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Patterning of a compound eye on an extinct dipteran wing

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We have discovered unexpected similarities between a novel and characteristic wing organ in an extinct biting midge from Baltic amber, *Eohelea petrunkevitchi*, and the surface of a dipteran's compound eye. Scanning electron microscope images now reveal vestigial mechanoreceptors between the facets of the organ. We interpret *Eohelea*'s wing organ as the blending of these two developmental systems: the formation and patterning of the cuticle in the eye and of the wing.

Typically, only females in the genus carry this distinctive, highly organized structure. Two species were studied (*E. petrunkevitchi* and *E. sinuosa*), and the structure differs in form between them. We examine *Eohelea*'s wing structures for modes of fabrication, material properties and biological functions, and the effective ecological environment in which these midges lived. We argue that the current view of the wing organ's function in stridulation has been misconstrued since it was described half a century ago.

Keywords: Evo-devo; amber; stridulation; ectopic eye

1. INTRODUCTION

A novel feature in a genus of extinct biting midges (Diptera: Ceratopogonidae) preserved in Baltic amber is the unique oval cuticular patch on their wings (figures 1*a* and 2*a*). Two species of *Eohelea*—*Eohelea sinuosa* and *Eohelea petrunkevitchi*—exemplify distinct wing patch morphologies (though the wing patches share features of patterning and position). In *E. sinuosa*, the wing organ resembles the teeth of a file, whereas in *E. petrunkevitchi*, the wing organ has been previously described as honey-comb like [1,2]. This study presents low-vacuum scanning electron microscope (SEM) images of *E. petrunkevitchi*'s wing organ that now reveal its similarity to the surface of a compound eye. We find uniform dimples around each 'facet' that agree in position and diameter with the mechanoreceptors on the surface of a dipteran's eye.

Novel traits may arise from old genes used in new ways. These traits can be interpreted in terms of the differences in well-defined genetic pathways and the co-option [3] of such networks to produce new morphologies. Researchers have found a conserved

developmental programme in sensory organs with different structures and functions (e.g. the compound eye, Johnston's organ, and stretch receptor [4]). Halder *et al.* [5] showed that ectopic eyes in *Drosophila* could be expressed on the wing (as well as the legs, and thorax) by targeting expression of the homeobox gene *eyeless* (*ey*) into a developing imaginal disc. Perhaps *E. petrunkevitchi*'s wing organ is a natural example of this experiment; however, *ey* involvement is speculative, as only the most apical parts of a compound eye appear on the wing.

The wing organ's position on the wing is consistent between individual specimens, and the left and right wings exhibit nearly identical structures. In the absence of a cladogram based on characters that are independent of the wing organ, all we can conclude is that the wing organs (a derived state in the tribe) have retained several aspects of lens fabrication, with more features demonstrable in *E. petrunkevitchi*.

If natural selection shaped the wing organs, then it should be possible to propose an adaptive advantage that favoured their appearance and fine tuning. Alexander Petrunkevitch established the genus in 1957 [6], and suggested that the wing organ was used for stridulation (he named the type *E. stridulans*, now synonymized with *E. sinuosa*, because the file-type morphology of this species resembles a cricket's stridulatory structure). However, the wing organ is located at the distal edge of the wing (a location not known in other stridulatory systems), and only female *Eohelea* are known to carry the organ. Meanwhile, male *Eohelea* have lost the primary sensory organs for perceiving sound and air movements: their antennal plume and Johnston's organ [1,2]. Such a loss is known to occur in other biting midge genera [7], where it accompanies a change in the organism's mating behaviour (from a swarm-based system, in which females fly into a swarm of males, to a substrate-based system, where a male finds a female in a specific location). Instead of sound production, we suggest a possible alternative function for the wing organ: light reflection.

2. MATERIAL AND METHODS

(a) Amber material

Specimens of *Eohelea* used for this study come from the Harvard Museum of Comparative Zoology and Mount Holyoke College (collection of S.R.). All specimens examined come from Baltic amber dating, at earliest, to the Eocene (approx. 60–40 Myr ago). Biting midges in amber were prepared for observation according to the methods described by Szadziewski [2]. Specimens belonging to the Mount Holyoke collection were cut-down using a jewellery saw (size 8), ground with coarse-grained abrasive paper (no. 200–300) along a plane, and polished with waterproof abrasive paper (no. 500–800).

(b) Specimens

(See table 1 for specimen measurements).

MHC_01: *E. petrunkevitchi* female.

Specimen captured with left wing bent downward, right wing extended. Body brown.

MHC_02: *E. petrunkevitchi* female.

Specimen also captured with left wing bent downward, right wing extended. Body brown.

MHC_03: *E. sinuosa* female.

Specimen partially pulled apart, most probably during resin encapsulation. Body brown.

HRVD_8876: *E. sinuosa* female and male.

Two specimens (female and male) of *E. sinuosa* post copulation. The female midge appears to be sucking haemolymph from a puncture to the male's head (a common practice in insectivorous midge species).

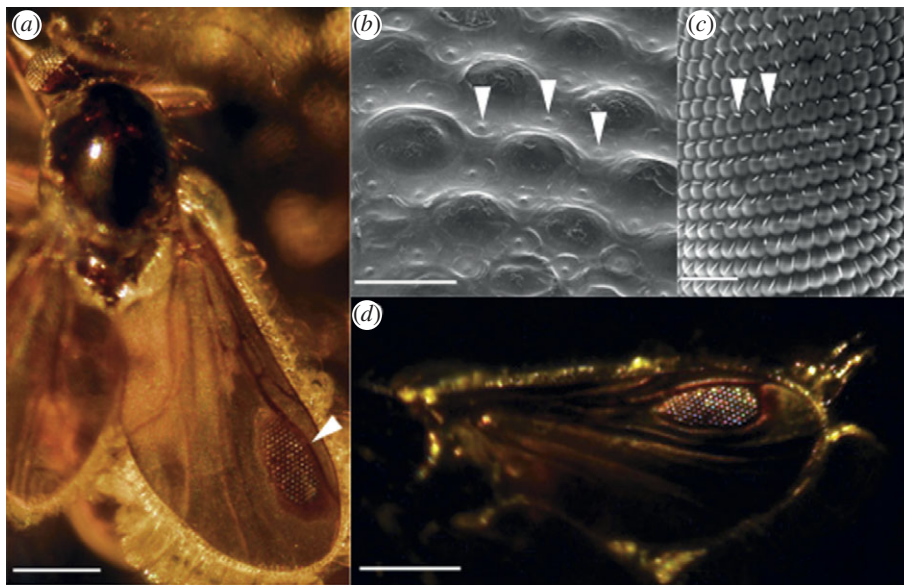


Figure 1. (a) Dorsal view of specimen *E. petrunkevitchi* female (MHC_01) showing wing organ of right wing (white arrow). Scale bar, 250 μm . (b) SEM image of *E. petrunkevitchi*'s wing organ (MHC_02). The 'facets' are convex. Around the border of each facet is a ring of what appears to be vestigial remnants of the mechanoreceptors that are present between the ommatidia in compound eyes (white arrows). Scale bar, 10 μm . (c) SEM image of *Drosophila melanogaster* eye. White arrows point to mechanoreceptors. Scale bar, 50 μm . (d) Light reflection from right wing organ of *E. petrunkevitchi* (MHC_01). Scale bar, 250 μm .

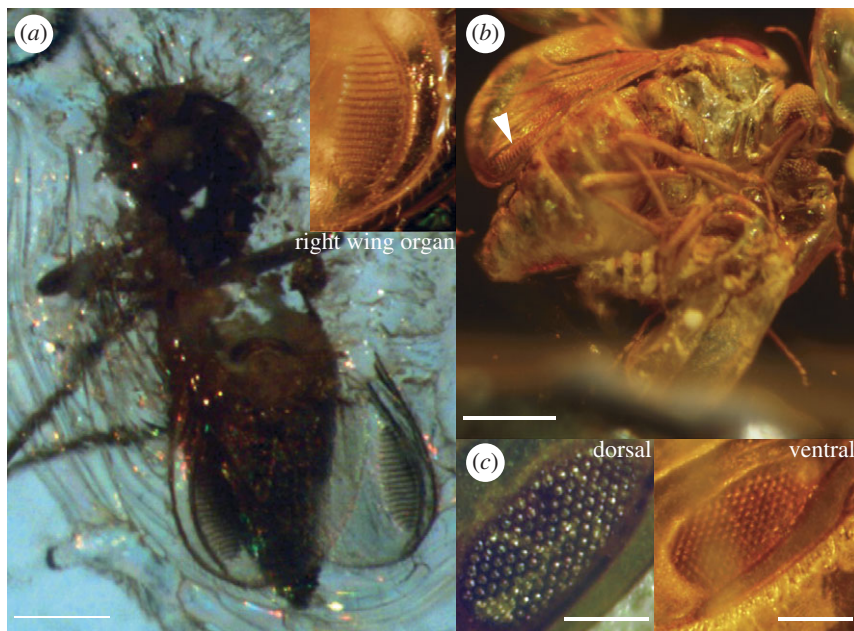


Figure 2. (a) Dorsal view of specimen *E. sinuosa* female (MHC_03). Scale bar, 250 μm . (b) 'An intimate embrace, part two' (*E. sinuosa*, HRVD_8876). The female has inserted her proboscis into the head of the male. White arrow indicates female's wing organ—the male is without a wing organ, and his genitalia appear open. Scale bar, 250 μm . (c) Close-ups of dorsal and ventral view of right wing organ of *E. petrunkevitchi* female (MHC_01). Dorsal structure is convex, while ventral structure is indented (ventral side does not reflect light). Scale bars, 10 μm .

- Female: body brown.
- Male: body brown. Wing organ absent. Genitalia appear open.

(c) SEM

The wing organ of a specimen of *E. petrunkevitchi* (MHC_02) had been exposed and was prepared for scanning electron imaging in low-vacuum mode (FEI Quanta 200). Low-vacuum standards were an acceleration voltage of 20 kV, pressure of 0.60 Torr and a spot

size of 3. The same standards were used for SEM preparation of the *Drosophila* eye.

(d) Light reflection

To observe light reflection from wing organs, the specimens were placed on a revolving microscope stage (Nikon E600—objective magnification of 0.1 x) and were rotated beneath a bright beam of white light (fibre-optic light guide, with a homemade pinhole

Table 1. Measurements obtained from images of only the *Eohelea* specimens referenced in this paper (average diameter of the wing organ is 0.24 mm). (*Eohelea*'s compound eye facet measurements (diameter of 10 μm) are comparable to the wing organ's ridge spacing in *E. sinuosa* (10 μm) and the 'facet' diameter of the wing organ in *E. petrunkevitchi* (10 μm .)

	MHC_01	MHC_02	MHC_03	HRVD_8876
specimen description	<i>E. petrunkevitchi</i> female	<i>E. petrunkevitchi</i> female	<i>E. sinuosa</i> female	<i>E. sinuosa</i> female and male
body length (mm)	1.42	1.3	1.2	1.2
wing length (mm)	1.04	0.96	0.8	0.8
wing organ length (mm)	0.25	0.24	0.24	0.21
wing organ width (mm)	0.11	0.12	0.09	0.09
number of ridges on wing organ	19	19	17	15
spacing between ridges (μm)	—	—	10	10
number of facets on wing organ	127	121	—	—
diameter of facets (μm)	10	9.5	—	—
spacing between facets (μm)	5	5	—	—
tibial comb length (μm)	—	—	2.5	—

aperture). Video images (Olympus DP71 diagnostic software) of the wing organ were captured as the specimen moved under the light.

3. RESULTS

Table 1 shows measurements obtained from images taken from the *Eohelea* specimens (average diameter of the wing organ is 0.24 mm). *Eohelea*'s compound eye facet measurements (diameter of 10 μm) are comparable to the wing organ's ridge spacing in *E. sinuosa* (10 μm) and the 'facet' diameter of the wing organ in *E. petrunkevitchi* (10 μm). Figure 2 illustrates this correlation.

SEM images of *E. petrunkevitchi*'s wing organ reveal uniformly patterned dimples around each 'facet' that agree in position and diameter with the mechanoreceptors on the surface of a dipteran's compound eye (see figure 1*b,c* for more detail). These are, to our knowledge, the first SEM images of the wing organ to have been obtained.

The topography of the wing organ is undulatory and altered the reflective patterning of the wing membrane. A still image from the video recording of the rotating wing shows light reflected from the dorsal surface of the wing organ (figure 1*d*). The ventral side of the wing organ (figure 2*c*) did not reflect light. The wing organ of *E. sinuosa* was also tested for light reflection, and while the results were less conclusive, this wing organ does bear a series of micro-ridges running perpendicular to the ridges [1] that resemble a diffraction grating.

(a) Notes on possibility of sound production

If these midges generated sound, they most probably scraped their hind leg tibial combs along the wing organ (a common cleaning behaviour in nematocerans). Differences in the number of ridges would have produced different frequencies of sound waves. Frequency estimates based on a model introduced by Turner [8], which states that the diameter of any sound emitter should be greater than the wavelength of sound that it produces, predict a high frequency (453 kHz)—far beyond any recorded for a living

insect. It is unlikely that the wing organ was used for stridulation.

4. DISCUSSION

Alexander Petrunkevitch was prepared to see the similarity in *E. sinuosa*'s wing organ to a stridulatory structure—he began his career in August Weismann's laboratory studying the anatomy of the stridulatory organs in male crickets; however, it was shape, not size or mechanical properties that guided his interpretation. In other comments and observations of the wing organ, anatomy has remained the basis of all discussion [1,2,9].

Eohelea's wing organ appears to have reflective properties. Many insects, including midges in *Eohelea*'s tribe (Ceratopogonini), are attracted to light signals, and even fall prey to carnivorous plants such as sundews [10–12]. Females within the Ceratopogonini often prey upon their mates after copulation, (figure 2*b*) and also prey on other insects for protein, such as male mayflies (insects that are attracted to light [7]).

Some insects have thickened wing regions (e.g. pterostigma in odonates) that allow them to glide through the air, and spend less energy moving their wings. Dipterans (and other neopterans) use asynchronous muscles to attain high wing-beat frequencies, and it would be surprising if the wing organ were a convergence on features of insects with different flying mechanisms; however, the wing organ may have played an unknown aerodynamic role in the midge's life.

The wing organ of *Eohelea* lies above the anterior-posterior boundary of the dipteran wing—a region of the wing with unique gene expression patterns. Because *E. petrunkevitchi*'s wing organ appears to be composed only of cuticle, it is not an ectopic eye. Instead, it was probably patterned at the end of a developmental cascade (when cuticle lays down).

The anatomical peculiarities of the novel wing structure in *Eohelea* can be read as a story of diet, mating habits and sensory modalities that comprise a way of life last seen 40 Ma. Evo-devo's first round of

success is based on recognizing similarities common to a wide array of organisms, e.g. the roles of *Hox* genes throughout the metazoans. It is also important to consider the restructuring and extension that can occur at the ends of these genetic cascades to yield novel derived features that enable new ways of living (and as a consequence, new species). Our interpretation of the wing organ suggests that it occurs as a late developmental change that involved the sharing of steps in two pathways (wing and eye development). This novelty is probably tied to an alteration in mate recognition (males drawn to a new stimulus—possibly light reflected from the female's wing organ), and it provides a novel way for female *Eohelea* to lure prey.

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