BIOLOGY LETTERS

rsbl.royalsocietypublishing.org

Research

Cite this article: Sun P, Mhatre N, Mason AC, Yack JE. 2018 In that vein: inflated wing veins contribute to butterfly hearing. *Biol. Lett.* **14**: 20180496.
http://dx.doi.org/10.1098/rsbl.2018.0496

Received: 13 July 2018
Accepted: 24 September 2018

Subject Areas:
behaviour, neuroscience, biomechanics

Keywords:
insect, hearing, sound, laser vibrometry, wing veins, butterfly

Author for correspondence:
Jayne E. Yack
e-mail: jayneyack@cunet.carleton.ca

Electronic supplementary material is available online at http://dx.doi.org/10.6084/m9.figshare.c.4252370.

THE ROYAL SOCIETY
PUBLISHING

Physiology

In that vein: inflated wing veins contribute to butterfly hearing

Penghui Sun[1], Natasha Mhatre[2], Andrew C. Mason[2] and Jayne E. Yack[1]

[1]Department of Biology, Carleton University, Nesbitt Building, 1125 Colonel By Drive, Ottawa, ON, Canada K1S 5B6
[2]Department of Biological Sciences, University of Toronto at Scarborough, Scarborough, Toronto, ON, Canada M1C 1A4

NM, 0000-0002-3618-306X; JEY, 0000-0001-9383-6437

Insects have evolved a diversity of hearing organs specialized to detect sounds critical for survival. We report on a unique structure on butterfly wings that enhances hearing. The Satyrini are a diverse group of butterflies occurring throughout the world. One of their distinguishing features is a conspicuous swelling of their forewing vein, but the functional significance of this structure is unknown. Here, we show that wing vein inflations function in hearing. Using the common wood nymph, *Cercyonis pegala*, as a model, we show that (i) these butterflies have ears on their forewings that are most sensitive to low frequency sounds (less than 5 kHz); (ii) inflated wing veins are directly connected to the ears; and (iii) when vein inflations are ablated, sensitivity to low frequency sounds is impaired. We propose that inflated veins contribute to low frequency hearing by impedance matching.

1. Introduction

Insects have a rich diversity of hearing organs that function in a variety of tasks including locating mates, evading predators and coordinating social interactions [1]. To achieve these tasks, insects need to evaluate the amplitude, frequency and temporal patterns of sounds [2]. The small size of insects presents challenges to achieving these tasks and consequently, insect ears have evolved unique specializations [3]. Here, we investigate how inflated wing veins in butterflies function to enhance hearing.

Satyrinae are a large subfamily of approximately 2400 butterfly species belonging to the family Nymphalidae. Many Satyrinae possess ears at the base of their forewings [4–6], but little is known about the characteristics of their hearing. A distinguishing feature of Satyrinae, particularly within the tribe Satyrini, is a conspicuous ‘inflated’, ‘dilated’ or ‘swollen’ vein on each forewing [7–9]. However, the function of these prominent structures is unknown. We propose that they function in hearing based on their morphological proximity to the ear and the known function of air cavities in insect ears. Using a Satyrini, the common wood nymph (*Cercyonis pegala*), we first describe the morphology of the eardrum and characterize its vibration properties. We then test the hypothesis that swollen wing veins function in hearing—specifically in the tuning and sensitivity of the eardrum’s response to sound. We predict that (i) there is a physical connection between the ear and the swollen vein and (ii) ablating the swollen vein will impair hearing.

2. Material and methods

(a) Animals

Cercyonis pegala butterflies were collected from their natural habitat near Ottawa and Perth, Ontario, Canada between July and August 2016 and 2017 (see electronic supplementary material). Specimens used for laser vibrometry were stored in glassine

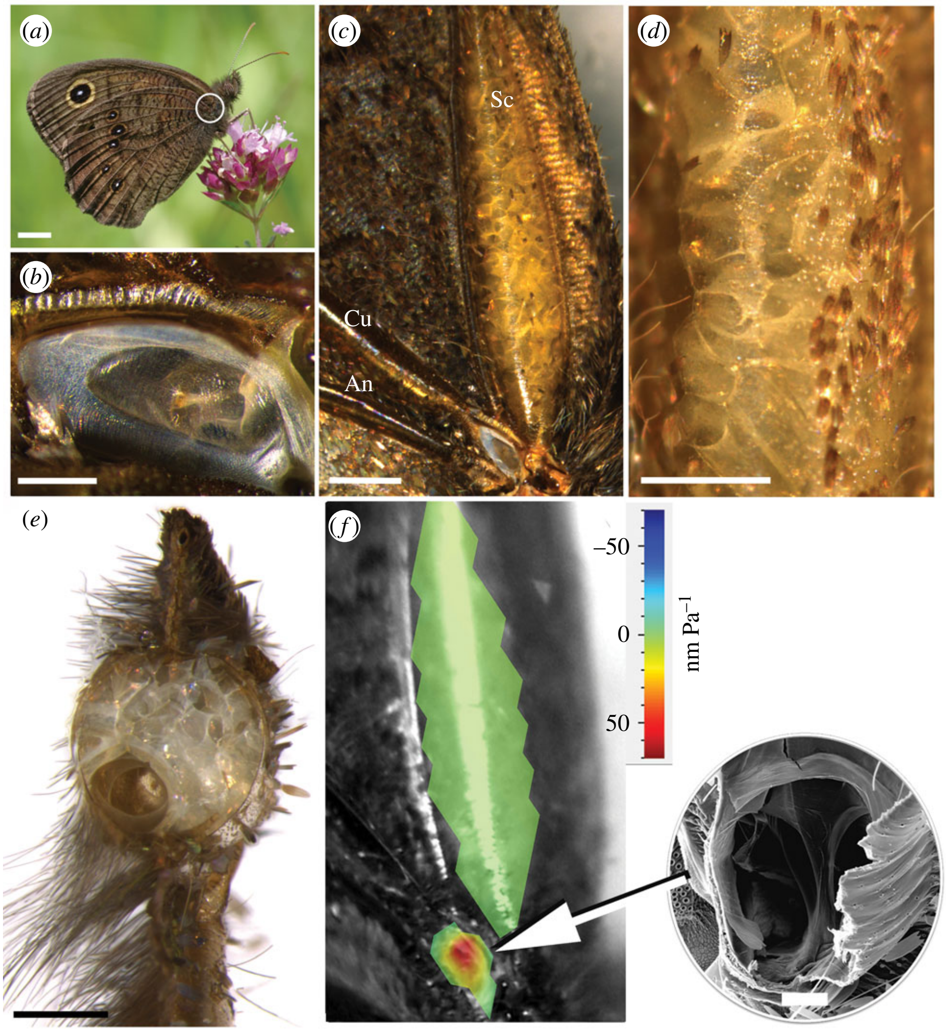

Figure 1. Ear and wing vein morphology of *C. pegala*. (*a*) Butterfly in resting position. A white circle marks the location of the ear. Scale bar: 5 mm. (*b*) Light micrograph of right tympanal membrane. Scale bar: 200 µm. (*c*) Forewing showing enlarged subcostal (Sc) vein, as well as cubital (Cu) and anal (An) veins. Tympanal ear is seen at the wing base. Scale bar: 1 mm. (*d*) Internal structure of Sc vein viewed through the cuticle. Scale bar: 500 µm. (*e*) Cross-section of the Sc vein. Scale bar: 500 µm. (*f*) Laser scan of Sc vein and tympanal membrane depicting displacement at 4.8 kHz. Inset: Scanning electron micrograph of the opening connecting the tympanal chamber and Sc vein. Scale bar of inset: 100 µm.

envelopes at approximately 8°C for up to 6 days prior to conducting experiments. A total of 30 specimens were used; seven males and seven females for laser vibrometry and all 30 for morphology, including the same samples used in vibrometry.

(b) Tympanal and wing vein morphology

Tympanal membrane and forewing vein morphology were examined in 15 males and 15 females. Photographs were taken using a light microscope (Leica DMC4500) equipped with a camera (Leica M205C) and tympanal surface area was measured (Leica Application Suite 4.8.0). Scanning electron micrographs (SEMs) were used to image the tympanal membrane and the connectivity between the tympanal chamber and the subcostal vein.

(c) Laser vibrometry and acoustic stimuli

Vibration measurements were made with a scanning laser Doppler vibrometer (Polytec PSV 400) coupled with an OFV-505 sensor fitted with a close-up unit. Anesthetized butterflies were mounted on a rotatable metal platform attached to a steel rod. The forewing and abdomen were immobilized, and wing-scales removed to expose the tympanum. For each butterfly, the entire ear membrane was scanned, using approximately 150–200 measurement points on a grid. Vibrations of the tympanic membrane were measured in response to acoustic stimuli: 160 ms periodic chirps from 0.75 to 20 kHz with a frequency resolution of 6.25 Hz (see electronic supplementary material). The sound pressure level (SPL) was measured using a calibrated microphone (Bruel and Kjær: 4138) coupled to a pre-amplifier (Nexus: 2690). Acoustic and vibrational data were digitized at a sampling rate of 51.2 kHz. The magnitude, phase and coherence of each membrane's displacement were plotted as a frequency response at each scan point, which was calculated from measurements of the membrane's velocity at each point (see electronic supplementary material). Video animations of the membrane vibration were created using PSV software (see electronic supplementary material).

Vibration properties of the tympanal membrane with the vein intact were measured for each individual. Following the initial scan, the inflated subcostal vein ipsilateral to the ear was ablated by making a longitudinal cut to open the ventral surface (electronic supplementary material, figure S1) and the vibration pattern of the tympanum was remeasured.

(d) Data analysis

Comparison of tympanal vibration characteristics between sexes was done using two-sample *t*-tests with unequal variances. The magnitude of the membrane transfer function (at point of highest displacement) was plotted using Matlab R2017b (v.9.3.0.713579) and fitted to a damped simple harmonic oscillator (SHO) model. Parameters for an SHO, such as resonance frequency, were estimated and fitted using Matlab's Curve Fitting toolbox, and corresponding displacements at resonance

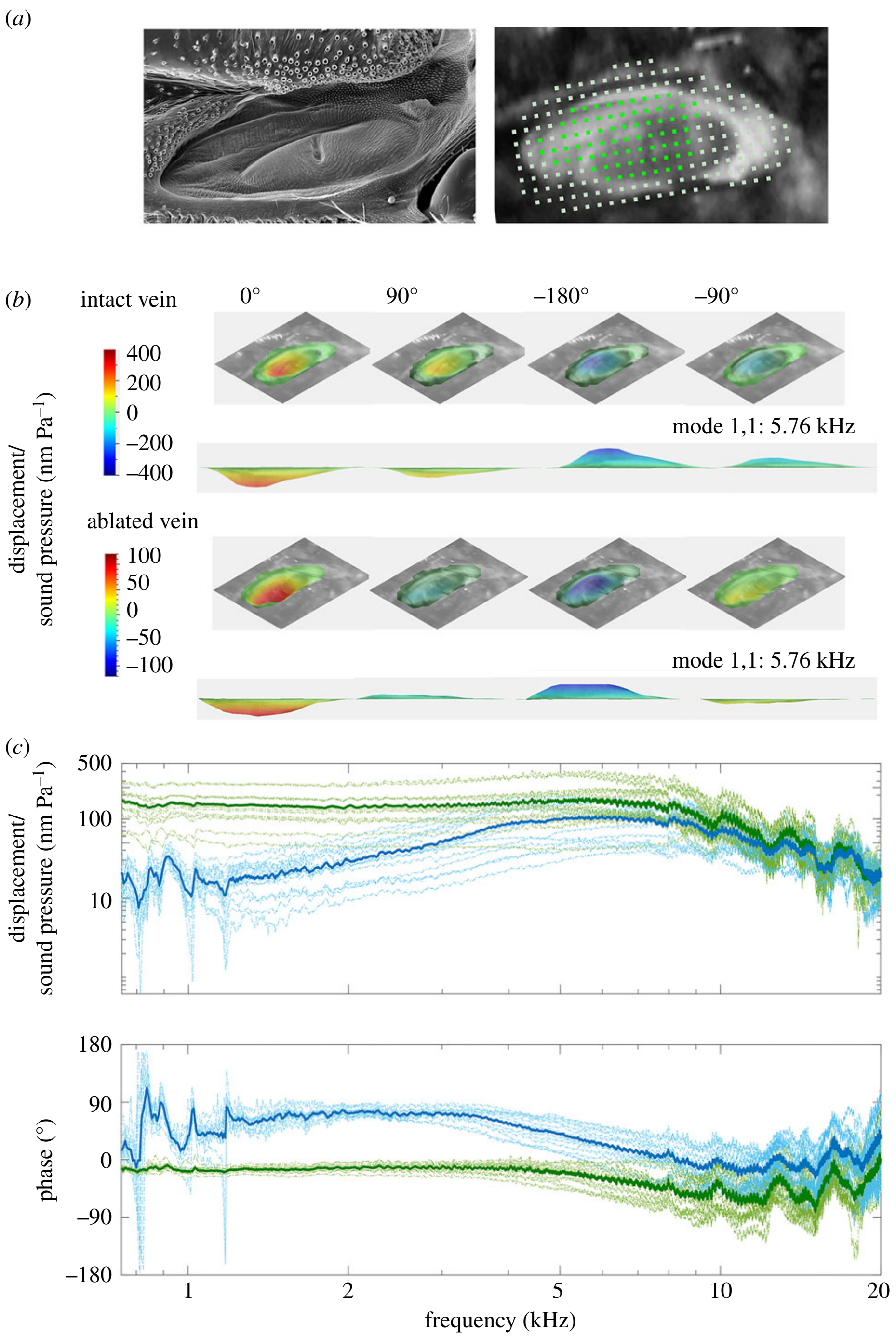

Figure 2. Vibrational response of tympanal membrane in *C. pegala*. (*a*) Scanning electron micrograph of tympanal membrane (left) and laser scanning grid (right). (*b*) Tympanal displacement per unit SPL in response to 5.7 kHz (male #8, resonant frequency) when inflated vein is intact (top) or ablated (bottom). Each is shown at 4 phases of the oscillation. Red: outward deflection, green: inward deflection. Note the difference in scale between the two conditions. (*c*) Displacement (top) and phase (bottom) of tympanal membrane relative to frequency for intact (green) and ablated (blue) conditions. ($N = 14$, solid lines: mean).

frequency were calculated from the fitted models. Mean resonance frequency and mean displacement at resonance frequency were compared between the intact and ablated conditions using paired two-sample *t*-tests.

3. Results

(a) Morphology

Cercyonis pegala has a well-developed tympanal ear located at the base of the ventral forewing (figure 1*a*). The membrane is oval-shaped and is bordered by a chitinous ring (figure 1*b*,*c*). Both males and females have well-developed tympanal membranes, with surface areas of 0.243 ± 0.040 and 0.274 ± 0.032 mm^2, respectively. Surface areas did not differ between sexes despite differences in body size (electronic supplementary material, table S2). The subcostal vein is visibly enlarged (figure 1*a*,*c*; electronic supplementary material, figure S2) and physically connected to the tympanal chamber (air space beneath the tympanum) (figure 1*f*). The enlarged vein contains an internal network of tissue that forms a honeycomb-like configuration (figure 1*c*–*e*).

(b) Mechanical response of tympanum

The mechanical response of the eardrum in *C. pegala* was measured in seven males and seven females with intact veins (figures 1*f* and 2, and table 1). There were no significant

Table 1. Tympanal membrane responses in intact and ablated condition.

parameter	intact ($n = 14$)[a]	ablated ($n = 14$)[a]	p-value[b]
mean resonance frequency (kHz) (±s.d.)	7.78 ± 1.21	8.08 ± 1.55	$t = -1.3450$, d.f. $= 13$, $p = 0.1008$
mean displacement at resonance frequency (nm Pa^{-1}) (±s.d.)	156 ± 50.1	98.1 ± 48.0	$t = 3.5980$, d.f. $= 13$, $p = 0.0016$
mean maximum displacement (nm Pa^{-1}) (±s.d.)	204 ± 99.3	122 ± 53.6	$t = 4.5040$, d.f. $= 13$, $p = 0.0006$
mean frequency at maximum displacement (kHz) (±s.d.)	4.39 ± 3.01	7.64 ± 3.36	$t = -2.5801$, d.f. $= 13$, $p = 0.0228$

[a] $N = 7$ males, 7 females.

[b] Significant difference between intact and ablated conditions, determined using paired Student's *t*-test for unequal variances ($p < 0.01$ taken as significant).

differences between sexes (electronic supplementary material, table S1 and figure S3). The tympanum is most responsive to low frequency sounds (less than 5 kHz) and behaves like a low-pass filter. The response is flat below 5 kHz and decreases between 5 and 8 kHz, before falling steeply beyond 8 kHz (figure 2*c*). Mean maximum displacement is 182 ± 102 nm Pa^{-1} and frequency at maximum displacement is 4.39 ± 3.01 kHz (figure 2*b* and table 1). The phase change is very gradual around the resonance frequency (7.8 ± 1.2 kHz), which is itself higher than the frequency of maximal displacement. Taken together, this indicates that the ear is a highly damped and non-resonant system, which is adapted to respond equally to a broad range of low frequency sounds.

When the subcostal vein is ablated, the membrane shows reduced sensitivity overall (electronic supplementary material, movie S1), but particularly to sounds at the lower end of this frequency range (0.75–5 kHz) (figure 2 and table 1). The response amplitude is no longer flat, is erratic for frequencies from 0.5 to 1.5 kHz and then steadily increases, exhibiting a maximum at 5.99 kHz. The mean maximum displacement and the mean displacement of the membrane at resonance frequency were both significantly lower in the ablated condition (figure 2 and table 1), although the mean resonance frequency of the membrane in the ablated condition remained unchanged (table 1). The spatial pattern of response amplitude also remains similar. These results show that ablating the inflated vein does not change the mechanical properties of the membrane, yet results in reduced sensitivity to lower frequency sounds. Our data clearly show that the inflated vein is connected to and functionally active in the *C. pegala* auditory system. The data also suggest that the vein inflation is crucial to developing the flat frequency response observed in the ears.

4. Discussion

Tympanal ears were first described in Satyrini butterflies more than 100 years ago [4], but until now, the tuning characteristics of these ears had not been studied. Our results from a diurnal Satyrini species, *C. pegala*, show that the tympanal membrane is broadly tuned to low frequency sounds (less than 7 kHz). Sensitivity to low frequency sounds concurs with one behavioural study of Satyrini species *Erebia euryale* and *E. manto* (125 Hz–16 kHz) [10], and neurophysiological studies of non-Satyrini species, *Caligo eurilochus* (1–4 kHz) and *Morpho peleides* (1–5 kHz) [11–13]. The functional significance of hearing in butterflies is not fully understood, but evidence to date indicates that they detect sounds of diurnal predators, including bird flight and calls that overlap with hearing sensitivity of butterflies [10,13,14].

While our results clearly support the hypothesis that inflated veins function to enhance hearing by increasing sensitivity to low frequency sounds, a number of details remain to be resolved. The ear may function either as a pressure or a pressure difference receiver, as sound may only reach the tympanal membrane by an external path, or may have a yet undiscovered path. Inflated veins may contribute to hearing by acoustic impedance matching, whereby the volume of air trapped within the inflated ear would allow the ear to respond to low frequencies that would otherwise cancel out from an absence of a gradient [15]. The honeycomb-like structures within the inflated vein may also contribute to enhancing hearing, perhaps functioning in damping the membrane response like honeycomb sandwich panels in buildings to provide noise transmission loss [16]. Our results show that inflated veins provide butterflies with a unique mechanism of auditory frequency tuning, with unusually 'flat' frequency responses that may have implications for novel acoustic technology.

This study resolves a century-old conundrum concerning the function of inflated wing veins in butterflies. We show that they function in hearing. Small insects face physical challenges in hearing low frequencies [3,15,17]. Thus, vein inflations may occur in other smaller species of butterflies. Alternatively, vein inflations may evolve in species that benefit from enhanced low frequency hearing, perhaps owing to habitat or predator differences. Hearing in butterflies is widespread, but at present little is known about the function and evolution of these sensory organs. Further experimental and comparative studies are essential to better understand the acoustic sensory ecology of these ecologically important insects.

Ethics. This work complies with ethical guidelines in Canada.

Data accessibility. The datasets supporting this article have been uploaded to the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.m5b784h [18].

Authors' contributions. P.S. conducted laser vibrometry trials, data analysis and co-drafted the manuscript. N.M. conducted laser vibrometry trials and advised on data analysis. A.C.M. advised on laser vibrometry trials and analysis. J.E.Y. contributed to experimental design and co-drafted the manuscript. All authors contributed to the writing, agreed to be held accountable for the content and approved the final version of the manuscript.

Competing interests. We have no competing interests.

Funding. Funding was provided by the Canadian government to J.E.Y. (NSERC, Innovation Canada) and A.C.M. (NSERC).

Acknowledgements. We thank Chelsea Stunden for SEM images, Jeff Skevington, Michael Olsen, Peter Hall, Rick Cavasin and Ross Layberry for help with specimen collection, and Rick Cavasin for his photographic contribution.

References

1. Yager DD. 1999 Structure, development, and evolution of insect auditory systems. *Microsc. Res. Technol.* **47**, 380–400. (doi:10.1002/(SICI)1097–0029(19991215)47:6<380::AID-JEMT3>3.0.CO;2-P)
2. Mason AC, Pollack GS. 2016 Introduction to insect acoustics. In *Insect hearing*, Springer Handbook of Auditory Research vol. 55 (eds GS Pollack, AC Mason, AN Popper, RR Fay), pp. 1–15. Cham, Switzerland: Springer International Publishing.
3. Windmill JFC, Jackson JC. 2016 Mechanical specializations of insect ears. In *Insect hearing* (eds GS Pollack, AC Mason, AN Popper, RR Fay), pp. 17–48. Cham, Switzerland: Springer International Publishing.
4. Vogel R. 1912 Uber die chordotonalorgane in der wurzel der schmetterlingsflugel. *Z. Wiss. Zool.* **100**, 210–244.
5. le Cerf F. 1926 Contribution à l'étude des organs sensoriels des Lépidoptères. *Encyclop. Ent.* **3**, 133–146.
6. Minet J, Surlykke A. 2003 Auditory and sound producing organs. In *Handbook of zoology: moths and butterflies* (ed. NP Kristensen). Berlin, Germany: Walter de Gruyter.
7. Ford EB. 1945 *Butterflies*. London, UK: Collins.
8. Ackery PR, de Jong R, Vane-Wright RI. 1998 The butterflies: Hedyloidea, Hesperoidea, and Papillionoidea. In *Handbook of zoology — arthropoda: insecta* (ed. NP Kristensen), pp. 263–301. Berlin, Germany: Walter de Gruyter.
9. Miller LD. 1968 The higher classification, phylogeny and zoogeography of the Satyridae (Lepidoptera). *Mem. Amer. Ent. Soc.* **24**, 10–11.
10. Ribarič D, Gogala M. 1996 Acoustic behaviour of some butterfly species of the genus *Erebia* (Lepidoptera: Satyridae). *Acta Entomol. Slov.* **4**, 5–12.
11. Lucas KM, Windmill JFC, Robert D, Yack JE. 2009 Auditory mechanics and sensitivity in the tropical butterfly *Morpho peleides* (Papilionoidea, Nymphalidae). *J. Exp. Biol.* **212**, 3533–3541. (doi:10.1242/jeb.032425)
12. Lucas KM, Mongrain JK, Windmill JFC, Robert D, Yack JE. 2014 Hearing in the crepuscular owl butterfly (*Caligo eurilochus*, Nymphalidae). *J. Comp. Physiol. A* **200**, 891–898. (doi:10.1007/s00359-014-0933-z)
13. Mikhail A, Lewis JE, Yack JE. 2018 What does a butterfly hear? Physiological characterization of auditory afferents in *Morpho peleides* (Nymphalidae). *J. Comp. Physiol. A.* (doi:10.1007/s00359-018-1280-2)
14. Fournier JP, Dawson JW, Mikhail A, Yack JE. 2013 If a bird flies in the forest, does an insect hear it? *Biol. Lett.* **9**, 20130319. (doi:10.1098/rsbl.2013.0319)
15. Michelsen A, Larsen ON. 2008 Pressure difference receiving ears. *Bioinsp. Biomim.* **3**, 1–18. (doi:10.1088/1748-3182/3/1/011001)
16. Wen-Chao H, Chung-Fai N. 1997 Sound insulation improvement using honeycomb sandwich panels. *Appl. Acoust.* **53**, 163–177. (doi:10.1016/S0003-682X(97)00033-9)
17. Bennet-Clark HC. 1998 Size and scale effects as constraints in insect sound communication. *Phil. Trans. R. Soc. Lond. B* **353**, 407–419. (doi:10.1098/rstb.1998.0219)
18. Sun P, Mhatre N, Mason A, Yack J. 2018 Data from: In that vein: Inflated wing veins contribute to butterfly hearing. Dryad Digital Repository. (doi:10.5061/dryad.m5b784h)