlinear mechanical sound processing generates distinct distortion-product otoacoustic emissions (Kössl et al. 2008). DPOAEs have been previously reported in three species of Noctuoidea: *Ascalapha odorata*, *Empyreuma pugione*, *Ptilodon cucullina* (Coro and Kössl 1998; Kössl et al. 2007) and one species of Geometroidea, *Urania boisduvalii* (Barro 2001). The present study extends DPOAE recordings to 31 moth species from a neotropical location. Threshold curves based on a $2f_1 - f_2$ iso-level criterion allowed us to define the

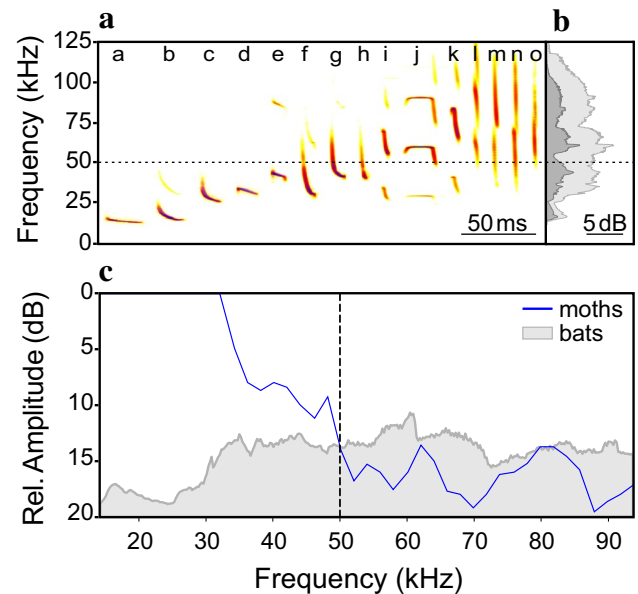


Fig. 3 Spectral correlation between moths' hearing range and the Cuban echolocation assemblage. **a** Spectrograms of representative search calls of each insectivorous Cuban bat: a, *Eumops ferox*; b, *Nyctinomops macrotis*; c, *Tadarida brasiliensis*; d, *Molossus molossus*; e, *Mormopterus minutus*; f, *Eptesicus fuscus*; g, *Nycticeius cubanus*; h, *Lasiurus pfeifferi*; i, *Pteronotus macleayii*; j, *P. parnellii*; k, *P. quadridens*; l, *Mormoops blainvillei*; m, *Nyctiellus lepidus*; n, *Natalus primus*; o, *Macrotus waterhousii*. **b** Non-weighted average power spectrum (dark gray) and spectrum weighted by the "predation factor" (see text, light gray). **c** Median threshold curve estimated from 31 species of moths is correlated with the weighted spectrum of bat echolocation

hearing range for each species. Our study shows a high diversity of frequency tuning with maximum sensitivity at frequencies between 36 and 94 kHz (or higher, since the recording system did not go above 94 kHz).

Diversity begins with the design of the ears. There are many structural differences among the ears of the three sampled superfamilies (Minet and Surlykke 2003). Interspecific variations in the mechanical properties of the tympanic membrane, the internal cavities and the external tympanic recess may be correlated with differences in auditory tuning characteristics (Adams 1972). Differences in tuning might also be related to the structural variations between entire scolopidia, or their components (Yack 2004). From our results, it is not possible to determine whether the position of the tympanic organ or the number of sensory cells are related to the overall frequency response. Moths from the superfamilies Geometroidea and Pyraloidea, both with abdominal ears and four scolopidia (Minet and Surlykke 2003), showed a tendency to be selective to the high frequencies (see Fig. 2). Previous vibrometric, electrophysiological and

behavioral studies support this observation for pyralid moths (Pérez and Zhantiev 1976; Spangler 1987; Heller and Krahe 1994; Rodríguez et al. 2005). However, best sensitivities at frequencies below 50 kHz have also been described in both superfamilies (Surlykke and Filskov 1997; Takanashi et al. 2010). In order to evaluate these parameters, it will be necessary to increase the sample size for each taxonomic group. It is worth noting, moreover, that there is a great deal of diversity in hearing range among the noctuids that have been studied, all of them with metathoracic ears and only two scolopidia.

All audiograms were broadly tuned as is expected for neotropical moths having to cope with a diverse bat assemblage (Fullard 1982). Broad threshold curves increase the chance of detecting potential predators emitting signals with different spectral content. Tuning curves estimated from DPOAE recordings are comparable in overall sensitivity to neuronal thresholds, but intraspecific comparisons reveal slight differences. As an example, for the species *A. odorata* the minimum threshold was 42 dB SPL, which is higher than the threshold previously defined (around 30 dB SPL) by electrophysiological recordings (Fullard 1984; Pérez and Coro 1985). In insects, $2f_1 - f_2$ DPOAE usually can be elicited with stimuli levels near the species-specific auditory threshold (Kössl et al. 2008). However, DPOAEs are faint sounds whose detectability depends on the background noise. In our experiments, a threshold equivalent to 15 dB SPL was used based on the mean background noise level plus two standard deviations. In order to avoid the frequency up-tuning that appears at the acoustically stimulated moths' tympanum (Windmill et al. 2006; Mora et al. 2015), we did not use multiple stimulus presentations and response averaging. Instead, a 42 ms single stimulus was presented. The high background noise level precludes us from evaluating $2f_1 - f_2$ response below 15 dB SPL, resulting in lower sensitivities (higher minimum thresholds).

Previous studies in moths from diverse locations have consistently found highest sensitivity at frequencies between 20 and 60 kHz (Fullard 1982; Fullard et al. 1997; ter Hofstede et al. 2013). The Cuban moth community exhibited a slightly different response spectrum when assessed for DPOAE responses. A wide diversity of DPOAE audiograms demonstrates that most Cuban moths have best sensitivities to frequencies above 50 kHz. Methodological issues could be related to these differences. However, our results are in accordance with Fullard's observations regarding tropical locations; the increased use of higher frequencies by the echolocating bat communities appears to be matched by the greater sensitivities of sympatric moths in the high frequency range (Fullard 1982). A great diversity of bats using a variety of echolocation strategies imposes different sensory requirements to sympatric moths. Mormoopid bats are predominant in Cuba and

constitute the principal source of the high frequencies that put predation pressure on tympanate moths (Silva 1979; Mancina et al. 2007).

We found a high incidence of moths with threshold minima at about 60 kHz, close to the dominant second harmonic in the echolocation calls of *P. parnellii*. During flight, the mustached bat compensates for upward Doppler shifts of echo frequency by lowering the frequency of the emitted calls, so that returning echoes are stabilized at a frequency that is slightly higher than 61 kHz (Keating et al. 1994). *P. parnellii* is the only New World species that has evolved high-duty cycle echolocation (Fenton et al. 2012). This species is abundant and widespread throughout Cuba (Silva 1979). It seems that the specific properties and resonances of the *P. parnellii* ear that make it most sensitive and sharply tuned to ~61 kHz are mirrored by a corresponding, although less sharply tuned, development in some moth ears. In short, our observations are consistent with the conclusion that the ears of many neotropical moths have evolved their frequency sensitivity to maximize detection and avoidance of mustached bats.

Hearing research in moths has been mainly based on a neurophysiological approach. The neuronal threshold curve parallels the behavioral or vibrational audiograms for some species (Skals and Surlykke 2000; ter Hofstede et al. 2011), and this is also expected from the mechanical evaluation of the tympanum from OAEs responses. Several studies in vertebrates have demonstrated a correlation between the minimal thresholds in the neuronal audiogram and lowest thresholds for distortion-product generation (Kössl 1992; Manley et al. 1993; Meenderink et al. 2005). In insects, too, $2f_1 - f_2$ distortion products reach maximal amplitudes at frequencies of highest auditory sensitivity (Coro and Kössl 1998; Kössl and Boyan 1998). However, the hearing range defined by electrophysiological recordings for species from the genera *Achaea*, *Heliothis*, *Leucania*, *Melipotis*, *Mocis*, *Ophisma*, *Spodoptera* (Agee 1967; Fullard 1994; Fullard et al. 1997) are not consistent with the spectral range that we found by evaluating DPOAE generation. Best frequencies for these genera previously reported from neurophysiological studies, are below 40 kHz, which contrasts to the tuning of DPOAE audiograms to higher frequencies (>50 kHz) found in the present study. Certain issues related to methodology may explain the discrepancies between the results. Exposing the auditory nerve requires dissection of the thorax and partial removal of the flight musculature (Roeder and Treat 1957). Furthermore, the previously cited authors recorded in decapitated moths with wings removed. In contrast, DPOAE recording permits noninvasive determination of the frequency dependence of ear sensitivity. This approach helps to preserve the mechanical properties of the acoustical system and keeps intact potential effectors associated with the tympanic organ. Simultaneous

recording of mechanical (DPOAE) and electrophysiological responses of the peripheral auditory system in specimens with intact reflex pathways could help to elucidate causes of this frequency mismatch.

Conclusions

Despite their morphological simplicity, the ears of moths show highly diverse tuning capabilities. The behavioral context in which they have evolved seems to be an essential factor to enrich the frequency-sound level space that is shaping the moths' tuning curves. The high density of Cuban bat fauna compared with temperate faunas places greater predation pressure at the higher frequencies of the spectrum. The ears of Cuban moths are tuned to the frequency range used by the collection of bats on the island. The overlap between the bats' echolocation frequencies and the hearing range of sympatric moths described for this neotropical community is comparable to the overlap previously found in temperate areas, but with a shifted spectral range. Future studies using noninvasive approaches are needed for reconciling DPOAE and neuronal recordings.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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