Mosquito eye design: conical rhabdoms are matched to wide aperture lenses

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SUMMARY

Night-active mosquitoes have photopigment-containing rhabdoms that take the form of a wide hollow cone, with the distal apex at the focus of the ommatidial lens. It is shown geometrically, and with the aid of a gelatin model, that such a structure can trap light over an angle of up to 60° , roughly equal to the width of the cone of light provided by the lens. This gives a gain in light-gathering power of up to nine times that of the more usual cylinder-shaped rhabdom with an acceptance angle of less than 25° .

1. INTRODUCTION

Most photoreceptors in both vertebrates and invertebrates have a photopigment-containing region that behaves as a cylindrical light-guide (Menzel & Snyder 1975). The angle over which such a cylinder accepts light is limited by the critical angle for total internal reflection, and hence the refractive index difference between the receptor and surrounding cytoplasm. Assuming geometrical optics, this angle is $2(n_{\text{in}}/n_{\text{out}})(90 - \theta_{\text{crit}})$, where θ_{crit} is the critical angle given by $\sin^{-1}(n_{\text{out}}/n_{\text{in}})$; see figure 1. For vertebrate rods and cones, with refractive indices in the range 1.39–1.41 (Sidman 1957) this acceptance angle is $32\text{--}38^\circ$, but for insect rhabdoms with lower refractive indices (1.36–1.37; Nilsson & Howard 1989) the acceptance angles are correspondingly narrower, between 20 and 25° . This means that for an insect with an apposition-type eye, nothing is gained by having a wide aperture lens that supplies the rhabdoms with a cone of light wider than 25° . This corresponds to an f number of about 2.3 for the corneal lens, a value which is close to that found in diurnal Dipteran flies (Stavenga *et al.* 1990). The eyes of nocturnal mosquitoes, however, have lenses with an almost hemispheric profile (figure 2) and these are capable of supplying a cone of light at least 60° wide (f number 0.9). This cannot be contained by a cylindrical rhabdom, and mosquito rhabdoms are indeed not cylindrical but take the form of a hollow cone with its apex at the focus of the lens. Conical rhabdoms have been described before in mosquitoes, (e.g. Mazokhin-Porshnyakov & Kazyakina 1978) but the significance of their shape was not previously realized. Here we show geometrically, and with the aid of a physical model, that a conical structure allows light from the outer zones of the lens to be trapped, increasing the light-gathering power by between four and nine times that of a simple cylinder (figures 3 and

Figure 1. Relationship between the acceptance angle of a receptor and the critical angle (θ_{crit}). n_{out} and n_{in} are refractive indices.

4). As a means of increasing sensitivity, this arrangement can be thought of as an alternative to the neural superposition system of advanced flies (Kirschfeld 1967; Nilsson & Ro 1995), and we discuss it in relation to the evolution of rhabdom function in the eyes of Diptera.

2. MOSQUITO EYE ANATOMY

Anopheles gambiae s.l. Giles is an important vector of malaria and other tropical diseases in sub-Saharan Africa (Gillies 1988; White 1974) and is active at night (Clements 1963). These mosquitoes show a behavioural optomotor response down to light levels between moonlight and starlight (Gibson 1995).

Figure 2. (a) Radial and (b) transverse sections through the lateral retina of a male *A. gambiae*, showing the hemispherical cornea and the conical structure of the rhabdoms. Scale bar 10 μm .

Like most other blood-feeding mosquitoes (Brammer 1970; Mazhokhin-Porshnyakov & Kazyakina 1978; Satō 1953, 1957) the eyes are small (0.5 mm) but have relatively large lenses (24–32 μm diameter), and the ommatidia have rhabdoms with a complex structure.

To examine ommatidial structure, 1 μm thick resin sections were made. The eyes were treated as for electron microscopy. Whole or part-dissected heads were fixed under partial vacuum using 3% buffered glutaraldehyde containing a trace of detergent. They were post-fixed in 1% OsO_4 , embedded in TAAB resin, sectioned and stained with 1% methylene blue. In radial sections of light-adapted eyes (figure 2a) the rhabdoms appear as inverted 'V's, with an extra element in some cases. Transverse sections (figure 2b) show that these structures are actually hollow cones, composed of six fused outer rhabdomeres and a central rhabdomere, which is presumably a vertical pair as in other dipterans (Trujillo-Cenóz & Melamed 1966; Hardie 1984) and is attached to one of the outer six for most of its length. In sections that are not exactly aligned with the ommatidial axis the rhabdom profiles provide a wide and confusing variety of conic sections, and it is possible that the 'acorn shape' of the rhabdoms described by Satō (1957) in *Culex pipiens* var *pallens* reflects this. In our sections of *A. gambiae* the rhabdoms widen continuously from distal to proximal, except perhaps for the last 1 or 2 μm (figure 2a), and are more accurately described by the word 'cone'. Although there may be differences between genera and species, the description of *Culex pipiens* var *molestus* rhabdoms by Mazhokhin-Porshnyakov & Kazyakina (1978) clearly

implies a conical structure, and it fits figure 2 perfectly. 'In sections across the middle part of the ommatidium is found a well marked rhabdom of seven rhabdomeres, of which six lie in the form of a circle around the asymmetrical seventh rhabdomere. They touch each other close to the crystalline cone, but are wide apart at the opposite (proximal) end.'

It is likely that the rhabdoms of culicine and anopheline mosquitoes have a similar design.

3. OMMATIDIAL OPTICS

In an ommatidium from *A. gambiae* with a 28 μm diameter lens, each rhabdom is about 5 μm wide at its blunt apex, 15 μm across the base, 20 μm from base to apex, and with cone walls 3–4 μm thick. These dimensions are large enough for geometrical optics to provide an adequate description without recourse to waveguide theory (Snyder 1979). The angle of the rhabdom cone, measured on sections from the base to the centre of the distal tip, is 39.1° (s.d. 4.4° , $n = 20$). The lenses are unusual in being almost hemispherical, and each supplies a cone of light about 60° wide, assuming that the focus is near the rhabdom tip. Paraxial optics suggest a focal point much deeper than this, assuming a lens with a homogeneous refractive index of 1.45 (Stavenga *et al.* 1990), but spherical aberration of the outer rays brings them to a focus much closer to the lens, giving a circle of least confusion about 4 μm wide, coincident with the rhabdom tip. The calculated focal length of the lens is 27.2 μm , but this reduces to about 17 μm when marginal rays are included. The latter figure implies that the rhabdom tip images an angle in space of 16.7° , roughly twice the interommatidial angle, which ranges from 6–10°. In dark-adapted eyes (fixed at 8 pm, 2 h after light off; see Satō *et al.* 1957) the rhabdom becomes shorter and fatter, and the aperture around the rhabdom tip widens to 12 μm , which enlarges the rhabdom's field of view to about 40° , increasing sensitivity almost six-fold at the expense of resolution.

4. AN OPTICAL MODEL OF THE RHABDOM

Optically realistic rhabdom models were made by casting gelatin in machined plastic (Perpex) moulds. The gelatin solution (23.5% w/w) solidifies to give a stiff jelly with a refractive index of approximately 1.37, close to that of a real rhabdom. A cylinder and a 30° hollow cone were produced, each 7 cm high, with walls 1 cm thick (figure 3). These were immersed in a transparent tank of water containing a small amount of eosin to make ray paths visible. A wide-aperture plano-convex (aspheric) lens was fixed with its convex surface in air, and its flat surface just below the water surface, imitating the situation in an ommatidium. The lens was illuminated from above with a parallel light beam, which produced a focus 35 mm below the lens, on the upper end of the model rhabdom. The lens provided a cone of light slightly more

Figure 3. Optical behaviour of lens-rhabdom systems, using gelatin rhabdom models. Outer surface of the glass lens is in air, with the inner surface and 'rhabdom' in water. Upper figures show side view and lower figures the light emerging from the base of the 'rhabdoms' onto a paper screen: (a) Cylinder with 50° beam; (b) 30° cone with 50° beam; (c) cylinder with beam reduced to 20°. Most of the light is lost from (a), but captured in (b) and (c).

than 60° wide. A piece of thin paper under the tank acted as a screen which displayed the light distribution at the base of the rhabdom model, and this was viewed from below, via a 45° mirror (figure 3). Figure 3a shows that a cylindrical rhabdom traps a proportion of the incident light, but spills most of it. The conical rhabdom (b) captures nearly all the light, with very little spillage either inside or outside the walls of the cone. In (c) the incident cone of light has been stopped down to 20°, and it is now evident that the cylindrical receptor captures all of it.

To contain a 60° cone of light a cylindrical structure would require a refractive index of 1.55, whereas the best estimates for insect rhabdoms give a value of 1.363 ± 0.005 (Nilsson & Howard 1989). When the cytoplasmic refractive index is 1.34, this gives a relative refractive index between inside and outside of 1.017, and provides an acceptance cone of 21°. As expected, such a rhabdom loses most of the light supplied by a 60° beam, but it retains a 20° beam (figures 3a, c and 4a). Figure 4 shows two solutions to the problem of light spillage. In figure 4b the receptor is a 21° cone. Seen in two-dimensional cross section, each vertical 'half' accepts light from the axis up to 21° from it, which gives a total acceptance angle of 42°, and for the whole three-dimensional rhabdom a four-fold increase in light capture compared with the cylinder

Figure 4. Diagrams showing the widest incident cone of light accepted by various structures with a refractive index of 1.017 relative to the surroundings: (a) a cylinder; (b) a hollow cone of angle 21°; (c) a hollow cone of angle 42° with a central cylinder. In (b) and (c) only part of the beam is emphasized for clarity.

(calculated from the relative areas of the cross section of the incident beams). This is essentially what is demonstrated in figure 3b. In figure 4c the cone is made 42° wide, with each 'half' accepting rays between 10.5° and 31.5° from the axis, and a central cylindrical element accepting the remainder. The total acceptance angle is 63°, giving nine times the light capture of the cylinder. This cone-plus-cylinder model corresponds very closely with the real rhabdoms, which also have cone angles close to 40°. The only small discrepancy is the asymmetric position of the central rhabdomere, which could cause some light loss. At worst, this would only be one-ninth of the total.

5. DISCUSSION

Receptors overcome the problem of light trapping in a variety of ways (Warrant & McIntyre 1991), but the conical solution has not been proposed before. Others include barrel-shaped receptors in decapod crustaceans, which retain light by total internal reflection (Bryceson & McIntyre 1983), and reflecting sheaths behind and around the rhabdom (Horridge *et al.* 1977). Whether arthropods other than mosquitoes employ the conical rhabdom strategy is not at present clear. Grenacher (1879) described cone-like rhabdoms in several insects including earwigs and tipulid flies. However, in the tipulids—Nematoceran relatives of the mosquitoes—it seems that the reasons for the complex rhabdom structure are not the same as in mosquitoes. The anatomy is somewhat different, because the rhabdomeres are not joined at the distal tip as in *A. gambiae*, and this permits the six outer and two inner rhabdomeres

to function independently (Nilsson & Ro 1995). In the light the pigmented iris beneath the lens closes so that only the central pair are illuminated, but in the dark the iris opens exposing all eight rhabdomeres to light. This increases sensitivity, but also greatly increases the acceptance angle of the ommatidium, thus reducing the eye's resolution, much as in mosquitoes. The principal difference is that in the mosquito eye the whole rhabdom is available for light capture, even in the light adapted state. This may be an advantage for an animal that is only active in light conditions that vary between dim and dark.

The advanced flies, Brachycera and Cyclorrhapha, all have an ommatidial optical arrangement known as 'neural superposition', in which the rhabdomeres are separated, and each rhabdomere in one ommatidium shares a field of view with one from a neighbouring ommatidium (Kirschfeld 1967; Hardie 1984). Axons from the receptors with the same field of view meet together in the first synaptic region (the lamina), resulting in a pooled photon signal that is six or seven times larger than that in any one receptor, but without compromise to the eye's resolution. Nilsson & Ro (1995) argue persuasively that the tipulid type of eye—where resolution is compromised by dark adaptation—could evolve into the advanced type with a suitable reorganization of the neural wiring between the retina and lamina. This is made more plausible by the fact that in the Bibionid flies (which are also Nematocerans) an intermediate type of neural organization does indeed exist (Zeil 1983; Melzer *et al.* 1997). Where might the mosquito eye fit into this evolutionary picture? One possibility is that it represents an even earlier stage than the tipulid eye. It is generally agreed that the ancestral compound eye had fused rhabdoms (Nilsson 1989) in which the individual rhabdomeric contributions join to form a single light-guiding rod; this is the situation in most insect and crustacean apposition eyes. In tipulids and all advanced flies, however, the rhabdom is of the 'open' type with separated rhabdomeres. The conical rhabdoms described here are joined distally but partially separated proximally, and it could be argued that this is an intermediate stage between ancestral (fused) and advanced (open) types. However, mosquitoes are not generally regarded as the most primitive of Nematocerans (Wood & Borkent 1989), and furthermore there are diurnal plant-feeding mosquitoes (e.g. the tribe Megarhinini = Toxorhynchitini) that have open rhabdoms with narrow rhabdomeres, not unlike those of advanced flies (Satô 1961). It thus seems more likely that the conical rhabdoms of nocturnal mosquitoes are a special adaptation to the need to capture as much light as possible in dim conditions, rather than a retained primitive condition.

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