An exceptionally well-preserved Eocene dolichopodid fly eye: function and evolutionary significance

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The exceptionally preserved eyes of an Eocene dolichopodid fly contained in Baltic amber show remarkable detail, including features at micrometre and submicrometre levels. Based on this material, we establish that it is likely that the neural superposition compound eye existed as far back as 45 Ma. The ommatidia have an open rhabdom with a trapezoidal arrangement of seven rhabdomeres. Such a structure is uniquely characteristic of the neural superposition compound eye of present-day flies. Optical analysis reveals that the fossil eyes had a sophisticated and efficient optical system.

Keywords: Baltic amber; compound eye; dolichopodid fly; optical analysis; neural superposition compound eye

1. INTRODUCTION

The morphology and function of living insect compound eyes have been the subject of intense study during the past century (e.g. Exner 1891; Land & Nilsson 2002). By contrast, principally because of the lack of soft-part preservation, the study of fossil eyes has been limited to descriptions of the morphology on trilobite eyes (e.g. Clarkson & Levi-Setti 1975; Gál *et al.* 2000; Clarkson *et al.* 2006), the description of the eye of a crustacean (Fröhlich *et al.* 1992) and a beetle larva (Duncan *et al.* 1998), and there is one paper that describes some (minimal) internal structure of the fossil fly eye embedded in Baltic amber (Mierzejewski 1976) and function of the corneal surface of a fly (Parker *et al.* 1998). The lack of specimens of well-preserved fossilized eyes, where the cells and their positions can be well recognized, has limited the direct comparison between eyes of extinct organisms and those of living relatives, and has constrained the functional analysis of the visual system of fossil organisms.

Amber deposits are known to contain cellular nuclei and mitochondria in insect tissues (Poinar & Hess 1982; Grimaldi *et al.* 1994; Kohring 1998), as well as chloroplasts with endoplasmic reticulum in plant tissues (Poinar *et al.* 1996; Koller *et al.* 2005). It is presumed that such an impressive level of preservation in amber was due to a combination of rapid dehydration, protection from biological decay by entrapment and *in situ* polymerization of cuticular waxes and internal lipids during early diagenesis (Stankiewicz *et al.* 1998), but there is also

possibility of fixation by low molecular weight volatiles in the fluid resin. Grimaldi & Engel (2005) have illustrated a complete red-coloured compound eye of the milichiid fly in Baltic amber (p. 58); they have also illustrated a swarm of male dolichopodid flies from Dominican amber (p. 530). The level of detail revealed in these studies indicates that fossil fly eyes in amber in general have the potential for internal soft-tissue preservation. The Mid Eocene (45 Ma) Yantarny deposit of the Kaliningrad district of Russia is famous for yielding exquisitely preserved insects and other arthropods and plants in resin—the Baltic amber (Weitschat & Wichard 1998; Keyser & Weitschat 2005). Here, we report the completely preserved visual system, including soft tissues, of a ‘long-legged fly’ (Dolichopodidae) from Baltic amber. We also discuss the functional and evolutionary significance of the fossilized eye.

2. MATERIAL AND METHODS

Two dolichopodid specimens, trapped together in Baltic amber, were studied (now deposited at the Campus Museum of Shizuoka University, nos SUM-CA0001 and SUM-CA0002). Both specimens retain reddish coloured compound eyes, along with other soft parts (figure 1a). One eye of one specimen (SUM-CA0001) was recovered by removing the surrounding amber matrix by levering (method of Mierzejewski 1976) and was coated in gold for study by scanning electron microscopy (SEM; JSM-6100). In order to examine internal structure, an eye from the second specimen (SUM-CA0002) was fixed in 2 per cent glutaldehyde with 2 per cent formaldehyde in 0.1 M sodium cacodylate buffer

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Figure 1. Dolichopodid fly eyes, Mid Eocene, Russia. (a,b,e) Specimen SUM-CA0001: (a) photomicrograph, right lateral view of specimen in amber; (b) SEM image of the surface of ommatidia showing the fly eye grating structure (fg). (c,d,f) specimen SUM-CA0002: (c) TEM image of a cross section of a single lens, showing the exocuticle (ex) and procuticle (pr); (d) TEM image of the exocuticle in figure (c), showing the eye grating structure (fg; Miller 1979). (e) SEM image of pigment screen. (f) TEM image of a cross section of the eye showing cuticular lenses (cl), primary pigment cells (ppc) and crystalline cone (cc).

(pH 7.4) and was fixed overnight in the solution. The specimen was subsequently post-fixed in 2 per cent osmium tetroxide for approximately 2.5 hours, dehydrated through an ethanol series and embedded in Spurr's resin and polymerized. Thin sections were obtained using an ultramicrotome and then stained with uranyl acetate and lead citrate and examined in a transmission electron microscope (TEM; Hitachi H-7100).

To elucidate the optical ability of the Eocene fly eye, we selected one TEM of a cross section of an ommatidium showing the widest diameter and symmetrical outer and inner outlines, thus representing the plane of, or a plane extremely close to, the optical axis. In the resultant model, we used the application of refractive index data for the cuticular lens (1.50) and crystalline cone (1.34) of a recent insect eye (Bernhard *et al.* 1963; Miller 1979).

3. MORPHOLOGY OF THE EYE

The compound eye (specimens SUM-CA 0001 and SUM-CA 0002, both left and right eyes) showed a distinctive red colour within and when extracted from the amber (figure 1a). The ommatidia are hexagonally packed in the anterior portion of the eye (figure 1b), and the average diameter of each corneal lens is 15 µm. In cross section, the cornea reveals prominent biconvex lenses with a five-layered exocuticle approximately 0.8 µm thick and a massive procuticle approximately 6.8 µm in thickness (figure 1c). The highest fidelity of detail is in the grating structure of the eye (Parker *et al.* 1998), which consists of a fingerprint-like pattern of ridges and sulci on each corneal lens surface (figure 1b). The ridges of the grating are approximately 80 nm high and

have a periodicity of 200 nm (figure 1d). Although irregular matrices fill the internal part of the eye, when the cuticular lenses and crystalline cones are removed, characteristic square mesh structures are apparent as the counterpart of the cones (figure 1e,f). Neighbouring cones are isolated from each other by primary pigment cells (figure 1e,f). The remains of primary pigment cells, filled with an electron-dense material and bound by an even more electron-dense membrane, enclose each fragmented crystalline cone (figure 1f). A rather irregular circular aperture (56 µm in diameter) opens at the base of the funnel-shaped pigment layer that consists of the neighbouring primary pigment cells, and is partly filled with photoreceptor fragments that extend deep into the holes (figure 1e). Observations using TEM indicate that the detailed morphology of the crystalline cone and the primary pigment cells is also preserved. Beneath each cone, there are four electron-dense nuclei of Semper cells surrounded by the pigment cells (figure 2a). The most conspicuous structure is the trapezoidal arrangement of photoreceptors, consisting of seven rhabdomeres (elliptical cross section 1–1.5 µm in diameter) that protrude towards the lumen in the ommatidium of each reticular cell (figure 2a,b). The reticular cells of each ommatidium are surrounded by six secondary pigment cells (figure 2a). Although none of the axons from the reticular cells are preserved, the components of the retina, such as the radially arranged reticular cells, their nuclei, trachea and secondary pigment cells, are completely preserved (figure 2d). The basement membrane, approximately 1.6 µm thick, is situated approximately 60 µm below the surface of the primary pigment cells (figure 2d).

Figure 2. Dolichopodid fly eye, Mid Eocene, Russia, specimen (SUM-CA0002). (a–d), TEM images of cross section of eye in orthogonal planes: (a) specimen showing crystalline cone (cc), primary pigment cells (ppc), electron-dense four-nuclei of Semper cells (nsc) and up to 10 clusters of seven rhabdomeres (rds); three rhabdomere clusters are enclosed by six secondary pigment cells (nspc, nuclei of secondary pigment cell); (b) a close-up of two clusters of rhabdomeres (rds) showing trapezoidal arrangement of seven rhabdomeres with neighbouring reticular cells that are clearly bounded by an electron-dense cell membrane; (c) a radial section showing the arrangement of ommatidia with cuticular lenses (cl), primary pigment cells (ppc) and crystalline cones (cc); (d) a radial section through ommatidia revealing the presence of basement membrane (bm), radially arranged long rhabdomeres (rds), trachea (tr), and secondary (spc) and primary (ppc) pigment cells.

To calculate the focal length of the system (cuticular lens+crystalline cone), the radii of curvature of both sides of the cuticular lens as shown in figure 1c were calculated based on the coordinate data of three homologous points on their outer surface, which were digitized along each surface profile on the computer image using a program written in VISUAL BASIC. The thickness of the cuticular lens was also measured on the image. As a result, we obtained the radii of curvatures of the outer lens surface (9.8 µm), the radii of curvatures of the inner lens surface (13.0 µm) and the thickness of the lens (8.2 µm). By using lens data, the refractive indices of the space in front of the cuticular lens (1.00), the cuticular lens (1.50) and the crystalline cone (1.34), together with the thick lens formula (e.g. see Stavenga 2003), we calculated that the focal distance of the lens is $f=16.7$ µm and that the distance between image focal point and the inner lens surface is 16.1 µm. The latter result indicates that diagenetical shrinkage of the fossil specimen (SUM-CA0002) has been negligible, because the distance between the vertex of the lens inner surface and the plane of the tip of the rhabdomere is approximately 13 µm.

4. THE MORPHOLOGICAL PROPERTY OF THE CORNEAL LENS

The dolichopodid fly had a typical dioptric apparatus, which consists of isolated ommatidia, short crystalline cones and trapezoidal arrangement of photoreceptors, as found in recent flies (Boschek 1971; Horridge *et al.* 1976).

In order to estimate the brightness of the captured image on a rhabdomere, an F -number was calculated: $F=f/D$, where f represents the focal length (16.1 µm) and D is the diameter of a lens (15 µm). The F -number of the fossil dolichopodid fly eye $F=1.1$ is slightly smaller than the $F=1.25 \pm 0.13$ derived for *Drosophila*, where the lens focal length is 20 ± 2 µm and the lens diameter is 16 µm (Stavenga 2003). The acceptance angle of a rhabdomere with diameter $D_r=1$ µm is $\Delta\rho_r=D_r/f=3.4^\circ$, slightly larger than the corresponding value for *Drosophila* ($2.9 \pm 0.3^\circ$, Stavenga 2003). The smaller lens of the fossil dolichopodid eye may be related to the presence of the grating in the front surface of the facet lens (Parker *et al.* 1998), which seems to be developed in strongly curved small corneal lenses (Meyer-Rochow & Stringer 1971). To conclude, the unique structure (the small diameter of corneal lens in combination with the fly eye grating) of the fossil fly eye

probably represents a compromise between function and structural/genetic constraints.

5. EVOLUTIONARY SIGNIFICANCE OF THE FOSSIL FLY EYE

Recent anatomical studies have shown that most insects possess a fused rhabdom, a structure in which the rhabdomeres of the usually nine photoreceptor cells of each ommatidium form a single optical waveguide. However, some diurnal extant flies, earwigs and water bugs have an open rhabdom with seven separate small rhabdomeres in one ommatidium (Osorio 2007). The fly rhabdom consists of eight rhabdomeres with six long, peripheral rhabdomeres (R1–6) and two short, central rhabdomeres (R7 and R8); the latter are positioned in tandem, thus creating one functional optical waveguide (figure 2a,b). The present-day flies have evolved for the last 200 Ma (Grimaldi & Engel 2005, p. 514). However, conclusions drawn from previous fossil eye studies are usually equivocal, because the specimens lack soft tissue that includes photoreceptors. The findings reported in the present paper indicate that dolichopodid flies, with a typical open rhabdom, have existed for at least 45 Ma. This is the first discovery, to our knowledge, of an open rhabdom in a fossil insect eye. The morphology and structural arrangement found therein are comparable with those found in the present-day 'long-legged flies' (Trujillo-Cenóz & Bernard 1972).

Modern Diptera (flies and mosquitoes) typically possess a so-called 'neural superposition eye' visual system, where the axons of the rhabdomeres from six neighbouring ommatidia converges onto a single cartridge in the lamina, the optical ganglion proximal to the retina, so that seven ommatidia simultaneously capture light from the same region in space, without loss of resolution (Kirschfeld 1967; Land & Nilsson 2002). The Eocene fly eye was not sufficiently preserved to provide any information on the neural wiring. However, it possesses long rhabdomeres (figure 2d), identical to those found in extant neural superposition eyes. In addition, this Eocene fly eye has a trapezoidal arrangement of the rhabdomeres (figure 2a,b), found only in the advanced brachyceran flies (Nilsson & Ro 1994). Zeil (1979, 1983) discovered symmetrically arranged open rhabdomeres in the nematoceran fly (Bibionidae). The nematoceran fly is considered to be more ancient than brachyceran (dolichopodid) fly, suggesting that the trapezoidal arrangement of rhabdomeres found in the dolichopodid fly is an apomorphic character.

The number and arrangement of each cell in an ommatidium of the fossil fly eye strictly corresponds to that of the present-day fly (Stavenga 1975). Many recent studies of eye evolution use present-day species to predict occurrences in geological time, but these must assume uniformity. The evidence demonstrated herein based on the Baltic amber fossil fly indicates that the cellular structure of the fly eye *did* exist 45 Ma, and supports previous claims on the conservation of the cellular organization of the eye (Meinertzhagen 1991; Osorio & Bacon 1994). Moreover, this study extends prior studies on cellular preservation in amber, by revealing a fidelity of delicate ultrastructural arrays in sensory cells that allow reconstruction of their function.

Phylogenetic studies of living species suggest that the open rhabdom has evolved independently in at least four insect groups (Osorio 2007), and the separation of the rhabdomeres is known to be affected by three genes mediated by a protein called *Spacemaker* (*Spam*) in *Drosophila* (Zelhof *et al.* 2006). It therefore follows that the *Spam* gene must have been present for at least 45 Ma.

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REFERENCES

- Bernhard, G. D., Miller, W. H. & Möller, A. R. 1963 Function of the corneal nipples in the compound eyes of insects. *Acta Physiol. Scand.* **58**, 381–382.
- Boschek, C. B. 1971 On the fine structure of the peripheral retina and lamina ganglionaris of the fly, *Musca domestica*. *Z. Zellforsch.* **118**, 369–409. (doi:10.1007/BF00331193)
- Clarkson, E. N. K. & Levi-Setti, R. 1975 Trilobite eyes and the optics of Des Cartes and Huygens. *Nature* **254**, 663–667. (doi:10.1038/254663a0)
- Clarkson, E. N. K., Levi-Setti, R. & Horváth, G. 2006 The eyes of trilobites: the oldest preserved visual system. *Arthropod. Struct. Dev.* **35**, 247–259. (doi:10.1016/j.asd.2006.08.002)
- Duncan, I. J., Briggs, D. E. G. & Archer, M. 1998 Three dimensionally mineralized insects and millipedes from the Tertiary of Riversleigh, Queensland, Australia. *Palaeontology* **41**, 835–851.
- Exner, S. 1891 *Die Physiologie der facettierten Augen von Krebsen und Insecten*. [English transl. by Hardie R. C. 1988. *The physiology of the compound eyes of insects and crustaceans*]. New York, NY: Springer.
- Fröhlich, F., Mayrat, A., Riou, B. & Secretan, S. 1992 Structures rétinienues phosphatisées dans l'œil géant de *Dollocaris*, un crustacé fossile. *Ann. Paléontol.* **78**, 193–204.
- Gál, J., Horváth, G., Clarkson, E. N. K. & Haiman, O. 2000 Image formation by bifocal lenses in a trilobite eye? *Vision Res.* **40**, 843–853. (doi:10.1016/S0042-6989(99)00216-3)
- Grimaldi, D. & Engel, M. S. 2005 *Evolution of the insects*. New York, NY: Cambridge University Press.
- Grimaldi, D., Bonwich, E., Delannoy, M. & Doberstein, S. 1994 Electron microscopic studies of mummified tissues in amber fossils. *Am. Mus. Novit.* **3097**, 1–31.
- Horridge, G. A., Mimura, K. & Hardie, R. C. 1976 Fly photoreceptors. III. Angular sensitivity as a function of wavelength and the limit of resolution. *Proc. R. Soc. Lond. B* **194**, 151–177. (doi:10.1098/rspb.1976.0071)
- Keyser, D. & Weitschat, W. 2005 First record of ostracods (Crustacea) in Baltic amber. *Hydrobiologia* **538**, 107–114. (doi:10.1007/s10750-004-5941-5)
- Kirschfeld, K. 1967 Die Projektion der optischen Umwelt auf das Raster der Rhabdomere im Komplexauge von *Musca*. *Exp. Brain Res.* **3**, 248–270. (doi:10.1007/BF00235588)
- Kohring, R. 1998 REM-Untersuchungen an harzkonserverten Arthropoden. *Entomol. Gen.* **23**, 95–106.
- Koller, B., Schmitt, J. M. & Tischendorf, G. 2005 Cellular fine structures and histochemical reactions in the tissue of a cypress twig preserved in Baltic amber. *Proc. R. Soc. B* **265**, 121–126. (doi:10.1098/rspb.2004.2939)
- Land, M. F. & Nilsson, D.-E. 2002 *Animal eyes*. Oxford, UK: Oxford University Press.

- Meinertzhagen, I. A. 1991 Evolution of the cellular organization of the arthropod compound eye and optic lobe. In *Vision and visual dysfunction*, vol. 2 *Evolution of eye and visual system* (eds J. R. Cronly-Dillon & R. L. Gregory), pp. 341–363. London, UK: Macmillan Press.
- Meyer-Rochow, V. B. & Stringer, I. A. N. 1971 A system of regular ridges instead of nipples on a compound eye that has to operate near the diffraction limit. *Vision Res.* **33**, 2645–2647. (doi:10.1016/0042-6989(93)90223-J)
- Mierzejewski, P. 1976 On application of scanning electron microscope to the study of organic inclusions from the Baltic amber. *Ann. Soc. Géol. Pologne* **46**, 291–295.
- Miller, W. H. 1979 Ocular optical filtering. In *Handbook of sensory physiology*, vol. VII/6A (ed. H. Autrum), pp. 69–143. Berlin, Germany: Springer.
- Nilsson, D.-E. & Ro, A.-I. 1994 Did neural pooling for night vision lead to the evolution of neural superposition eyes? *J. Comp. Physiol. A* **175**, 289–302. (doi:10.1007/BF00192988)
- Osorio, D. 2007 Spam and the evolution of the fly's eye. *BioEssays* **29**, 111–115. (doi:10.1002/bies.20533)
- Osorio, D. & Bacon, J. P. 1994 A good eye for arthropod evolution. *BioEssays* **16**, 419–424. (doi:10.1002/bies.950160610)
- Parker, A. R., Hegedus, Z. & Watts, R. A. 1998 Solar-absorber anti-reflector on the eye of an Eocene fly (45 Ma). *Proc. R. Soc. B* **265**, 811–815. (doi:10.1098/rspb.1998.0364)
- Poinar Jr, G. O. & Hess, R. 1982 Ultrastructure of 40-million-year-old insect tissue. *Science* **215**, 1241–1242. (doi:10.1126/science.215.4537.1241)
- Poinar, H. N., Melzer, R. R. & Poinar Jr, G. O. 1996 Ultrastructure of 30–40 million year old leaflets from Dominican amber (*Hymenacea protera* Fabaceae: Angiospermae). *Experientia* **52**, 387–390. (doi:10.1007/BF01919546)
- Stankiewicz, B. A., Poinar, H. N., Briggs, D. E. G., Evershed, R. P. & Poinar Jr, G. O. 1998 Chemical preservation of plants and insects in natural resins. *Proc. R. Soc. B* **265**, 641–647. (doi:10.1098/rspb.1998.0342)
- Stavenga, D. G. 1975 Optical qualities of the fly eye: an approach from the side of geometrical, physical and waveguide optics. In *Photoreceptor optics* (eds A. W. Snyder & R. Menzel), pp. 126–144. New York, NY: Springer.
- Stavenga, D. G. 2003 Angular and spectral sensitivity of fly photoreceptors. II. Dependence on facet lens *F*-number and rhabdomere type in *Drosophila*. *J. Comp. Physiol. A* **189**, 189–222. (doi:10.1007/s00359-003-0390-6)
- Trujillo-Cenóz, O. & Bernard, G. D. 1972 Some aspects of the retinal organization of *Sympycnus lineatus* Loew (Diptera Dolichopodidae). *J. Ultrastruct. Res.* **38**, 149–160. (doi:10.1016/S0022-5320(72)90089-5)
- Weitschat, W. & Wichard, W. 1998 *Atlas der Pflanzen und Tiere im Baltischen Bernstein*. Munchen, Germany: Pfeil.
- Zeil, J. 1979 A new kind of neural superposition eye: the compound eye of male Bibionidae. *Nature* **278**, 249–250. (doi:10.1038/278249a0)
- Zeil, J. 1983 Sexual dimorphism in the visual system of flies: the free flight behaviour of male bibionidae (Diptera). *J. Comp. Physiol. A* **150**, 379–393. (doi:10.1007/BF00605028)
- Zelhof, A. C., Hardy, R. W., Becker, A. & Zuker, C. S. 2006 Transforming the architecture of compound eyes. *Nature* **443**, 696–699. (doi:10.1038/nature05128)