

Ultrasonic hearing in nocturnal butterflies

Hedyliids have ultrasound-sensitive ears on their wings to help them avoid bats.

Ultrasonic hearing is common in moths, which rely on it for defence and communication^{1,2}, but it has never been demonstrated in butterflies. Here we describe a new type of ultrasound-sensitive ear that we have discovered in an unusual group of nocturnally active, neotropical butterflies, the Hedyloidea. Hedyliids have ears on their wings and respond to ultrasound by making flight manoeuvres to avoid bats. On the basis of phylogenetic and comparative anatomical evidence, we propose that hearing in Lepidoptera and day flight in butterflies both result from an intense selection pressure imposed by echolocating bats more than 50 million years ago.

Diurnality has traditionally been used to distinguish butterflies from moths, and underlies a major difference in the sensory worlds of these two groups. Butterflies are predominantly diurnal and rely on a well-developed visual system for communication and predator detection³. Although it has been proposed that some butterflies have low-frequency hearing^{4,5}, ultrasonic hearing is unknown for this taxon. In contrast, most moths have tympanal ears that function primarily to detect echolocation calls used by insectivorous bats to locate and track their prey¹.

The Hedyloidea, which have previously been identified as moths, are now believed to be the closest relatives, and possibly the 'living ancestors', of modern-day butterflies (Papilionoidea, Hesperoidea)^{6,7}. The foregoing modifications of Hedyloidea have been suggested to function in hearing or scent production⁶, but until now they had not been studied in living individuals. Because hedyliids fly at night and are therefore exposed to bats, we predicted that they should have ultrasound-sensitive ears and exhibit bat-avoidance behaviour.

Our anatomical investigations of *Macrosoma heliconiaria*, collected live on Barro Colorado Island, Panama, have revealed a tympanal ear⁴ of intriguing design. The eardrum (tympanal membrane) is nestled within a tympanic cavity at the narrow end of a canal that resembles the pinna of a rabbit ear (Fig. 1a,b). The thin (1 µm) membrane stretches over a large air-filled chamber and has three vibration-sensitive chordotonal sensory organs attached to separate regions of its inner surface, similar to the frequency-discriminating ears of acridid grasshoppers⁸. The ears move with the wing and so may be efficient at localizing sounds during flight. Comparative anatomical studies indicate that ears are

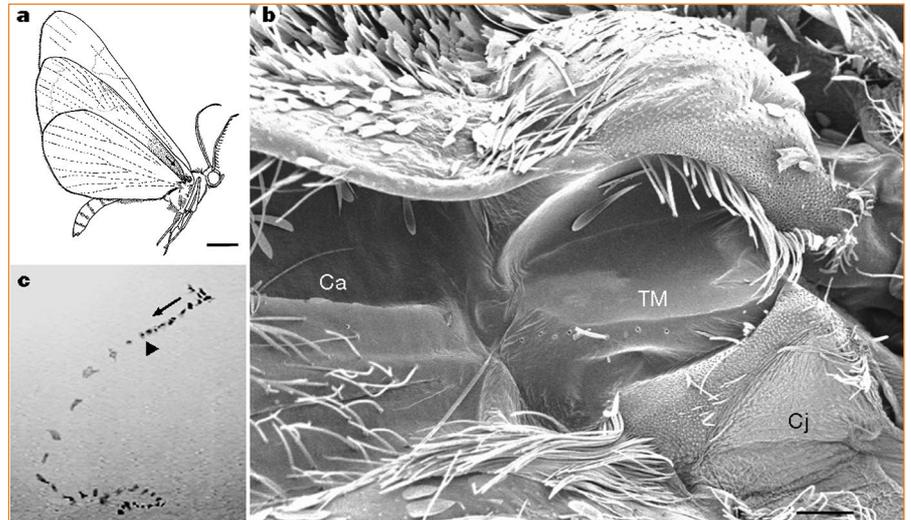


Figure 1 Hedyliid butterflies have ultrasound-sensitive ears that allow them to evade bats in flight. **a**, Lateral view of *M. heliconiaria*, showing the location of the right ear. An arrow points down the canal to the tympanic cavity where the tympanic membrane (not shown) resides. Scale bar, ~3 mm. **b**, Scanning electron micrograph of the right tympanic cavity showing the tympanic membrane (TM), ear canal (Ca) and an accessory membrane, the conjunctivum (Cj). Scale bar, 120 µm. **c**, Consecutive video images (41 frames at 30 frames per s; 13 before and 28 after the stimulus onset) of a free-flying *M. heliconiaria* responding to a short (~250 ms), high-frequency (25 kHz), high-intensity (>100 dB) sound. Arrow, flight direction; arrowhead, stimulus onset.

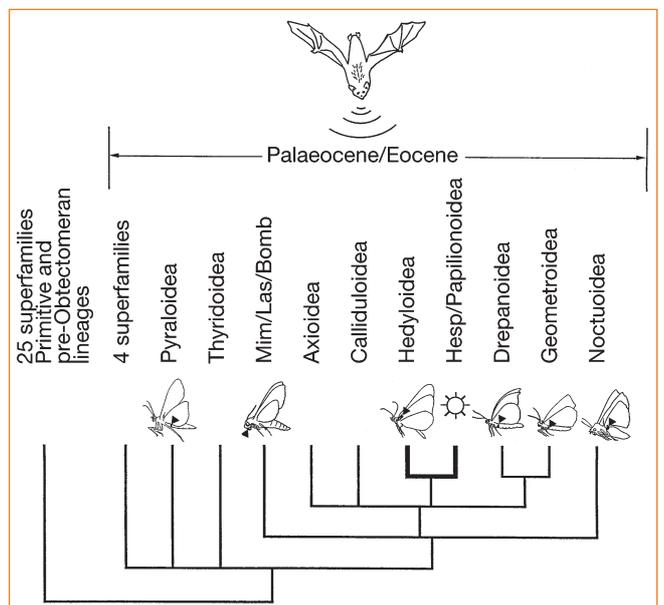
widespread throughout the Hedyloidea (about 40 species), and are equally developed in both sexes.

When stimulated with an intense ultrasonic stimulus, hedyliids perform one of several flight manoeuvres (Fig. 1c), including steep dives or climbs, upward or downward loops, spirals and horizontal sweeps. These responses, shown through ablation studies to be governed by the tympanal ears, are unpredictable in their direction, and are characterized by short latencies

(45.4 ± 9.35 ms) and substantial increases (400.1%) in flight speeds. These features are of selective advantage to an insect in a close-range encounter with a bat⁹.

The discovery of hearing in the Hedyloidea provides an insight into the evolution of lepidopteran ears and diurnality in butterflies. Mapping ultrasonic hearing onto a phylogeny of the Lepidoptera¹⁰ shows that it has evolved independently at least six times, all within the obtectomeran lineage (Fig. 2). Current fossil evidence indicates that the

Figure 2 The presence of hearing mapped onto a lepidopteran phylogeny¹⁰. Ultrasound-sensitive ears occur on a variety of body parts (arrowheads) reflecting multiple evolutionary origins. All ears, known to function primarily as bat-detectors, occur within the Obtectomera. We propose that hearing in extant Lepidoptera originated as a response to selection pressures imposed by echolocating bats in the late Palaeocene, and that echolocating bats were a driving force for the large, species-rich butterfly groups Hesperoidea and Papilionoidea (Sun symbol) to move into the daytime. The butterfly superfamilies are highlighted in bold. Earlessness and nocturnalism are the plesiomorphic (ancestral) characteristics of the Lepidoptera.



principal obtectomeran groups originated around the late Palaeocene epoch (~60 million years ago)¹⁰, by which time microchiropteran bats had evolved echolocation¹¹. We conclude that predation by bats imposed a great selection pressure on the evolution of ears in Lepidoptera.

Butterflies are the largest and most diverse group of diurnal Lepidoptera, and the selection pressures generally proposed for their diurnality include various physiological and ecological factors, but not selection pressure by bats. Given the significant impact of bats on other obtectomeran taxa, we suggest that diurnality in non-hedylid butterflies was also an anti-bat strategy, promoted by selection for individuals that avoided bats by appearing during the day. The butterfly, in effect, was therefore 'invented' by the bat.

Is the earless condition of other (non-hedylid) butterflies primitive or secondarily derived? Consider the Vogel's organ, a forewing structure of unknown function that is distributed sporadically with varying degrees of development among certain Papilionoidea^{12,13}. Our comparative anatomical studies show that the hedylid ear and Vogel's organ are homologous structures. Given the current placement of the Hedy-

loidea as a sister-group to the Papilionoidea and Hesperoidea, it is possible that Vogel's organ is a degenerate 'bat detector'. Our discovery may help to bring to light the evolutionary origin of this group of butterflies.

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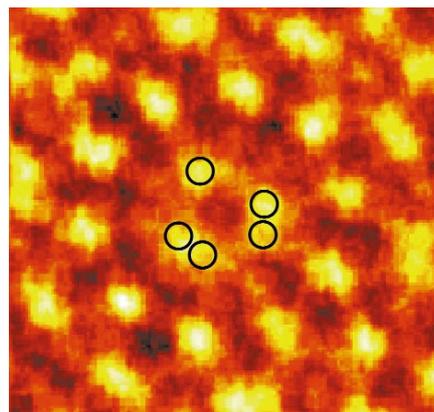


Figure 2 Cluster showing broken decagonal symmetry within the central ring, but there are still ten columns present, five of which have higher intensities (yellow), indicating high TM occupancy, whereas the other five show high Al occupancy (red). One of the five TM columns is a single column; the others form closely spaced column pairs, very similar to those in the outer 2-nm ring.

decagonal symmetry for the entire cluster (Fig. 1a) which enables the decagons to have identical subtiles, as suggested by the Gummelt coverage model, ensuring that the overlap rules required by this model are satisfied.

However, it is hard to see how a structure with such extensive broken symmetry can be energetically stable. Figure 1b shows a typical Z-contrast image of clusters that do not have strong broken symmetry. In a Z-contrast image, the intensity is directly correlated with the mean-square atomic number (Z), so that transition metal (TM) columns are seen with much higher intensity than Al columns.

The superimposition in Fig. 1c shows that the structure of the decagon proposed by Steinhardt *et al.* does not match our Z-contrast image in significant ways: the four proposed TM columns causing the broken symmetry are definitely absent from our image. In addition, our image reveals the presence of ten closely spaced TM column pairs in the outermost ring of the cluster, as indicated by double blue arrows in Fig. 1b. These are only single TM columns in the model of Steinhardt *et al.*

Turning to the central ring, our image clearly shows its underlying ten-fold symmetry. It is seen as a ring, with an intensity varying between that of an Al column (red) and a TM column (yellow). This is inconsistent with Steinhardt *et al.*'s triangular arrangement. This intensity pattern shows that there are ten closely spaced atomic columns around the central ring with a composition intermediate between that of an Al column and a TM column. However, there are many clusters with broken symmetry in the central ring.

Figure 2 is a typical Z-contrast image of such a cluster where the intensity in the central ring shows broken decagonal symmetry. The intensity distribution shows that

Alloys

Atomic structure of the quasicrystal $Al_{72}Ni_{20}Co_8$

Steinhardt *et al.*¹ reported experimental evidence in support of the coverage model^{2–4} by presenting a structure for the high-perfection decagonal quasicrystal $Al_{72}Ni_{20}Co_8$. The coverage model describes the decagonal quasicrystals by a single type of tile, a decagon, the basic 2-nm cluster of which is allowed to overlap so as to cover the surface. Although their Z-contrast image (also referred to as a high-angle annular dark-

field image) supports the coverage model, we find that the atomic structure they propose for the decagon has significant shortcomings which are inconsistent with our Z-contrast images.

The correct atomic structure of the decagon is critical to understanding how and why quasicrystals form. We have investigated the same high-perfection decagonal quasicrystal $Al_{72}Ni_{20}Co_8$ (provided by A. P. Tsai) by Z-contrast imaging, but at a higher spatial resolution (~0.13 nm) than Steinhardt *et al.*¹

The main feature in the proposed structure of the decagon¹ is the extensive broken

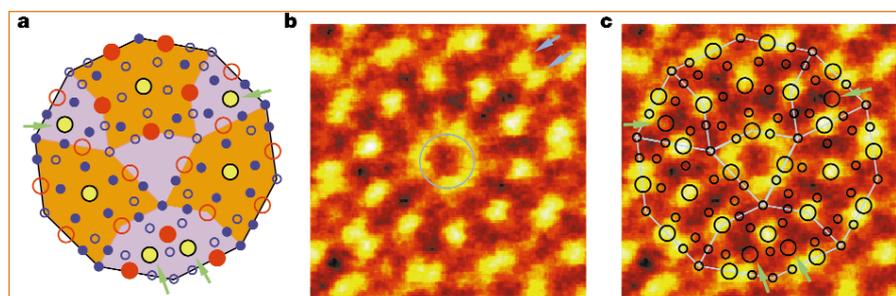


Figure 1 Comparison of the proposed "best-fit candidate model"¹ for the 2-nm cluster of the high-perfection decagonal quasicrystal $Al_{72}Ni_{20}Co_8$ with our higher resolution Z-contrast image. **a**, The proposed model contains broken decagonal symmetry for the entire cluster: see, for example, the four yellow columns indicated by green arrows. **b**, Z-contrast image of a 2-nm cluster of the same high-perfection decagonal quasicrystal $Al_{72}Ni_{20}Co_8$ along the ten-fold axis. The bright yellow features show the locations of highest intensity corresponding to high transition-metal (TM) occupancy. Al columns have lower intensity and are seen as red features: see, for example, the ten red spokes around the central ring. These spokes connect the central ring to the ring of ten TM columns (yellow) and show the basic ten-fold symmetry of the cluster. **c**, The model cluster of Steinhardt *et al.* superimposed on our Z-contrast image shows that their four proposed TM columns indicated by green arrows are not present.