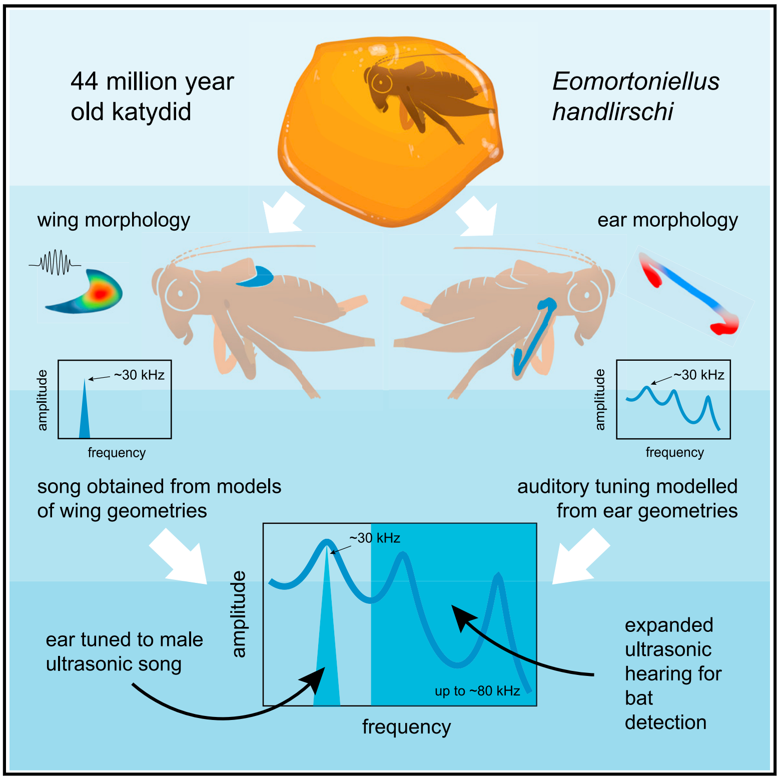
Article

Current Biology

# An Eocene insect could hear conspecific ultrasounds and bat echolocation

## Graphical abstract



## Highlights

- A 44-million-year-old amber fossil katydid reveals exquisite ear preservation
- Biophysics of wings reveals this species utilized ultrasounds for communication
- Modeling of auditory range demonstrates tuning to male sexual signal, as well as to bat cries
- Ultrasound discrimination in insects was established by the Eocene

## Authors

Charlie Woodrow, Emine Celiker, Fernando Montealegre-Z

## Correspondence

charlie.woodrow@ebc.uu.se (C.W.),
fmontealegrez@lincoln.ac.uk (F.M.-Z.)

## In brief

Woodrow et al. show auditory tuning to male acoustic signals and extended ultrasonic hearing for predator detection in the ear of an Eocene katydid. This remarkable fossil pushes back the evolution of complex auditory processing in insects and suggests that acoustic communication strategies in katydids diversified during the emergence of echolocating bats.

Woodrow et al., 2023, Current Biology *33*, 1–12
December 18, 2023 © 2023 The Authors. Published by Elsevier Inc.
https://doi.org/10.1016/j.cub.2023.10.040

CellPress

Please cite this article in press as: Woodrow et al., An Eocene insect could hear conspecific ultrasounds and bat echolocation, Current Biology (2023), https://doi.org/10.1016/j.cub.2023.10.040

Current Biology

CellPress
OPEN ACCESS

Article

# An Eocene insect could hear conspecific ultrasounds and bat echolocation

**Charlie Woodrow,**[1,2,*] **Emine Celiker,**[3,4] **and Fernando Montealegre-Z**[1,5,*]
[1]University of Lincoln, School of Life and Environmental Sciences, Joseph Banks Laboratories, Green Lane, Lincoln LN6 7DL, UK
[2]Uppsala University, Department of Ecology and Genetics, Evolutionary Biology Centre, Norbyvägen 18 D, 752 36, Uppsala, Sweden
[3]University of Dundee, Division of Mathematics, School of Science and Engineering, Nethergate, Dundee DD1 4HN, UK
[4]University of Leicester, School of Engineering, University Road, Leicester LE1 7RH, UK
[5]Lead contact
*Correspondence: charlie.woodrow@ebc.uu.se (C.W.), fmontealegrez@lincoln.ac.uk (F.M.-Z.)

https://doi.org/10.1016/j.cub.2023.10.040

## SUMMARY

Hearing has evolved independently many times in the animal kingdom and is prominent in various insects and vertebrates for conspecific communication and predator detection. Among insects, katydid (Orthoptera: Tettigoniidae) ears are unique, as they have evolved outer, middle, and inner ear components, analogous in their biophysical principles to the mammalian ear. The katydid ear consists of two paired tympana located in each foreleg. These tympana receive sound externally on the tympanum surface (usually via pinnae) or internally via an ear canal (EC). The EC functions to capture conspecific calls and low frequencies, while the pinnae passively amplify higher-frequency ultrasounds including bat echolocation. Together, these outer ear components provide enhanced hearing sensitivity across a dynamic range of over 100 kHz. However, despite a growing understanding of the biophysics and function of the katydid ear, its precise emergence and evolutionary history remains elusive. Here, using microcomputed tomography (μCT) scanning, we recovered geometries of the outer ear components and wings of an exceptionally well-preserved katydid fossilized in Baltic amber (∼44 million years [Ma]). Using numerical and theoretical modeling of the wings, we show that this species was communicating at a peak frequency of 31.62 (± 2.27) kHz, and we demonstrate that the ear was biophysically tuned to this signal and to providing hearing at higher-frequency ultrasounds (>80 kHz), likely for enhanced predator detection. The results indicate that the evolution of the unique ear of the katydid, with its broadband ultrasonic sensitivity and analogous biophysical properties to the ears of mammals, emerged in the Eocene.

## INTRODUCTION

Katydids (Orthoptera: Tettigoniidae), notable for their unique sound production and hearing organs, diversified in the early Eocene 60–40 million years ago (mya)[1]; but their ancestors were singing and hearing over 200 million years (Ma) earlier.[2,3] While the first singing orthopterans utilized low-frequency sounds for their acoustic signals, extant katydids are known to communicate using calling songs that range from ∼600 Hz to over 150 kHz in frequency.[4,5] These features place katydids among the first animals to use sound and among the most acoustically diverse group of organisms on the planet.[6] Katydids produce sound using specialized regions on the tegmina (forewings). These signals are usually produced by the male to attract a mate.[7–9] A hardened wing edge (plectrum) on one wing rubs against a row of cuticular teeth (stridulatory file) on the other wing to produce vibrations enhanced by specialized wing cells, which are radiated as airborne sound.[7,9–11] Recent works have demonstrated our ability to infer the acoustic signals of extinct katydids, thanks to allometric scaling parameters of their sound-production organs.[2,3,12] The scaling of the sound-production structures dictate the frequency composition of the call[13] and the teeth distribution and wing resonance its tone, making it possible to infer several characters of the sounds of extinct species based on wing geometry.[2,3,14] As a result, this system has offered enhanced understanding of the sounds produced by insects since before the Jurassic, which are rapidly aiding in our understanding of the evolutionary drivers of acoustic signal composition.[2,3] However, less is known about the evolution of the hearing systems of these insects.

Katydid ears, located in each foreleg, are used to detect these conspecific acoustic signals, as well as the sounds of their predators.[15–18] Hearing in katydids follows the same canonical steps of hearing in mammals, with outer ear structures for sound collection, a middle ear for converting tympanal vibrations into fluid vibrations, and an inner ear organ for frequency analysis.[19–21] These ears can receive sound on the external tympanal surface directly or on the internal surface via a narrowing ear canal (EC), defining them as pressure-difference receivers (Figure 1A).[22–24] The EC is derived from one of the fore-femoral branches of the respiratory system and connects with a modified thoracic spiracle (Figure 1B). It is well known from empirical measurements and numerical modeling that the EC typically has a broad tuning, with enhanced responses at the frequency

Please cite this article in press as: Woodrow et al., An Eocene insect could hear conspecific ultrasounds and bat echolocation, Current Biology (2023), https://doi.org/10.1016/j.cub.2023.10.040

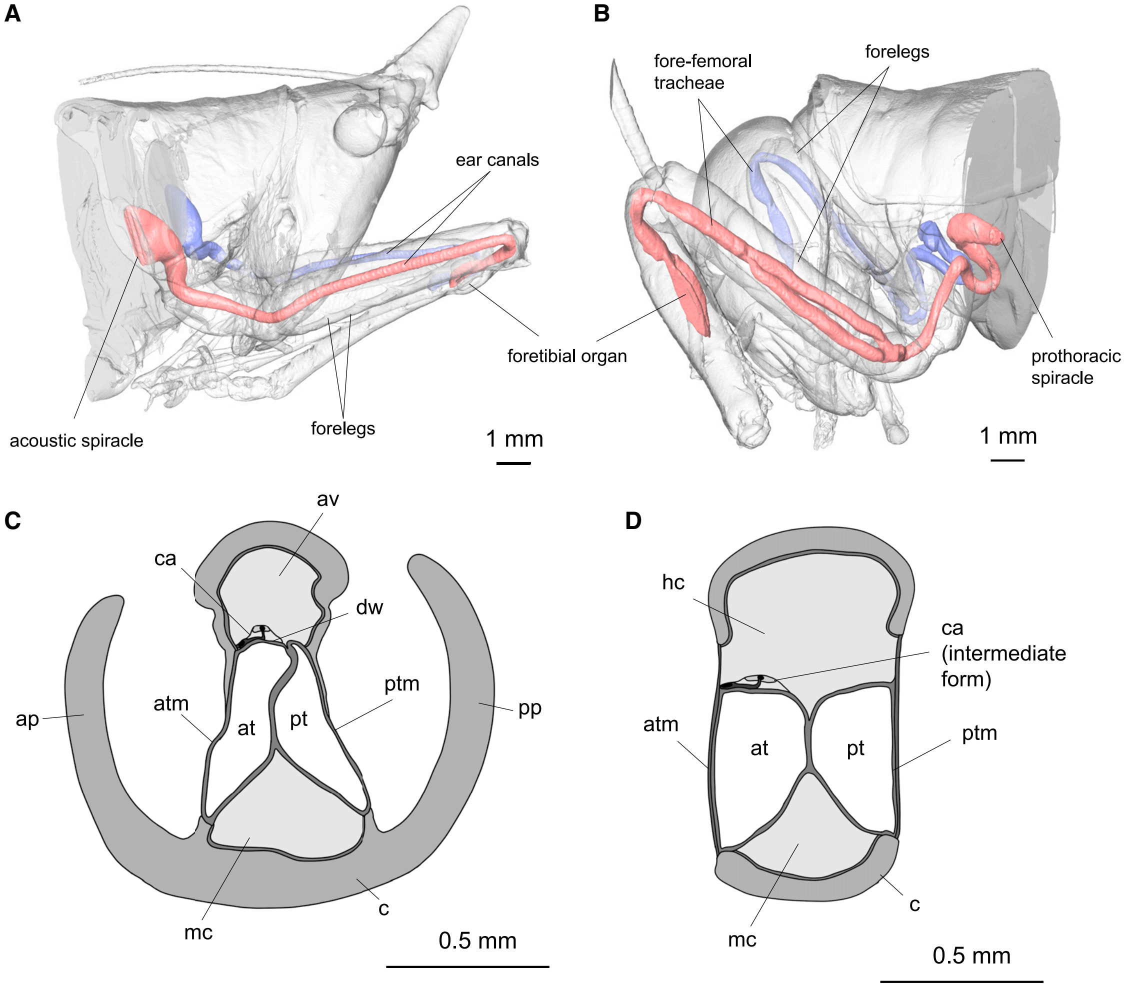


**Figure 1. Many features of the katydid hearing system are specialized compared with relict outgroups**

(A) Modern katydid (*Copiphora gorgonensis*) µCT reconstruction showing ECs.

(B) Relict katydid relative (*Cyphoderris monstrosa*) µCT reconstruction showing fore-femoral tracheae, unspecialized for sound reception.

(C) *C. gorgonensis* foretibial organ in cross-section.

(D) *C. monstrosa* foretibial organ in cross-section.

Abbreviations are as follows: ap, anterior pinna; at, anterior tracheal branch; atm, anterior tympanum; av, auditory vesicle; c, cuticle; ca, crista acustica; dw, dorsal wall; hc, haemolymph channel; mc, muscle channel; pp, posterior pinna; pt, posterior tracheal branch; ptm, posterior tympanum. Illustration by C Woodrow.

of the species-specific acoustic signals in extant forms,[16,25] and it is most often observed to follow the shape and function of an exponential horn.[16,23,24,26,27] At the external entrance to the ear, many katydids also have pinnae covering the tympana (Figure 1C).[28,29] Relict species that diverged from extant katydids in the Jurassic, lack pinnae (Figure 1D), as do many extinct katydids, suggesting that these appeared later in katydid evolution. Pinnae were initially believed to function for tympana protection or for directional hearing,[15,29] but they have recently been shown to help extend the hearing range into the extreme ultrasonics (the limitation of the EC) for enhancing the detection of bat echolocation.[30] The emergence of pinnae coincides with the formation of a tonotopic fluid-filled inner ear (auditory vesicle) for enhanced frequency analysis across a large dynamic range.[31] The outer ear structures are fundamental for establishing the overall auditory range of the katydid and function together to make the ear a uniquely wide-spectrum sound receiver. However, the evolutionary history of this hearing system is still not well understood, and no studies have been able to connect sound production and sound reception in katydids through deep time because of a lack of materials and methodologies. More widely across the animal kingdom, investigating hearing and sound production in extinct organisms is challenging and often based on indirect measurement parameters. For example, existing studies predicting hearing ranges in extinct mammals do so based on scaling relationships and extrapolation, using indicators such as mass and cochlea geometry.[32–34] More direct measurements of auditory tuning in extinct organisms, on the other hand, has not yet been possible.

Although rare, insect ears have been observed in the fossil record[2,3,35] and date back to over 150 mya in katydids.[2] However, these ears are known only from compression fossils where inferences of auditory tuning have not yet been possible. Here, we report exceptionally well-preserved ears in an amber-embedded fossil of a katydid from the Eocene (∼44 Ma old), *Eomortoniellus handlirschi* (*E. handlirschi*) (Zeuner[36]). This specimen, currently housed at the London Natural History Museum (NHM), represents the only known adult katydid to be preserved in amber, at a crucial moment in the evolutionary arms race of insect ears, the diversification of echolocating bats.[37–40] Using microcomputed tomography (μCT) analysis of this specimen, combined with numerical and statistical modeling, we show that the ears of *E. handlirschi* were biophysically tuned to the frequency of the conspecific ultrasonic calling song (∼32 kHz), which functions as a sexual signal for competition and mate attraction. We demonstrate that in addition to conspecific tuning, the ears were also capable of responding acoustically to higher-frequency sounds in the range 60–90 kHz. Anatomically, the ears show all the indicators of the extant katydid hearing system, complete with outer, middle, and inner ear structures, indicating sophisticated hearing based on tonotopy (frequency mapping), traveling waves, and acoustic triangulation. The pinnae, on the other hand, show an intermediate geometry compared with extant forms. Combined, this evidence supports a hypothesis that the wide-spectrum receiver of the katydid was established in the Eocene, during a period of acoustic signal diversification.

## RESULTS

### The sophisticated ear of the katydid was established in the Eocene

The outer ear of the extant katydid consists of two separate inputs. In most species, the dominant input is an acoustic spiracle that delivers sound via an EC running through the forelegs to the internal tympanum surface (Figure 1A).[22–24] Externally, the two tympana can also receive sound directly on their surface, often via auditory pinnae (Figure 1C). At the level of the foretibial organ, the EC divides into two asymmetric branches (Figure 1C). The anterior branch is the larger of the two, widening along its dorsal edge forming a structure known as the dorsal wall (DW).[41] The DW is curvilinear in geometry and narrows toward the distal end of the ear.[20] The DW supports the cochlear organ of the katydid, comprising a set of mechanosensory cells forming the *crista acustica* (CA) and in many species, a fluid-filled cavity known as the auditory vesicle (Figure 1C). While closely related outgroups such as the Prophalangopsidae show a primitive CA (Figure 1D), they lack the morphological specialization of a closed auditory vesicle seen in extant katydids.[41] Instead, they process frequency at the most peripheral level through tonotopy of the tympanum, transmitting the vibrations laterally from tympanum to primitive CA via the DW.[31]

*E. handlirschi* is a small katydid of the subfamily Lipotactinae.[36,42] The holotype presented here is a male and measures just 8.98 mm from head to tip of the abdomen. The μCT scan revealed several notable features of the ear and sound-production organs of this species. First, the ECs are preserved in their entirety (Figure 2A). The fortunate preservation of both ECs, down to the level of the tympana, has almost certainly been achieved during the inclusion process, whereby the coniferous tree resins that captured the insect have traveled through the ECs, possibly enhanced by the struggling of the insect, resulting in a cast of the ECs, which later hardened in the same manner as the resins surrounding the animal (Figures 2E and S2). The left and right ECs are 8.69 and 7.63 mm long, respectively, comparable to modern members of the subfamily (Figure S1). Disparity between the two EC lengths will be expected from different positioning of the legs or minor taphonomic distortions. The acoustic spiracles are open and measure 0.037 mm$^2$ (left) and 0.038 mm$^2$ (right) (Figure 2A). At the level of the foretibial organ, the tympana are well preserved and visible through the amber (Figure 2B). The EC branches asymmetrically as in modern forms (Figure 2C). The anterior branch is flattened and curvilinear along the dorsal surface forming the DW, indicating the presence of a fully functional CA rather than a CA homolog (Figure 2C), which enhances tonotopic traveling waves. The CA has a maximum length of 350 μm and maximum width of 80 μm. Although there is no correlation between CA length and the absolute number of auditory cells,[43] we could infer a theoretical maximum number of cells based on existing measurements of CA cell lengths.[44] Few studies have provided precise measurement of the cell lengths of the CA. Based on measurements in Celiker et al.,[26,44] which have an average (± SD) cell length of 20.16 ± 9.9 μm, we suggest that the CA of *E. handlirschi* could have held a maximum of 17–18 sensory cells. This is within the lower range of the number of auditory sensilla in modern katydids, which ranges from 12 to 116.[43] Based on experimental data in modern katydids where the CA has been investigated experimentally and numerically, it is likely that the length of the CA of *E. handlirschi* would be capable of transducing mechanical vibrations over a range of at least 50 kHz.[44,45] The tympana are encapsulated by cuticular pinnae that are less developed than in extant katydids (Figure S1), only covering the ventral half of the tympana (Figure 2C). The cavities formed by the pinnae, which were defined as the volume from the top of the pinna to the dorsal edge of the tympanum (Figure 1E), are small, averaging 0.00635 mm$^3$.

### *E. handlirschi* produced ultrasounds for communication

The forewings are also well preserved and are near symmetrical, resembling modern members of the subfamily Lipotactinae (Figure 2D).[46] The posterior branch of the posterior cubitus (CuPb) wing vein, which holds the stridulatory file for sound production, is visible and has a length of 1.36 mm. Like the extant Lipotactinae, *E. handlirschi* has a stridulatory area with a large and well-defined mirror.[46] Through the amber, it is possible to measure the area of the mirror, the harp (h1 region), and the length of the CuPb vein.[46–48] The mirror area is 0.34 mm$^2$, and the h1 harp area 1.22 mm$^2$. In extant katydids and their relatives, it has been shown that the stridulatory file length can confidently predict calling song frequency,[2,3,12,14,48,49] based on an equation proposed by Montealegre-Z et al.[48] (Table 1). The file of *E. handlirschi* is 1.36 mm, and predicted calling song peak frequencies of 31.24 and 33.46 kHz are obtained from these phylogenetically generalised least squares (PGLS) and ordinary least squares (OLS) models, respectively (Table 1). In extant katydids, the effective vibrating area on the right wing can also be used to accurately infer song frequency, as the natural frequency of the emitted vibration depends on this area. In some of the living species, this area is reduced to the mirror, but in others, it covers mirror and harp.[48] When considering both the mirror and harp regions (1.46 mm$^2$), the predicted calling song frequency of *E. handlirschi* offered by

Please cite this article in press as: Woodrow et al., An Eocene insect could hear conspecific ultrasounds and bat echolocation, Current Biology (2023), https://doi.org/10.1016/j.cub.2023.10.040

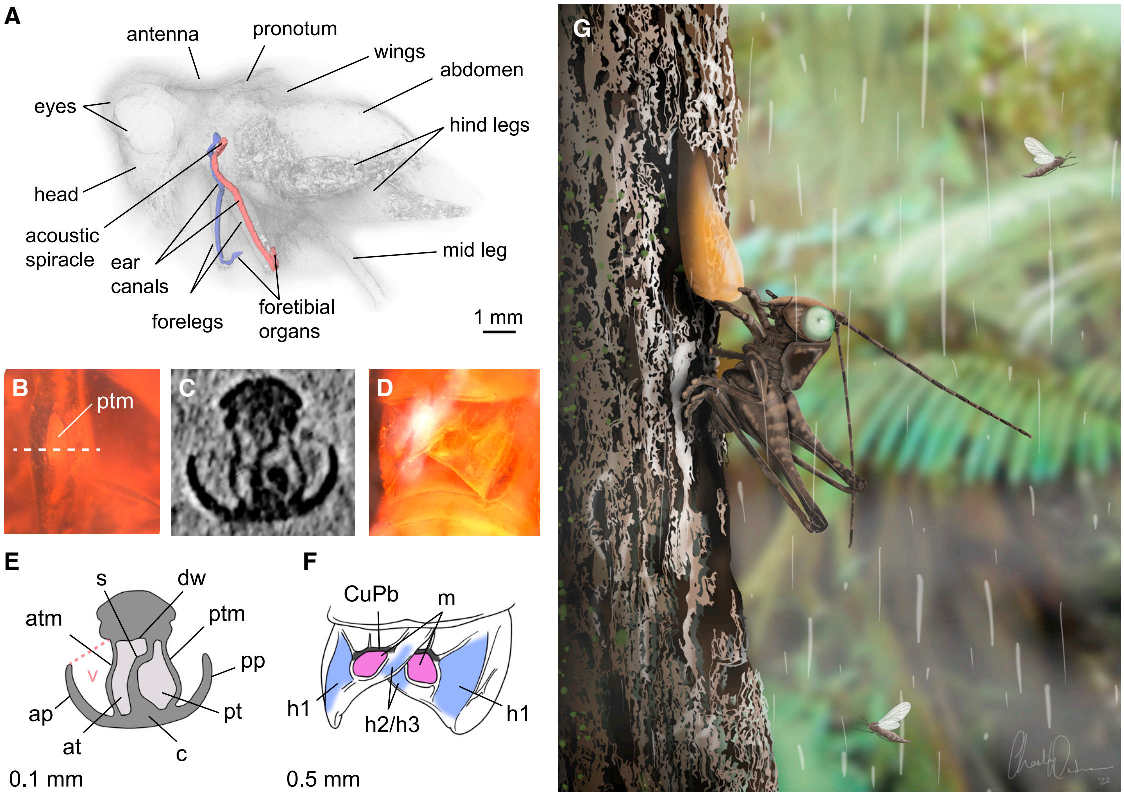


**Figure 2. Remarkable preservation of the ears and wings of *Eomortoniellus handlirschi* provides insights into the evolution of katydid communication**

(A) *E. handlirschi* male habitus μCT reconstruction showing ECs.

(B) View of the anterior tympanum and external ear structures through amber, with dotted line indicating cross-section in (C).

(C) μCT cross-section of the foretibial ear.

(D) Forewings and associated sound-production regions through amber.

(E) Illustrated diagram of (C).

(F) Illustrated diagram of (D).

(G) Reconstruction of *E. handlirschi* moments before encapsulation in tree resins.

Abbreviations are as follows: ap, anterior pinna; at, anterior tracheal branch; atm, anterior tympanum; c, cuticle; ca, crista acustica; dw, dorsal wall; h1–3, harp regions; m, mirror; pp, posterior pinna; pt, posterior tracheal branch; ptm, posterior tympanum; s, septum; v, volume. Illustration in (F) modified from Gorochov.[42] Illustrations in (E) and (G) by C Woodrow. See also Figures S1 and S2.

further PGLS and OLS models from Montealegre-Z et al.[48] is 27.82 and 33.01 kHz, respectively (Table 1). To investigate the vibration of the forewings in *E. handlirschi*, a digital reconstruction of the wing (Figure 2F) was produced and a finite element mesh was constructed on the geometry (Figure 3A). The wings of this species are near symmetrical. The μCT scans did not provide high enough resolution for three-dimensional (3D) reconstruction of the wings; thus, the right wing that was more visible through the amber was used as a reference to construct a 3D geometry. The wing was given material properties based on measurements in extant species (see STAR Methods). The active region of the wing was considered as the mirror and harp, as suggested by the aforementioned model, and the anal field, which is thin in this subfamily and lies close to the stridulatory file (Figure 3A). The lateral field was not included in the model as this folds around the body as in modern species. Including these components in the model results in a natural wing resonance of 32.52 kHz (Figure 3B), which is similar to the frequency predicted using the mirror and harp area, as well as stridulatory file length. The vibrating area is primarily represented by the mirror, anal field, and h1 region, as suggested for living members of the subfamily.[46] The size of the area that vibrated within this region in the model was ~1.36 mm$^2$, which by using the aforementioned models of wing vibrating area would predict a frequency of 28.9 (PGLS) or 34.42 (OLS) kHz (Table 1).

The average prediction of calling song frequency in *E. handlirschi* from these seven different estimation methods is 31.62 (± 2.27) kHz (Table 1). Modern Lipotactinae produce acoustic signals peaking at ~30–35 kHz, with energy expanding about 30 kHz in the range 20–50 kHz.[46,50] *E. handlirschi* was almost certainly utilizing a similar communication mechanism and dominant frequency to extant members of this subfamily.

### The ear of *E. handlirschi* was biophysically tuned to the male acoustic signal

Numerical simulation of auditory tuning in katydid ECs has been demonstrated through various recent studies in modern species using accurate 3D geometries.[24,26,51] Here, we used two

**Table 1. Estimates of calling song frequency in *E. handlirschi***

| Measurement parameter | Measurement (x) | Slope (m) | Intercept (c) | $f_c$ (kHz) |
|---|---|---|---|---|
| File length (mm) Corrected for phylogeny | 1.36 | −0.97 | 3.74 | 31.24 |
| File length (mm) Uncorrected for phylogeny | 1.36 | −1.04 | 3.83 | 33.46 |
| Theoretical vibrating area ($mm^2$) Corrected for phylogeny | 1.46 | −0.54 | 3.53 | 27.82 |
| Theoretical vibrating area ($mm^2$) Uncorrected for phylogeny | 1.46 | −0.59 | 3.72 | 33.01 |
| Model vibrating area ($mm^2$) Corrected for phylogeny | 1.36 | −0.54 | 3.53 | 28.90 |
| Model vibrating area ($mm^2$) Uncorrected for phylogeny | 1.36 | −0.59 | 3.72 | 34.42 |
| Numerical model resonance (kHz) | – | – | – | 32.52 |
| Average (± SD) | – | – | – | 31.62 (± 2.27) |

For all estimates of $f_c$: $ln(f_c) = m \times \ln(x) + c$, where ln, natural logarithm. Equations from Montealegre-Z et al.[48]

numerical methods to describe the tuning of the EC: an eigenfrequency analysis that is used to describe the natural resonances of the EC[26,52] and a frequency analysis to investigate the acoustic gain and filtering properties of the EC as sound reaches the tympana.[24,26] These models demonstrate that the left and right ECs show fundamental resonances at frequencies of 30.0 and 30.6 kHz, respectively (Figures 4A and 4B). Both ECs also show second and third harmonic resonances at ~58 and ~89 kHz, respectively (Figures 4C–4F). This tuning of the EC results in an increase in sound pressure at the tympanum by 10.92 dB sound pressure level (SPL) in the left EC and 2.96 dB SPL in the right EC around the frequency of the species' calling song (Figures 4G and 4H). The frequency analysis revealed that as sound reaches the tympana via the ECs, the greatest acoustic pressure increase is near 30 kHz (Figure 4I). The discrepancy in sound pressure increase between the ECs likely results from minor differences in preservation such as the position of the legs and extent to which the tree resins filled the EC, causing minor differences to the overall trachea geometry. We assume that based on inferences of pressure gains in extant species, the left EC is a more accurate representation of the pressure gain, although future work should investigate the effect of leg position on the katydid EC frequency response. Despite this, both ECs show increased pressure gains around 30 kHz. From the frequency analysis and geometry, we can conclude that the ECs do not resemble or function as exponential horns, like in most modern species,[16,23,24] but instead can be described to function as linear resonators, with a fluctuating but decreasing gain after the first harmonic at ~30 kHz. This linear resonator may represent an intermediate form between the ancestral branched trachea (unfunctional for sound propagation; Figure 1B) and the exponential horn EC (specialized for broadband sound reception; Figure 1A), although some living species also utilize linear resonators.[52] This functional anatomy resembles that of the acoustic trachea in field crickets, which offers a gain of up to 10 dB at its resonant frequencies, despite lacking an exponential geometry.[53] The ECs of *E. handlirschi* are also capable of enhancing sounds at higher frequencies, with harmonic resonances around 50 and 80 kHz.

In addition to modeling hearing through the EC, hearing was also investigated through the external auditory input to the tympana via auditory pinnae. In many katydids, pinnae aid in high-frequency sound reception,[28,30] with matched resonances obtainable through either numerical modeling and 3D printing and scaled experimentation.[30] These previous studies have shown pinnae to function as acoustic amplifiers for sounds in the range of 60 to over 150 kHz.[30,52] In *E. handlirschi*, the pinnae show unique intermediate forms between katydid ancestors and the pinnae of extant species. The peak pinnae resonances through numerical modeling and experiments on scaled 3D printed models were 452 and 421 ± 3 kHz, respectively, far exceeding the functional range of the ECs or pinnae in living species[30] (Figures 5A and 5B). In living species of the same subfamily Lipotactinae, the pinnae resonances are much lower in frequency, peaking at 175 ± 3 kHz (Figures 5C and 5D). In the extant *Copiphora gorgonensis* (subfamily Conocephalinae), resonances are even lower, peaking at 111.13 ± 4.24 (Figures 5E and 5F).[30] Across further species where resonance has been measured, peak values are in a similar range.[30,52] In the living lipotactines, the formation of a closed cavity provides a much more coherent resonance, and thus the level of gain at the resonant frequency increases to nearly 20 dB (Figure 5). The frequency response of pinnae can be explained by the equation for a Helmholtz resonator.[30] In such resonators where all other parameters remain fixed, resonant frequency decreases with increasing volume but increases with increasing size of the opening to the cavity. This means we could describe resonance here between living and extinct species, based on the ratio of cavity volume to slit area. In the fossil *E. handlirschi*, this ratio is 0.676; and in living species *L. maculatus*, it is 0.632. The similarity in these ratios indicates that the resonance is down mostly due to differences in scale, whereby *L. maculatus* has lower-frequency resonances simply because its ear is larger (Table S1), and larger structures have lower-frequency resonances.

Furthermore, the arrangement of the ears of the extinct *E. handlirschi* suggests that their tympana were functional pressure-difference receivers, capable of receiving sound on both their external and internal surfaces, as in extant katydids and crickets, for enhanced directional hearing.[27] This differs from their sister clades, as evidenced by living Prophalangopsidae with leg tracheae lacking an acoustic function.[31] In such pressure-difference receiver, the mechanical displacement of the tympanum is dictated by the inputs to both sides of the ear[54,55] and is usually aided by reduced velocity of sound propagating within the EC.[56] This velocity will depend on the radius of

Please cite this article in press as: Woodrow et al., An Eocene insect could hear conspecific ultrasounds and bat echolocation, Current Biology (2023), https://doi.org/10.1016/j.cub.2023.10.040

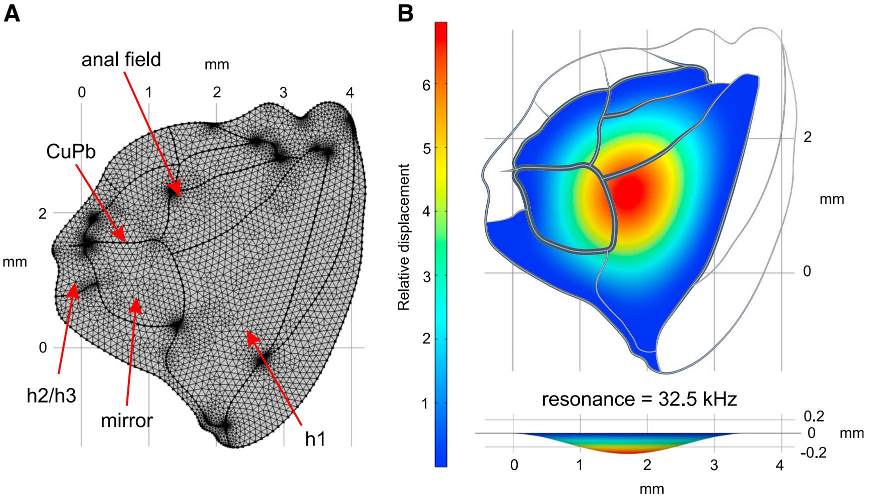


**Figure 3. The forewings of *Eomortoniellus handlirschi* functioned for the production of ultrasounds**

(A) Finite element mesh used in model, with key regions of the wing labeled.

(B) Fundamental frequency (first-order harmonic) of the forewing and the corresponding eigenmode.

the EC, which averages 67.5 μm in *E. handlirschi*, and based on calculations from an existing study would result in a propagation velocity of around 180 m/s,[56] compared with free field sound propagation of 343 m/s. From a numerical simulation of sound propagation velocity in the right EC, this is supported by a velocity of 186.21 m/s (for model conditions and assumptions, see STAR Methods). This strongly suggests that *E. handlirschi* utilized a pressure-difference receiver (acoustic resistor) based on a delayed internal input.[54]

## DISCUSSION

### Insights from a 44-million-year-old ear

Reconstructing hearing in any extinct animal is challenging, as fossils rarely preserve their soft tissue components.[57] For example, the most common way to infer auditory ranges in extinct vertebrates is cochlea length, which is possible through measurements of the bony matrix.[58–60] However, this method cannot directly inform us of important auditory ranges and their relevancies in different ecological circumstances. Here, we were able to recover wing and ear anatomies for biophysical reconstruction of acoustic signals and hearing ranges in a ~44- to 42-million-year-old katydid.[42] Our analysis and current knowledge of the biomechanics of sound production in extant katydids, including members of the closest living subfamily (Lipotactinae),[46] suggest that *E. handlirschi* produced a broadband song with a dominant component around 31.62 (± 2.27) kHz, making this species the oldest evidence of ultrasound in katydids, which informs us that ultrasonic communication in these insects was fully established by the Eocene. Other katydid fossils from the Eocene are known, but these forms were larger and utilized lower-frequency sounds (e.g., *Pseudotettigonia amoema* and *P. leona*, 10–11 kHz).[14,48,61] The song of *E. handlirschi* resembles the songs of the extant Lipotactinae in frequency. For example, the extant *L. alienus* and *L. kabili* produce broadband songs with dominant frequencies of 36.9 and 35.5 kHz, respectively.[46] Near-symmetrical forewings, as observed in the Lipotactinae, extinct *Pseudotettigonia*,[14,61] and Jurassic katydids,[3] seem to be the ancestral state of wings in the Orthoptera. For low-frequency singers, such as the Jurassic katydids and extant field crickets, this anatomy is key for producing efficient pure tone sexual signals. However, for higher frequencies, such pure tone signals are difficult to generate using coupled wing resonators.[62] This is owing to the asymmetric nature of the stridulatory mechanism: while one wing is stimulated along the file, the other is stimulated via the scraper. The scraper wing will have a single point of energy entry, but the file wing will have various points of energy entry as the scraper moves along the file, producing oscillations of varying phase, which interfere with those of the plectrum wing, creating instances of destructive interference.[62,63] This means that ultrasound production with near-symmetrical wings is challenging, and in modern species with near-symmetrical wings, the ultrasounds produced are usually broad in their frequency composition.[64,65] Therefore, the song of *E. handlirschi* would have likely contained broad-frequency components around a spectral peak of 32 kHz, like in extant Lipotactinae species whose peak song frequencies are known to range from 28.7 to 36.9 kHz[46]—a consequence of both wings contributing to high-frequency sound radiation and utilizing both the mirror and the harp for sound production, as described in some extant katydids with near-symmetrical wings.[48,62]

Considering the calculation of calling song frequency and the presented numerical results of the EC dynamic range, we provide evidence that the ear of *E. handlirschi* was tuned to the male song through EC resonances around the calling song frequency (~32 kHz), as in modern katydids.[16,24,25] Although our inferences for sexual selection are limited because no adult female specimens are known, we can confidently assume that based on extant species, the female would not produce sound.[46] In addition, few examples of EC sexual dimorphisms are known,[26,66] although ear dimorphisms in other katydid taxa suggest more research is needed.[67,68] We hypothesize that the female shows similar auditory tuning and that ultrasonic sexual communication in katydids was established by the Eocene. Crucially, this fossil informs us that the foreleg trachea was functional as an EC, having already transitioned from the precursory bifurcated respiratory trachea observed in relict forms (e.g., Figure 1B). The ECs also offer evidence of higher-frequency hearing capabilities, with the ability to provide acoustic pressure gains for sounds around 50 and 80 kHz.

Looking at the external input to the auditory system, the reduced auditory pinnae of *E. handlirschi* suggest that pinnae were emerging during the Eocene, as the pinnae are in an intermediate stage between covered tympana seen in most extant forms and ancestral exposed tympana forms (observed in extinct Haglidae,[2] living Prophalangopsidae,[12,31] most Gryllidae, and extinct Tettigoniidae[14]). Later, in the final stage of the Eocene, there is compression fossil evidence of fully formed

Please cite this article in press as: Woodrow et al., An Eocene insect could hear conspecific ultrasounds and bat echolocation, Current Biology (2023), https://doi.org/10.1016/j.cub.2023.10.040

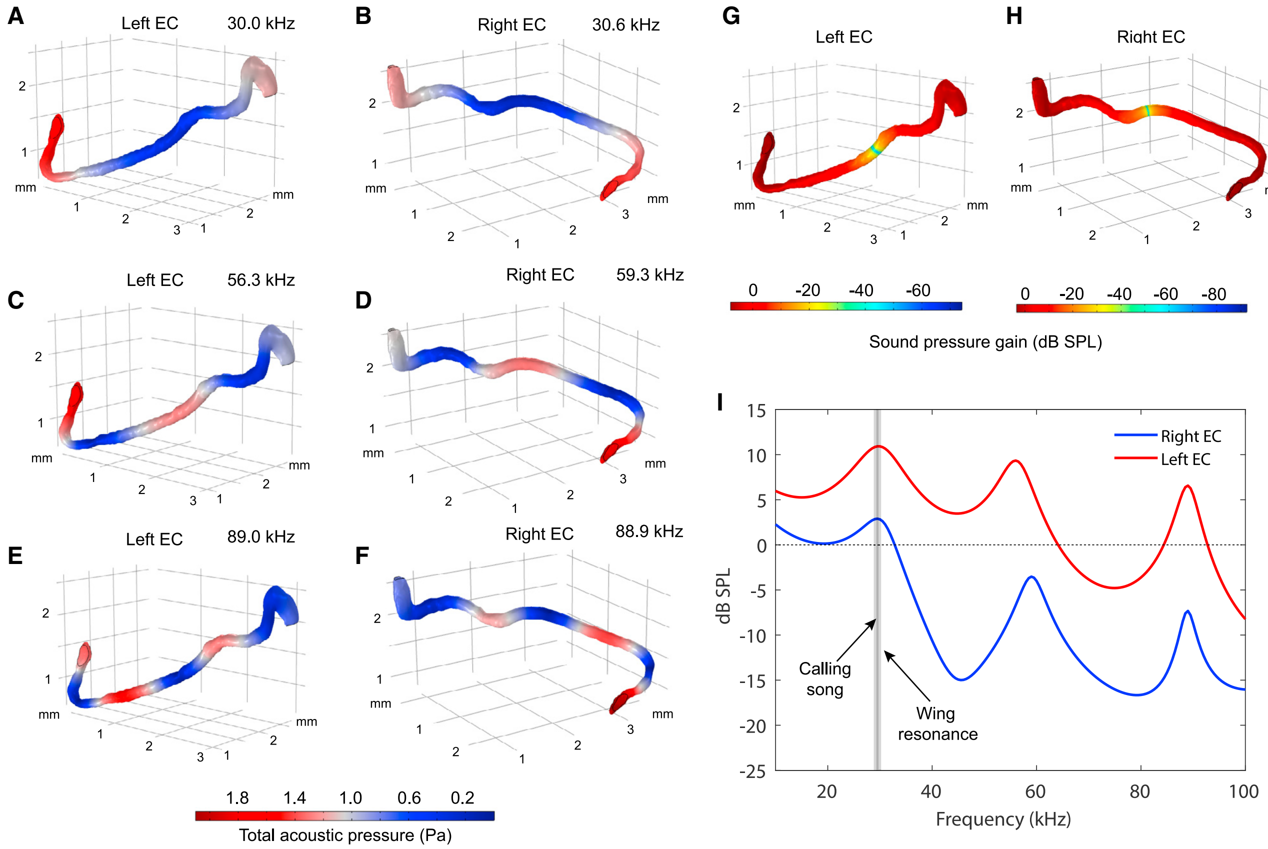


**Figure 4. Tuning of the ear canals of *Eomortoniellus handlirschi* supports both mate detection and higher-frequency predator detection**

(A) Fundamental frequency (first-order harmonic) of the left EC and the corresponding eigenmode.
(B) Fundamental frequency (first-order harmonic) of the right EC and the corresponding eigenmode.
(C) Second-order harmonic of the left EC and the corresponding eigenmode.
(D) Second-order harmonic of the right EC and the corresponding eigenmode.
(E) Third-order harmonic of the left EC and the corresponding eigenmode.
(F) Third-order harmonic of the right EC and the corresponding eigenmode.
(G) Sound pressure difference with respect to the incident sound wave inside the left EC during stimulation with a sound wave at 30 kHz.
(H) Sound pressure difference with respect to the incident sound wave inside the right EC during stimulation with a sound wave at 30 kHz.
(I) Sound pressure increase (decrease) aided by the ECs, measured at the tympana of both ears; note how the greatest sound enhancement is at 30 kHz.
The color in (A)–(H) represents the spatial distribution of sound pressure inside the lumen of the EC.

pinnae in the Conocephaline katydid *Orchelimum placidum* (34 Ma[69]). At the emergence of the Lipotactinae, the major divisions within the Tettigoniidae were already established.[1] The occurrence of fully developed pinnae in the extant Lipotactinae but only partial pinnae in the extinct *E. handlirschi* suggests that pinnae evolved from intermediate to full geometry independently in this subfamily. A robust phylogenetic analysis should be conducted to further investigate pinnae evolution, but we hypothesize that they have emerged multiple times.

Experimental and numerical data revealed that the pinnae display high-frequency resonances like in extant katydids.[30] In extant neotropical species, high-frequency resonances function to passively amplify sympatric bat echolocation signals in the range of ~60–150 kHz.[30,52] However, the resonances observed in *E. handlirschi* from both numerical and experimental measurements exceed 400 kHz, surpassing the echolocation frequencies of any known bat species,[40] and these resonances are likely an artifact of the geometry of the pinnae. In this species, pinnae likely serve for protection of the tympana, as observed in other ultrasonic insect ears.[70,71] For example, the ear of the noctuid moth *Feltia subgothica*—which has ears for ultrasonic detection—has tympana as thin as 0.4 μm[72]; and the anterior tympanum of the eneopterine cricket *Lebinthus bitaeniatus*, which has independently evolved pinnae, is as thin as 0.35 μm[70]; and in many neotropical katydids, the tympana are similarly thin.[73] The thinnest area of the tympanum of the locust *Schistocerca gregarial*, on the other hand, which does not process such high-frequency ultrasounds, is significantly thicker at ~1 μm.[74] Protection of thin tympana could have been the original function of pinnae after transitioning to high-frequency conspecific communication, as listening to high frequencies with tympanal ears requires thin tympana.[70,75] However, the pinnae resonances have rapidly changed in the Lipotactinae from *E. handlirschi* to modern forms, providing pressure gains at frequencies as low as 100 kHz. This change in resonance was achieved through modifications to pinna geometry and size, and while further research into pinnae evolution is required, we provide evidence that tympanum

Please cite this article in press as: Woodrow et al., An Eocene insect could hear conspecific ultrasounds and bat echolocation, Current Biology (2023), https://doi.org/10.1016/j.cub.2023.10.040

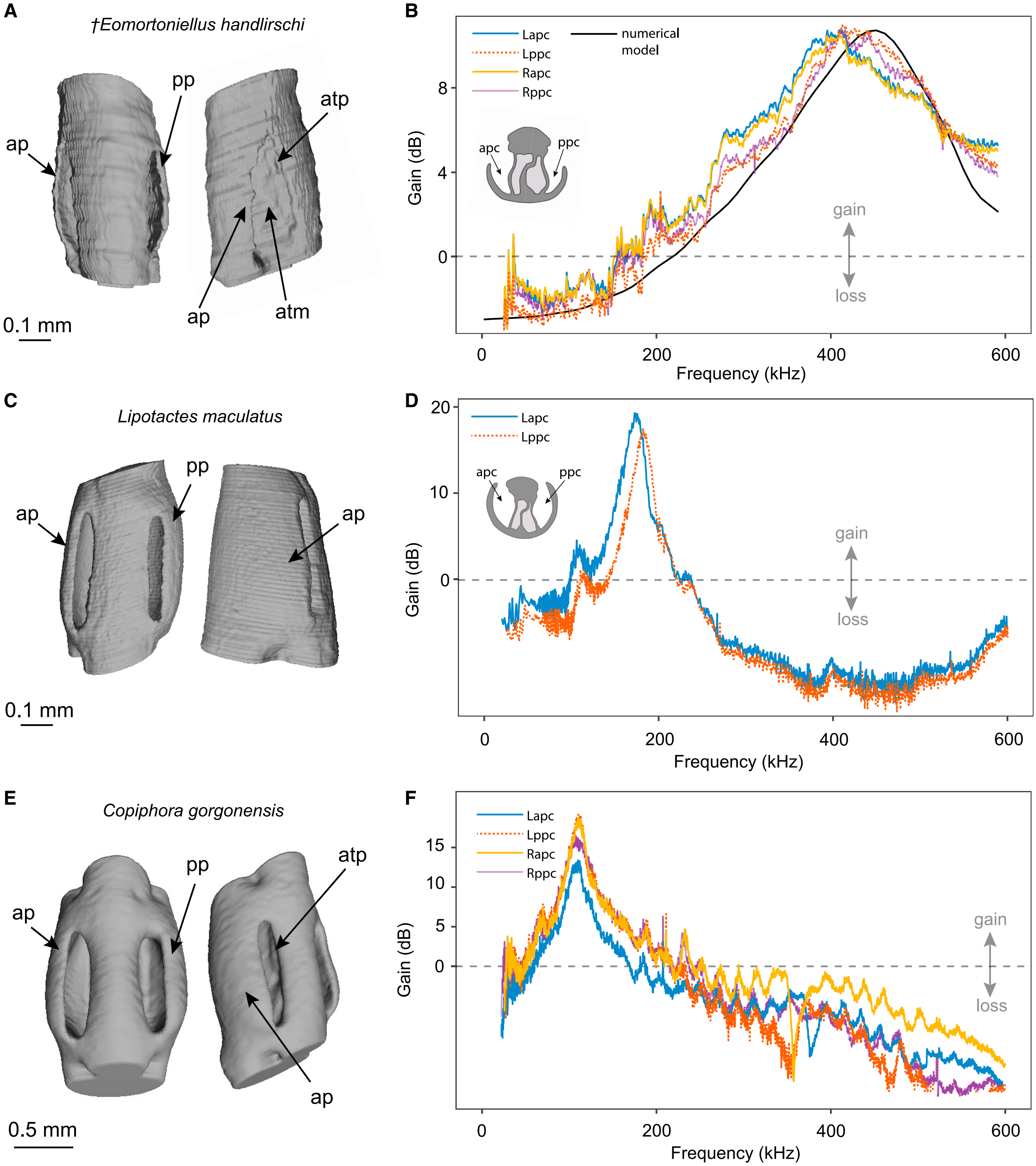


**Figure 5. Pinnae in katydids did not initially evolve for predator detection**

(A) Pinnae anatomy in *Eomortoniellus handlirschi* in dorsal and anterior view.

(B) Measurements of sound pressure gain from numerical and 3D printed model data in *E. handlirschi*.

(C) Pinnae anatomy in *Lipotactes maculatus* in dorsal and anterior view.

(D) Measurements of sound pressure gain from 3D printed model data in *L. maculatus*.

(E) Pinnae anatomy in *Copiphora gorgonensis* in dorsal and anterior view.

(F) Measurements of sound pressure gain from 3D printed model data in *C. gorgonensis*. A cross-section of the ear of *C. gorgonensis* is shown in Figure 1C.

Abbreviations are as follows: Lapc, left anterior pinna cavity; Lppc, left posterior pinna cavity; Rapc, right anterior pinna cavity; Rppc, right posterior pinna cavity. Methodologies for numerical and 3D printed pinnae resonances follow from Pulver et al.[30] See also Figure S1 and Table S1.

Please cite this article in press as: Woodrow et al., An Eocene insect could hear conspecific ultrasounds and bat echolocation, Current Biology (2023), https://doi.org/10.1016/j.cub.2023.10.040

protection and predator detection have been key drivers of these modifications.

## Implications for the predator-prey arms race of katydids and bats

In light of recent evidence supporting a theory of an arms race between the ancestral katydid lineage and mammalian acoustics,[2] it seems increasingly likely that there have been unique long acoustic interactions between orthopteran and mammalian lineages through time. This arms race may have started through katydids increasing their acoustic signal frequencies beyond the predator upper hearing limit and the predators evolving ears that are more capable of higher-frequency eavesdropping.[2,76,77] Although *E. handlirschi* offers first evidence of ultrasounds in extinct katydids, high-frequency songs extending up to 16 kHz are known and have been established for at least 150 Ma.[2] It was not until the early Cretaceous, over 100 Ma later, that therian mammal eavesdropping would be possible for ultrasounds.[78] The earliest known ancestor of modern bats (52.5 Ma) was nocturnal, insectivorous, and arboreal but unable to perform laryngeal echolocation.[79] At this time, eavesdropping on ultrasonic acoustic signals may have represented a key foraging method. In the early Eocene following the evolution of laryngeal echolocation in bats 52–50 Ma, ancestral reconstructions of cochlear geometry suggest that the hypothetical modern bat common ancestor rapidly expanded this upper frequency hearing limit to ~100 kHz,[80] and its echolocating call would have been in the range of ~40–65 kHz.[80] It was soon after this point in the arms race that *E. handlirschi* was frozen in time. This fossil supports these predictions of bat echolocation frequency, as *E. handlirschi* shows no acoustic pinnae adaptations to bat detection above 80 kHz, but it could certainly detect this modern bat common ancestor through its ECs. Importantly, this early bat predator could also hear *E. handlirschi* for predation. The sophisticated katydid ear with its cochlea-like anatomy was established at this time (as evidenced in this report, Figures 2C and 2E), indicating that discrimination between conspecific and predator ultrasounds occurred through tonotopically organized auditory sensilla and traveling waves for frequency mapping.[19,20,31] Later, chiropterans began experimenting with even higher-frequency ultrasounds that would eventually exceed 200 kHz in some living species.[80] At such frequencies, the limit of sound transmission gain through the EC is exceeded,[24] and resonating pinnae, already established for protecting a thin tympanum, may have started to function for ultrasound reception in katydids with naturally lower-frequency pinnae resonances. The external pinna as an auditory input should be favored for predator detection over the EC, as we confirm here that the narrow geometry of the EC produces a delay in sound propagation, like in extant species,[56] which would curtail a rapid anti-predator response. The switch to higher-frequency signals was likely favored by bats to increase the spatial resolution of their echolocation to capture insects based on their shape rather than the ancestral eavesdropping method.[40] This increased spatial resolution may have become more important for insectivorous bats predating on katydids that had evolved pure tone signals, because pure tones are challenging to localize with the mammalian ear.[81,82] This is because in the mammalian cochlea, spectral differences that provide directional hearing are limited by a quality factor (center frequency to bandwidth ratio; Q) of about 9–13,[81,82] and pure tone songs of many neotropical katydids show Q > 10, making them hard to localize. Katydids are able to localize the high Q songs of conspecifics at the mechanical level due to their pressure-difference receiver ears that are intrinsically directional, favoring both pure tone and broadband signal detection.[55] *E. handlirschi* also offers evidence of loss of flight and miniaturization in response to the emergence of bats, which may have led to higher-frequency songs through allometry of the vibrating areas on the tegmina.[57]

Overall, *E. handlirschi* offers a range of new insights into communication strategies of katydids through time, filling key gaps in our understanding of acoustic signal diversification. Future work should aim to locate the female of the species and identify other katydid taxa in tree resins to advance our understanding of signaler and receiver evolution through deep time.

## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - ○ Lead contact
  - ○ Materials availability
  - ○ Data and code availability
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
  - ○ Holotype
  - ○ Brief description
- METHOD DETAILS
  - ○ μCT scanning and measurements
  - ○ Estimation of calling song frequency
  - ○ 3D printing and pinnae experiments
  - ○ Numerical modelling
- QUANTIFICATION AND STATISTICAL ANALYSIS

## SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2023.10.040.

## ACKNOWLEDGMENTS

We thank Lil Stevens, Beulah Garner, Rich Howard, and Ben Price at NHM London for coordinating the specimen loan. This study was funded by a European Research Council grant ERCCoG-2017-773067 (to F.M.-Z. for the project “The Insect Cochlea”) and an NSF-NERC grant NSF DEB-1937815-NE/T014806/1 (to F.M.-Z.).

## AUTHOR CONTRIBUTIONS

Conceptualization, C.W. and F.M.-Z.; methodology, C.W., E.C., and F.M.-Z.; investigation, C.W. and E.C.; visualization, C.W.; funding, F.M.-Z.; project administration and supervision, F.M.-Z.; writing – original draft, C.W.; writing – review & editing, C.W., E.C., and F.M.-Z.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

## INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

Received: September 2, 2023
Revised: October 8, 2023
Accepted: October 21, 2023
Published: November 13, 2023

## REFERENCES

1. Mugleston, J.D., Naegle, M., Song, H., and Whiting, M.F. (2018). A comprehensive phylogeny of Tettigoniidae (Orthoptera: Ensifera) reveals extensive ecomorph convergence and widespread taxonomic incongruence. Insect Syst. Divers. *2*, 1–27. https://doi.org/10.1093/isd/ixy010.
2. Xu, C., Wang, B., Wappler, T., Chen, J., Koplov, D., Fang, Y., Jarzembowski, E.A., Zhang, H., and Engel, M.S. (2022). High acoustic diversity and behavioral complexity of katydids in the Mesozoic soundscape Chunpeng. Proc. Natl. Acad. Sci. USA *119*, 1–8. https://doi.org/10.1073/pnas.2210601119.
3. Gu, J.-J., Montealegre, Z.-F., Robert, D., Engel, M.S., Qiao, G., and Ren, D. (2012). Wing stridulation in a Jurassic katydid (Insecta, Orthoptera) produced low-pitched musical calls to attract females. Proc. Natl. Acad. Sci. USA *109*, 3868–3873. https://doi.org/10.1073/pnas.1118372109.
4. Heller, K.-G. (1995). Acoustic signalling in palaeotropical bushcrickets (Orthoptera: Tettigonioidea: Pseudophyllidae): does predation pressure by eavesdropping enemies differ in the Palaeo- and Neotropics? J. Zool. *237*, 469–485. https://doi.org/10.1111/j.1469-7998.1995.tb02775.x.
5. Sarria-S, F.A., Morris, G.K., Windmill, J.F., Jackson, J., and Montealegre-Z, F. (2014). Shrinking wings for ultrasonic pitch production: hyperintense ultra-short-wavelength calls in a new genus of Neotropical katydids (Orthoptera: Tettigoniidae). PLoS One *9*, e98708. https://doi.org/10.1371/journal.pone.0098708.
6. Song, H., Amédégnato, C., Cigliano, M.M., Desutter-Grandcolas, L., Heads, S.W., Huang, Y., Otte, D., and Whiting, M.F. (2015). 300 million years of diversification: elucidating the patterns of orthopteran evolution based on comprehensive taxon and gene sampling. Cladistics *31*, 621–651. https://doi.org/10.1111/cla.12116.
7. Lutz, F.E., and Hicks, W.R. (1930). An analysis by movietone of a cricket's chirp (Gryllus assimilis). Am. Museum Novit. 1–14.
8. Nielsen, E.T., and Dreisig, H. (1970). The behaviour of stridulation in Orthoptera ensifera. Behaviour *53*, 1689–1699. https://doi.org/10.1017/CBO9781107415324.004.
9. Montealegre-Z, F., and Mason, A.C. (2005). The mechanics of sound production in *Panacanthus pallicornis* (Orthoptera: Tettigoniidae: Conocephalinae): the stridulatory motor patterns. J. Exp. Biol. *208*, 1219–1237. https://doi.org/10.1242/jeb.01526.
10. Nielsen, E.T., and Dreisig, H. (1970). The behavior of stridulation in Orthoptera ensifera. Behaviour *37*, 205–251.
11. Morris, G.K. (1999). Song in arthropods. In Encyclopedia of Reproduction, *4*, G. Davey, ed. (Academic Press), pp. 508–517.
12. Woodrow, C., Baker, E., Johnsson, T., and Montealegre, Z.F. (2022). Reviving the sound of a 150-year-old insect: the bioacoustics of *Prophalangopsis obscura* (Ensifera: Hagloidea). PLoS One *17*, e0270498. https://doi.org/10.1371/journal.pone.0270498.
13. Chivers, B.D., Jonsson, T., Soulsbury, C.D., and Montealegre-Z, F. (2017). Structural biomechanics determine spectral purity of bush-cricket calls. Biol. Lett. *13,* 0–5. https://doi.org/10.1098/rsbl.2017.0573.
14. Rust, J., Stumpner, A., and Gottwald, J. (1999). Singing and hearing in a tertiary bushcricket. Nature *399*, 650. https://doi.org/10.1038/21356.
15. Autrum, H. (1963). Anatomy and physiology of sound receptors in invertebrates. In Acoustic Behaviour of Animals, R.G. Busnel, ed. (Elsevier), pp. 412–433.
16. Nocke, H. (1975). Physical and physiological properties of the tettigoniid ("grasshopper") ear. J. Comp. Physiol. *100*, 25–57. https://doi.org/10.1007/BF00623929.
17. Bailey, W.J. (1990). The Ear of the Bushcricket. In the Tettigoniidae: Biology, Systematics and Evolution, W.J. Bailey, and D.F.C. Rentz, eds. (Springer), pp. 217–247.
18. Bailey, W.J. (1993). The tettigoniid (Orthoptera: Tettigoniidae) ear: multiple functions and structural diversity. Int. J. Insect Morphol. Embryol. *22*, 185–205. https://doi.org/10.1016/0020-7322(93)90009-P.
19. Montealegre-Z, F., Jonsson, T., Robson-Brown, K.A., Postles, M., and Robert, D. (2012). Convergent evolution between insect and mammalian audition. Science *338*, 968–971. https://doi.org/10.1126/science.1225271.
20. Palghat Udayashankar, A.P., Kössl, M., and Nowotny, M. (2012). Tonotopically arranged traveling waves in the miniature hearing organ of bushcrickets. PLoS One *7*, e31008. https://doi.org/10.1371/journal.pone.0031008.
21. Warren, B., and Nowotny, M. (2021). Bridging the gap between mammal and insect ears – a comparative and evolutionary view of sound-reception. Front. Ecol. Evol. *9*, 1–16. https://doi.org/10.3389/fevo.2021.667218.
22. Heinrich, R., Popov, V.V., and Kalmring, K. (1993). Direction-dependent spectral sensitivity and interaural spectral difference in a dolphin: evoked potential study. J. Acoust. Soc. Am. *93*, 3490–3495. https://doi.org/10.1121/1.405679.
23. Hoffmann, E., and Jatho, M. (1995). The acoustic trachea of Tettigoniids as an exponential horn: theoretical calculations and bioacoustical measurements. J. Acoust. Soc. Am. *98*, 1845–1851. https://doi.org/10.1121/1.413371.
24. Celiker, E., Jonsson, T., and Montealegre-Z, F. (2020). The auditory mechanics of the outer ear of the bush cricket: a numerical approach. Biophys. J. *118*, 464–475. https://doi.org/10.1016/j.bpj.2019.11.3394.
25. Lewis, D.B. (1974). The physiology of the tettigoniid ear I: the implications of the anatomy of the ear to its function in sound reception. J. Exp. Biol. *60*, 821–837.
26. Celiker, E., Woodrow, C., Rocha-Sánchez, A.Y., Chivers, B.D., Barrientos-Lozano, L., and Montealegre-Z, F. (2022). Beyond the exponential horn: a bush-cricket with ear canals which function as coupled resonators. R. Soc. Open Sci. *9*, 220532. https://doi.org/10.1098/rsos.220532.
27. Jonsson, T., Montealegre-Z, F., Soulsbury, C.D., Robson Brown, K.A., and Robert, D. (2016). Auditory mechanics in a bush-cricket: direct evidence of dual sound inputs in the pressure difference receiver. J. R. Soc. Interface *13*, 20160560. https://doi.org/10.1098/rsif.2016.0560.
28. Mason, A.C., Morris, G.K., and Wall, P. (1991). High ultrasonic hearing and tympanal slit function in rainforest katydids. Naturwissenschaften *78*, 365–367. https://doi.org/10.1007/BF01131611.
29. Bailey, W.J., and Stephen, R.O. (1978). Directionality and auditory slit function: a theory of hearing in bushcrickets. Science *201*, 633–634. https://doi.org/10.1126/science.201.4356.633.
30. Pulver, C.A., Celiker, E., Woodrow, C., Geipel, I., Soulsbury, C.D., Cullen, D.A., Rogers, S.M., Veitch, D., and Montealegre-Z, F. (2022). Ear pinnae in a neotropical katydid (Orthoptera: Tettigoniidae) function as ultrasound guides for bat detection. eLife *11*, 1–31. https://doi.org/10.7554/eLife.77628.
31. Woodrow, C., Pulver, C., Song, H., and Montealegre, Z.F. (2022). Auditory mechanics in the grig (*Cyphoderris monstrosa*): tympanal travelling waves and frequency discrimination as a precursor to inner ear tonotopy. Proc. Biol. Sci. *289*, 20220398. https://doi.org/10.1098/rspb.2022.0398.
32. Gleich, O., Dooling, R.J., and Manley, G.A. (2005). Audiogram, body mass, and basilar papilla length: correlations in birds and predictions for extinct archosaurs. Naturwissenschaften *92*, 595–598. https://doi.org/10.1007/s00114-005-0050-5.
33. Manley, G.A. (2000). Cochlear mechanisms from a phylogenetic viewpoint. Proc. Natl. Acad. Sci. USA *97*, 11736–11743. https://doi.org/10.1073/pnas.97.22.11736.

Please cite this article in press as: Woodrow et al., An Eocene insect could hear conspecific ultrasounds and bat echolocation, Current Biology (2023), https://doi.org/10.1016/j.cub.2023.10.040

34. Mennecart, B., deMiguel, D., Bibi, F., Rössner, G.E., Métais, G., Neenan, J.M., Wang, S., Schulz, G., Müller, B., and Costeur, L. (2017). Bony labyrinth morphology clarifies the origin and evolution of deer. Sci. Rep. *7*, 13176. https://doi.org/10.1038/s41598-017-12848-9.

35. Plotnick, R.E., and Smith, D.M. (2012). Exceptionally preserved fossil insect ears from the Eocene Green River Formation of Colorado. J. Paleontol. *86*, 19–24. https://doi.org/10.1666/11-072.1.

36. Zeuner, F.E. (1936). The recent and fossil Tympanophorinae (Tettigoniidae, Saltatoria). Proc. R. Entomol. Soc. Lon. B *85*, 287–302.

37. Hoy, R.R. (1992). The evolution of hearing in insects as an adaptation to predation from bats. In The Evolutionary Biology of Hearing (Springer), pp. 115–129. https://doi.org/10.1007/978-1-4612-2784-7_8.

38. Conner, W.E., and Corcoran, A.J. (2012). Sound strategies: the 65-million-year-old battle between bats and insects. Annu. Rev. Entomol. *57*, 21–39. https://doi.org/10.1146/annurev-ento-121510-133537.

39. Benavides-Lopez, J.L., ter Hofstede, H., and Robillard, T. (2020). Novel system of communication in crickets originated at the same time as bat echolocation and includes male-male multimodal communication. Sci. Nat. *107*, 9. https://doi.org/10.1007/s00114-020-1666-1.

40. Zou, W., Liang, H., Wu, P., Luo, B., Zhou, D., Liu, W., Wu, J., Fang, L., Lei, Y., and Feng, J. (2022). Correlated evolution of wing morphology and echolocation calls in bats. Front. Ecol. Evol. *10*, 1031548. https://doi.org/10.3389/fevo.2022.1031548.

41. Vavakou, A., Scherberich, J., Nowotny, M., and van der Heijden, M. (2021). Micromechanics in a miniature hearing organ - insights from the bush-cricket. Proc. Natl. Acad. Sci. USA *118*, e2105234118, https://doi.org/10.1073/pnas.2105234118.

42. Gorochov, A.V. (2010). New and little-known orthopteroid insects (Polyneoptera) from Fossil resins: communication 4. Paleontol. J. *44*, 657–671. https://doi.org/10.1134/S0031030110060080.

43. Strauß, J. (2019). What determines the number of auditory sensilla in the tympanal hearing organs of Tettigoniidae? Perspectives from comparative neuroanatomy and evolutionary forces. J. Orthopt. Res. *28*, 205–219. https://doi.org/10.3897/jor.28.33586.

44. Celiker, E., Woodrow, C., Mhatre, N., and Montealegre-Z, F. (2022). A numerical approach to investigating the mechanisms behind tonotopy in the bush-cricket inner-ear. Front. Insect Sci. *2*, 1–12. https://doi.org/10.3389/finsc.2022.957385.

45. Sarria-S, F.A., Chivers, B.D., Soulsbury, C.D., and Montealegre-Z, F. (2017). Non-invasive biophysical measurement of travelling waves in the insect inner ear. R. Soc. Open Sci. *4*, 1–11. https://doi.org/10.1098/rsos.170171.

46. Tan, M.K., Ingrisch, S., Wahab, R.B.H.A., Japir, R., and Chung, A.Y.C. (2020). Ultrasonic bioacoustics and stridulum morphology reveal cryptic species among Lipotactes big-eyed katydids (Orthoptera: Tettigoniidae: Lipotactinae) from Borneo. Syst. Biodivers. *18*, 510–524. https://doi.org/10.1080/14772000.2020.1769223.

47. Béthoux, O. (2012). Grylloptera - a unique origin of the stridulatory file in katydids, crickets, and their kin (Archaeorthoptera). Arthropod Syst. Phylogeny *70*, 43–68. https://doi.org/10.3897/asp.70.e31747.

48. Montealegre-Z, F., Ogden, J., Jonsson, T., and Soulsbury, C.D. (2017). Morphological determinants of signal carrier frequency in katydids (Orthoptera): a comparative analysis using biophysical evidence of wing vibration. J. Evol. Biol. *30*, 2068–2078. https://doi.org/10.1111/jeb.13179.

49. Jones, P.L., Page, R.A., Hartbauer, M., and Siemers, B.M. (2011). Behavioral evidence for eavesdropping on prey song in two Palearctic sibling bat species. Behav. Ecol. Sociobiol. *65*, 333–340. https://doi.org/10.1007/s00265-010-1050-9.

50. Tan, M.K., Duncan, J., Wahab, R.B.H.A., Lee, C.-Y., Japir, R., Chung, A.Y.C., Baroga-Barbecho, J.B., Yap, S.A., and Montealegre-Z, F. (2023). The calling songs of some katydids (Orthoptera, Tettigonioidea) from the tropical forests of Southeast Asia. J. Orthoptera Res. *32*, 1–24. https://doi.org/10.3897/jor.32.84563.

51. Celiker, E., Jonsson, T., and Montealegre-Z, F. (2020). On the tympanic membrane impedance of the katydid *Copiphora gorgonensis* (Insecta: Orthoptera: Tettigoniidae). J. Acoust. Soc. Am. *148*, 1952. https://doi.org/10.1121/10.0002119.

52. Woodrow, C., and Montealegre-Z, F. (2023). Auditory system biophysics in a new species of false-leaf katydid (Tettigoniidae: Pseudophyllinae) supports a hypothesis of broadband ultrasound reception. Zool. Anz. *304*, 94–104. https://doi.org/10.1016/j.jcz.2023.04.002.

53. Michelsen, A., and Löhe, G. (1995). Tuned directionality in cricket ears. Nature *375*, 639. https://doi.org/10.1038/375639a0.

54. Michelsen, A., and Larsen, O.N. (2008). Pressure difference receiving ears. Bioinspir. Biomim. *3*, 11001. https://doi.org/10.1088/1748-3182/3/1/011001.

55. Michelsen, A., and Larsen, O.N. (1978). Biophysics of the ensiferan ear - I. Tympanal vibrations in bushcrickets (Tettigoniidae) studied with laser vibrometry. J. Comp. Physiol. *123*, 193–203. https://doi.org/10.1007/BF00656872.

56. Veitch, D., Celiker, E., Aldridge, S., Pulver, C., Soulsbury, C.D., Jonsson, T., Woodrow, C., and Montealegre-Z, F. (2021). A narrow ear canal reduces sound velocity to create additional acoustic inputs in a microscale insect ear. Proc. Natl. Acad. Sci. USA *118*, https://doi.org/10.1073/pnas.2017281118.

57. Yoshida, J., Kobayashi, Y., and Norell, M.A. (2023). An ankylosaur larynx provides insights for bird-like vocalization in non-avian dinosaurs. Commun. Biol. *6*, 152. https://doi.org/10.1038/s42003-023-04513-x.

58. Sakagami, R., and Kawabe, S. (2020). Endocranial anatomy of the ceratopsid dinosaur Triceratops and interpretations of sensory and motor function. PeerJ *8*, e9888. https://doi.org/10.7717/peerj.9888.

59. West, C.D. (1985). The relationship of the spiral turns of the cochlea and the length of the basilar membrane to the range of audible frequencies in ground dwelling mammals. J. Acoust. Soc. Am. *77*, 1091–1101. https://doi.org/10.1121/1.392227.

60. Manoussaki, D., Chadwick, R.S., Ketten, D.R., Arruda, J., Dimitriadis, E.K., and O'Malley, J.T. (2008). The influence of cochlear shape on low-frequency hearing. Proc. Natl. Acad. Sci. USA *105*, 6162–6166. https://doi.org/10.1073/pnas.0710037105.

61. Greenwalt, D.E., and Rust, J. (2014). A new species of *Pseudotettigonia* Zeuner (Orthoptera: Tettigoniidae) with an intact stridulatory field and reexamination of the subfamily Pseudotettigoniinae. Syst. Entomol. *39*, 256–263. https://doi.org/10.1111/syen.12050.

62. Jonsson, T., Montealegre-Z, F., Soulsbury, C.D., and Robert, D. (2021). Tenors not sopranos: bio-mechanical constraints on calling song frequencies in the Mediterranean field-cricket. Front. Ecol. Evol. *9*, https://doi.org/10.3389/fevo.2021.647786.

63. Montealegre, Z.F. (2005). Biomechanics of musical stridulation in katydids (Orthoptera: Ensifera: Tettigoniidae): an evolutionary approach. PhD thesis (University of Toronto).

64. Morris, G.K., Aiken, R.B., and Kerr, G.E. (1975). Calling songs of *Neduba macneilli* and *N. sierranus* (Orthoptera: Tettigoniidae: Decticinae). J.N.Y. Entomol. Soc. *83*, 229–234.

65. Morris, G.K., and Pipher, R.E. (1967). Tegminal amplifiers and spectrum consistencies in Conocephalus nigropleurum (Bruner), Tettigoniidae. J. Insect Physiol. *13*, 1075–1085.

66. Hemp, C., Montealegre-Z, F., Woodrow, C., and Heller, K. (2023). Bushcrickets with very special ears and songs – review of the East African Phaneropterinae genus *Dioncomena* Brunner von Wattenwyl, 1878, with notes on its biogeography and the description of new species. Dtsch. Entomol. Z. *70*, 221–259. https://doi.org/10.3897/DEZ.70.100804.

67. Hare, R.M., and Simmons, L.W. (2021). Sexual selection maintains a female-specific character in a species with dynamic sex roles. Behav. Ecol. *32*, 609–616. https://doi.org/10.1093/beheco/arab005.

68. Bailey, W.J., and Römer, H. (1991). Sexual differences in auditory sensitivity: mismatch of hearing threshold and call frequency in a tettigoniid (Orthoptera, Tettigoniidae: Zaprochilinae). J. Comp. Physiol. A *169*, 349–353. https://doi.org/10.1007/BF00206999.

69. Scudder, S.H. (1890). The Tertiary Insects of North America, Thirteenth Edition (Rep. U.S. Geol. Surv. Territ.).

70. Schneider, E.S., Römer, H., Robillard, T., and Schmidt, A.K.D. (2017). Hearing with exceptionally thin tympana: ear morphology and tympanal membrane vibrations in eneopterine crickets. Sci. Rep. *7*, 15266. https://doi.org/10.1038/s41598-017-15282-z.

71. Fullard, J.H. (1984). External auditory structures in two species of Neotropical notodontid moths. J. Comp. Physiol. *155*, 625–632. https://doi.org/10.1007/BF00610848.

72. Ghiradella, H. (1971). Fine structure of the noctuid moth ear. I. The transducer area and connections to the tympanic membrane in *Feltia subgothica* haworth. J. Morphol. *134*, 21–45. https://doi.org/10.1002/jmor.1051340103.

73. Montealegre-Z, F., and Robert, D. (2015). Biomechanics of hearing in katydids. J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol. *201*, 5–18. https://doi.org/10.1007/s00359-014-0976-1.

74. Stephen, R.O., and Bennet-Clark, H.C. (1982). The anatomical and mechanical basis of stimulation and frequency analysis in the locust ear. J. Exp. Biol. *99*, 279–314. https://doi.org/10.1242/jeb.99.1.279.

75. Windmill, J.F.C., Fullard, J.H., and Robert, D. (2007). Mechanics of a "simple" ear: tympanal vibrations in noctuid moths. J. Exp. Biol. *210*, 2637–2648. https://doi.org/10.1242/jeb.005025.

76. Morris, G.K., Mason, A.C., Wall, P., and Belwood, J.J. (1994). High ultrasonic and tremulation signals in neotropical katydids (Orthoptera: Tettigoniidae). J. Zool. *233*, 129–163. https://doi.org/10.1111/j.1469-7998.1994.tb05266.x.

77. ter Hofstede, H., Voigt-Heucke, S., Lang, A., Römer, H., Page, R., Faure, P., and Dechmann, D. (2017). Revisiting adaptations of neotropical katydids (Orthoptera: Tettigoniidae) to gleaning bat predation. Neotrop. Biodivers. *3*, 41–49. https://doi.org/10.1080/23766808.2016.1272314.

78. Manley, G.A. (2012). Evolutionary paths to mammalian cochleae. J. Assoc. Res. Otolaryngol. *13*, 733–743. https://doi.org/10.1007/s10162-012-0349-9.

79. Simmons, N.B., Seymour, K.L., Habersetzer, J., and Gunnell, G.F. (2008). Primitive Early Eocene bat from Wyoming and the evolution of flight and echolocation. Nature *451*, 818–821. https://doi.org/10.1038/nature06549.

80. Davies, K.T.J., Maryanto, I., and Rossiter, S.J. (2013). Evolutionary origins of ultrasonic hearing and laryngeal echolocation in bats inferred from morphological analyses of the inner ear. Front. Zool. *10*, 2. https://doi.org/10.1186/1742-9994-10-2.

81. Heffner, R.S., and Heffner, H.E. (1992). Evolution of sound localization in mammals. In The Evolutionary Biology of Hearing, *232* (Springer), pp. 691–715.

82. Jones, D.L., and Ratnam, R. (2023). Are frog calls relatively difficult to locate by mammalian predators? J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol. *209*, 11–30. https://doi.org/10.1007/s00359-022-01594-7.

Please cite this article in press as: Woodrow et al., An Eocene insect could hear conspecific ultrasounds and bat echolocation, Current Biology (2023), https://doi.org/10.1016/j.cub.2023.10.040

## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Biological samples | | |
| †*Eomortoniellus handlirschi* Zeuner[36] amber fossil | London Natural History Museum | NHMUK In. 29119 |
| Software and algorithms | | |
| NRecon v.1.6.9.18 | Bruker Corporation, Billerica, MA, USA | https://www.bruker.com/en.html |
| Amira-Aviso v.6.7 | Thermo Fisher Scientific, Waltham, Massachusetts, USA | https://www.thermofisher.com/se/en/home/electron-microscopy/products/software-em-3d-vis/avizo-software.html |
| COMSOL Multiphysics v.5.6 | COMSOL Multiphysics, Burlington, MA). | https://www.comsol.com/ |
| CHITUBOX 64 | Chitubox, Guangdong, China | https://www.chitubox.com/en/index |

### RESOURCE AVAILABILITY

#### Lead contact

Further information and requests for resources should be directed to the lead contact, Fernando Montealegre-Z (fmontealegrez@lincoln.ac.uk)

#### Materials availability

Raw tomographic images from micro-CT scans are available from the authors upon request and have been submitted to MorphoSource (pending at time of publication) for future open access use. Numerical models available from authors upon request.

#### Data and code availability

Specimen used in this study is accessioned at the London Natural History Museum (NHMUK, In. 29119). There are no known ethical considerations regarding the collection of this specimen. No custom codes were used for data analysis. Experimental data provided within main text or supplemental information sections. µCT scans of *E. handlirschi are* available from C Woodrow upon request and have been submitted to MorphoSource and are pending accession codes. All key data is presented in the main paper or supplemental information. Raw data is available upon request.

### EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

†*Eomortoniellus handlirschi* Zeuner[36]

#### Holotype

NHMUK, In. 29119, inclusion of almost complete adult male; Baltic amber; Eocene, Lower Oligocene, East Prussia, Germany.

#### Brief description

This specimen has been subject to thorough descriptions, thus a redescription at this stage is not required.[36,42]. Unique features of this specimen include the intact tegmina, with specialised sound producing structures visible, and the tympana, which are recessed into distinct cavities characteristic of the Tettigoniidae. The specimen sits to one edge of a large (~20mm) block of Baltic amber, which is cloudy in appearance around the specimen. The hind tibiae have been lost, probably during the extraction or polishing process.

Please cite this article in press as: Woodrow et al., An Eocene insect could hear conspecific ultrasounds and bat echolocation, Current Biology (2023), https://doi.org/10.1016/j.cub.2023.10.040

## METHOD DETAILS

### μCT scanning and measurements

To investigate the anatomy of the auditory pinnae of *E. handlirschi*, the specimen was μCT scanned under agreement during a loan from NHM London (PAL 2022-526 PI). The specimen was mounted in a custom-built polystyrene holder with a brass base and positioned in a SkyScan 1172 μCT scanner (Bruker Corporation, Billerica, MA, USA; voxel size 5 μm, voltage 55 kV, current 180 μA, exposure 200 ms, rotation step 0.2°). μCT projection images were constructed to produce orthogonal slices with NRecon (v.1.6.9.18, Bruker Corporation, Billerica, MA, USA). For 3D segmentation, the slice data was imported into Amira-Aviso 6.7 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and the ear manually selected using the magic wand tool every 3 slices, followed by interpolation to connect the selected geometries and generate a 3D surface. μCT data of *E. handlirschi* was also compared to that of *Lipotactes maculatus*, to facilitate discussion between extinct and modern Lipotactinae.

### Estimation of calling song frequency

To estimate calling song frequency ($f_c$), the length of the CuPb vein along the mirror containing the stridulatory file was measured. This was measured for the left tegmen where CuPb is fully visible through the amber. Stridulatory file length has been shown to correlate with peak song frequency,[48] whereby $ln(f_c) = -0.97 * ln(\text{file length}) + 3.74$, and *ln* is the natural logarithm. Thus, calculating $f_c$ based on the maximum length of CuPb should predict the minimum calling song frequency.

### 3D printing and pinnae experiments

Standard Tessellation Language (STL) files (a 3D geometry file type) of the pinnae of *E. handlirschi* and *L. maculatus* were built from the μCT data. Pinnae STLs were then 3D printed using a Mars Elegoo Pro 2 3D Printer (Elegoo Inc, Shenzhen, China). Models were printed using grey photopolymer resin (exposure parameters: 20 s first layer, 5 s normal layers) with a solidification wavelength of 405 nm. When printing was complete (about 1 hr 30 min), models were washed in 100% isopropyl alcohol, rinsed in cold water, then exposed to UV light in an Elegoo Mercury Plus curing station (Elegoo Inc, Shenzhen, China) for 10 min. 3D models were printed to be ~30 x larger than the real ear. Models were then stimulated with a loudspeaker with a calibrated B&K Type 4182 probe microphone (Brüel & Kjær, Nærum, Denmark) inside the anterior and posterior pinnae cavities (apc and ppc respectively) to record pinna cavity resonance. The effective tested frequency range of the stimulus after considering the scaling factor of the 3D print was 20600 kHz, with all frequencies delivered at the same intensity (60 dB). This way, any resonances in the system would be identified as peaks in the frequency spectrum of the microphone response. For full details of the methodology see Pulver et al.[30] Three measurements ($F_0$, $F_{min}$, and $F_{max}$) were extracted from the frequency spectra.

### Numerical modelling

To investigate the natural frequencies and modes of resonance (eigenfrequencies and eigenmodes) of the ECs, the precise geometries of the ECs obtained through μCT scanning and 3D reconstruction were imported into the simulation toolbox COMSOL (v5.6, COMSOL Multiphysics, Burlington, MA). The geometry (.stl files) were imported with default simplification tolerance. The material inside the EC was selected to be air and given the properties of air under default temperature settings (293.15 K). Edges for the acoustic spiracle and two tympana were defined manually within the geometry using the 'create entities' function.

The eigenfrequencies and eigenmodes were calculated from the solution of the Helmholtz equation in the pressure acoustics node of COMSOL Multiphysics, v. 5.6. The acoustic spiracle was given the plane wave radiation boundary condition; a class of non-reflecting boundary conditions which permits the passage of a plane wave without resistance, and accurately reflects ports such as the opening to a tube where the medium inside and outside the tube are the same (COMSOL Multiphysics, v5.6 Reference Manual, 2018). The EC wall was assigned a sound hard boundary condition, so that the normal derivative of pressure was zero at the wall. The tympana were assigned an impedance boundary condition as described in Celiker et al.[24,51]

The variational form of the defined problem was solved using the finite element method on a problem specific finite element mesh, constructed as in Celiker et al.[24,51] Eigenvalues were solved for using the ARPACK eigenfrequency solver inbuilt in COMSOL Multiphysics.

The same problem was also considered in the frequency domain, where the incident wave used had a magnitude 1 Pa and frequency range 10-100 kHz, with a resolution of 0.5 kHz. The sound pressure was recorded at the tympanic membranes and showed the change in pressure magnitude of the sound wave after travelling through the EC.

A further frequency domain model, following the protocol in Pulver et al.[30], was solved to investigate auditory pinnae resonances.

Next, a further eigenfrequency analysis was employed to obtain the natural frequencies and modes of resonance (eigenfrequencies and eigenmodes) of the forewings. The forewing was built as a.dxf file in Adobe Illustrator (Adobe Inc., San Jose, California, United States) and imported into the xy plane of COMSOL (v5.6, COMSOL Multiphysics, Burlington, MA). The wing membrane and veins were given a Young's Modulus of 9 GPa, Poisson's ratio of 0.33, and a Density of 1200 kg/m$^3$. The aforementioned method and was used to obtain the eigenmode at the theoretical vibrating region of the wing (mirror, harp, and anal areas).

Finally, to calculate the propagation velocity in the EC, the solution of the wave equation was considered in the EC, in real time. An incident wave of magnitude 1 Pa and frequency 30 kHz was modelled to enter the EC through the spiracle. The EC wall and tympana were assigned the sound hard boundary and impedance conditions as described above, respectively. The solution showed the movement of the sound wave inside the EC, and we recorded the time it took to reach the tympana. Since the distance travelled

Please cite this article in press as: Woodrow et al., An Eocene insect could hear conspecific ultrasounds and bat echolocation, Current Biology (2023), https://doi.org/10.1016/j.cub.2023.10.040

by the sound wave is equivalent to the length of the EC, the speed was calculated as *speed* = *distance/ time*[64]. The problem was considered in the time domain, using the *Pressure Acoustics, Transient* node of COMSOL Multiphysics, v. 5.6. The solution was obtained by the finite element method for the space variables as described above. The Generalized alpha method, available in COMSOL, was used for the time variable. The time step was taken as $\Delta t = 5/(30\ \text{kHz})\ /1000 = 1.67\text{e-}8$ s, for five cycles at 30 kHz.

## QUANTIFICATION AND STATISTICAL ANALYSIS

Quantification of geometries were conducted in Amira-Aviso 6.7 (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Statistical analyses were not performed in this study as its findings were descriptive, numerical, and based on a sole fossil specimen (n = 1).