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# What does a butterfly hear? Physiological characterization of auditory afferents in *Morpho peleides* (Nymphalidae)

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## Abstract

Many Nymphalidae butterflies possess ears, but little is known about their hearing. The tympanal membrane of butterflies typically comprises distinct inner and outer regions innervated by auditory nerve branches NII and NIII and their respective sensory organs. Using the Blue Morpho butterfly (*Morpho peleides*) as a model, we characterized threshold and suprathreshold responses of NII and NIII. Both are broadly tuned to 1–20 kHz with best frequencies at 1–3 kHz, but NIII is significantly more sensitive than NII. The compound action potentials (CAPs) of both branches increase their first peak amplitudes and areas in response to higher sound levels. NII and NIII differed in their suprathreshold CAP responses to sound frequencies, with stronger responses to 1–3 and 4–6 kHz, for NIII and NII respectively; results that are consistent with tympanal membrane mechanics. These results indicate that butterflies are capable of amplitude and frequency discrimination. Both auditory branches responded to playbacks of the flight and calls of predatory birds. We propose that the ears of butterflies, like those of many vertebrate prey such as some rabbits and lizards, function primarily in predator risk assessment.

**Keywords** Butterfly · Ear · Sensory · Neurophysiology · Predator detection

## Introduction

Butterflies are popular insects for behavioural and ecological research, and commonly used as models to study animal contests (Kemp and Wiklund 2004; Takeuchi 2017), migration (Guerra and Reppert 2015), mimicry (Su et al. 2015), and conservation (Oostermeijer and Swaay 1998; Kharouba et al. 2014). A full appreciation of an animal's behaviour requires an understanding of its sensory ecology, and for butterflies, research in this field has focused on visual and chemosensory systems (Hallberg and Poppy 2003; Warrant et al. 2003). While many butterflies possess ears, little is known about what they are capable of hearing, and how hearing plays a role in their survival.

Our understanding of an animal's acoustic sensory ecology is constructed from research on comparative ear

morphology, as well as from behavioural and neurophysiological responses to sounds. Tympanal ears in butterflies occur at the base of each forewing in species of the large family Nymphalidae (Minet and Surlykke 2003; Yack 2004). With few exceptions, eared butterflies are diurnally active and do not produce acoustic communication signals. Therefore, tympanal hearing is unlikely to function in pair formation, aggression, bat detection, or host localization, the primary reported functions for hearing in other insects (Stumpner and von Helversen 2001; Strauss and Stumpner 2015; Mason and Pollack 2016). Like most other insect tympanal ears, butterfly ears comprise a tympanal membrane, chordotonal sensory organs associated with the membrane, and air chambers beneath the membrane to detect pressure waves (Minet and Surlykke 2003; Yack 2004). Unlike other insect ears, butterfly tympanal membranes comprise distinct inner and outer regions, each associated with multi-celled sensory organs (e.g., Yack et al. 2000; Lucas et al. 2009), but the functional significance of this unusual morphology is unknown. There is little evidence at present for behavioural responses to sound in diurnal butterflies. Ribaric and Gogala (1996) reported wing flicking and flight responses to low-frequency sounds, with a best frequency (BF) at 1 kHz. It has been proposed that butterflies hear the calls or

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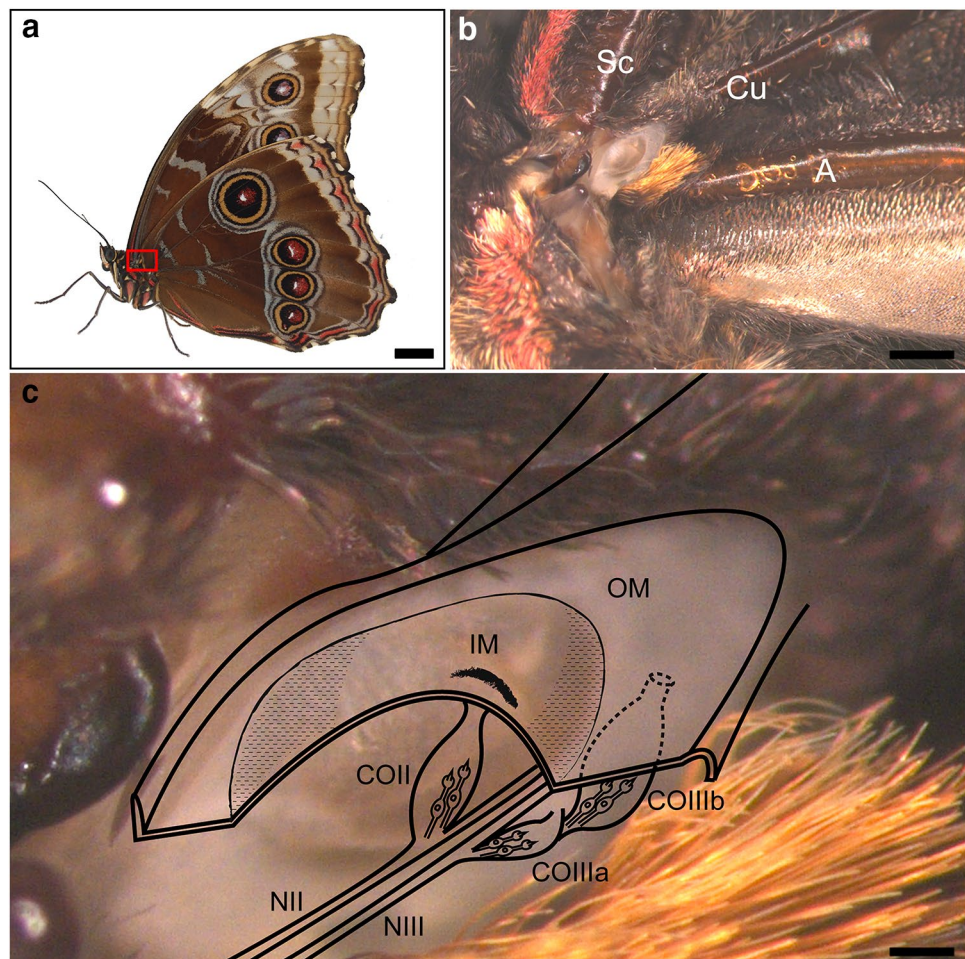


flight sounds of avian predators (Ribaric and Gogala 1996; Fournier et al. 2013), but this hypothesis requires testing. Neurophysiological recordings from a few species show that ears are tuned to low-frequency sounds between 500 Hz and 20 kHz, with best frequencies between 1 and 6 kHz (Yack et al. 2000; Mahony 2006; Lane et al. 2008; Lucas et al. 2009, 2014). However, these studies are limited in that they focused on minimum threshold tuning curves of only one of the two auditory branches. The primary roles of any insect auditory system are to detect, discriminate, and localize biologically relevant sounds (Mason and Pollack 2016). These tasks are achieved by gathering information about the spectral content, amplitude and temporal patterning of acoustic stimuli, which in turn is represented in the spiking output of populations of auditory cells (Stumpner and von Helversen 2001; Mason and Pollack 2016). To understand the hearing capabilities of a butterfly, it is necessary to characterize the threshold and suprathreshold responses of the cell populations in both auditory nerve branches innervating the ear.

The Blue Morpho, *Morpho peleides*, is an excellent model to study butterfly hearing. The species is representative of most eared Nymphalidae, being diurnally active, having no

known sound production, and having well-developed tympanal ears (Lane et al. 2008; Lucas et al. 2009). This species also has a large body size, facilitating neurophysiological recordings from auditory nerves that are difficult to access. The tympanal membrane consists of a dome shaped inner membrane surrounded by a flatter outer membrane (Fig. 1). Separate chordotonal organs (CO) innervate each of these tympanal regions by two auditory nerve branches. The inner membrane is associated with CO II with ~10–15 scolopidia (primary sense cells) and is innervated by auditory branch NII. The outer membrane is associated with two chordotonal organs, CO IIIa and CO IIIb, with ~10–12 and ~15–20 scolopidia, respectively, and these are both innervated by auditory branch NIII (Lane et al. 2008; Lucas et al. 2009) (Fig. 1). It is not known how the chordotonal organs differ in their responses to sound, but predictions can be made based on a previous laser vibrometry study in *M. peleides* (Lucas et al. 2009). That study reported greater overall displacements at best frequency for the outer membrane compared to the inner membrane. In addition, the tympanal membrane vibrated in two distinct modes depending on frequency range. At lower frequencies (< 5 kHz), the vibration was

**Fig. 1** Tympanal ear of the Blue Morpho butterfly (*M. peleides*). **a** Butterfly in natural resting position showing the location of the ear on the ventral surface of the forewing (indicated by red rectangle). **b** Close-up of the ear and surrounding wing veins: subcostal (Sc), cubital (Cu), and anal (A). **c** Enlargement of the tympanal membrane, with schematic drawings of the auditory nerve branches NII and NIII, and chordotonal organs COII, COIIIa, and COIIIb. Scale bars = 1 cm in **a**; 1 mm in **b**; 100  $\mu$ m in **c**



focused on the outer membrane, while at higher frequencies (> 5 kHz), the entire membrane vibrated. These results suggested that *M. peleides* is capable of amplitude and frequency discrimination, which could provide information about the distance or location of a sound source, such as a predator (see “Discussion”; Mason and Faure 2004). However, the extent to which this information is transduced and encoded by the nervous system remains unclear.

In this study we ask, “What does a butterfly hear?” by characterizing and comparing the responses of both auditory nerve branches. We test the hypotheses that NII and NIII respond differently to sound amplitude and frequency by comparing whole nerve audiograms and compound action potential (CAP) responses to suprathreshold stimuli. We also tested the hypothesis that butterfly ears are capable of assessing predation risk using playbacks of predator sounds.

## Materials and methods

### Animals

*Morpho peleides* were purchased from London Pupae Sales (Oxfordshire, UK) (Permit: P-2011-04393). Pupae were housed in mesh cages in a greenhouse at Carleton University, Ottawa, Canada, where they were exposed to natural light conditions, temperature fluctuations of 25–35 °C, and humidity levels of 55–75%. Butterflies were provided with orange slices *ad libitum* and used for experiments within 1–6 days following emergence.

### Neurophysiology recording setup

Previously described methods were performed to expose and record from the auditory branches NII and NIII (Lane et al. 2008; Lucas et al. 2009). The main mesothoracic wing nerve, IIN1c, and its three branches; NI (non-auditory), NII and NIII were exposed by positioning the butterfly on modeling clay, securing with wire holders, and removing the wing tegula and overlying membrane. Electrolytically sharpened stainless steel hook electrodes were used in a single-ended configuration (with the ground electrode in the abdomen) to record from NII or NIII. Once a stable signal was obtained from one branch, the other two were cut to ensure that neural activity originated only from the branch under study. Neural signals were amplified by a GRASS P-55 preamplifier (West Warwick, RI, USA) and displayed on a Tektronix (TDS2002C) (Beaverton, OR, USA) digital oscilloscope, and also monitored through an audio monitor. Both the sound stimuli and neural responses were recorded as .wav files at a sampling rate of 88.2 kHz using a Fostex FR-2: Field Memory Recorder (Akishima, Tokyo, Japan) for offline analysis. All recordings were

performed within a Faraday cage lined with acoustic foam (1.2 m × 0.9 m × 0.8 m).

### Tuning curves and amplitude ramps

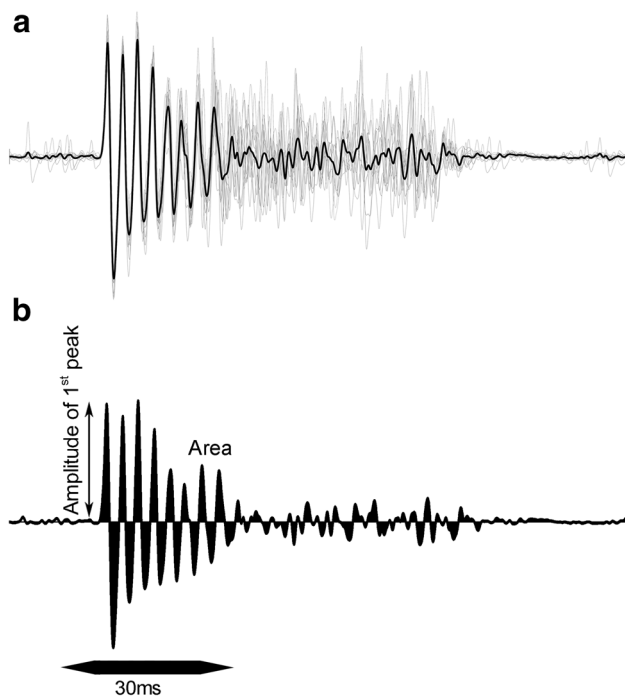
Sounds between 0.5 and 25 kHz were presented as 30 ms trapezoidal pulses (including 5 ms rise/fall linear ramps). Acoustic stimuli were synthesized using a Tucker Davis Technologies (TDT) (Alachua, FL, USA) RX6 digital signal processor and shaped using the TDT PC software (RpvdsEx, v. 5.4). Sound frequencies between 0.5 and 3 kHz were generated and amplified by a TDT SA1 stereo power amplifier and broadcast from a generic woofer positioned 1 m from the specimen ipsilateral to the side of the recording. Sound frequencies between 4 and 25 kHz were attenuated using a TDT PA5 programmable attenuator and broadcast from a CTS tweeter (KSN1167A) (Boston, MA, USA) at 30 cm from the specimen ipsilateral to the side of the recording. Both the woofer and tweeter were calibrated by calculating the sound level in dB SPL at the specimen using continuous sine waves measured by a Brüel and Kjær Type 2239 sound-level meter and a Brüel and Kjær Type 4135 microphone and Nexus Type 2610 measuring amplifier.

Threshold tuning curves were constructed using previously described methods (Yack et al. 2000; Lane et al. 2008; Lucas et al. 2009). Neural responses were evoked by gradually increasing the sound amplitude until a neural response that was above the background activity and in synchrony with the sound pulse appeared on the oscilloscope. Threshold was determined as the lowest sound pressure level that consistently elicited a response ( $\geq 90\%$  of the time) for sound frequencies presented at 1 kHz intervals. Test frequencies were presented in random sequence, and upon completion of the audiogram, the first three test frequencies were repeated and thresholds re-measured. The tuning curve was only included in the results if these re-tested thresholds were within  $\pm 3$  dB of the original thresholds; this ensured that any variation in threshold was not due to changes in the animal's condition.

The analysis of neural responses was performed using custom-Matlab scripts (The Mathworks, Natick, MA, USA). Tuning curves were compared for BF, threshold at BF, sharpness, and overall sensitivity. ‘Sharpness’ of tuning curves was quantified by the  $Q_{10\text{dB}}$  factor (Bennet-Clark 1999), BF divided by the bandwidth of the tuning curve 10 dB above threshold. Overall sensitivity was quantified as the area above tuning curves under 70 and 80 dB SPL. Best frequency (BF), threshold at BF,  $Q_{10\text{dB}}$  factors, and overall sensitivity of NII and NIII were compared using Mann–Whitney *U* tests. The significance level for all statistical tests was 0.05.

Suprathreshold activity of NII and NIII was measured by recording the compound action potential (CAP) in response

to selected sound frequencies (1, 2, 3, 4, 6, and 8 kHz) played at increasing sound levels. A CAP is the sum of multiple single action potentials firing in a nerve branch. At each frequency, ten sound pulses (30 ms trapezoidal pulses as described above) were played at 1 s intervals for a given amplitude, and this was repeated at +2 dB increments from 60 to 85 dB SPL. Neural responses to each identical set of ten pulses were averaged (Fig. 2), and for each averaged trace, we measured the neural activity 100 ms prior to the stimulus onset (pre-stimulus) and 100 ms following stimulus onset (post-stimulus). The strength of the response was quantified by measuring both the amplitude of the first peak and the area of the CAP (Fig. 2). The first peak of the CAP (after stimulus onset) was identified by the first peak in the signal with amplitude of at least 4× the standard deviation of the pre-stimulus recording (approximating a 99.99% confidence interval). The CAP area was calculated from the rectified signal (absolute value) by subtracting the area in the pre-stimulus window from that in the post-stimulus window. Averages for both CAP peak and CAP area were calculated for each sound level and frequency. These data were then interpolated and smoothed by convolving with a 2D gaussian (Matlab) to generate heat maps of neural response (normalized to peak response in each panel).



**Fig. 2** Compound action potential (CAP) recorded from NIII in response to a 30 ms, 2 kHz, sound stimulus: **a** ten individual CAPs (grey) and the averaged CAP (black) from a single animal. **b** Measurements from the averaged CAP included the amplitude of the first peak and area (shaded area)

## Playback of bird sounds

To test how ears respond to predator sounds, bird flight and vocalization sounds were played while recording from NII or NIII. Flight sounds of blue jays (*Cyanocitta cristata*) had been previously recorded with an Earthworks QTC40 microphone (4 Hz–40 kHz  $\pm$  1 dB, Milford, NH, USA) at a sampling rate of 192 kHz (see Fournier 2011). These flight sounds are representative of other insectivorous birds in that they are broadband with most energy between 500 Hz and 10 kHz (Fournier 2011; Fournier et al. 2013). Vocalizations of the jacamar (*Galbula ruficauda*), a predator of *Morpho* butterflies (Young 1971), were obtained from the Xeno-canto database (Xeno-canto 2016). These vocalizations comprise a series of notes that function in mate selection and territoriality (Hilty 2003). Jacamars call throughout the day (Hilty 2003), and feed on *Morpho* spp. butterflies (Young 1971). Sounds were played back to auditory nerve preparations using an Avisoft ScanSpeak speaker and Avisoft USG Player 116 (Berlin, Germany). Sound levels were adjusted, such that the loudest sound in the playback sequence was set to 10, 20 and 30 dB above threshold.

## Results

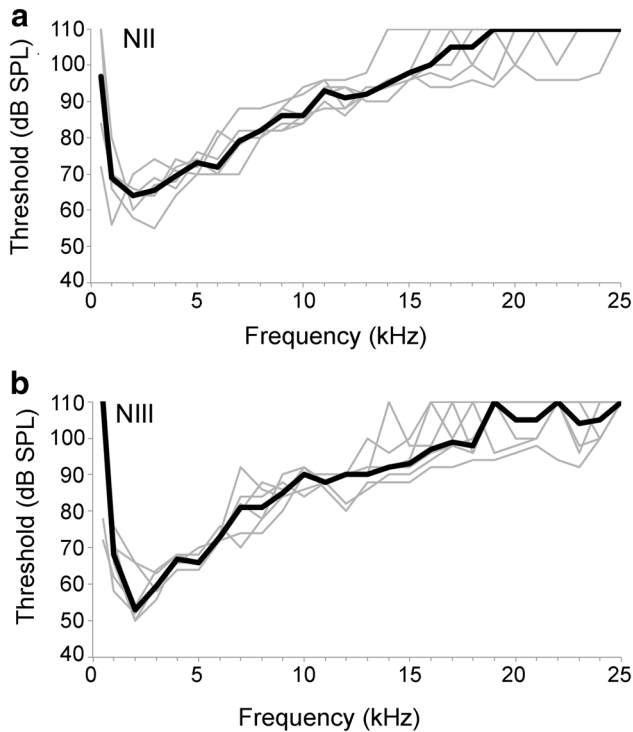
### Audiograms

Tuning curves for NII and NIII are shown in Fig. 3. Both responded to sounds between 1 and 20 kHz, with BFs between 1 and 3 kHz (Fig. 3; Table 1). BFs did not differ significantly between the two branches. However, NIII is significantly more sensitive than NII at BF with median minimum thresholds of 53 and 62 dB SPL respectively (Fig. 3; Table 1). The increased sensitivity of NIII compared to NII was also reflected in the total area falling below 70 dB and 80 dB, which was significantly greater for NIII (Table 1). Both were broadly tuned with mean  $Q_{10dB}$  values of 0.71 (NII) and 0.90 (NIII); these did not differ significantly between nerve branches (Table 1).

### Suprathreshold CAP Responses

A previous laser vibrometry study reported that the tympanal membrane of *M. peleides* vibrated in two distinct modes depending on the sound frequency (Lucas et al. 2009). Deflection envelopes along a transect of the membrane (Fig. 4a) show that in response to higher frequencies (e.g., 6.5 kHz), the NII attachment point (indicated as XII) shows a greater response than does the attachment point of NIII (indicated as XIII). In contrast, at lower frequencies (e.g., 2.5 kHz), the vibration is focused on the attachment point of NIII (indicated as XIII). To determine how this mechanical





**Fig. 3** Auditory tuning curves for *M. peleides*. Response thresholds in six individuals are shown in grey, with median thresholds in black for NII (a) and NIII (b) nerve branches

tuning influences neural tuning, suprathreshold responses of NII and NIII were quantified for increasing sound levels at frequencies between 1 and 8 kHz. Heat maps of CAP peak amplitude and CAP area (Fig. 4b–e) show that in general, both NII and NIII responded to increasing sound levels by increasing both the amplitude of the first peak and the area of the CAP. However, these suprathreshold responses reveal distinct frequency tuning between NII and NIII that was not evident in the threshold tuning curves.

The broad frequency response of NIII, with a maximum near 1–2 kHz (Fig. 4c, e) is consistent with the broad

mechanical response of the outer membrane to lower frequencies. Furthermore, the more selective responses to higher frequencies exhibited by NII (Fig. 4b, d) is consistent with the larger deflections of the inner membrane at frequencies greater than 4 kHz. These results show that the two nerve branches respond differently to sound frequencies, corroborating the previous results on tympanal membrane mechanics.

**Responses to natural sounds**

Both auditory branches responded to playbacks of bird flight and vocalizations, but NIII was most responsive (Fig. 5). NIII responded in a bursting pattern to the downstrokes of bird flight (Fig. 5a). The CAP increased in peak amplitude (375%) and area (240%) (*N*=4) when sound levels of wing downstrokes were played 20 dB above threshold (Fig. 5a). Responses from NII were consistently more “noisy” with smaller CAP peaks and did not represent the down stroke pattern accurately in the few recordings that were obtained (data not shown). NIII clearly responded to Jacamar calls (Fig. 5b) with an increase in CAP peak amplitude (376%) and area (151%) (*N*=4) when the playback was 20 dB above minimum auditory thresholds of individual butterflies. NII responded consistently but weakly to jacamar vocalizations, perhaps owing to the low-frequency nature (2 kHz) of the vocalizations and the reduced sensitivity of NII at those frequencies.

**Discussion**

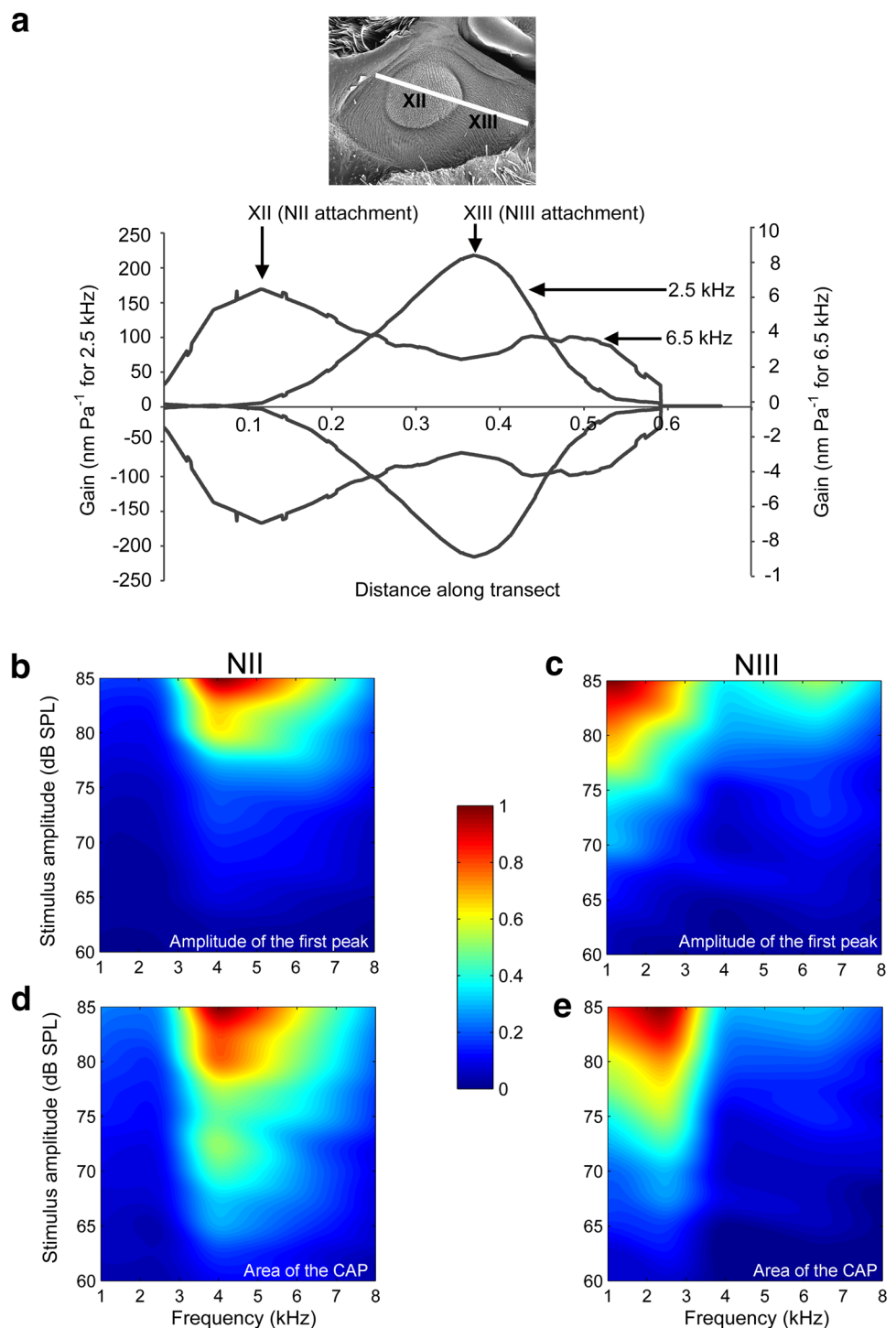
This study characterizes the hearing capabilities of a butterfly by recording from both auditory branches in the Blue Morpho. The ears are broadly tuned to low-frequency (1–20 kHz) sounds and capable of amplitude and frequency discrimination. We propose that hearing in diurnal butterflies functions in predator detection.

**Table 1** Tuning curve measurements for tympanal nerve branches NII and NIII in the Blue Morpho butterfly (*M. peleides*)

Best frequency (BF) (kHz)		Threshold at BF (dB SPL)		$Q_{10dB}$		Area under 70 dB SPL (arbitrary units)		Area under 80 dB SPL (arbitrary units)	
NII	NIII	NII	NIII	NII	NIII	NII	NIII	NII	NIII
1	2	56	54	1.00	0.95	49	160	67	100
2	2	64	50	0.31	1.17	57	176	68	96
3	2	55	52	1.03	0.77	173	214	97	109
3	3	64	63	0.53	0.51	38	64	56	75
2	3	64	58	0.68	0.75	51	132	79	98
2	2	60	50	0.71	1.25	59	184	55	96
$P=0.81^a$		$P=0.04$		$P=0.29$		$P=0.02$		$P=0.03$	

<sup>a</sup>Statistical comparisons between nerve branches were conducted using Mann–Whitney *U* tests

**Fig. 4** Tympanic membrane (TM) mechanics and CAP responses of NII and NIII to different sound frequencies. **a** Displacement envelopes showing amplitude gain along the TM in response to sounds played at 2.5 and 6.5 kHz (adapted from Lucas et al. 2009). In the scanning electron micrograph of the TM, the transect measured is shown as a white line and XII and XIII indicate attachment points of chordotonal organs innervated by NII and NIII respectively. The left y-axis of the plot corresponds to the gain at 2.5 kHz, and the right axis corresponds to the gain at 6.5 kHz. The mechanical response varies with frequency. At lower frequencies the membrane vibrates mostly around XIII, but at higher frequencies the membrane vibrates more at XII. **b–e** Averaged CAP amplitude–frequency plots of NII and NIII (5 butterflies for each branch). The amplitude of the first peak (**b, c**) and the area of the CAP (**d, e**) are normalized to the maximum response (colour bar in center applies to all panels). NII responds best to suprathreshold stimuli of frequencies between 4 and 6 kHz, whereas NIII responds best to suprathreshold stimuli below 4 kHz



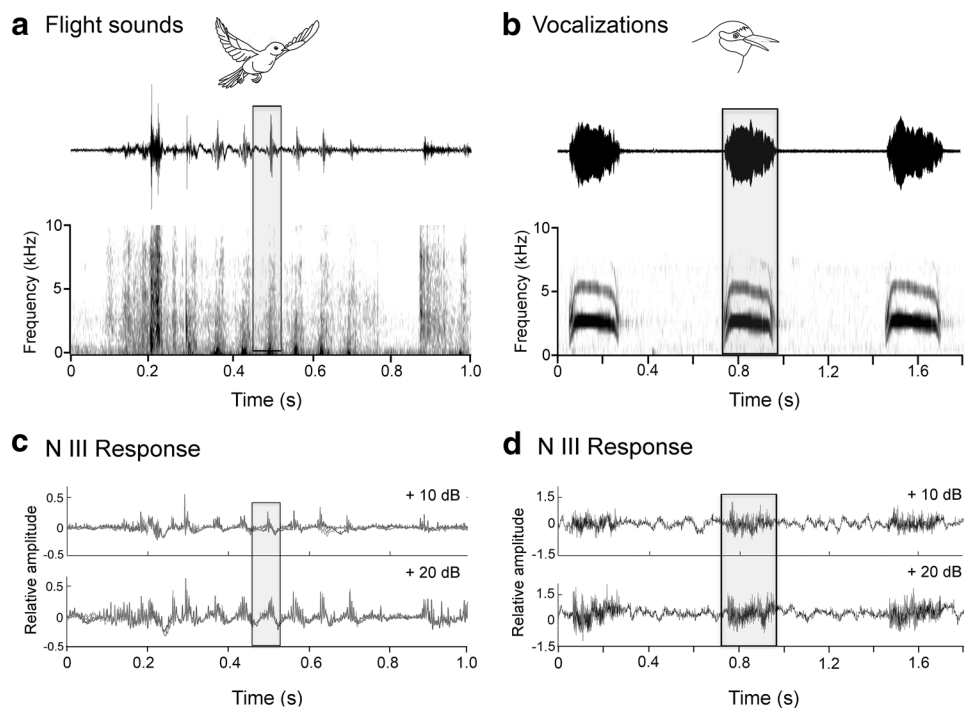
**Auditory threshold tuning curves**

NII and NIII have similarly shaped threshold tuning curves, but NIII is more sensitive. Both are broadly tuned to 1–20 kHz with greatest sensitivity at 1–3 kHz. Similar broad tuning to low-frequency sounds has been reported in other non-calling insects including some mantids (Yager 1996), grasshoppers (Lehmann et al. 2010) and other

butterfly species (Swihart 1967; Lucas et al. 2014), as well as in sound producing insects whose hearing sensitivity is mismatched to their calling songs (Mason 1991; Yack et al. 2000). In these examples, the significance of low-frequency hearing sensitivity is unknown but proposed to function in predator detection. NIII is significantly more sensitive than NII, which may be attributable to larger amplitude displacements of the outer membrane (Lucas et al. 2009).



**Fig. 5** Averaged NIII responses to playbacks of bird flight and vocalizations. **a** Sound waveform and corresponding spectrogram of the flight sounds of a Blue jay (*C. cristata*). There are ~8 wing beat cycles, with downstrokes being the loudest. One wing beat cycle is shaded. **b** Sound waveform and corresponding spectrogram of a call of a rufous-tailed jacamar (*G. ruficauda*). One call is shaded. **c** NIII responses to bird flight played at 10 and 20 dB above the auditory threshold of the butterfly. The neural response corresponding to the wing beat cycle in **a** is shaded. **d** NIII responses to bird call at sound levels 10 and 20 dB above threshold. The neural response corresponding to one call cycle in **b** is shaded



Amplitude discrimination by populations of auditory cells with similar tuning has been shown in Lepidoptera, Coleoptera, and Orthoptera, and proposed to extend the dynamic range of hearing, and to enable discrimination between close and distant sound sources (e.g., Yager and Spangler 1995; Greenfield 2002; Surlykke et al. 2003; Mason and Faure 2004). The proposed functional significance for such tuning characteristics in butterflies is discussed below (see “What does a butterfly hear?”).

### Suprathreshold responses of NII and NIII

The CAP response increased in peak amplitude and area for both NII and NIII across all sound frequencies. The NII CAP is the aggregate response of an estimated 10–15 scolopidia in COII, and the NIII CAP is the aggregate response of ~10–12 and ~15–20 scolopidia in COIIIa and COIIIb, respectively (Lane et al. 2008; Lucas et al. 2009). Based on this morphology, the NIII CAP might be expected to have a larger peak and area due to the larger number of cells in NIII and the separation of the two chordotonal organs in the ear. However, a number of factors can affect CAP characteristics, including the number and the sizes of axons and their temporal firing patterns, so our data cannot address this issue directly (Adam 1977a, b; Schul 1999). The relative contributions of individual sensory receptors to the CAP should be further studied by recording intracellularly from individual sensory cells.

Our results support the hypothesis that NII and NIII differ in their suprathreshold frequency tuning. At frequencies

below 3 kHz, the suprathreshold response of NIII exceeds that of NII, but from 4 to 8 kHz, NII exceeds that of NIII. This result corroborates a previous laser vibrometry study in *M. peleides* showing that at frequencies below 4 kHz, vibrations were focused on the outer membrane (innervated by NIII), whereas at frequencies above 4 kHz, the inner membrane (innervated by NII) vibrated more relative to the outer membrane (Lucas et al. 2009). Lucas et al. (2009) concluded that the complex topography of the tympanal membrane allows for two distinct vibrational modes. Thus, the frequency dependent responses seen in our neurophysiology study likely are attributable to tympanal membrane mechanics. The proposed functional significance of frequency tuning for butterflies is discussed below.

### What does a butterfly hear?

Although tympanal ears are widespread in Nymphalidae butterflies, the function of their hearing remains unknown. Most species do not produce sounds, which rules out conspecific communication. Hearing for bat detection is also unlikely as most species are diurnally active, and their ears are tuned to sound frequencies of 1–6 kHz (Lane et al. 2008; Lucas et al. 2009, 2014) much lower than those used by echolocating bats (Ratcliffe 2009). The best-supported hypothesis is that butterfly ears function to detect diurnally active predators, and specifically, insectivorous birds that are major predators of butterflies (e.g., Pinheiro 1996; Pearce-Higgins 2010).

One specific hypothesis is that butterflies detect incidental sounds of approaching predators, such as bird flight sounds

(Fournier et al. 2013). Vertebrate prey use sound cues to assess predation risk (e.g., Magrath et al. 2007; Haff and Magrath 2010), but aside from bat detection, the role of insect hearing in predator detection is poorly understood. A few lines of evidence support this hypothesis for butterflies. First, diurnal butterflies are sensitive to low-frequency sounds (1–6 kHz) that are within the range of passive sounds made by an approaching predator such as flight or rustling leaves (Goerlitz et al. 2008; Fournier et al. 2013). Butterfly ears exhibit tuning similar to the ears of some lizards and rabbits, which use their hearing primarily to assess predation risk (see Heffner and Masterton 1980; Cantwell and Forrest 2013). Second, butterfly ears are broadly tuned with  $Q_{10\text{dB}}$  values below 1.0, which is characteristic of hearing that functions primarily in predator detection (see Yager and Spangler 1995). Third, as shown by our playback studies, the auditory cells are capable of following wing beat patterns. Fourth, the butterfly could potentially determine if a predator is approaching by monitoring changes in sound amplitude and bandwidth through different firing patterns of NII and NIII. Based on detection thresholds, butterflies should be able to detect an approaching predator at distances of at least 2.5 m (Fournier et al. 2013). To further test the hypothesis that hearing functions to detect passive sounds of predators, further neurophysiological and behavioural responses to predator sounds or live predators should be characterized.

An alternative but related hypothesis is that butterflies eavesdrop on predator communication signals (Ribaric and Gogala 1996). Vertebrate prey assess predation risk by attending to communication signals of predators, or alarm calls of other species (e.g., Ito and Mori 2010; Barrera et al. 2011; Cantwell and Forrest 2013), but there is little evidence for this in insects. Our results support this hypothesis by demonstrating that *M. peleides*' ears respond to the calls of one of their major predators, the jacamar. The vocalizations of most birds fall within the range of 1–5 kHz, matching the tuning of their ears (Dooling et al. 2000). Interestingly, the two frequency bands of the bird call presented in our experiment match the frequency responses of NII and NIII. While this is a single example of a *Morpho* predator, it could be argued that broad tuning allows for increased detection of harmonics in predator calls. The expected behavioural responses to bird calls might be to remain stationary if a butterfly is at rest, or to land, or fly in the opposite direction if the butterfly is flying.

## Conclusion

We provide insights into what a butterfly hears by recording from both auditory nerve branches of *M. peleides*. The ears are tuned to low-frequency sounds, are capable of amplitude and frequency discrimination, and respond to sounds

of avian predators. The neurophysiological characteristics of butterfly ears share similarities with the ears of many vertebrate prey, such as some lizards and rabbits, which use their hearing primarily for predator detection. Predation imposes an important selection pressure on the behaviour of butterflies, but the role of hearing for predator detection has not been explored. We propose that the function of low-frequency hearing for predator risk assessment is underappreciated for insects and warrants further investigation.

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**Author contributions** AM designed the experiments, collected the neurophysiology data, analyzed the results, and contributed to manuscript writing. JL helped with experiment design, data analysis, and manuscript revisions. JY designed the experiments, helped with data collection, and contributed to manuscript writing.

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## Compliance with ethical standards

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

**Ethical approval** All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

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