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Fundamental differences in the optical structure of the eyes of nocturnal and diurnal mosquitoes

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Abstract We have studied the anatomy and optics of the eyes of a range of mosquito species from the wholly dark-active blood-feeding *Anopheles gambiae* to the diurnal plant-feeder *Toxorhynchites brevipalpis*. Consistent with studies by Satô in the 1950s, we find that dark-active and crepuscular species have short fused rhabdoms with a conical construction. This maximises the amount of light the rhabdoms receive from the almost hemispherical wide-aperture lenses. *Toxorhynchites*, on the other hand, has long narrow rhabdomeres that are separated from each other over their entire length, and so resemble the open rhabdoms of advanced flies (Brachycera and Cyclorrhapha). These findings are confirmed by studies of the pseudopupil, whose form indicates the layout of the rhabdomere tips in the focal plane of each ommatidial lens. In anopheline species the pseudopupil is a single undivided ellipse, indicating a fused rhabdom structure, whereas in *Toxorhynchites* there is a ring of six outer elements surrounding a central one. This means that each rhabdomere views a separate direction in space, and our measurements indicate that, as in higher Diptera, adjacent rhabdomeres share their fields of view with one of the rhabdomeres in the immediately adjacent ommatidia. This in turn means that in the diurnal type of mosquito eye there is a basis for neural superposition, but the fused construction of anopheline rhabdoms precludes this. The *Aedes* species studied were similar to *Anopheles* but with lenses of less extreme aperture, and *Sabethes cyaneus*, a diurnal blood-feeder, was intermediate in structure, with fused conical rhabdoms in the centre of the eye and unfused rhabdomeres around the periphery.

Key words Mosquito · Eye · Vision · Neural superposition · Optics

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Introduction

The life-styles of mosquitoes cover a very wide range of feeding habits and light environments, from wholly nocturnal blood-feeding species such as *Anopheles gambiae*, the scourge of sub-saharan Africa, to the large but innocuous diurnal nectar feeder *Toxorhynchites brevipalpis*, from Australia and New Guinea. The average light intensities experienced by these insects during their periods of activity differ by a factor of at least 10^6, the difference between sunlight and moonlight, and it is reasonable to suppose that the structure of the eyes should reflect this difference. In a recent study (Land et al. 1997) we found that *A. gambiae* has almost hemispherical facet lenses, providing a cone of light to the rhabdom about 60° wide, and consequently a very bright image. A simple cylindrical rhabdom can only contain, by total internal reflection, a beam 20–25° wide, and the rhabdoms of *A. gambiae* have an unusual conical shape that allows them to accept the much wider cone provided by the lens. The rhabdoms themselves are short but thick, and both the ommatidial acceptance angle (~17° light adapted, ~40° dark adapted) and the inter-ommatidial angle (~8°) are large by comparison with other dipterans. All this is consistent with an apposition eye that is adapted for high sensitivity in dim environments, where high photon capture takes precedence over fine resolution (Kirschfeld 1974; Land 1981, 1989).

Whilst studying *A. gambiae* (Land et al. 1997) we made an exploratory examination of *T. brevipalpis* at the other end of the range of light environments and found it to be very different. The resolution was much better, with inter-ommatidial angles of about 3°. The lenses were of small diameter relative to their focal length, providing narrower cones of light, less than 30° wide. Of particular interest was the fact that the rhabdoms were of the open type, consisting of the typical dipteran six outer and two central rhabdomeres; these were long, narrow and cylindrical. Open rhabdom eyes in higher Diptera are associated with neural superposition, a system that provides a high effective photon capture rate without compromising resolution, by pooling in the lamina inputs from receptors in neighbouring ommatidia that share the same field of view (Kirschfeld 1967; Hardie 1984). It thus appears that within the mosquitoes there is an interesting range from classical apposition eyes to neural superposition eyes, and that this may give useful insights into the origins of the neural superposition mechanism itself.

Other nematoceran flies so far studied all have the open rhabdom arrangement, but it seems that it is put to different uses in different groups (tipulids: Ro and Nilsson 1994; chaoborid midges: Melzer et al. 1997; bibionids: Zeil 1983). In tipulids the arrangement is used to provide extra dynamic range, with the outer six rhabdomeres occluded during the day by an iris (Ro and Nilsson 1994), an arrangement also found in earwigs, some waterbugs and beetles (Nilsson and Ro 1994). In chaoborids and bibionids the arrangement is more like neural superposition in the higher dipterans, except that it is not rhabdomeres from the nearest neighbouring ommatidia that contribute to the image in the lamina, but oblique next-but-one neighbours in the enlarged dorsal eyes of male bibionids (Zeil 1983), and both nearest neighbours *and* next-but-one neighbours in chaoborids (Melzer et al. 1997). This is also similar to the neural configuration in the eyes of female bibionids (*Dilophus*, Zeil 1983), although there the rhabdomere pattern is closest to a nearest-neighbours arrangement. In this study we found that in *T. brevipalpis* the rhabdomere spacing fitted a nearest-neighbours arrangement very closely, making it very similar to the pattern in the advanced flies (Cyclorrhapha and Brachycera). This paper provides the evidence for this, based on anatomical and optical measurements.

In the course of this study we encountered the remarkable series of papers on mosquito eyes by Shôzô Satô. These were written in the 1950's and early 1960's, and published in a rather inaccessible source (Science Reports of Tohoku University). They describe with great precision the anatomy of the eyes of a variety of mosquitoes from all light environments, as well as accounts of dark and light adaptation. Satô did not comment on either the optics of the eyes, nor the rhabdom structure – this was a decade before the neural superposition system was elucidated – but he did describe and accurately illustrate both the fused rhabdoms of the dark-living species and the open rhabdoms of diurnal species. In this paper we have examined a further six species, in addition to the six studied by Satô, to try to establish the full range of ommatidial morphologies across the whole Culicidae family. The only other account of mosquito optics so far, apart from our own study (Land et al. 1997) is that of Muir et al. (1992), and this is limited to a discussion of acuity and sensitivity in *Aedes aegypti*. Here we provide an analysis of the optics of the eyes of *A. gambiae* and *T. brevipalpis* using both anatomical data and studies of the pseudopupil.

Materials and methods

A. gambiae and *A. stephensi*, and *T. brevipalpis* were all obtained from cultures produced by the London School of Hygiene and Tropical Medicine. *Sabethes cyaneus* came from cultures in Ohio State University USA, and *Aedes detritus* and *punctor*, and *Culiseta litorea* were collected from sites in the UK. They were kept in a moist cage with a normal dark/light cycle for a maximum of 3 days before use.

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. At least three series of sections were made for histological measurements on each species.

Pseudopupils were photographed at different depths under a variety of lighting conditions. Normal-eyed mosquitoes have very dark eyes, with no visible pseudopupil, and antidromic illumination (i.e. illumination from below or behind the head) was used to produce a luminous pseudopupil in which the light travels up the rhabdoms or rhabdomeres and is emitted from their distal tips (e.g. Franceschini 1975). It was not easy to obtain good images, but when the pseudopupils could be photographed they provided consistent measurements (Fig. 7a, c). A strain of *Anopheles stephensi* with reduced pigmentation was available to us, and this gave an easily visible pseudopupil when lit from the side (Fig. 7b).

Results

The eye as a whole

Mosquito eyes of all types have been described by Satô in his many publications, and whilst there is little need for further description it will be helpful to give a brief synopsis of his main conclusions. Figure 1 shows three of Satô's illustrations of the eyes of *Aedes japonicus* (Satô 1953a), a species not strongly adapted to extreme light or dark conditions. Figure 1a shows male and female eyes. With the exception of diurnal mosquitoes of the genus *Toxorhynchites* the females have slightly larger eyes in all genera studied. In *A. japonicus* the average head widths of males and females are 0.75 and 0.85 mm, respectively, and the corresponding facet numbers 419 and 516. There are also modest differences in facet diameter (males 19.0–26.7 µm, females 19.5–28.8 µm). The male antennae are larger than the female, which is typical for mosquitoes.

Figure 1b shows that facet size is not uniform across the eye, the facets being largest in the antero-ventral region, and smallest in the posterior and dorsal regions. This is an unusual distribution amongst insects, where

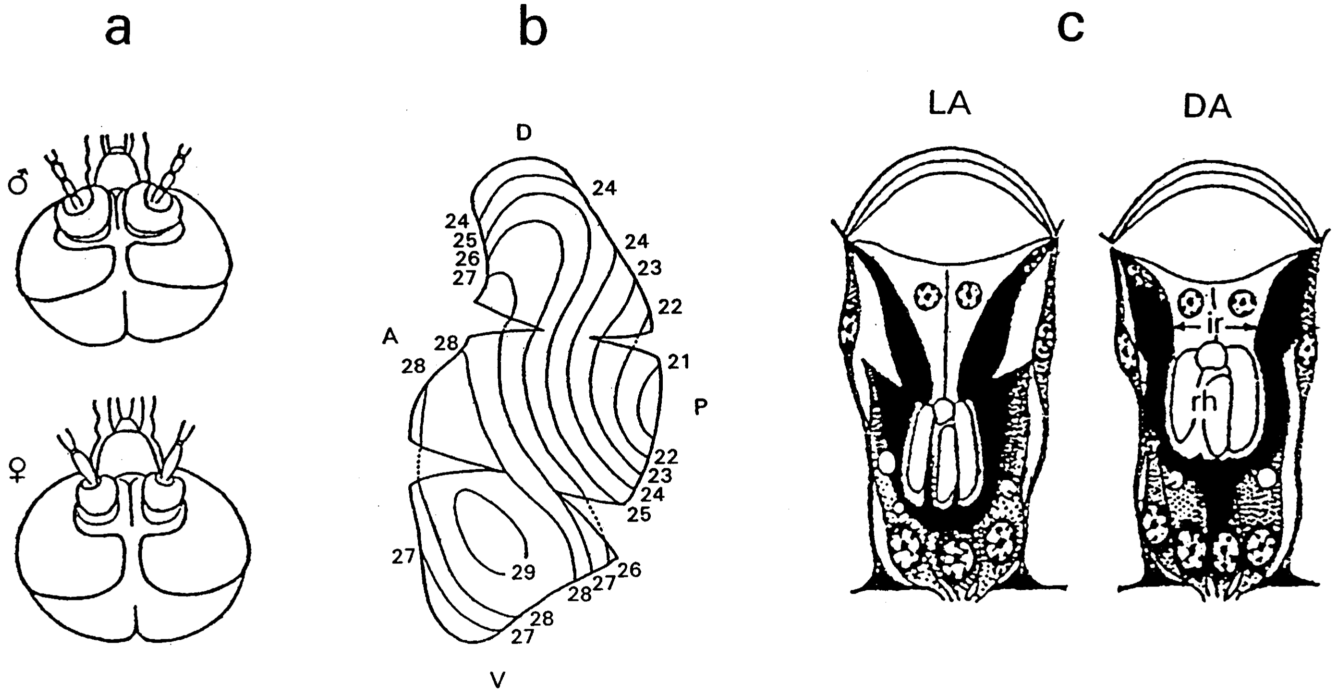

Fig. 1a–c *Aedes japonicus*. Three figures from Satô (1953a) showing the principle characteristics of the mosquito eye. **a** Female eyes are usually larger than those of males. Head widths about 0.75 mm in male and 0.85 mm in female. **b** Non-uniform distribution of facet sizes across the eye (left eye of female, numbers give diameters in μm); the largest facets point downwards. **c** In dark adaptation the iris (*ir*) and the rhabdom (*rh*) widen and the rhabdom moves towards the lens

the largest facets are nearly always in the antero-dorsal quadrant (Land 1989, 1997). Large ventral facets are typical of other mosquitoes, however, with the exceptions of the diurnal blood-feeder *Armigeres subalatus* (Satô 1960) and diurnal plant-feeder *Megarhinus* (= *Toxorhynchites*) *towadensis* (Satô 1961) where the distribution is more uniform.

Figure 1c illustrates a single ommatidium of *A. japonicus* in light and dark-adapted conditions. The ommatidium consists of a corneal lens which focuses an image via the aqueous crystalline cone onto the region of the distal rhabdom tip. As in other Diptera, the rhabdom is made up of contributions from eight receptors, six of which form a peripheral ring, and a central in-line pair which is usually somewhat eccentric with respect to the ring. Black pigment surrounds the crystalline cone and rhabdom, and forms an iris ring just above the distal rhabdom tip. Unlike the situation in tipulids (Williams 1980; Nilsson and Ro 1994), the iris does not obscure the outer receptors, even in the light-adapted condition (Land et al. 1997). The main differences between light- and dark-adapted ommatidia concern the location of the rhabdom and the diameter of the iris. In the light the rhabdom lies relatively deep, and the iris is narrow, typically <5 μm. In the dark the rhabdom moves much closer to the lens, and the iris opens to a diameter of >10 μm. These adaptations increase the light flux through the rhabdom mainly by increasing its acceptance angle – the region of external space over which it collects light. There are also minor changes to the dimensions and shape of the rhabdom. In day-flying mosquitoes such as *Toxorhynchites* spp. the adaptational changes are very slight; for example in *T. towadensis* the iris only expands from 6 to 9 μm, and there is no change in the radial position of the distal end of the rhabdom (Satô 1961), but in all dim-light mosquitoes the changes are similar to those illustrated here for *A. japonicus*, or even greater.

Differences in ommatidial structure

We examined the eyes of six mosquito species ranging from the nocturnal blood feeder *A. gambiae* to the diurnal plant feeder *T. brevipalpis*. Drawings derived from the mean dimensions of ten ommatidia from each species are shown in Fig. 2. Ommatidial dimensions are given in Table 1, together with Satô's measurements which cover a comparable range of species. It is immediately clear from Fig. 2 that, although all ommatidia contain the same components, there are major differences in the proportions of the different parts. These differences appear to be related to the light conditions under which the mosquitoes are active, with the most nocturnal species on the left and most diurnal on the right. In the sections that follow we have concentrated on the two species (*A. gambiae* and *T. brevipalpis*) that represent the extremes of light regime. All other species are, in various ways, intermediate between these two.

Table 1 Anatomical dimensions of mosquito ommatidia (light adapted females: mean values only). Numbers in square brackets refer to individual rhabdomeres. Crepusc. = crepuscular

Species (all light-adapted females)	Head width (mm)	Facet number	Facet diameter (µm)	Cornea to rhabd tip (µm)	Rhabdom max diam (µm)	Rhabdom length (µm)	Rhabdom iris diam (µm)	Light regime
Aedes detritus	0.94		23.0	30.3	8.6	29.2	7.7	Crepusc.
Aedes japonicus (Satô 1953a)	0.85	516	26.0	30.8	9.2	13.8	2.8	Mixed
Aedes punctor	0.93		25.0	30.6	6.9	18.5	5.4	Crepusc.
Anopheles gambiae	0.61		28.0	29.4	15.4	22	6.0	Nocturnal
Anopheles sinenis (Satô 1953b)	0.77	622	20.3	26.0	8.0	15	2.5	Crepusc.
Armigeres subalbatus (Satô 1960)	0.92	907	20.0	26.5	7.0	20.3	4.5	Diurnal
Culex pipiens (Satô 1957)	0.84	532	23.1	28.5	8.5	14.3	2.3	Nocturnal
Culex (*Lutzia*) *vorax* (Satô 1959)	1.11	629	32.4	37.0	12.5	18.8	4.8	Nocturnal
Culiseta litorea	0.97		30.6	34.2	13.8	26.2	7.7	Nocturnal
Sabethes cyaneus	0.79		17.5	31.3	4.3 [1.3]	69.2	3.5	Diurnal
Toxorhynchites brevipalpis	1.31		22.4	42.9	7.7 [1.2]	80	6.2	Diurnal
Megarhinus (= *Tox.*) *towadensis* (Satô 1961)	1.49	2175	25.0	41.5	9.0 [1.5]	85	5.3	Diurnal

Focal length and image position

In compound eyes that operate in air, most of the refraction occurs at the outer air/chitin surface of the cornea, but the second surface of the lens does have some power. Provided the lens and the material in the image space are homogeneous, the focal length (f) of the system can be obtained from the thick lens formula, the appropriate form of which is: $1/f = (n_1 - n_0)/r_1 + (n_2 - n_1)/r_2 - s(n_1 - n_0)(n_2 - n_1)/n_1 r_1 r_2$, where n_0, n_1 and n_2 are the refractive indices of the outside medium (air), the lens, and the fluid in image space, r_1 and r_2 are the radii of curvature of the front and rear surfaces of the corneal lens, and s is their axial separation. No attempt was made here to measure the lens refractive index (n_1). We have used the value of 1.43 that Stavenga et al. (1990)

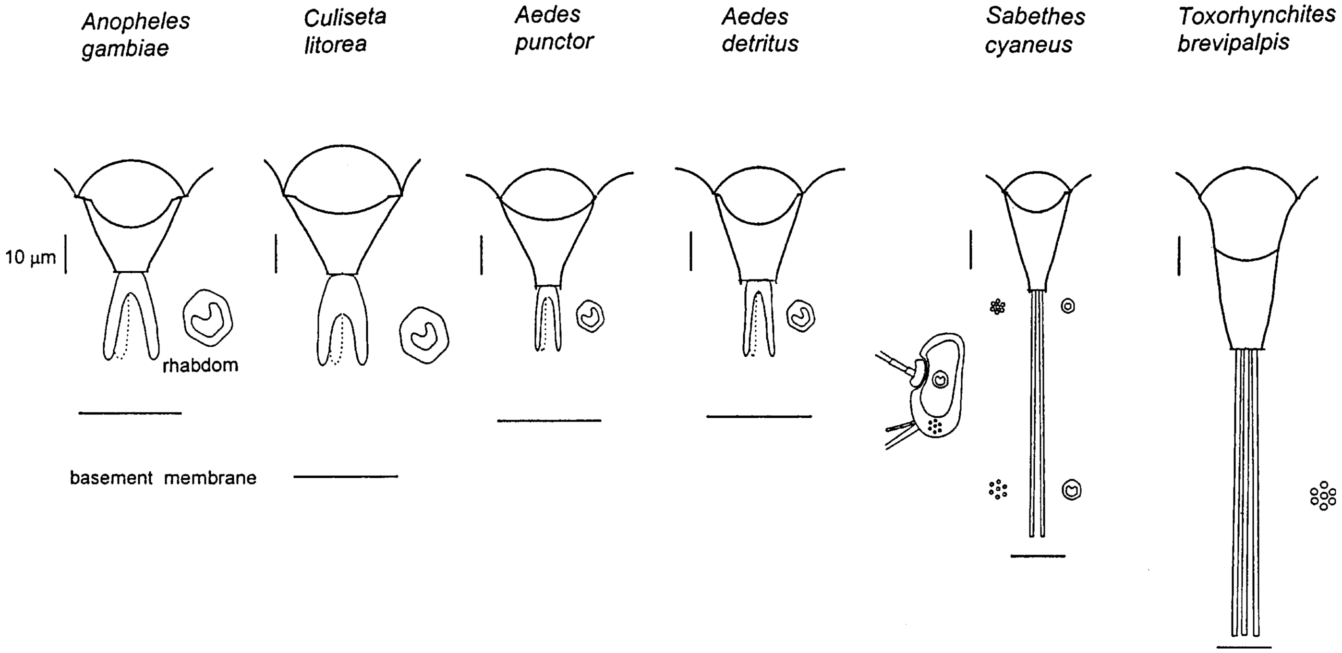

Fig. 2 Ommatidial dimensions in six species of mosquito, ranging from fully nocturnal (*left*), crepuscular (*centre*) to diurnal (*right*). *Inserts* show the rhabdom cross-sections; the inward-pointing bulge (*dotted line* on main figures) indicates the position of rhabdomeres 7 and 8. The left hand four species all have conical fused rhabdoms, *T. brevipalpis* has open rhabdoms, and *S. cyaneus* has both, as indicated on the inset, *left* (see also Fig. 5)

obtained for the material of comparable diameter lenses in blowflies, and we assume an index of 1.34 for n_2. Measurements were made on ten ommatidia from the centre of the eye of females of each of the two extreme species, *A. gambiae* and *T. brevipalpis*. Mean values for r_1, r_2 and s were 14.4, −13.0 and 17.4 μm (28-μm-wide facets), and 13.8, −12.1 and 23.6 μm (22.4-μm-wide facets) for the two species, respectively (standard errors were in the range 1.5–2.5 μm). These yield almost identical focal lengths of 30.1 and 30.2 μm. The focal lengths specify image magnification ($I = f\tan\theta$, where I is image size and θ is the angular size of the object), but do not give image position. This has to be found by applying the surface formula $n'/v - n/u = (n' - n)/r$ to each surface of the lens in turn [if rays come from the left, n and n' are the refractive index to the left and right of each surface, and u and v are the object and image distances at each surface using a Cartesian (right positive) sign convention]. This method puts the focal point in *A. gambiae* 42.3 μm behind the front surface of the cornea, and in *T. brevipalpis* at exactly the same distance, 42.3 μm.

This coincidence would be unremarkable except for the fact, obvious from Fig. 2, that the distal tip of the rhabdom in *A. gambiae* is much closer to the cornea than it is in *T. brevipalpis*. Table 1 indicates that in a 28-μm-wide facet in *A. gambiae* the cornea to rhabdom distance is 29.4 μm, 13 μm inside the focal point. However, the same figure for a 22.4-μm-wide facet in *T. brevipalpis* is 42.9 μm, which is only 0.6 μm beyond the focal point, well within measurement error. Essentially the image in *T. brevipalpis* is in focus on the rhabdom tips, but in *A. gambiae* the focal point is well beyond them, by enough to make the image seriously out of focus. It appears from Fig. 2 and Table 1 that the eyes of other nocturnal mosquitoes are also likely to be out of focus. The probable reason for this is that the very wide relative aperture of the lenses of nocturnal mosquitoes results in a large amount of spherical aberration, so that the best image of a point source is a wide circle of confusion situated much nearer to the lens than the image for rays close to the axis (Fig. 3). In *A. gambiae* the lenses are nearly hemispheric, although pigment behind the lens limits the emerging beam to a width of about 65°; by comparison, the beam emerging from a *T. brevipalpis* lens is only about 30° wide. The construction in Fig. 3 shows that the difference in image quality that results from this aperture difference is quite dramatic. In *A. gambiae* the smallest blur circle is 4 μm across and situated 11.6 μm in front of the focus for rays close to the axis; this corresponds closely in size and location to the width and position of the distal rhabdom tip, and it represents an angle of 12° at the nodal point. In *T. brevipalpis*, on the other hand, the best blur circle is only 0.4 μm in diameter, 3.2 μm in front of the focal point, and smaller than the diameter of one rhabdomere tip (1.5 μm). Thus, *T. brevipalpis* has an eye capable of high resolution, whereas *A. gambiae* has sacrificed resolution for sensitivity, with a strategy that actually makes use of the severe spherical aberration of the wide aperture lens.

Image brightness

The amount of light reaching the focal plane of a lens system from an extended source is determined by $(A/f)^2$ (Kirschfeld 1974; Land 1981), where A is the aperture and f the focal length (the posterior nodal distance in a defect-free system). Histology indicates that the aperture is slightly smaller than the facet diameter (see Fig. 5 a, c below), by a factor of about 0.85, so that for a 28-μm facet in *A. gambiae* the aperture is 23.8 μm, and for a 22.4-μm facet in *T. brevipalpis* it is 19.0 μm. It is the distance from the receiving plane to the nodal point that matters in the brightness calculation, since that determines the area over which the light beam is spread, and as we have just seen this is a considerably shorter distance

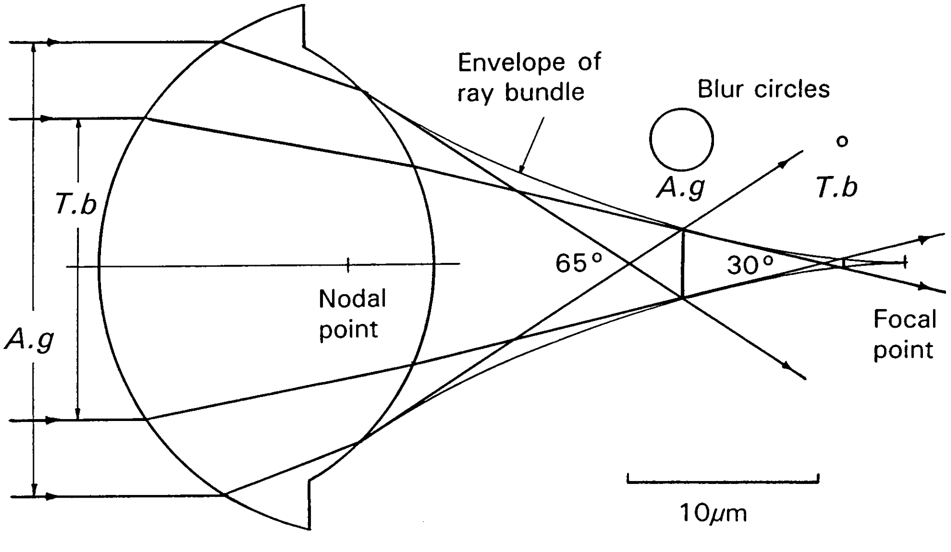

Fig. 3 Optical diagram of a mosquito lens, showing the effects of uncorrected spherical aberration. The wide aperture of *Anopheles gambiae* (*A.g*) produces a 65° cone of light. Because of the over-focusing of the outermost rays, the best image of a point source is a blur circle 4 μm wide, 11.6 μm in front of the calculated focus. The much smaller relative aperture of *Toxorhynchites brevipalpis* (*T.b*) yields a 30° cone, and a 0.4° blur circle, 3.2 μm from the calculated focus

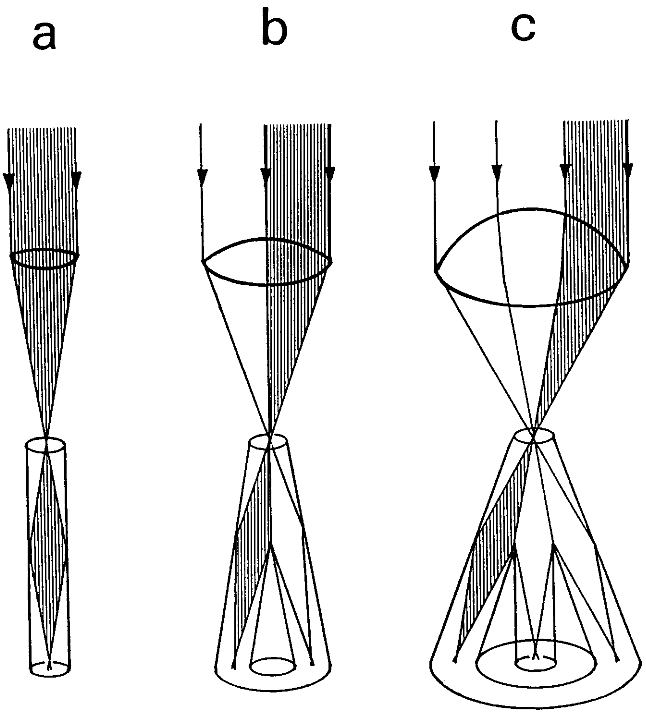

Fig. 4a–c Conical rhabdoms can accept wider cones of light than cylindrical ones. **a** A cylinder will only trap a 21° wide cone of light by total internal reflection, if its refractive index is 1.365. **b** A hollow cone of angle 21° will trap a 42° cone of light. **c** A 42° hollow cone with a central cylinder will trap a 63° cone of light (see Land et al. 1997)

than the focal length itself especially in *A. gambiae*. Making use of the results of Fig. 3, the appropriate values for *f* are (30.1 − 11.6) = 18.5 μm for *A. gambiae* and (30.2 − 3.2) = 27 μm for *T. brevipalpis*. Using these figures, the corresponding values of $(A/f)^2$ are $(23.8/18.5)^2 = 1.66$ and $(19/27)^2 = 0.50$. This indicates that the difference in image brightness between nocturnal and diurnal mosquitoes is a factor of about 3.3.

During dark adaptation in nocturnal mosquitoes the rhabdom tip widens and moves even closer to the lens. Satô's figure (Fig. 1c) shows that in *Aedes japonicus* in the dark the whole rhabdom moves a further 10 μm or so towards the lens, and in other species such as *Culex pipiens* the distal movement is even greater. This will again increase the brightness of the image by decreasing the effective value of *f*, but the image will become even more blurred from a combination of spherical aberration and defocus. This is unimportant, however, because the increase in iris diameter has already made it impossible for the rhabdom to respond to any high spatial frequency information in the image.

Rhabdom size and shape

The rhabdoms of nocturnal and diurnal mosquitoes differ in shape, width, length, the degree of fusion of the rhabdomeres, and the diameter of the iris through which they are illuminated (Table 2, Fig. 2). Nocturnal rhabdoms in the light-adapted state have the shape of a blunt hollow cone with its apex distally, formed from the six outer rhabdomeres. The central pair of rhabdomeres is attached to the inner wall of one of the outer six (see inserts on Fig. 2). The rhabdom is surrounded by black pigment which also fills the inside of the cone. In the day-active *T. brevipalpis* the rhabdomeres are not fused, even at the distal tip (Fig. 5d). They form long, narrow cylinders, and although they are more separated at the base than at the distal tip, the 'cone angle' (arbitrarily defined as the subtense of the proximal ends of the outer rhabdomeres at the centre of the distal tip) is only about 5°, as opposed to about 30° for *A. gambiae* and *Culiseta litorea*, and 20° for the two *Aedes* species. As demonstrated in a previous paper (Land et al. 1997) the function of the conical shape is to trap, in a minimal volume, all the light provided by the lens. The problem is that a cylinder with a refractive index of 1.363, the best current estimate for insect rhabdoms (Nilsson and Howard 1989), will only trap a cone of light 21° wide, when surrounded by cytoplasm, and that falls far short of the roughly 65° cone supplied by the corneal lenses in *A. gambiae*. An answer to the problem is to intercept the light not with a cylinder but a conical rhabdom, which will accept a cone of light of 42°, or better still a conical structure with a central cylinder, which will accept light over 63° (Fig. 4). From Fig. 2 it appears that the wide aperture lenses of the nocturnal mosquitoes are more or less matched to the wide rhabdom cones, and the narrow aperture lenses of the diurnal *Toxorhynchites* and *Sabethes* species supply light to rhabdomeres that are essentially cylindrical, as in the eyes of higher Diptera (Stavenga et al. 1990). The logic of Fig. 4 implies that the angle of the rhabdom cone should be narrower than the incident cone of light by 21°, and that seems to be approximately borne out by the ommatidia illustrated in Fig. 2.

Another feature of the rhabdoms that varies with light regime is the ratio of length to width, or 'aspect ratio'. Rhabdoms of nocturnal mosquitoes are wide but short, and those of diurnal species are long and narrow. The increased width of the nocturnal rhabdoms is easy to understand. A wide converging beam of light enters the distal part of the fused rhabdom, and this has to be absorbed. This requires the rhabdoms to be both conical (see Fig. 4) and thick walled. The fact that nocturnal rhabdoms are short, however, is much more difficult to comprehend. The proportion of light reaching the distal end of a rhabdom that is subsequently absorbed depends on the absorption coefficient *k* and the length *x*. For monochromatic light this an exponential function $(1 - e^{-kx})$, and for white light a slightly different function $[kx/2.3 + kx)]$ (Land 1981, Warrant and Nilsson 1998). In either case the longer the rhabdom the more light is absorbed, subject to a law of diminishing returns. For rhabdomeric structures *k* is about 1% per micrometer, which means that a 15-μm rhabdom will absorb slightly less than 15% of the light reaching it. On the face of it this is a shocking waste of the light won at such cost by the wide aperture optical system. Rhabdomeres of diurnal mosquitoes, conversely, have longer rhabdomeres capable of receiving 50% or more of the light reaching them. This appears to be the wrong way round; the nocturnal rhabdoms should be maximizing their catch of available photons. Possible reasons are given in the Discussion.

Acceptance angles and the role of the iris

The pigment ring that surrounds and to some extent overlies the distal rhabdom tip has the function of determining the acceptance angle of the rhabdom ($\Delta\varrho_r$, in the terminology of Snyder 1979), i.e. the angle in outside space over which the rhabdom receives light, if diffraction and other lens defects are ignored. This angle is d/f (radians) or 57.3 d/f (degrees), where d is the iris diameter and f the effective focal length, which as we have seen is shorter in nocturnal species than the focal length given by the thick lens formula. For a light-adapted *A. gambiae,* $d = 6$ μm and $f = 18.5$ μm, giving an acceptance angle of 18.6°. In the dark the iris increases in diameter to 12 μm, increasing this angle to 37°, or possibly more as the rhabdom also moves further towards the lens. In *T. brevipalpis* the situation is more complex because each rhabdomere is separated from its neighbours, and the appropriate value for $\Delta\varrho$ is the subtense of a single rhabdomere at the nodal point. d here is 1.5 μm, and f is 27 μm, so that $\Delta\varrho$ is 3.2°, nearly five times smaller than in *A. gambiae*.

Inter-ommatidial angles

The resolution of an eye is determined by the quality of the optics and by the density with which the receptors sample the environment. In the apposition eyes of insects, it is the inter-ommatidial angle ($\Delta\phi$) that determines the sampling density. The smaller this is the greater the spatial sampling frequency of the mosaic ($1/2\Delta\phi$). The best way to determine $\Delta\phi$ is by measuring the movement of the pseudopupil across the eye as the head is rotated (e.g. Horridge 1978). However, most mosquito eyes are so dark that the pupil is invisible and cannot be located even with 'antidromic' illumination from beneath the head. An alternative is to measure the angle between ommatidial axes from histological sections, a method that can be quite accurate provided such factors as skewness of marginal ommatidia, and possible distortion by shrinkage are taken into account (see Stavenga 1979). Using this method, values for $\Delta\phi$ between 6.5 and 9.5° (mean 8°) were obtained for oblique rows near the centre of the eye of *A. gambiae* (♀). A pseudopupil was visible in a red-eyed mutant of *A. stephensi* (♀), which is very similar to *A. gambiae*. The pseudopupil moved across ten facets along a vertical row on rotating the head through 70°, giving an inter-ommatidial angle of 7°, consistent with the anatomical estimates. Estimates of $\Delta\phi$ for *T. brevipalpis* (♀) from histological sections of oblique central facet rows gave values between 2 and 3.5° (mean 2.8°). The great difference between *A. gambiae* and *T. brevipalpis* is explained by the much larger head of the latter, and the smaller facets. The only other estimate of a mosquito inter-ommatidial angle available is 6.2° for *A. aegypti*, measured by Muir et al. (1992) using a histological method.

In a perfectly spherical eye of radius r with ommatidial axes perpendicular to the surface, the inter-ommatidial angle along facet rows is A/r (radians), where A is the corneal facet diameter. Mosquito eyes are not perfect spheres, but head-width (h) can be expected to scale with average eye radius, so that the expression $\Delta\phi = A/kh$ should hold for compound eyes in general, and where eyes are the same shape, as is approximately the case for mosquitoes, the value of k should be the same across species. For the three species for which estimates of $\Delta\phi$ are available, k lies between 3.9 and 5.7 (mean 5.0). Thus, the formula $\Delta\phi = A/5h$ (degrees) should give a reasonable approximation for the average inter-ommatidial angle in mosquito eyes. The non-uniform distribution of facet sizes across mosquito eyes (Fig. 1b) strongly suggests that there are regional variations in resolution or sensitivity or both. However, no study has yet been made of these variations.

In an eye adapted to bright light conditions it would be expected that the receptor acceptance angle ($\Delta\varrho$) and the inter-ommatidial angle would be similar, but in dim conditions $\Delta\varrho$ would be larger than $\Delta\phi$ by a factor of 2 or more in order to acquire more light (Snyder 1979; Land 1997). In *A. gambiae* the ratio $\Delta\varrho/\Delta\phi$ is 2.3 in the light rising to about 4.6 in the dark, and in *T. brevipalpis* it is 1.1. These values are entirely consistent with eyes whose optical structure is adapted to dim and bright light, respectively.

Open and closed rhabdoms: the pseudopupil

In *Anopheles gambiae*, *Culiseta litorea* and the two *Aedes* species in Fig. 2 all the rhabdomeres are fused together, and tests with a model rhabdom (Land et al. 1997) indicate that there is little if any possibility of resolution within the image between different parts of the rhabdom. In *T. brevipalpis*, however, the rhabdomeres are separated from each other at all levels including the distal tip (Fig. 5c, d), so that provided there is adequate resolution in the image at the rhabdom tip – as indeed seems to be the case (Fig. 3) – each rhabdomere should receive light from a separate region of surrounding space. From Satô's (1961) photographs the situation in *T. towadensis* appears to be the same. *Sabethes cyaneus*, which is also diurnal, has a particularly interesting eye containing rhabdoms of two kinds (Figs. 2, 5e). Both are long and narrow, but those in the central regions of the eye are fused for most or all of their length, whereas those in the peripheral five to ten rows and in the large downward-pointing region are unfused, as in *Toxorhynchites* sp.

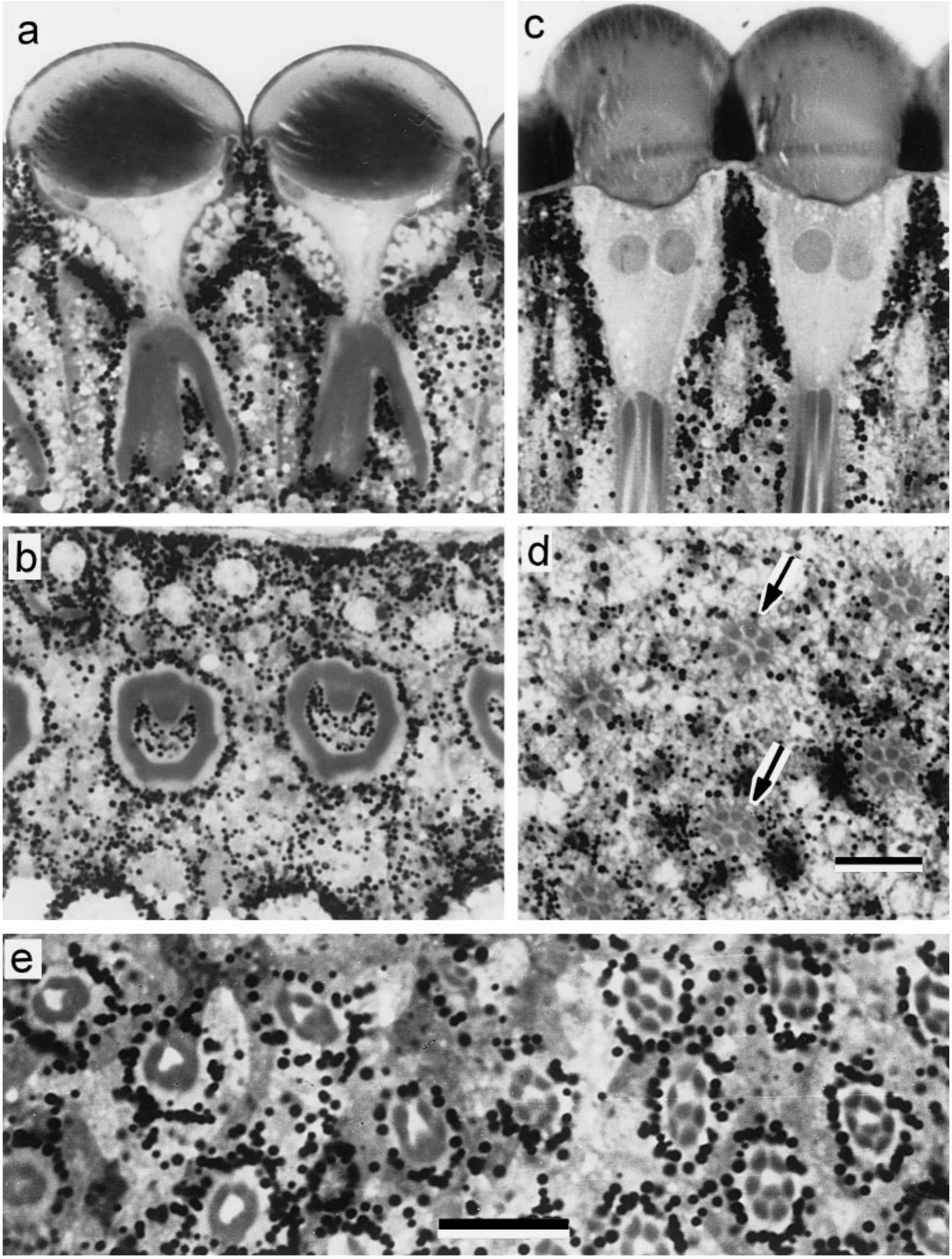

Fig. 5 Details of the ommatidia of *A. gambiae* (**a, b**), *T. brevipalpis* (**c, d**), and *Sabethes cyaneus* (**e**). **a** and **c** are radial sections through the centre of two ommatidia, and they show the fused rhabdoms of A. gambiae, and the separated rhabdomere tips of *T. brevipalpis*. **b** is a cross-section of the proximal region of a rhabdom, showing that the structure in **a** should be interpreted as a cone, with the internal addition of a strut formed from rhabdomeres 7 and 8. The section in **d** is taken at the extreme distal tip of the rhadbomeres, and shows the separated 6 + 1 structure (*arrows*). 10-μm scale on **d** applies to **a–d.** **e** Retina of ***S. cyaneus*** in the region of transition between closed rhabdoms (*left*) and open rhabdoms (*right*). Scale: 10 μm

There seem to be both ‘dark’ and ‘light’ type systems in the same eye. *Armigeres subalbatus* is also diurnal, and has elongated rhabdoms intermediate in shape between those of the *Aedes* and *Toxorhynchites* spp. (Satô 1960). It is clear from Satô’s illustrations that the rhabdomeres are separated in the proximal region, but it is less obvious whether or not they are joined at the distal tip, which is the crucial part for determining whether there is spatial resolution within the rhabdom. Thus, it seems that amongst mosqitoes it is only some day-active species that have open-rhabdom eyes like those of higher dipterans such as *Musca* and *Calliphora*.

One way of demonstrating the optical differences between nocturnal and diurnal eyes is to examine the pseudopupil. In insects with light-coloured eyes, such as grasshoppers, this is a black dot that appears to move round the eye, as the viewer rotates the animal (see Stavenga 1979; Land 1981). Optically the pseudopupil is made up of the images of the rhabdom tips and other structures in the focal plane of each lens, superimposed on each other at the local centre of curvature of the eye. Figure 6a, which represents a nocturnal mosquito, shows how this occurs. Rays emerging from the centres of the rhabdoms in many ommatidia all seem to be coming from a single deep-lying point, and similarly the edges of the pigment rings around the rhabdoms are imaged in the same plane, but to one side. The image of the deep-pseudopupil in the eye of *Anopheles stephensi* (Fig. 7b) shows just these features. The central bright structure is the image of the rhabdom tip, and it is surrounded by an obviously hexagonal array of dark pigment cells. There are even 'secondary' pseudopupils about six lens diameters away from the central image, which represent the images of the pigment in neighbouring ommatidia.

An important conclusion from the photographs of *A. stephensi* and *A. gambiae* (Figs. 7a, b) is that there is no trace of subdivision within the pseudopupil image of the rhabdom, which confirms that the rhabdoms are fused and do not resolve the image further. In contrast to this, the deep pseudopupils from *T. brevipalpis* (Fig. 7c) are clearly divided into subunits corresponding to the rhabdomeres in the focal plane. There may just be a ring of six, or these may surround a central rhabdomere, which reflects the rather variable appearance of the

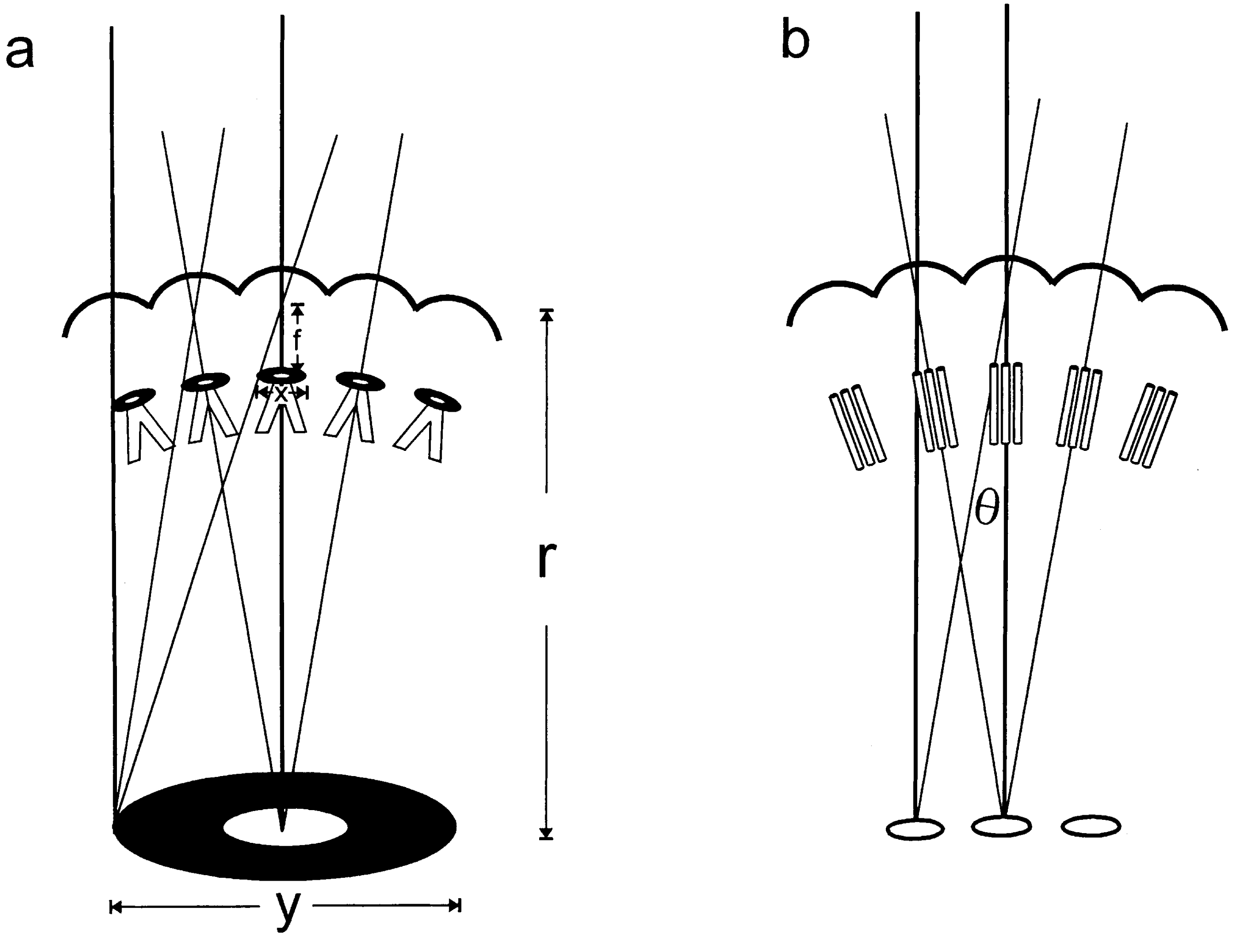

Fig. 6a, b Geometry of the pseudopupil. **a** The deep pseudopupil is a magnified image of the objects in the focal plane of each facet lens, formed by the superposition of corresponding rays from many ommatidia. x width of pigmented region in ommatidium; f focal length of facet lens; y observed width of pseudopupil image; r local radius of curvature of eye. Note that $x/f = y/r$. Further details in text. **b** In the neural superposition eyes of higher Diptera, and the eye of *Toxorhynchites*, the outer rhabdomeres in each ommatidium image the same field as the central ones in neighbouring ommatidia. This produces a deep pseudopupil in which the images of the rhabdomeres are the same distance apart as the facet lenses themselves. θ is both the inter-ommatidial and inter-rhabdomere angle

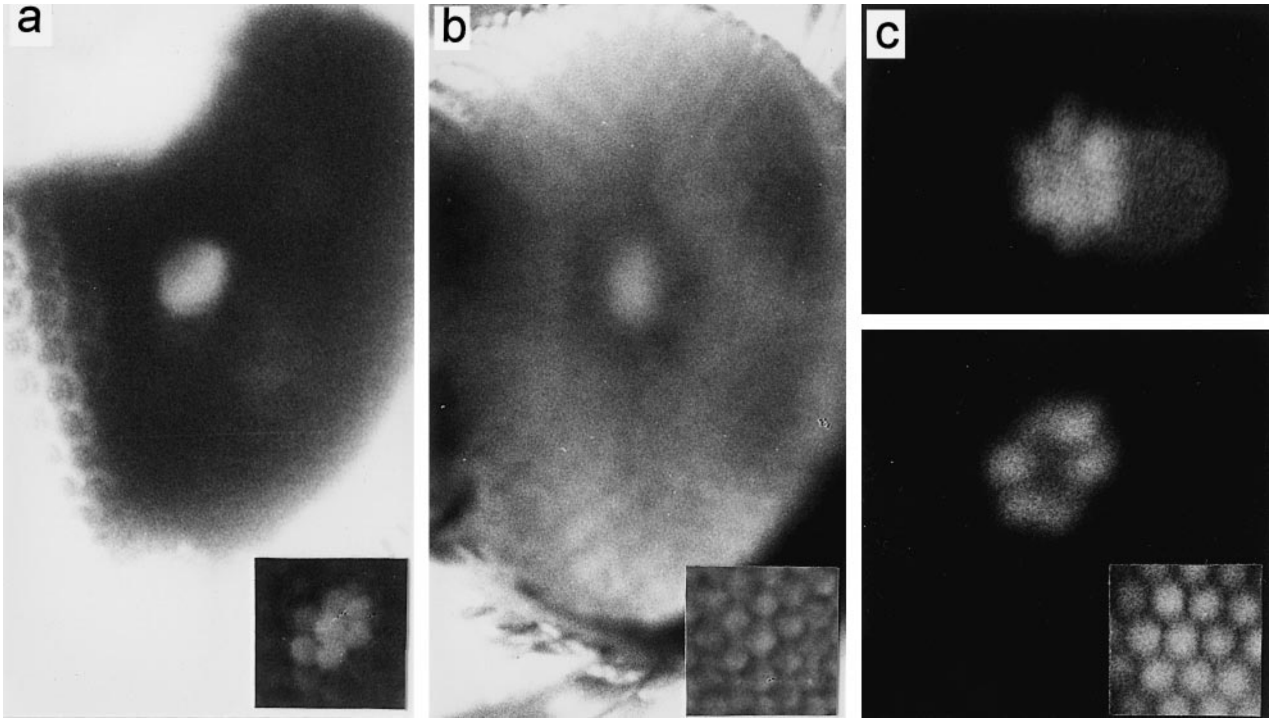

Fig. 7a–c Deep pseudopupils of mosquitoes. **a** Antidromic psuedopupil of *A. gambiae*. **b** Sidelit pseudopupil of red-eyed form of *A. stephensi*, showing ring of pigment around the central (light) rhabdom. **c** Antidromic pseudopupil of *T. brevipalpis* showing separate images of individual rhabdomeres. The *inserts* in each photograph show the corresponding facet lenses photographed at the level of the cornea (dimensions in Table 1). Note the correspondence in **c** between the spacing of the facets and the rhabdomeres in the pseudopupil

central rhabdomere pair seen histologically. The separate rhabdomere images in the pseudopupil are a hallmark of the neural superposition eyes of higher Diptera (Franceschini 1975), and of other eyes with an open rhabdom structure (Nilsson and Ro 1995). In Fig. 7 the photographs of *A. gambiae* and *T. brevipalpis* were both obtained using 'antidromic' illumination, in which the head is illuminated from below, and light travels up the rhabdoms or rhabdomeres, emerging from their distal tips. No pseudopupil was visible with ordinary diffuse lighting.

Figure 6a shows that the pseudopupil can be used to obtain useful information about the resolution of the eye. From similar triangles it can be seen that the angular subtense of objects in the focal plane, at the nodal point of the lens, is x/f, which is the same as the subtense of the pseudopupil image at a depth equal to the radius of the eye (y/r). It is also easy to show from the geometry of Fig. 6a that the width of the pseudopupil *measured in ommatidial diameters* (A) gives the angular width of the focal plane structures in multiples of the inter-ommatidial angle $\Delta\phi$. Thus, in Fig. 6a, the width of the light oval representing the rhabdom tip of *A. gambiae* is very close to $2A$, so that the rhabdom acceptance angle $\Delta\varrho$ is approximately equal to $2\Delta\phi$, a conclusion already arrived at from histology and optical construction. In higher Diptera the optical situation is as shown in Fig. 6b, where the angle between rhabdomere tips at the nodal point of each lens (θ) is equal to the inter-ommatidial angle. This means that the central rhabdomere in one ommatidium images the same direction in space (thick lines) as one of the peripheral rhabdomeres in each of the neighbouring ommatidia. An important question is whether this is also true of day-active mosquitoes such as *T. brevipalpis*. A simple prediction from Fig. 6b is that the distance between the rhabdomere images in the pseudopupil should be equal to the separation of facet lenses, and this is indeed borne out. The rhabdomere images in Fig. 7c are separated by 1.09 ± 0.02 (SE) times the average lens centre separation, which is very close to the value of 1 predicted for a 'nearest neighbours' visual field overlap as in higher Diptera. The same relationship can be deduced from anatomy. Figure 5c, d shows that the separation of the distal rhabdomere tips is approximately 1.6 μm and the distance from the rhabdomere tips to the inferred position of the nodal point in these facets in 32 μm, giving an angular rhabdom separation of 2.9°, very close to the independent estimate for inter-ommatidial angle (2.8°) obtained earlier. The parallel with higher Diptera is interesting because in other nematocerans the situation is different (see Discussion).

Discussion

Eyes for use in light and dark conditions

The anatomical differences between mosquitoes adapted to different light regimes are profound, and are summarized in Table 2. They are of two main kinds: those that increase the amount of light provided by the optical system, and those that involve the rhabdom's ability to trap that light. Thus, in the nocturnal mosquitoes the hemispherical corneal lens and short cornea-to-rhabdom distance both contribute to the brightness of the image at the rhabdom tip. The wide, fused, conical rhabdoms ensure that the light in the image is effectively absorbed. Generally speaking, adaptations to diurnal conditions are the reverse of those for nocturnality. However, the change from fused to unfused rhabdoms is more than just a variation in parameters of shape and size, and has fundamental consequences for the way the retina should connect to the lamina; these are discussed in the next section. It is also interesting that whereas the nocturnal species have large changes with light/dark adaptation (Satô 1953a, 1957, Satô et al. 1957), the diurnal species do not (Satô, 1961). This suggests that the nocturnal species can function over a range of intensities – at least into crepuscular conditions – but that the diurnal species are confined to daylight.

The most surprising feature of Tables 1 and 2 is that the more diurnal species have much longer rhabdoms than the nocturnal species, the latter being only about 15 μm long, and therefore only able to absorb a quite small fraction of the light that enters them. To see what effect this has on the overall sensitivity of the eye (*S*) we can apply the standard formula, which gives the ratio of the number of photons absorbed by a receptor or rhabdom to those emitted by a standard source. This is (Land 1981): $S = (\pi/4)^2 (A/f)^2 d^2 (1 - e^{-kx})$, where A is the aperture diameter, f the effective focal length, d the rhabdom or rhabdomere diameter, k the absorption coefficient of the photopigment in the rhabdom and x the rhabdom length. Using figures derived in the Results section we have for *A. gambiae*: $S = 0.62\ (23.8/18.5)^2\ 6^2\ (1 - e^{-0.01 \cdot 22})$, and for *T. brevipalpis*: $S = 0.62\ (19/27)^2\ 1.5^2 \cdot 7\ (1 - e^{-0.01 \cdot 80})$. If we ignore the last term relating to rhabdom length then a light-adapted *A. gambiae* works out to be 8 times more sensitive than *T. brevipalpis* (36.9 compared with 4.8), and a dark-adapted *A. gambiae* is approximately 32 times more sensitive. However, the difference in receptor length means that *A. gambiae* only absorbs 20% of the light entering the rhabdom, but *T. brevipalpis* absorbs 55% (assuming an absorption coefficient 0.01, i.e. 1% per μm). This reduces the respective light-adapted sensitivities to 7.4 and 2.6, a ratio of less than 3:1 [the use of the alternative formula of Warrant and Nilsson (1998) for white light gives lower overall values, but the same ratio]. Thus, although the optical modifications of nocturnal mosquitoes are really quite impressive, the sensitivity they produce is seriously undermined by the short length of the rhabdoms. We must therefore suppose that there is some very good reason for having short rhabdoms in the dark.

Table 2 Principal differences between nocturnal and diurnal mosquitoes

Nocturnal	Diurnal
Facet lenses almost hemispheric	Facet lenses with a smaller arc
Short cornea to rhabdom distance	Long cornea to rhabdom distance
Wide fused rhabdoms	Narrow unfused rhabdomeres
Conical rhabdoms	Cylindrical rhabdomeres
Short rhabdoms	Long rhabdomeres
Large interommatidial angles	Small interommatidial angles
Strong adaptation changes	Weak adaptation changes

A suggestion that may go some way to explaining this odd result concerns the role of spontaneous photopigment isomerizations (Dan-Eric Nilsson, personal communication). These occur infrequently, but are related to the volume of the photoreceptor, and so their number increases as a function of receptor length. In the dark, when there are few photo-isomerizations, there will come a light level where the spontaneous isomerizations exceed those due to photon captures, and the receptor will no longer produce a useful signal. With the appropriate assumptions, this can lead to a situation that favours shorter receptors as conditions become dimmer. Supporting this idea, some crepuscular spiders have eyes with large lenses but very short receptors (Land and Barth 1992). On the other hand, a well-researched series of dung beetle eyes (genus *Onitis*) shows exactly the opposite tendency: the more nocturnal beetles have longer rhabdoms (McIntyre and Caveney 1998).

A second possibility is that there is simply not enough space for long conical rhabdoms, as the bases of the cones will meet each other if the rhabdoms become much longer than they are in *A. gambiae* (see Fig. 5a, b). It is not clear, however, that the cone needs to maintain its initial wide angle. With the light trapped in the cone it should be possible to straighten the sides into a more cylindrical shape without loss, as in a conventional light guide. Indeed, the rhabdoms in some bugs do have this shape (Walcott 1971), and they are much longer (80 μm) than those of *A. gambiae*. Thus, there remains no really satisfactory explanation for the short length of nocturnal mosquito rhabdoms.

Mosquito eyes and the origins of neural superposition

The eyes of advanced flies (Muscomorpha) have a unique system for increasing the size of the received photon signal without compromising resolution, designated 'neural superposition' (Kirschfeld 1967). The system works by pooling the signals from receptors in adjacent ommatidia at the level of the first optic ganglion – the lamina. In brachyceran flies the six peripheral receptors in each ommatidium image the same region in space as the central receptor pair in each neighbouring ommatidium. This means that if signals from the appropriate receptors from the neighbouring ommatidia join together beneath the central ommatidium, the joint signal will be six or in some cases seven times larger than that of any one receptor, but there will be no loss of resolution as all the receptors have the same field of view. The other ways of securing a better photon signal are either to increase the receptor diameter, or to pool inputs from adjacent ommatidia which have different fields of view. However, either of these solutions leads to decreased resolution. The advanced dipteran arrangement requires a complicated organization of the neural 'wiring' between the retina and lamina, to ensure that each receptor axon finds its proper destination. The appropriate wiring does indeed exist (Braitenberg 1967; Strausfeld 1971). How this arrangement originated, however, is still a matter of conjecture (Shaw 1989; Melzer et al. 1997).

As pointed out in the Introduction, optical studies and mappings of retina-lamina projections have been carried out in some nematocerans – more closely related to the ancestral branch of the Diptera (see Shaw 1989). However, in none of these (tipulids, bibionids and chaoborid midges) is there a straightforward nearest-neighbour overlap between the fields of view of the rhabdomeres in adjacent ommatidia, as in the Muscomorpha. Thus, the finding of just this arrangement in diurnal mosquitoes such as *Toxorhynchites* is of particular interest. It immediately suggests that these mosquitoes ought to have connections from the retina to lamina that permit an advanced form of neural superposition, and it will be interesting to find out whether this is true, and how it is achieved. It makes no biological sense to have an optical arrangement that is right for neural superposition, and then not to exploit it by having inappropriate connections. Nilsson and Ro (1994) first suggested that neural superposition eyes could be derived from nocturnal eyes in which extensive pooling of inputs from neighbouring ommatidia occurred, by a selective reduction in the extent of such pooling. Similarly, Melzer et al. (1997) pointed out that it is possible to arrive at a connection system appropriate to nearest neighbour neural superposition by reducing the array of multiple collaterals found in midge receptors to just the one required to connect with the appropriate neighbouring laminar cartridge. Perhaps that is what has occurred here. There is no suggestion, from the taxonomic position of mosquitoes within the Diptera, that the arrangement we are likely to find here is directly ancestral to the system in higher Diptera, but it provides an important parallel, and from a functional point of view it is a missing link in the evolutionary scheme. Almost as intriguing are the nocturnal mosquitoes, as they are one of a very small number of dipterans known to have fused rhabdoms, making their eyes rather atypical versions of the ordinary apposition type of eye. This is perhaps more closely related to the situation in the mecopteroid ancestor of the Diptera. Again, it will be interesting to see what neural connexions are associated with this design.

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