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Light guides in the dorsal eye of the male mayfly

By G. A. Horridge, F.R.S., L. Marčelja and R. Jahnke

Department of Neurobiology, Research School of Biological Sciences, Australian National University, P.O. Box 475, *Canberra City, A.C.T. Australia*

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[Plates 1–6]

(i) The dorsal eyes are sensitive to ultraviolet light, which is focused by the corneal lens into crystalline cones in the region where these taper progressively to columns across the clear zone. The action of these columns as light guides can be observed in fixed eyes embedded in polymerized resin. In life the light guide part of the column is surrounded by watery non-cellular haemolymph.

(ii) Shadowing the eye surface with a thin wire (three facets wide) while recording from individual receptor units shows that ultraviolet light reaches each receptor by its own facet as in an apposition eye, and not, as in a superposition eye, by a group of many facets.

(iii) As shown by the dye Lucifer Yellow injected from a microelectrode, the electrophysiological unit consists of all seven retinula cells in the rhabdom region. Consistent with this tight coupling of retinula cells there is no polarization sensitivity. The peak spectral sensitivity of all single units is at 345–365 nm in the ultraviolet. The acceptance angle is 2.0–2.5°. The sensitivity at the spectral peak to a point source on the optical axis of the unit is poor compared to that in other insects tested with the same equipment.

(iv) The acceptance angles ($\Delta\rho$) in the dorsal eye are at the theoretical minimum for the facet diameter and wavelength from diffraction theory. Ultraviolet vision, therefore, has made possible a reduction in facet size but the interommatidial angle $\Delta\phi$ is greater than expected from the optimum sampling theory of the diffraction limited compound eye. In fact $\Delta\rho \approx \Delta\phi \approx 2°$.

(v) The dorsal eye is effectively a foveal region with greater sampling density and narrower receptive fields but less overlap of fields than the lateral eye.

(vi) The square cones and yellow screening pigment strongly suggest that there is superposition by reflexion of yellow light that spreads between ommatidia across the clear zone. This yellow light might photoreisomerize the visual pigment. Attempts to prove this theory during the recording from single units have so far failed but no better function for the clear zone has been suggested.

Introduction

The dorsal part of the compound eye of the male mayfly *Atalophlebia* is spectacular in appearance. As can be inferred from the square facets and long cones tapering to tracts across the watery clear zone, the optical system is undoubtedly interesting,

but not yet understood. The little that is known of the physiology of the receptors shows that the dorsal eye is sensitive only to the ultraviolet (u.v.) part of the spectrum. The dorsal eye is clearly concerned with finding the female because it occurs only in the male, but little is known of the behaviour that must have governed the natural selection of such a complex structure. Let us summarize what is already known of these four aspects of the eye.

In gross anatomy the dorsal eye is separate from the lateral eye, which has hexagonal facets. The dorsal eye has square facets, each with an obvious convex surface. The dorsal eye of *Atalophlebia* surveys a large angle of the sky on each side. In contrast, in the *Cloëon* group of mayflies (Baetoidea) the dorsal eye of the male is shaped like a cake with a rather flat top and it views a smaller segment of the sky.

In structure, the dorsal eye has a wide clear zone but we already have strong indications against the two possible mechanisms of forming a superposition image at rhabdom level. The possibility of a reflecting superposition eye appears unlikely because the corneal facets are strongly convex and therefore they must act as strong lenses with a focus within the crystalline cone. The refracting superposition mechanism also appears to be ruled out because the crystalline cones have long progressively tapering tips which are surrounded by bags of yellow pigment that would absorb u.v. rays crossing between ommatidia from the cones. Furthermore, the rhabdom layer is protected by a layer of yellow pigment which makes a superposition mechanism impossible. With no alternative explanation available, it was suggested that each ommatidium acts separately like an apposition eye by focusing axial u.v. rays into the cone and then into the retinula cell column which functions as a light guide (Horridge & McLean 1978). But such an eye could function equally well with the rhabdoms directly attached to the cone tips. To explain the clear zone, therefore, it was suggested in the same paper that the longer wavelengths pass through the peripheral yellow screening pigments and increase sensitivity by the photoreisomerization of visual pigment. Possibly the mechanism is widespread (Hamdorf *et al.* 1973; Horridge 1976; Schneider *et al.* 1978) but there has been no experimental demonstration of this effect on the u.v. visual pigment in the mayfly, and we have no other example of an eye in which the superposition principle has been applied only to the photoreisomerization. Quite clearly, however, if the threads of higher refractive index than their surroundings provide the light path across the clear zone between the cone tips and the rhabdom in each ommatidium, a function must be found for such an anatomical arrangement, for otherwise the rhabdoms could lie directly at the cone tips.

Two seasons' work with microelectrodes now allows us to describe the physiological properties of the visual units. As shown by the intracellular marking with the dye Lucifer Yellow, the visual units are the ommatidia and not the individual retinula cells. An experiment in which the facets are shaded with a thin (45 μm diameter) wire shows that the visual system in the ultraviolet has no superposition, and therefore the tract across the clear zone is a light guide. The small fields and relative insensitivity compared to other eyes are consistent with the light guide action of the individual retinula cell columns across the clear zone, and the light guides are demonstrated directly in eyes embedded in resin.

Materials and methods

Atalophlebia A (figure 1, plate 1), as described in the previous paper (Horridge & McLean 1978), was caught in the late larval stages in the upper reaches of Ginninderra Creek, Canberra. The larvae emerge as subimagos in an aquarium and wait for a further day before the final moult to the imago.

Recording from the units of the retina was by standard techniques. The electrodes must be sharp and of resistance greater than 150 MΩ for recording from this eye. The electrodes were filled with potassium acetate or with Lucifer Yellow by capillary action from the inside, and when filled with dye the resistance often exceeded 250 MΩ. Care must be taken to avoid the preparation drying out. The eye is soft, and least mechanical damage is caused by making a tiny hole through the cornea at the extreme posterior edge of the eye near the cuticle. Recordings can then be made from ommatidia that look out dorsally from the centre of the eye. Units were marked by passing negative current pulses of 3 nA of 1 s duration at intervals of 3 s for 15 to 20 min until the normal response was reduced to 50% of its value to a standard flash. The eye was then fixed for 2 h in 2.5% glutaraldehyde in 0.1 M sucrose in 0.1 M cacodylate buffer (pH 7.1), dehydrated and embedded in Araldite by standard procedures. Sections 2 μm thick each containing a unit marked by the Lucifer Yellow dye were photographed with a Zeiss Photomicroscope set up for fluorescence microscopy (Stewart 1978).

For freeze fracture, eyes of male *Atalophlebia* were fixed for 2 h in the above fixative, washed, and perfused over 30 min with glycerol to a final concentration of 30% (by volume). The eyes were then prepared in a Baltzer freeze fracture apparatus by standard methods.

An eye map was made by photograhy of the pseudopupil at angular intervals of 5° across the eye after facets had been marked by dust (Horridge 1978).

Distribution of screening pigment was observed in unstained sections of material fixed only in glutaraldehyde without osmic acid post-fixation treatment. Pigment distribution was also observed in freeze fracture preparations of glutaraldehyde-fixed eyes, treated with dilute glycerine.

Methods of optical examination are described below in the relevant sections.

Results

Anatomy

The dorsal eye of the male *Atalophlebia* (figure 1*a*) is unusual when compared with the eyes of other insect groups. The cell types and their layout (figure 2) have been described from a study by electron microscopy (Horridge & McLean 1978) but the relation between structure and function is more easily understood at a smaller scale, as illustrated in figure 3, plate 2, figure 4, plate 3, figure 5, plate 4, and figure 6.

The cornea

The facets of the dorsal eye are square, in contrast to the hexagonal facets of the lateral eye, and therefore we are alerted to the possibility that this is a reflecting

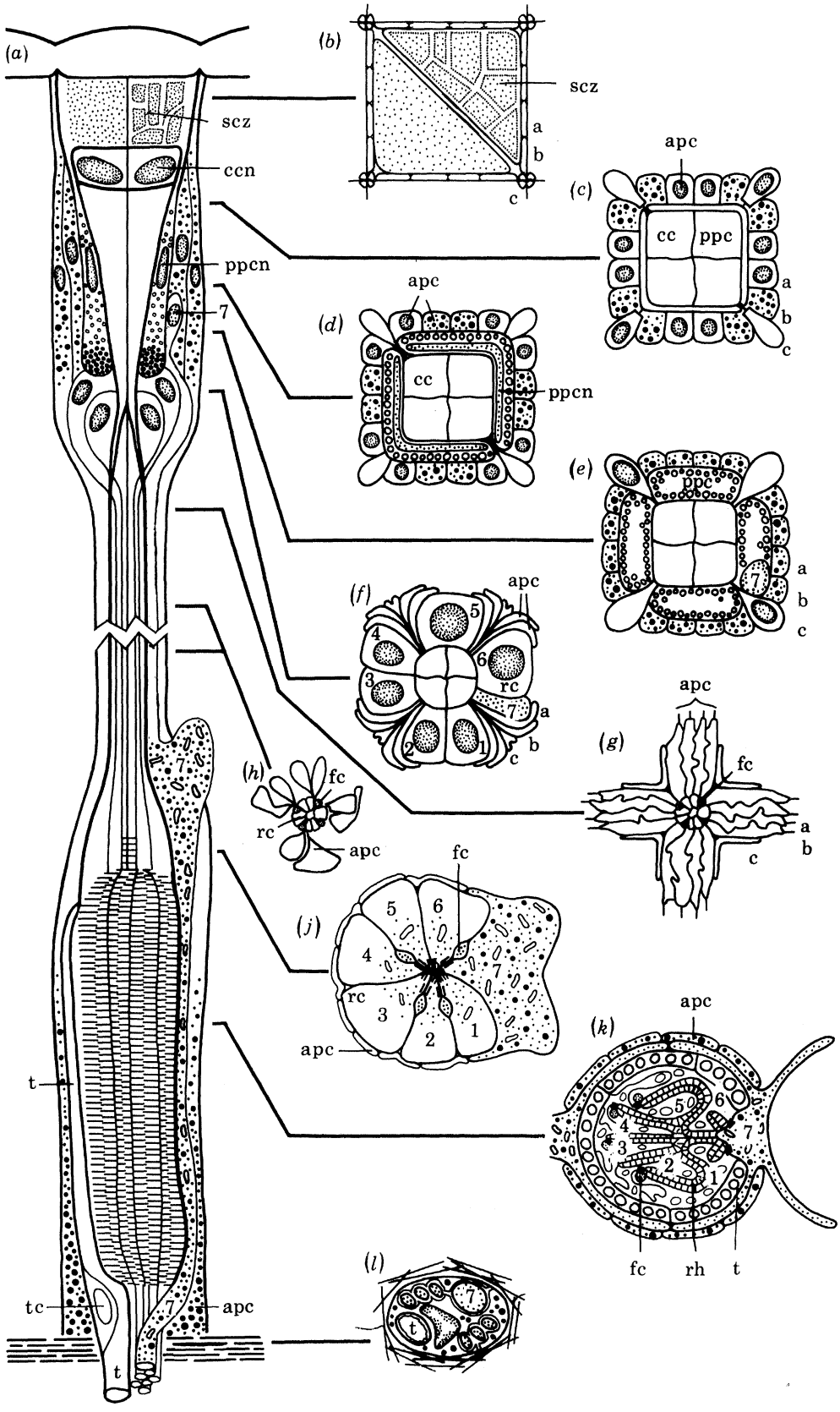


FIGURE 2. For description see opposite.

superposition eye (Vogt 1975; Land 1976). This mechanism, however, cannot be important in sharp vision in *Atalophlebia* because the corneal facets are strongly convex with a radius of curvature of 30 μm so that they must form a first focus well within the cone far from the rhabdom layer. For the measurement of the focal length of the corneal lens, see below. The inner surface of the cornea is relatively flat (figures 3, 4*a*).

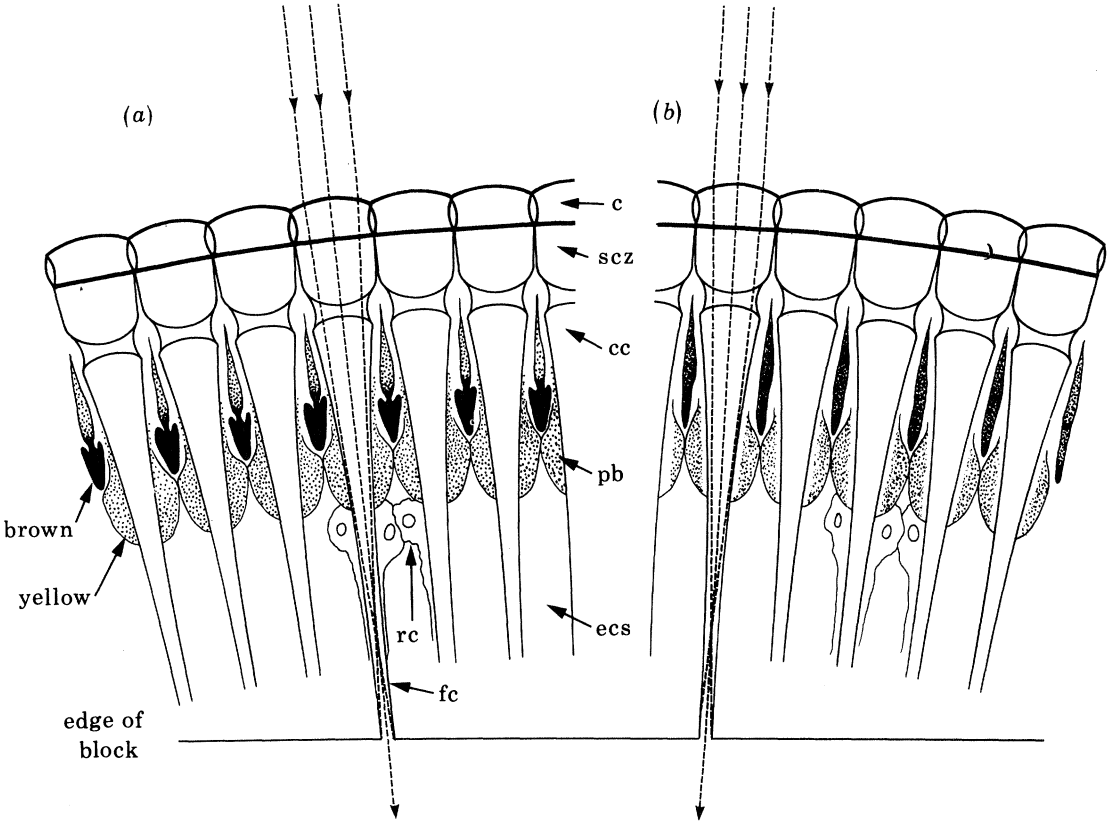


FIGURE 6. A drawing of a longitudinal section of the cone region showing pigment locations in (*a*) the day and (*b*) the night state. Compare with figure 4. It is also evident from this diagram that the cut ends of the light guides are illuminated in a group that moves progressively across the clear zone when a point source is moved in the periphery (see figure 23).

FIGURE 2. An ommatidium of the dorsal male eye. (*a*) Longitudinal section in the day state, with transverse section (*b*)–(*l*) at the levels shown. (*b*) The subcorneal zone, scz, composed of two principal pigment cells. (*c*), (*d*) Crystalline cone and four types of pigment cells in a constant pattern across the eye. (*e*) The region where principal pigment cells are filled with yellow grains. (*f*) Retinula cell bodies 1–6 surround the narrow neck of the cone. (*g*) The retinula cell column is supported by a square pattern of accessory pigment cell processes. (*h*) The retinula cell column surrounded by extracellular space. (*j*) The region where cell 7 is enlarged and filled with yellow pigment. (*k*) The rhabdom region, surrounded by a sheath of trachea, by processes projecting from cell 7 and by a layer of pigmented cells. (*l*) Seven axons, of which no. 7 is the largest, pass through the basement membrane with a tracheole.

The subcorneal zone

Between the cornea and the four cone cells is a fluid-filled zone (scz in figures 2*a*, *b*, 6) that is not found in most insect groups. The location and varied width of this zone suggests that it acts as an adjustable spacing element which can change the position of the cone relative to the cornea and therefore move the focus within the cone, so that sensitivity and receptor field size can be adjusted simultaneously. This zone is part of the two principal pigment cells, the other ends of which are in contact with the retinula cell bodies. Some form of feedback from the primary visual cells to a focusing mechanism is therefore possible with this anatomical arrangement.

The cone

From the functional point of view the most remarkable features of the cones are their square outline in cross-section and their progressive taper to narrow columns which traverse the clear zone (figure 4). The middle part of the cone was illustrated previously as round in section (Horridge & McLean 1978, pl. 2, 3), but in our most recent material, fixed differently, the cone is usually a square prism along its whole length (figure 5). When examined by freeze fracture, the cone is square in section, but no technique has revealed a reflecting structure along the faces of the prism (figure 7, plate 5). In the day eye, yellow pigment grains lie close against the outside surface of a long length of the cone (figures 4, 7), but in the night eye they are more dispersed, which must allow more u.v. light to pass down them. Whether this is optically significant is not known.

In transverse section the cone is homogeneous, as shown by stains for protein, by electron or light microscopy, and by examination of frozen sections of cones in an interference microscope. We have seen no evidence of a refractive index gradient along the radius of the cone. Along its length, however, the cone consistently becomes denser as it narrows, as shown by stains for protein in the electron or light microscope. This increase in density must represent an increase in refractive index which effectively reduces the effect of the taper because internal reflection occurs more readily in the narrower denser region than it would if the whole cone were homogeneous. The four cone cell processes extend across the clear zone to the rhabdom, surrounded by the seven necks of the retinula cells and accompanied for a part of the way by thin flat leaves of accessory cells (figures 2*g*, 5 (lower part)).

Pigment cells around the cone

As previously described in detail (Horridge & McLean 1978), there is a complex square pattern of pigment cells between the cones (figure 2), best appreciated in a transverse section across the eye (figure 5).

A pair of principal pigment cells surrounds each cone and forms a bag of yellow pigment against each face of the cone where it narrows to a width of 5 μm, as shown by pb in figure 6. The location of this yellow pigment suggests that it serves two functions. First, it must be a barrier to u.v. rays that would cross between

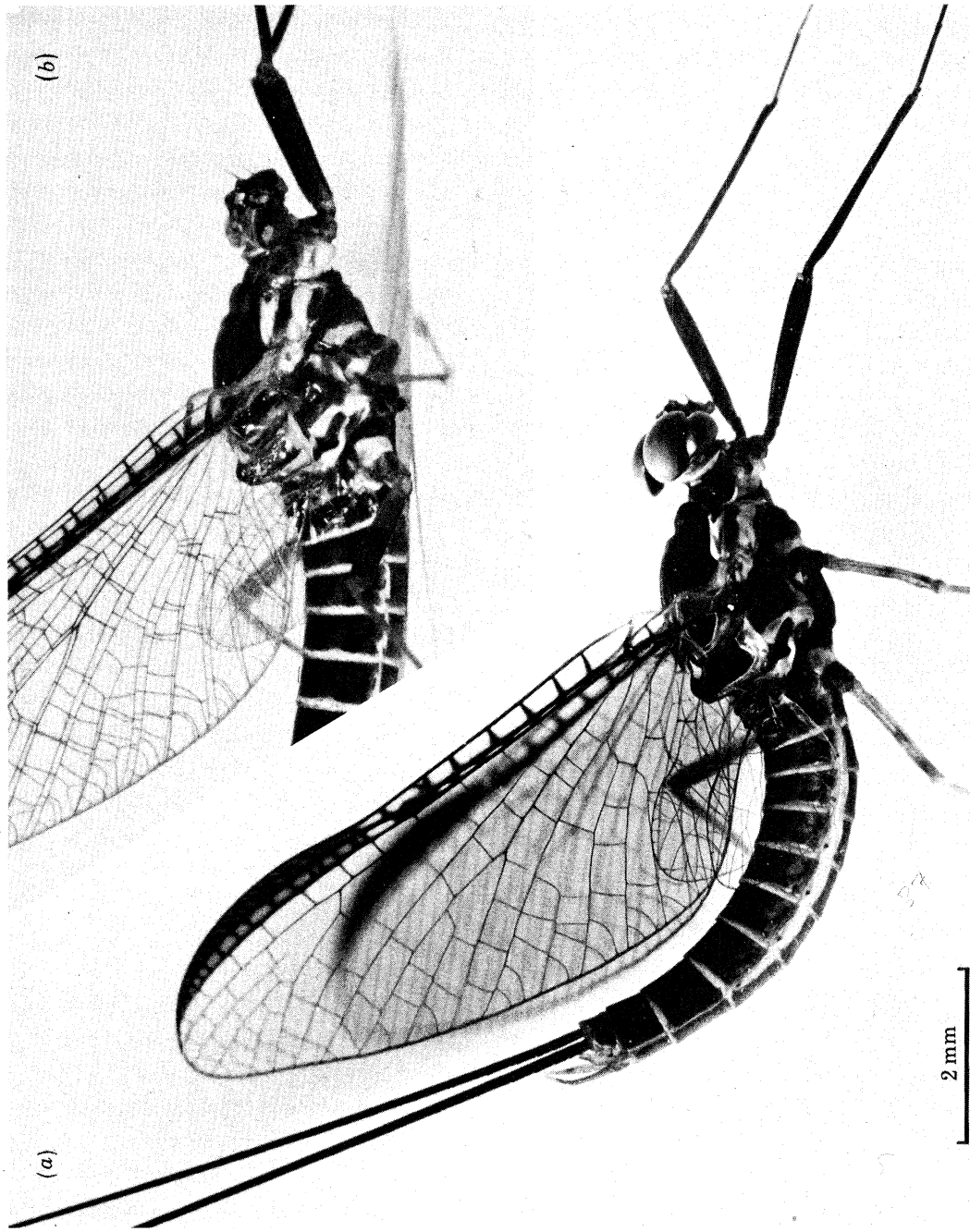


FIGURE 1. The mayfly *Atalophlebia* in the resting posture. (*a*) Male with dorsal and lateral eyes. (*b*) Female with only the lateral eyes.

(*Facing p.* 30)

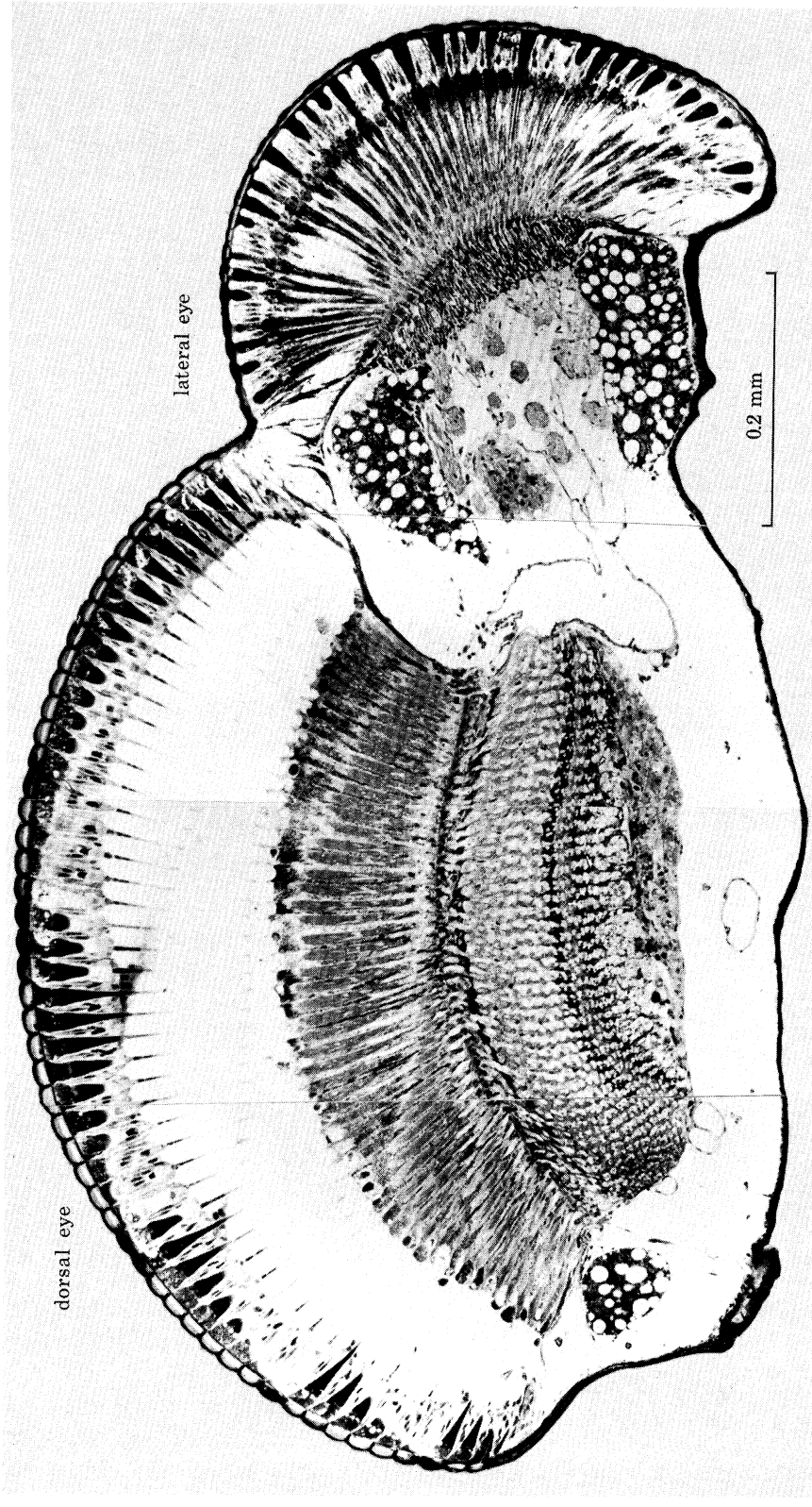


FIGURE 3. A section almost in a vertical plane transverse to the head through the dorsal and lateral eye on one side in the male *Atalophlebia*. The difference between the two parts of the eye lies in the clear zone and in the elongated cones of the dorsal eye. See also figure 19.

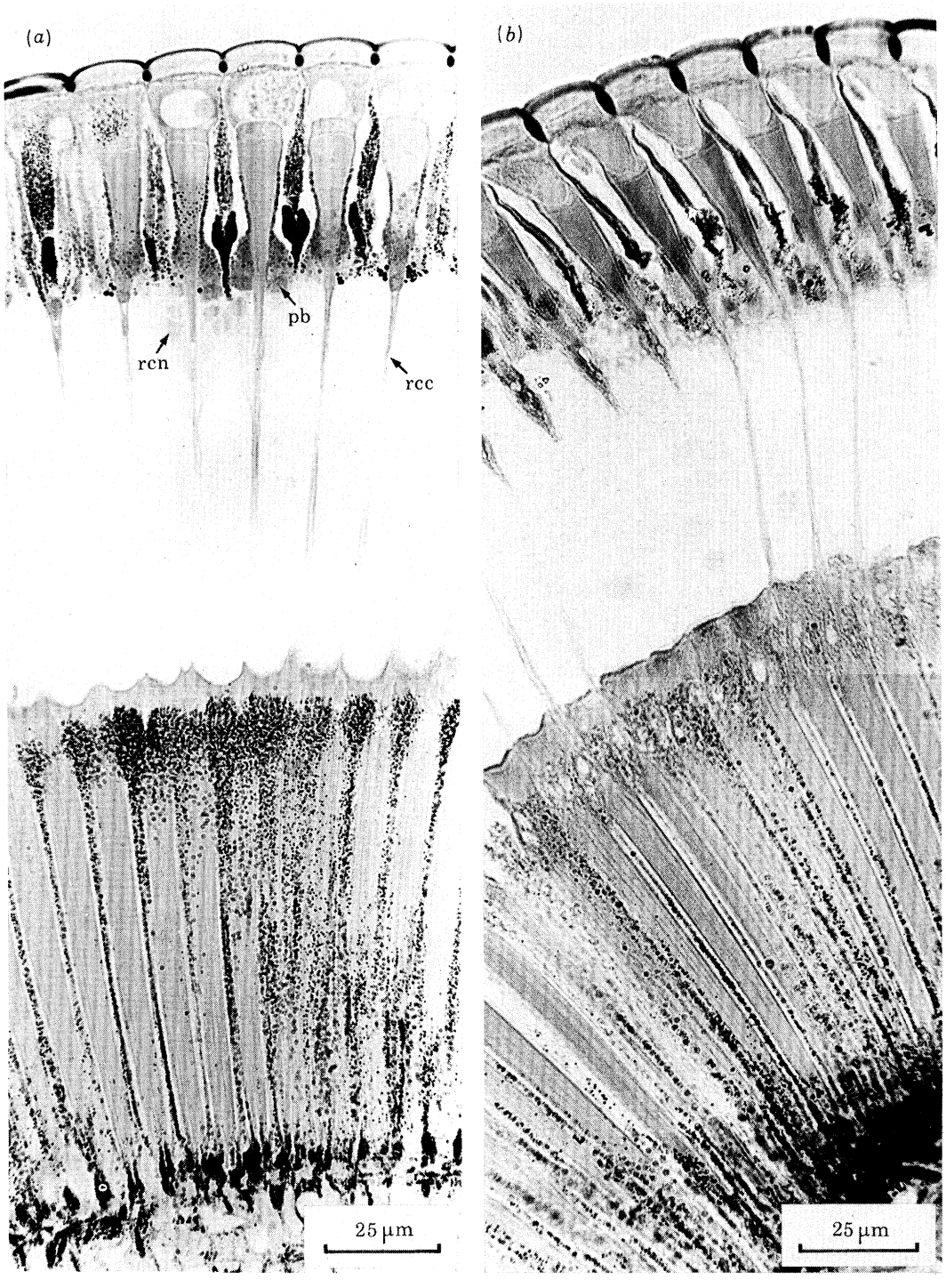


FIGURE 4. Unstained longitudinal sections through the cone and rhabdom regions of the dorsal eye of *Atalophlebia* in (*a*) the day and (*b*) the night states, showing the pigment locations in unstained sections and the difference in position of the yellow and the brown pigments in the two states. These eyes were fixed with glutaraldehyde then processed without osmic acid and embedded by standard procedures. Compare with figure 6.

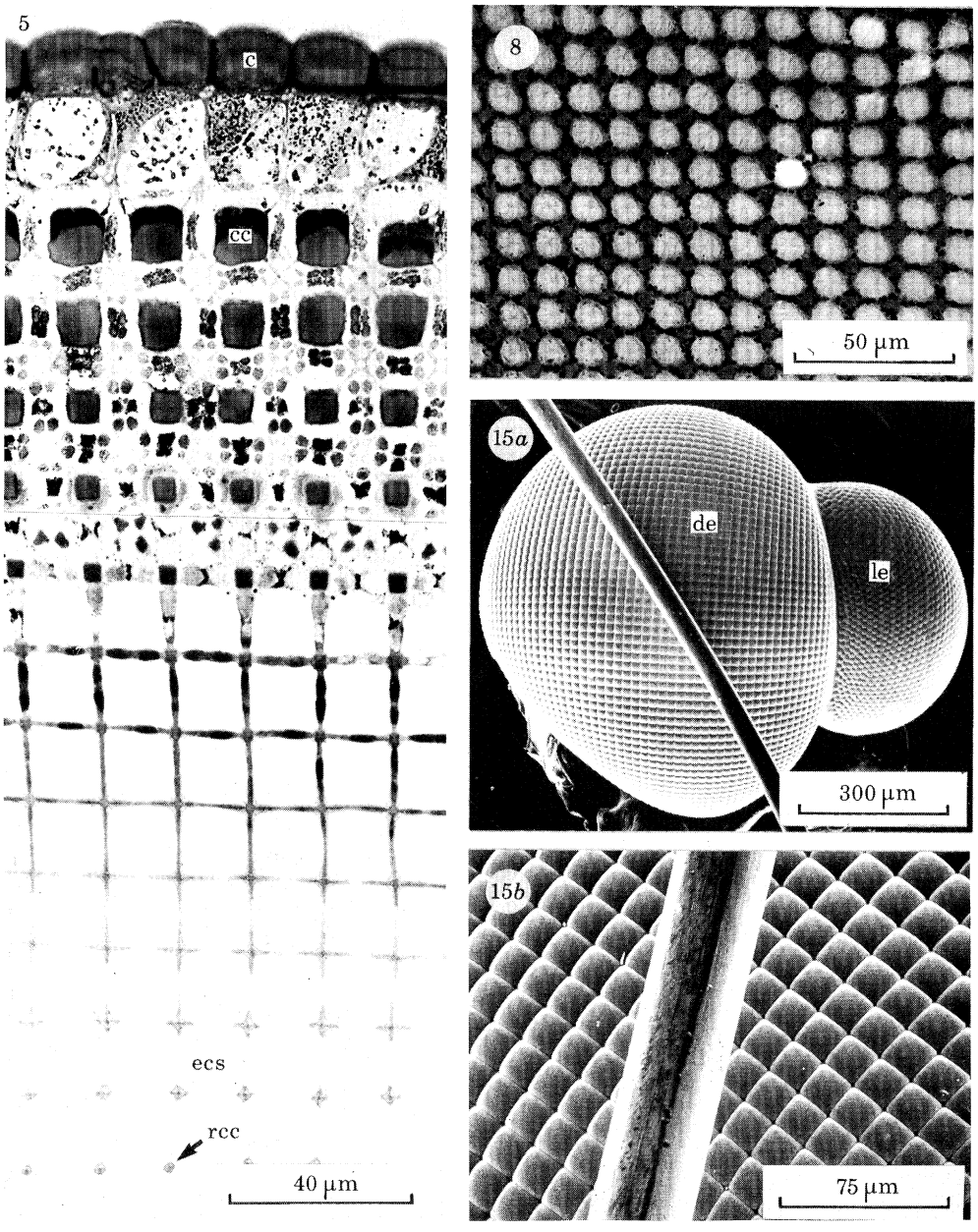
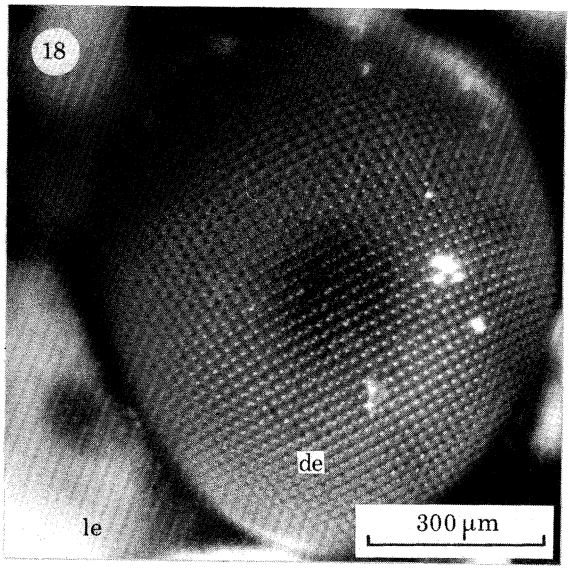


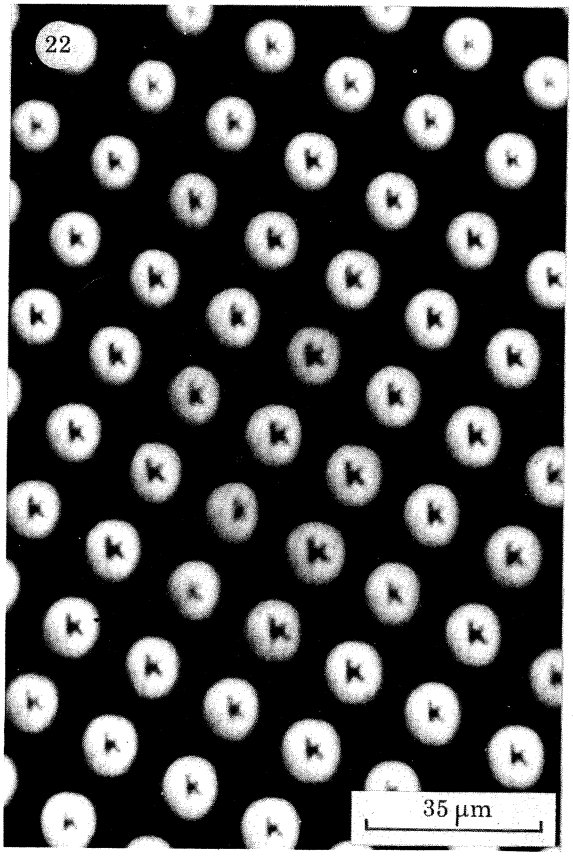
FIGURE 5. Transverse section through the dorsal eye of *Atalophlebia* in the day state, showing the arrangement of the components of the ommatidium at each level. For interpretation at each level see figure 2.

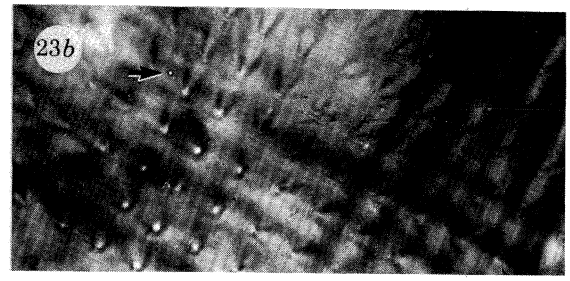
FIGURE 8. A unit marked with Lucifer Yellow consists of all seven retinula cells of one ommatidium. The filled axon of cell 7 is clearly seen outside the disc formed by the other six cells. Compare with level (*k*) in figure 2.

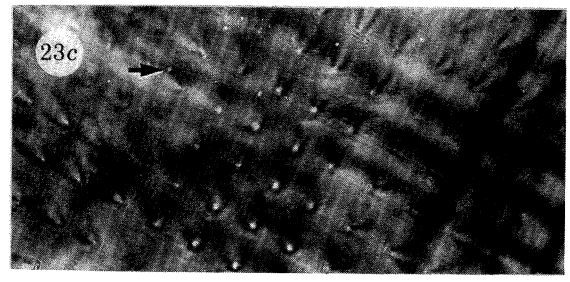
FIGURE 15. (*a*) The experimental arrangement in which a thin wire is moved across the surface of the eye, in the orientation that yielded the records in figure 16. This is a picture taken by scanning electron microscopy of a fixed eye that had been embedded and then chipped out from the block of Araldite to avoid the problems of shrinkage. (*b*) The same at higher magnification.

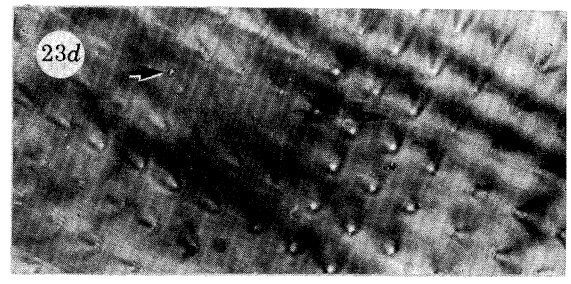
FIGURE 7. Transverse section through the square neck of the cone where the yellow pigment is located in the principal pigment cells at level (*e*) in figure 2. The section was prepared by freeze fracture then etching of an eye that had been lightly fixed in glutaraldehyde and infiltrated with dilute glycerol. The cone is surrounded by absorbing pigment, not reflecting structures.

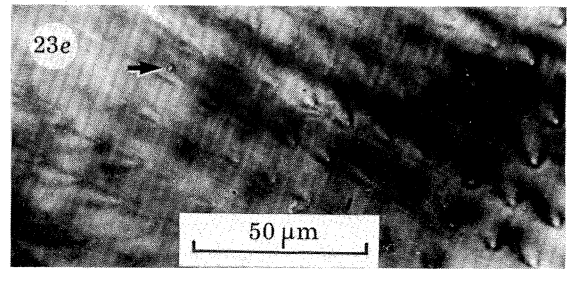












FIGURES 18, 22 AND 23. For description see opposite.

ommatidia and spread towards the clear zone, and secondly it may attenuate u.v. light passing down the cone by hindering total internal reflexion. This pigment is therefore the main stop in the peripheral eye for u.v. vision. Three types of accessory pigment cells are distinguished by their position (Horridge & McLean 1978, p. 140) and by colour (figure 6). All three types a, b and c shown in figure 2*c* have a yellow pigment (least in type c), but type a also has a dense brown pigment in a position that would prevent visible and u.v. light entering the eye between the cones and escaping the optical systems altogether (figures 4–6).

Possible reflecting structures

From the anatomy, something can be said about the optical system. The inside surface of the cone must act as a total internal reflector because the cone is quite clearly denser than the immediately surrounding cytoplasm of the principal pigment cells. Yellow screening pigment on the outside surface of the cone surface (figure 7) must necessarily attenuate the u.v. light reflected within, and therefore radial movement of this pigment must vary the attenuation. There is no problem over the critical angle inside the cone, because even a 1 % (by mass) difference in protein concentration between inside and outside gives a complement of the critical angle of 2.71° (Barer 1957), which is quite adequate for the observed field widths.

That the square lattice of accessory pigment cells, shown so clearly as square boxes in the middle part of figure 5, is also an array of reflecting right angle corners has been considered. However, reflexions here would not be total internal reflexion, and special surfaces such as orientated crystalline plates would be required for reflexion into the less dense medium. So far no such structures have been revealed in a polarizing microscope and no crystals are seen in this region in an eye squash.

Screening pigment, day and night states

In the previous paper (Horridge & McLean 1978) we saw no changes because, like Burghause (1981) working on *Cloëon*, we did not then compare night and day eyes. In the day eye, irrespective of light or dark adaptation, there is yellow pigment around the cone down to the region at the level of the retinula cell nuclei where it is less than 5 μm wide. This pigment appears to lie in bag-shaped cells around the cone tip. The dense brown pigment lies about half way between the cones in a specific pattern, as seen in longitudinal section (figures 3, 4*a*, 6*a*).

DESCRIPTION OF PLATE 6

FIGURE 18. The dorsal eye and, out of focus and lighter coloured, the lateral eye, each with its pseudopupil. The photograph is taken from an anterior dorsal direction in which both parts of the eye are looking. The white chalk particles are facet markers.

FIGURE 22. Images in the corneal lenses formed by a letter K placed outside the eye, as in figure 20, for measurement of focal length.

FIGURE 23. The cut ends of the retinula cell columns in a square array in the clear zone. (*a*)–(*e*) As a light source is moved in steps of 5° outside the eye, the group of columns that are illuminated moves across the eye with the source, but the bright ends do not themselves move. The arrow indicates a reference point in the eye (in the form of a dirt spot) which remains fixed.

In the night eye the yellow pigment is more dispersed and is cleared away from the immediate surface of the neck of the cones. The brown pigment is now concentrated in a narrow strip midway between the cones (figures 4*b*, 6*b*). The first change can be expected to increase the u.v. light reaching the receptor, and the second will let more yellow light into the eye. The diurnal rhythm overrides the light–dark régime, in that the night state cannot be obtained by placing animals in the dark during the day.

There are two further zones of screening pigment in the rhabdom layer. There is at all times a layer of yellow pigment between the clear zone and the rhabdom layer, and also a brown pigment between the rhabdoms as described below.

Columns across the clear zone

The proximal ends of the three types of accessory pigment cells form a square lattice that stands out sharply in transverse section (figure 2, level g; figure 5, lower centre). There is a dense group of attenuated cells at each intersection of the pattern (figure 2, level g and h; figure 5). These supporting cells progressively drop out as we move towards the clear zone, leaving the square pattern of columns running freely across a non-cellular clear zone that consists only of fluid. Each column of the ommatidium at this level contains four thin cone cell processes, filled with microtubules, and seven long thin waist regions of retinula cells that have their nuclei around the cone tip (figures 2, 5). Such unsupported columns across the clear zone have been described only in male mayfly dorsal eyes. The clear zone in nocturnal insect eyes is normally filled with the processes of accessory pigment cells which have dilute contents and no pigment in the night state in the clear zone. In *Atalophlebia* dorsal eye, therefore, the refractive index of the clear zone is clearly the minimum possible, which is significant for their action as light guides.

The rhabdom layer

The rhabdom appears as a five-armed candelabra in transverse section, as previously described (Horridge & McLean 1978, pl. 5, facing p. 144), with the arms facing posteriorly. Whether or not any other function can be found for it, this extraordinary shape makes sense as a device for catching the yellow light that is scattered within the eye, as discussed below.

By day there are dark pigment grains of separate cells in a sleeve around the rhabdom outside the ring of tracheoles in each ommatidium, concentrated towards the clear zone (figure 4*b*). By night this sleeve of pigment moves away below the rhabdom layer (figure 4*b*). Even so, by night, any oblique rays within the eye are likely to be scattered by the sleeve of trachea that surrounds the rhabdom for most of its length inside the pigment sleeve (figure 2*k*).

Between the clear zone and the layer of rhabdoms is a barrier of yellow pigment that blankets the receptor region except where each column across the clear zone passes through it (figures 2*j*, 4). In the freshly opened eye the rhabdom layer looks yellow. The pigment lies mainly in the broad part of retinula cell 7 at this level (figure 2*j*) and by electron microscopy is seen as numerous empty grains in the cytoplasm of cell 7 (Horridge & McLean 1978, fig. 7*a*, pl. 6).

The interpretation of these details in terms of optical function is therefore as follows. If any ultraviolet light penetrates the eye between the cones, and is outside the lens–light guide pathway, the yellow pigment at the proximal side of the clear zone is in a position to stop it and protect the rhabdom layer. Being within the light guides, the focused u.v. light passes through this yellow pigment layer to the rhabdoms. Yellow light, however, that has come into the eye from any direction finds negligible barriers in the cone region or in the night eye in the rhabdom region. The sleeve of trachea around each rhabdom can have only one optical function, to prevent any u.v. light crossing between the rhabdom columns. For visible light, the eye is full of 'glare'; but we have to remember that in the ultraviolet there is a mechanism that retains the isolation of ommatidia right from the cornea to the basement membrane, as demonstrated by the narrow fields of the receptor units.

Electrophysiology of retinal units

The electroretinogram is very small, presumably because the rhabdom layer is separated from the cornea by a watery clear zone that appears to be continuous with the body fluid and also because the light source stimulates few receptors. The successful penetration of retinula units depends on locating exactly the right depth in the eye, where the retinula cells are mainly cytoplasm (figure 2, level j).

Intracellular marking

Units were penetrated with an electrode containing a solution of Lucifer Yellow CH and filled with the dye by electrophoresis. The dye enters all seven retinula cells of the ommatidium (figure 8), suggesting that during life they are coupled together electrically as a single unit. This coupling fits with the findings that the polarization sensitivity is negligible and spectral sensitivities are all the same. Dye could be traced in the axon of cell 7 (see figure 2*k*) through the basement membrane but the dye did not pass outwards across the clear zone along the very thin necks of the retinula cells to the retinula cell bodies which lie around the cone tip.

More significantly, the coupling of cell 7 with the other six retinula cells in the rhabdom region agrees with the earlier interpretation of the anatomy, that retinula cell 7 collects a current flow from all the other cells and acts as a path of rapid electronic spread directly to the medulla. Cell 7 has no rhabdom of its own but nevertheless it has the largest axon leaving the retina (figure 2*l*).

Absolute sensitivity

Other factors being equal, a superposition eye can be expected to be more sensitive than an apposition eye of similar dimensions, because in the former the rays from a point striking many facets are brought to a small number of rhabdoms within the eye whereas in the apposition eye only rays striking one facet are effective. Also, if an eye is adapted to operate in dim light, the gain in the transduction process, i.e. the depolarization per photon, can be expected to be greater. The combined effect of these adaptations is revealed by the stimulus intensity at which the dark-adapted receptors are in the middle of their working range.

Recordings from single units have been made with a variety of dark-adapted insects, with the same stimulating equipment. The light source was the end of a quartz light guide 0.5 mm in diameter at a distance of 12.5 cm from the eye, adjusted to the optical axis of the receptor. Representative responses are shown in figures 9–11. The curves of response amplitude in millivolts plotted against flash intensity reveal that the mayfly eye is not sensitive, compared with that of the fly *Calliphora* or that of the locust *Valanga* (figure 11). The lateral eye units of *Atalophlebia* are still less sensitive than the dorsal ones, by a factor of about 10 at the 50% response level, when both are measured at equivalent photon flux at the peaks of their own spectral sensitivity curves, i.e. the lateral eye measured at 552 nm, the dorsal at 345 nm. Facet size is, of course, a factor contributing to this difference in sensitivity. All measurements were made on the dark-adapted day eye, and the attenuating yellow pigment may also be a factor.

Bumps

A better measure of sensitivity is obtained by counting 'bumps', which are the quantal electrical responses to the effective absorption of single photons. Quite large bumps can be recorded in mayfly dorsal eye units (figure 9*a*), but in our data a flux of more than 50 photons per facet at 345 nm is required to yield one bump per second in the receptor unit, which is apparently of six retinula cells.

At peak wavelength for each eye, the most sensitive eye that we tested by counting bumps is the superposition eye of the aquatic beetle *Macrogyrus* in the night state, which catches in each receptor unit about twice as many photons as fall on a single facet. Next are the fly *Calliphora* and the locust *Valanga*, catching about 50% of the photons falling on a facet. By day the mayfly ommatidium catches only 2% of incident photons. Clearly the mayfly dorsal eye is not a specialization for *sensitivity* and its small facet area does not wholly account for the difference.

Spectral sensitivity

Spectral sensitivity of single units was measured over a range of wavelengths at intervals of 15 nm. All units had similar spectral sensitivity curves, with peak near 350 nm, falling to zero near 450 nm (figures 9*c*, 10*c*, 12), confirming the earlier observation from the electroretinogram that the dorsal eye is purely a u.v. eye (Horridge & McLean 1978, fig. 10, p. 145).

The lateral eye is quite different, with the peak of the spectral sensitivity near 535 nm and a low second peak in the ultraviolet (figure 10*d*). We have encountered one receptor type in the lateral eye but have made only a preliminary search, and the lateral eye may well have several spectral types.

Angular sensitivity

The angular sensitivity curves of single units were measured with a stimulus subtending 0.2° at the end of a quartz light guide on a cardan arm. Fields are approximately gaussian in shape, with a width at the 50% level of 2.0–2.5° in the best cells (figures 10*b*, 13*a*). The insertion of the electrode could possibly defocus the optics and so increase the field width but is unlikely to improve the focus. We

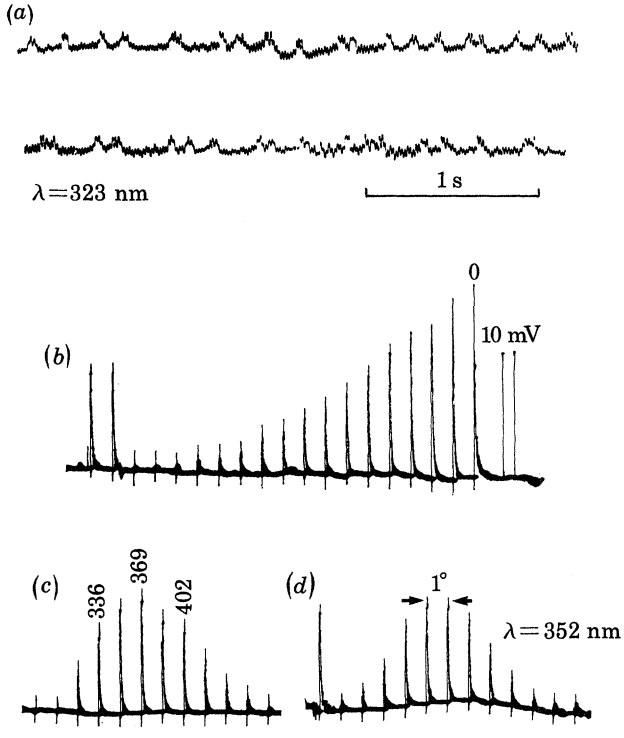


FIGURE 9. Electrical responses of a retinula unit. (*a*) Continuous record of bumps in response to continuous low intensity. (*b*) Responses to a series of intensities at 352 nm in steps of 0.2 logarithmic units of intensity (five such steps increases the intensity by a factor of 10), preceded by responses to two test flashes. The maximum intensity (response marked 0) was 1.55×10^{13} photons per square centimetre per second at the cornea. (*c*) Responses to a series of wavelengths (λ) of approximately similar intensities, each of which has been calibrated. The series was λ = 288, 303, 319, 336, 352, 369, 385, 402, 421, 436, 453, 473 nm, three of which are marked on the record. (*d*) Responses to a test flash followed by responses to a constant point source in steps of 1° passing through the previously determined axis of the cell.

can infer, therefore, that in the mechanically undamaged eye the acceptance angle is the best value, 2°. The factor that relates the width of the field to the behavioural response is the modulation of light in the receptor. For example an acceptance angle of 2° implies that a round black spot that subtends 2° at the eye against a bright background causes a maximum modulation of 50 % of the light at the receptor and would be easily seen (Horridge 1975). This calculation of modulation assumes that the retinula cell field is a symmetrical gaussian distribution.

Considering the possibility of interaction between adjacent ommatidia, the peculiar spread of cell 7 between two ommatidia (figure 2*k*) could possibly cause an elongation of the field of the receptor unit, but a careful search for this asymmetry led to nothing. The shape of the visual fields is apparently determined by conditions in the crystalline cone neck and not by any electrical interaction based on the asymmetrical anatomy of the retinula cells, especially cell 7.

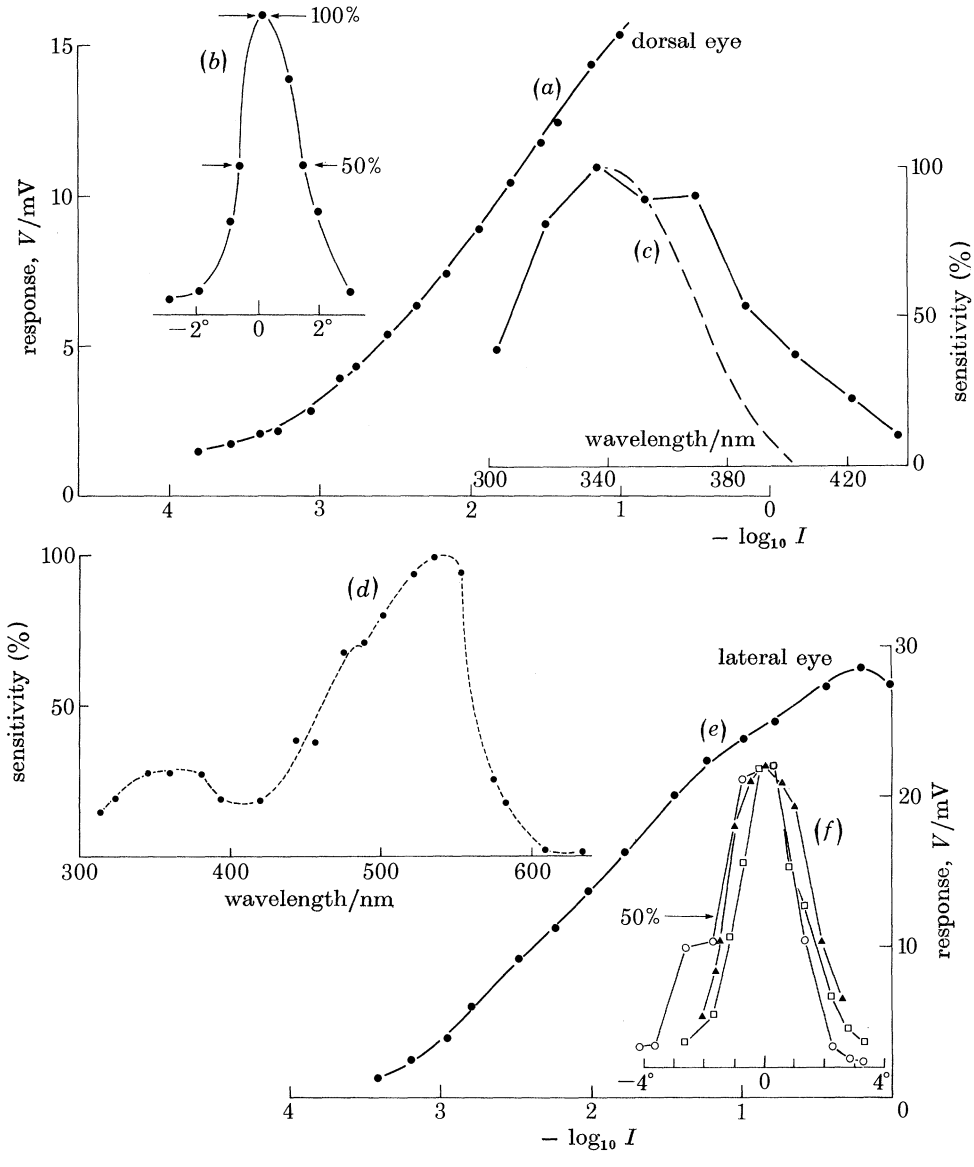


FIGURE 10. A series of results from a unit in the dorsal eye (*a*)–(*c*) and for comparison (*d*)–(*f*) in the lateral eye. (*a*) Peak response in millivolts plotted against log (flash intensity), the *V*–log *I* curve. (*b*) Angular sensitivity curve in the horizontal plane, with a width of 2.0° at the 50 % level. (*c*) Spectral sensitivity with peak near 340 nm, compared with the corresponding curve for a typical fly retinula cell 7 (dashed lines). (*d*) Spectral sensitivity of a lateral eye unit. (*e*) *V*–log *I* curve. (*f*) Three determinations of the angular sensitivity curve of lateral eye units.

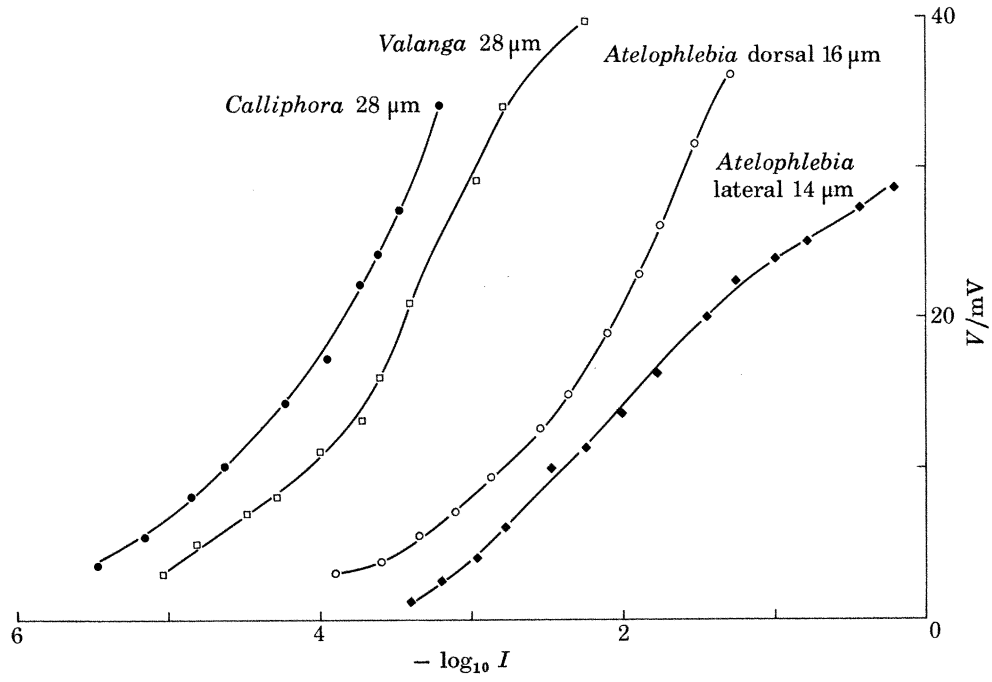


FIGURE 11. *V*–log *I* curves for typical units of (from left to right) the fly *Calliphora stygia* (wild type), with facet diameter 28 μm, the Australian locust *Valanga*, facet diameter 28 μm, the dorsal eye of *Atalophlebia*, facet diameter 16 μm, and the lateral eye of *Atalophlebia*, facet diameter 14 μm. The stimulus in each case was at the peak wavelength of the spectral sensitivity curve, and the intensities are corrected to give equal flux of photons per square centimetre per second at these different wavelengths. The relative positions of these *V*–log *I* curves therefore show relative sensitivities in terms of electrical response for comparable numbers of photons at the spectral peak.

Polarization sensitivity

Although many dorsal eye units were tested by rotating a Polaroid between the stimulus and the eye, none showed any sensitivity to the plane of polarization. For this test, Polaroid that is effective in the u.v. must be used. The result is consistent with the finding that Lucifer Yellow spreads between all seven cells in the rhabdom region, for if the retinula cells are coupled there will be a loss of the effect of the orientation of the microvilli of any one cell. Clearly the u.v. sensitivity is not related to an orientation behaviour dependent on the plane of polarization.

Spikes in the retinula responses

When responses reach an amplitude of about 10 mV, occasional units suddenly show spikes on the plateau of the response (figure 14). The spikes are each preceded by depolarizing postsynaptic potentials, and each spike is followed by an undershoot. The intensity range over which the spikes increase in frequency is about 1 logarithmic unit (tenfold), which suggests that the spikes originate in the lamina rather than the retina. At higher intensities the spikes are progressively

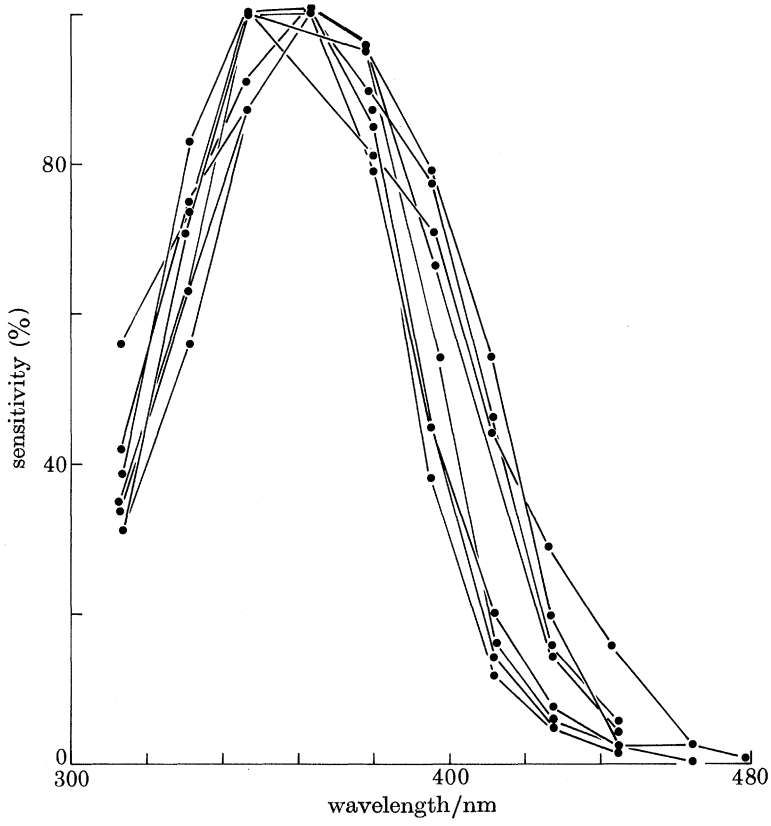


FIGURE 12. Spectral sensitivity curves of seven units of the *Atalophlebia* male dorsal eye.

replaced by a confusion of p.s.ps, resembling noise (figure 14*e*), which is again reduced at still higher intensities (figure 14*f*).

The small size of the spikes and the fact that they are rarely seen suggests that they are conducted electrotonically from far away, which also implies that they come from the lamina, and that they are attenuated when they reach the retina where they are superimposed on the receptor potential. There is also a notch on the rising phase, as seen in many insect retinula cells (e.g. in the locust: Matic & Laughlin 1981). The location of the notch is higher on the rising phase when the intensity is higher, as seen by comparing figure 14*a* with figure 14*b*. With a small increase in intensity the notch soon reaches the top of the rising phase, and it is seen no more at higher intensities. This observation that the notch on the rising phase has a latency component of its own which is distinct from the rising phase suggests that the notch represents the first of the series of spikes.

Apposition or superposition test

By usage the term apposition is applied to a compound eye where it is thought that light entering a facet affects only the receptors of the same ommatidium, whereas the term superposition is applied where a receptor cell can be excited by

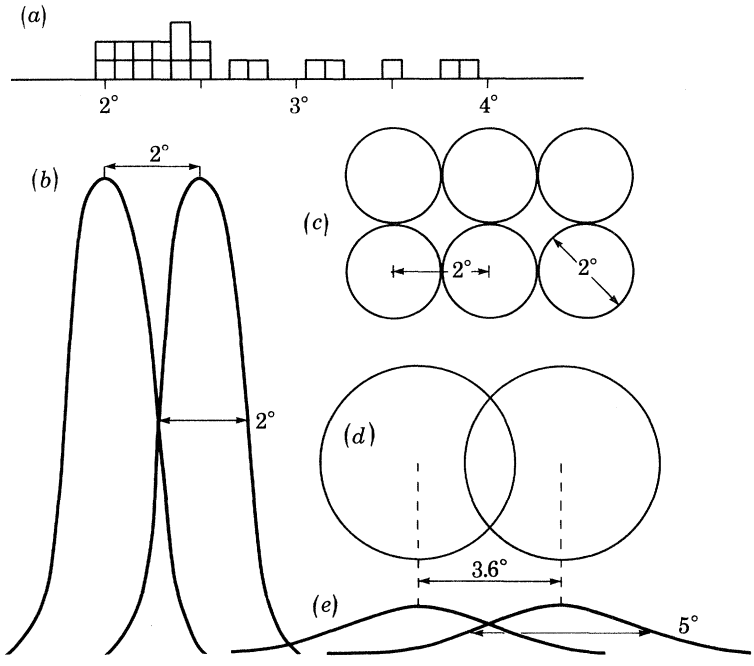


FIGURE 13. Fields and their overlap. (*a*) The distribution of the measured acceptance angles. (*b*) Two adjacent gaussian fields of the dorsal eye, based on the assumption that the narrowest examples in (*a*) are the best results. (*c*) A map of part of the dorsal eye with fields plotted as circles of diameter equal to the acceptance angle. The circles just touch in a square pattern. (*d*) Overlap of fields in the lateral eye, plotted as in (*c*) and to the same scale. (*e*) Overlap of gaussian fields in the lateral eye, to the same angular scale as in (*b*) and with 0.1 of the sensitivity to a point source on axis, as indicated by the position of the V–log I curves in figure 11.

light from one direction entering by many facets. The existence of many types of eyes that do not fit this sharp distinction is often ignored. At least three types of insect eyes, those of *Dytiscus* (Horridge *et al.* 1970), *Chauliognathus* (Horridge 1975) and *Chrysopa* (Horridge & Henderson 1976), have rhabdoms at two levels, suggesting that some retinula cells are apposition and others superposition, in the above usage of terms. Also, in the mayfly *Cloëon* (Horridge 1976) the rhabdom is at two levels in each retinula cell, so that an experiment designed to distinguish between apposition and superposition could be misleading.

In *Atalophlebia*, however, the anatomy is sufficiently clear for a simple experiment that rules out the superposition option. The method is to move a fine wire across the surface of the eye while recording from a single unit. The diameter of the selected wire is the width of three facets as a compromise between the mechanical stability of the wire and the shading of too many facets at once (figure 15). As the wire shadows one particular facet the response shows that the wire has cut out more than 70% of the light reaching the unit recorded from (figure 16*b*, *d*, *e*, *f*). The percentage of light shadow cut out by the wire is calculated from the V–log I curve, which is measured at the same time (figure 16). As the reader may easily

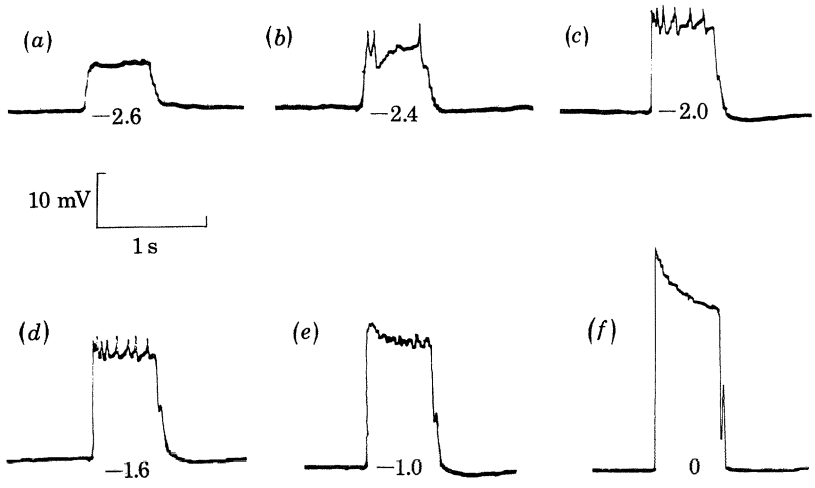


FIGURE 14. Small spikes in the retinula cell response, apparently efferent from the optic lobe but seen only occasionally. (*a*) The sustained response is the depolarization in response to a flash at the log. intensity of −2.6. (*b*)–(*d*) With increase in log. intensity in the range −2.4 to −1.6, the frequency of the spikes covers its full range over an intensity range that is small compared to that of the sustained depolarization. (*e*), (*f*) At higher intensities the spikes drop out.

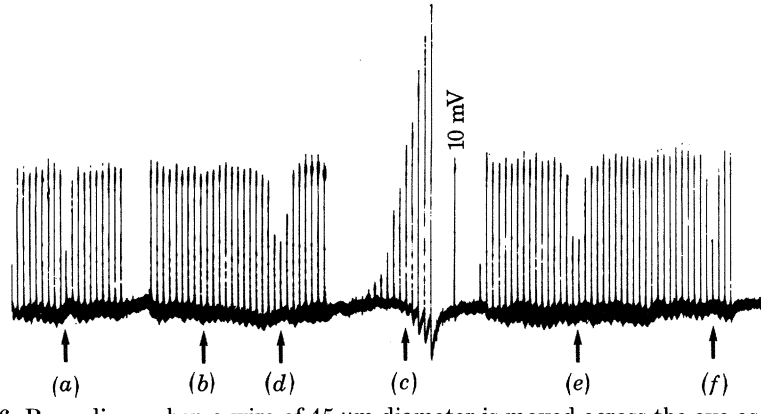


FIGURE 16. Recordings when a wire of 45 μm diameter is moved across the eye as in figure 15. (*a*) Test to find the ommatidium from which recordings are being made. (*b*) Responses with the wire over regions of the eye adjacent to the critical region. (*c*) Series of responses to intensity steps of 0.25 logarithmic units (i.e. four such equal steps increases the intensity by a factor of 10). (*d*), (*e*) Movement of the wire in steps of 25 μm. (*f*) Repetition of (*d*) in steps of 25 μm with the wire adjusted closer to the cornea.

see by moving one hand over this page, the distance moved across the eye over which the wire has any effect is the diameter of the wire plus the diameter of the facet. This distance is measured the more accurately the closer the wire is brought to the cornea by careful adjustment (figure 17*f*).

Even when centred over the unit recorded from, the wire obscures at most 75 %

of the light. A superposition image however, is ruled out, because in that case a thin wire moved across the eye surface has negligible effect.

This experiment is the converse of that used to demonstrate superposition optics in the skipper butterfly eye while recording from a single retinula cell; in that case a slit 60 μm wide was moved across the eye (Horridge *et al.* 1972, fig. 16, p. 468).

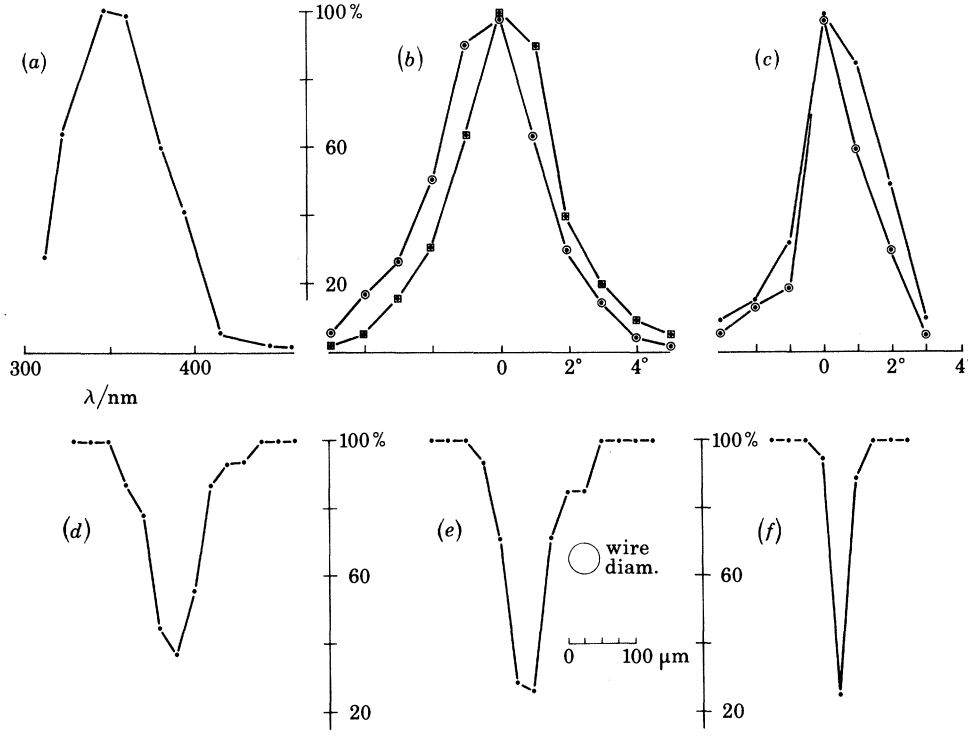


FIGURE 17. Calculated result of the experiment in which a wire 45 μm in diameter is moved across the eye surface in steps of 25 μm. First the unit was characterized without the wire. (*a*) Spectral sensitivity, (*b*) Angular sensitivity in the horizontal plane, two runs. (*c*) Angular sensitivity in the vertical plane with mean acceptance angle 2.2°, two runs. Then the shadowing effect of the wire was measured. (*d*) Decrease in effective intensity at the receptor when the shadow of the wire passes, calculated from (*c*) and (*d*) in figure 16. (*e*) Repeat of (*d*). (*f*) Repeat with the wire adjusted closer to the eye. The shadowing effects of the wire are calculated from records (*c*)–(*f*) in figure 16.

Interommatidial angle

A dark symmetrical pseudopupil is visible (figure 18, plate 6) and the interommatidial angle in all parts of the eye can be mapped by methods already described (Horridge 1978). Chalk particles are blown upon the eye surface to identify individual facets, and the eye is photographed at a series of regularly spaced angular intervals. The position of the centre of the pseudopupil shows which facet has its axis along the axis of the camera. The angle between the ommatidial axes can be

derived directly from the movement of the centre of the pseudopupil across a known number of facets as the eye is rotated.

In the central region of the eye the angle between adjacent ommatidial axes along a line of facets is 2.0°, the same as the acceptance angle (figure 19). In the lateral eye the degree of overlap is greater, strongly suggesting that the two parts of the eye are adapted to different visual functions.

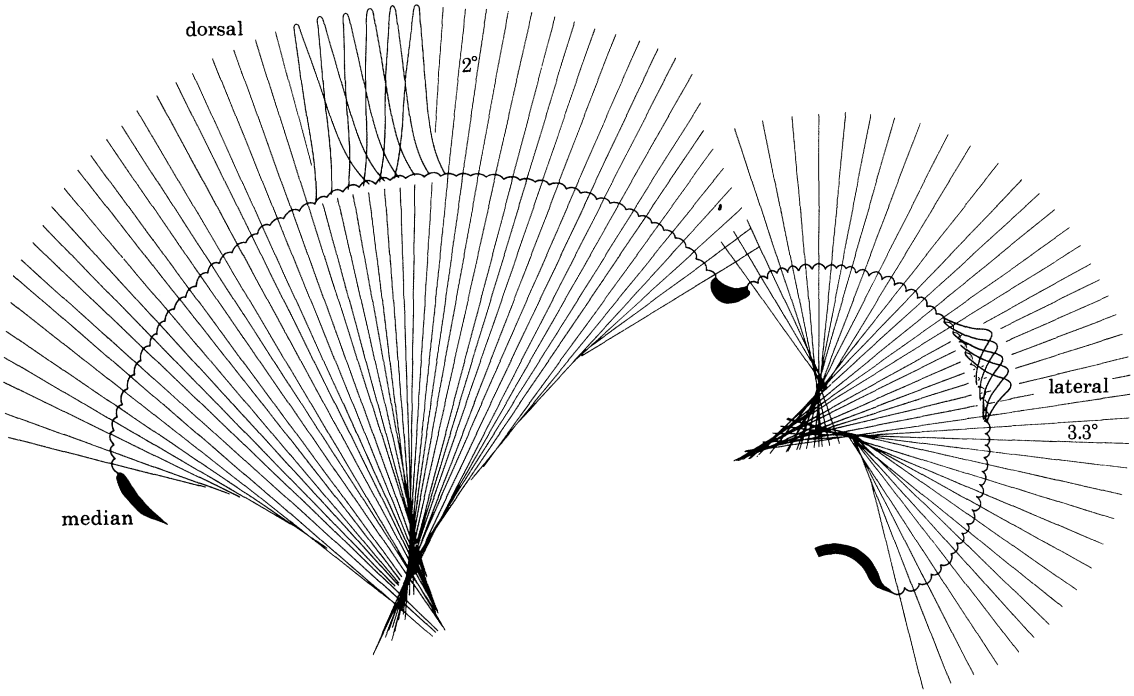


FIGURE 19. Diagram of axes and fields of ommatidia in a section through both eyes. This diagram is based upon a section as in figure 3, with intervals between facets drawn as if along a facet line, approximately in the normal postural position. Fields are narrower and overlap less in the dorsal eye than in the lateral part.

From the fields determined electrophysiologically we can now draw a diagram of the dorsal eye as a series of sampling stations, showing the overlap of the fields along a line of facets (figure 13*b*, *c*). The facet lines are approximately 45° to the main axes of the head (figures 15, 18). Then $\Delta\rho/\Delta\phi \approx 1$ in the central region of the dorsal eye as in some insects, e.g. large flies, butterflies, mantids and dragonflies (Horridge 1978, 1980). In the central and upward-looking region of the lateral eye of *Atalophlebia* the interommatidial angle is 3.3–3.6° and the acceptance angle is 2.9–3.5°. Therefore we are justified in regarding the dorsal eye as a specialized acute zone or foveal region, defining a fovea in a compound eye as a region of more receptors per unit solid angle.

Optics

Besides the anatomical description and the electrophysiological recordings from primary receptors, two observations on the optics of the eye have proved possible, both strengthening the idea that the eye functions in the u.v. by means of light guides crossing the clear zone, while yellow light floods the eye.

The normal eye

The pseudopupil occupies about 20° on the eye (figure 18), and therefore its whole dark area cannot be the hollow of the eye seen down the centres of cones, because the acceptance angles of the individual facets are only 2°. The size and appearance of the pseudopupil owes little to the corneal lens, as shown by coating the living eye with nail varnish, which neutralizes most of the power of the corneal lens. With a layer of varnish on the cornea the pseudopupil hardly changes in appearance or diameter and it therefore depends more on $\Delta\phi$ than $\Delta\rho$. Possibly the dark hue of the pseudopupil derives from the dark pigment between the cones. If that is so, we see light that reaches this pigment through the side of the cone and that is reflected back to the observer by the same path. On this model, only the black centre of the pseudopupil is the visually effective inside of the optical system.

By making a small hole in the side of the eye, most readily done when it is frozen, it is possible to look inside the clear zone with a dissecting microscope. By direct observation, it can be seen that a distant point source directed at the eye produces a broad patch of yellow light on the rhabdom layer, with little sign of focusing. The path of the u.v. light is, of course, not seen.

The isolated cornea

The cornea is readily cleaned out with a fine paint brush. It is then mounted with a drop of very dilute (10 % (by volume)) glycerine inside the cornea, on the underside of a cover slip over the hollow in a well slide, so that it can be examined on its inner surface under a compound microscope without drying out while the outer surface of the cornea looking at an object is not wetted (figure 20).

Each convex facet acts as a converging lens. To measure the focal length the cleaned cornea is mounted on the stage of a compound microscope with the condenser removed. Beneath the stage is an object consisting of a letter K printed on a piece of glass. The microscope is focused on the image of the K inside the cornea (figure 22, plate 6). The distance x from the focal plane *to the nodal point* (f in figure 21*b*) is given by the relation:

$$\text{image distance } x = \text{image size} \times \text{object distance/object size},$$

with the assumption that the object is at a relatively great distance.

The distance between the image and the cornea is measured directly on the vertical movement of the microscope. To calculate the position of the focal plane in the intact eye we assume the cornea is a convex–plane lens of refractive index 1.5 and that the refractive index of the cone is 1.4.

As indicated in figure 6, on a section of the eye, the focal plane lies at 85 μm from the external surface of the cornea, nicely within the narrow region of the cone

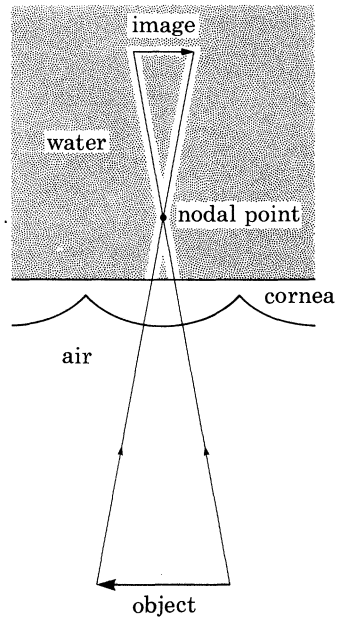


FIGURE 20. The formation of the image (seen in figure 22) by the corneal lens, which is inverted on the microscope stage. The image size is measured in water and therefore the nodal point lies inside the eye. If the object is relatively far away the focal length is the distance from the nodal point to the image.

tip, *for visible light*, and it will be a little closer to the cornea for u.v., depending on the dispersion. This result eliminates the possibility of a sharp focus of any wavelength on the rhabdom layer by reflecting optics, and the field of view measured with a point source is formed as in a typical apposition eye, in this case by convolution of the Airy disc with the distribution of the light-catching properties of the neck of the cone (figure 21).

Light guides in embedded eyes

Light guide action can be observed directly in dorsal eyes that are lightly fixed in glutaraldehyde and embedded in resin. The tissue is not treated with osmic acid. The block, embedded in Araldite, or preferably in glycol methacrylate, is sectioned transversely from the rhabdom layer in the direction of the cornea until the rhabdom layer is just cut away and the sections lie across the lower level of the clear zone. The remainder of the eye, consisting of cornea, cones and clear zone, is then pushed out of the resin block, and carefully checked to see that the surface of the cornea has come away clean from the resin (see figure 15). Because each facet is convex on the outside and flat on the inside, it remains a converging lens when the water within the eye is exchanged for resin (refractive index *ca.* 1.5). However, because the cone becomes impregnated with resin below the flat inner surface of the cornea, there is an increase of the focal length in the ratio 1.5/1.4 if the refractive index of the normal cone is 1.4 and if the impregnated cone is 1.5. Possibly, therefore, the resin *reduces* the effectiveness of the cone tip as a light guide

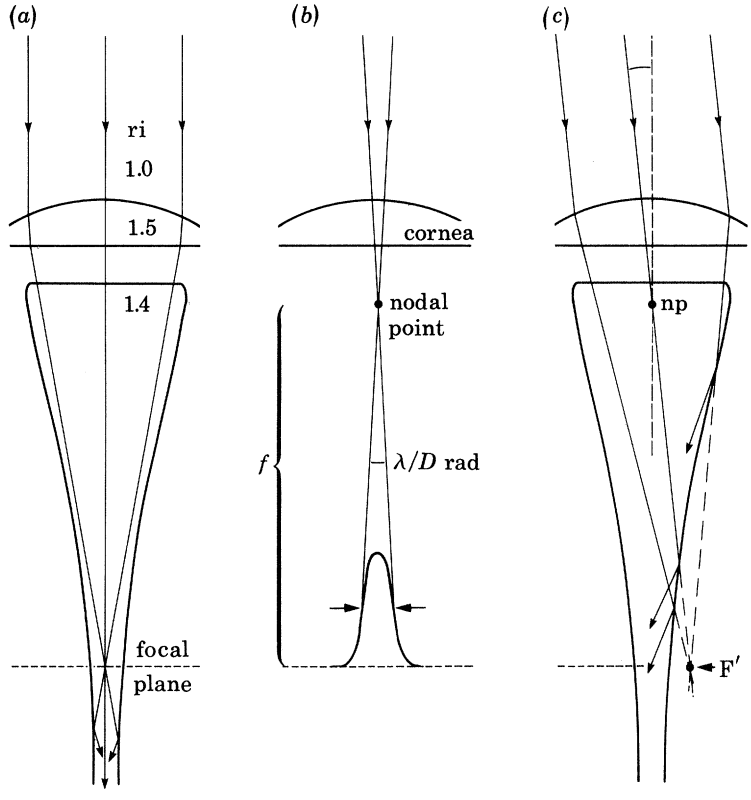


FIGURE 21. The orgin of the visual field of the ommatidium. (*a*) A parallel beam from a distant point source is focused in the neck of the cone in the focal plane. (*b*) The image of a point source is a point spread function (the Airy disc) subtending a minimum angle of λ/D radians at the nodal point, when the angular width is measured at the 50 % level (f, focal length). (*c*) When the point source is displaced, as in the measurement of the field, the focus moves out of the neck of the cone and rays no longer reach the receptors. The field is therefore the result of the convolution of the Airy disc with the effective capture cross-section of the neck of the cone.

by defocusing the lens. A positive result with an eye in resin therefore errs on the right side.

The embedded eye slice is now mounted cornea side up on a thin cover slip and inverted over a well slide so that the cut ends of the columns which cross the clear zone can be examined under a compound microscope. The microscope condenser is removed and the eye slice is illuminated by approximately axial light which enters the ommatidia orthodromically by the cornea. The light source is the end of a flexible light guide. With a × 10 objective one sees a spot of light behind each facet in the region of the focal plane of the corneal lens. This bright spot moves when the light source below the stage is moved, and clearly it is the real image of the source formed within each ommatidium by its own lens (figures 20, 22). With a × 40 or oil immersion objective, however, the columns crossing the clear zone in a square array can be readily seen, diverging towards the cones and held in place

as a square array in the resin. Depending on the focus of the microscope and the position of the light source, the columns can look dark or bright.

In one region of the eye a small group of the columns are bright when the high power objective is focused exactly on their cut ends (figure 23, plate 6). The light source on the outside of the eye is now moved sideways. What one sees is absolutely critical for understanding the eye function, and figures 3 and 6 may be helpful. The bright ends of the light guides remain stationary as the light source is moved, showing that they are not images. Instead, the ends of columns that were formerly dark, on the edge of the group that showed bright, now become bright. This happens on the side towards which the source is moved, and correspondingly columns on the other side of the group darken as the source moves away from their axis (figure 23*a–e*). Clearly the light guide action can be observed with visible light. The columns will necessarily act more effectively as light guides in the ultraviolet because the wavelength is shorter in relation to the diameter of the light guide, and they will also act more effectively in the normal eye when the lipids are present and water is the medium, because the refractive index difference between light guide and medium then will be greater than when impregnated with resin.

In the embedded eye we found no improvement in the light guide effect by using blue rather than white or yellow light; this is explained as an effect of leakage of the yellow pigment into the cones during the embedding process.

Absorption spectrum of the female's wings

As a possible reason why the dorsal male eye is sensitive to u.v. but not to visible light we considered the possibility that u.v. vision enables the male to see the female against the background of the sky more effectively than she can be seen by predators such as fish. Mayflies have transparent wings in both sexes; so this theory predicts that the female wings should absorb ultraviolet but should be transparent in the visible range. Unfortunately the prediction is not fulfilled. We examined the gauzy wings of many insects including mayflies in a spectrometer down to 200 nm but found none with marked absorbance in the u.v. The absorbance of *Atalophlebia* wings rises slightly in the range 200–300 nm, but there was no difference between males and females.

Discussion

Several different lines of investigation leave no doubt that the dorsal eye of the male *Atalophlebia* is a specialized acute region that operates by means of light guides that take ultraviolet light across the clear zone.

First, the progressive taper of the cone to a thin column across the clear zone contrasts strongly with the shape of the cone in a typical superposition eye, and it looks like a light guide. Secondly, the strong corneal lens and the position of its focal plane in the neck of the cone are compatible with the narrow angular sensitivity only if the column across the clear zone acts as a light guide. It is inconceivable that any other mechanism could preserve the excellent resolving power that is demonstrated by recording from single units. Thirdly, superposition in any form is ruled out by the shadow experiment with the wire and the layer

of yellow pigment overlying the rhabdom layer. In any case reflecting superposition could not yield narrow fields in the presence of the corneal lens, and refracting superposition is ruled out by the shape and homogeneity of the cone and by the distribution of yellow pigment around the narrowing part of the cone. The direct demonstration of light guide action in the fixed and embedded eye is then merely visual evidence of what has already been inferred for u.v. light.

Use of terms

An apposition compound eye is one where each ommatidium is always a separate optical unit, as in most Hymenoptera, Hemiptera, Orthoptera and Lepidoptera. Apposition eyes are not necessarily all well focused, especially in crepuscular species. A superposition eye is one where light crosses between ommatidia at least in the dark-adapted night eye as part of an optical system that increases sensitivity. We would not regard the light-adapted day eyes of moths and many beetles as apposition even though the ommatidia are optically separated by screening pigment. Superposition eyes need not be well focused either. The term 'clear-zone eye' has been introduced because it is *based upon anatomy and carries no connotation about function in eyes that have not been examined physiologically or optically*. The *Atalophlebia* dorsal eye illustrates that a clear-zone eye is not necessarily a superposition eye for the primary visual function. The term 'superposition' assumes the very point that is to be tested in examples where the anatomy of clear-zone compound eyes has turned out to be varied and subtle.

In various clear-zone eyes, the column of the ommatidium that crosses the clear zone consists of either the extensions of the four cone cells or the long necks of the retinula cells, or both. We usually have no idea whether it is 'crystalline' or not, and so it is safer to use the anatomical terms 'retinula cell column' and 'cone extension' rather than 'crystalline tract' which carries an assumption of high refractive index. Again, to call the structure a light guide in an untested eye type is to assume the very point at issue. These structures are light guides in *Atalophlebia* but the same function can hardly be assumed in other groups until investigated. For example, in skipper butterflies the retinula cell column has no light guide action (Horridge *et al.* 1972). Also there may be other forms of light guide in the moth *Ephestia* (Horridge & Giddings 1971) and in some night-flying beetles, e.g. *Gyrinus*, there is an elongated part of the rhabdom which looks as if it might be a light guide across the clear zone (Burghause 1976).

Ultraviolet vision and lens resolving power

The theoretical minimum angular width of the Airy disc (subtended at the nodal point, figure 21*b*) for an 18 μm aperture is 1.17° at the 50% intensity level for a wavelength 370 nm. Inevitably there is a compromise between the sensitivity and the conservation of the lens resolving power at the next stage, when the light is channelled into the light guide, as set out in several earlier papers (Snyder 1977; Horridge 1980). If all the light in the Airy disc is caught by the light guide the calculated acceptance angle $\Delta\rho$ is at least the full angular width across the base of the Airy disc, giving in our case $\Delta\rho' = 2.3°$. The measured value of $\Delta\rho = 2°$

therefore shows that there has been some sacrifice of sensitivity for the sake of resolution i.e. even with a point source on axis, the edges of Airy disc do not pass down the light guide. If we take the above values for *Atalophlebia* facet diameter and the measured value of $\Delta\rho = 2°$, and assume perfect optics, with the neck of the cone assumed to be an absorbing disc with a gaussian distribution of light capture, we find that the 50% capture level in the effective neck of the cone subtends an angle of 1.6° at the nodal point, using the theory set out in Snyder (1977, p. 165) or Horridge (1980, pp. 297–298). In fact an effective aperture of this size would be readily located in the narrow neck of the cone, and its exact size could be under the control of the surrounding yellow pigment.

The above calculation shows that the measured fields are close to the limit set by the facet diameter, wavelength, and the match between the absorbing aperture and the Airy disc. Therefore this eye certainly takes advantage of the greater resolving power afforded by the use of u.v. light. The visual task promoting natural selection is the search for the female against the sky. A large foveal region is an obvious advantage, as is u.v. vision to reduce facet size and so increase the number of sampling points. But we note that $\Delta\rho/\Delta\phi \approx 1$ and that the eye parameter ($D\Delta\phi = 0.66$ μm) is much greater than expected for a diffraction limited eye with $\lambda = 360$ nm (Snyder 1977; Horridge 1978). For the visual task of seeing a moving spot against a bright background, it is likely that the limiting factor is the modulation in the photoreceptor caused by a small dark spot against a bright background.

The overlap of visual fields in the dorsal eye, with the interommatidial angle equal to the acceptance angle, is in fact smaller than that in the fovea of many insects that are active in normal daylight. Examples are the locust (Horridge 1977, fig. 11), two Australian native bees, dragonflies and mantids (Horridge 1978, 1980). All the examples that have been mapped, however, are of eyes larger than those of mayfly. It appears that, even with small facets that are made possible by the use of u.v. light, the mayfly is only just capable of generating sufficient sampling points to cover the essential dorsal hemisphere in its visual world with $\Delta\rho \approx \Delta\phi$.

Exactly how the parameters of the retina are optimized by natural selection in the task of catching the female in flight is still in doubt because the compromise between having maximum number of sampling stations and maximum resolution of each is not understood for the particular task. Perhaps the best that we can do at the present time is to point out that, when the interommatidial and acceptance angles are equal, the intersection of adjacent fields is in the middle of the steep part of their boundaries so that a small contrasting object moving from one field to the next has a strong differential effect over a reasonable angular range.

The dorsal eye is a fovea

Both male and female *Atalophlebia* have lateral eyes with upwardly looking ommatidia, as shown in figure 18 by the position of the pseudopupil which can be seen on the lateral eye, with its optical axes parallel to those in the pseudopupil of the dorsal eye. The male has an additional and much greater eye region looking upwards (figure 19). The u.v. sensitivity and the greater facet size are signs of

greater lens resolving power than in the lateral eye. The interommatidial angle is also smaller in the dorsal than in the lateral eye. Like a fovea, the dorsal eye is an eye region for a special task, with greater lens resolving power and also greater spatial resolution conferred by the u.v. sensitivity. There is no indication, however, that the enlarged dorsal eye is a specialization for low light levels.

Divided eyes

Insects with divided eyes always have a specialized dorsal part of the eye looking dorsally and the more lateral part of the eye looking laterally, forwards and downwards. Examples are the two groups of mayflies with divided eyes, the owlflies, e.g. *Ascalaphus* (Neuroptera) (Schneider *et al.* 1978), some biting flies (Simuliidae) (Kirschfeld & Wenk 1976) and other flies, e.g. Bibionidae. Some dragonflies have an almost divided eye, with a dorsal region of larger facets of a brown or reddish colour. In all these examples the specialized dorsal part of the eye is used to spot and pursue small objects such as the female or the prey against the background of the sky, whereas the more typical part of the eye is used to see against a background of green undergrowth and disruptive contrasting objects. The lesson from *Atalophlebia* is that the enlarged dorsal part of the eye is a way of forming an acute zone of higher resolution. In the few examples studied, the dorsal ommatidia are blue- or u.v.-sensitive, presumably because the background is blue and also to increase resolution.

Why have a clear zone and light guides?

If the whole advantage of the dorsal eye is simply that it is a region of greater lens resolving power and spatial resolution, then the rhabdoms could be located at the cone tips as in the foveas of mantids or dragonflies and as in the lateral eye. The theory that there is superposition of yellow light, which is poorly focused but which regenerates the visual pigment by photoreisomerization (Horridge & McLean 1978), is the only adequate explanation that has been proposed for the light guides. This idea certainly agrees with the general observation that the lateral eyes are usually black or brown, and in different groups of mayflies the greater the development of the dorsal eye in the male the more likely it is to be red, or yellow in the extreme examples (Clifford 1981). We can suspect any yellow or red compound eye, including the red eyes of dipteran flies where the theory was proposed (Hamdorf *et al.* 1973), of being specialized for photoreisomerization. For the owlfly Schneider *et al.* (1978) proposed the same mechanism on the basis of the properties of the visual pigment. The superposition mechanism at long wavelengths is an obvious way of concentrating the available yellow light when the target is a black spot against the sky. The trouble is that we have not been able to confirm this theory so far by direct tests with microelectrodes on the retinula cells of *Atalophlebia*.

Ultraviolet vision in relation to behaviour

The usual interpretation is that the dorsal eye aids discrimination of the female as she flies through a swarm of dancing males. The proportion of u.v. in the

background light from the blue of the sky increases with the angle from the Sun and therefore is large as the Sun sets or rises. Clifford (1981) tells me that many field observations indicate that the enlarged dorsal eyes of many mayfly genera are not necessarily an adaptation to crepuscular activity, contrary to our earlier assumption (Horridge & McLean 1978). Male mayflies with large dorsal eyes perform their nuptial dance almost vertically up and down but males with less developed eyes dance at 45° to the vertical. With many exceptions, there is a tendency for baetids and leptophlebiids to swarm at various times of day, often in broad daylight, sometimes at a distance from water (Clifford *et al.* 1979). With the evidence that we have available little more can be said about the part played by the dorsal eyes or about u.v. vision in behaviour. It is now time for experiment in the field, for example study of dance behaviour of males with eyes partly painted over and investigation of the effect of yellow illumination on visual threshold in the ultraviolet. It now seems possible that the u.v. sensitivity is no more than an adaptation that increases the number of facets on the dorsal eye above that possible for visible light, with the same resolving power.

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Explanation of abbreviations used on figures

A	anterior	P	posterior
a,b,c,	types of accessory pigment cells	pb	pigment bag
apc	accessory pigment cell	ppc	principal pigment cell
c	cornea	ppcn	principal pigment cell nucleus
cc	crystalline cone	rc	retinula cell
ccn	cone cell nucleus	rcn	retinula cell
ecs	extracellular space	rcc	retinula cell column nucleus
de	dorsal eye	rh	rhabdom
fc	filament of the cone	ri	refractive index
le	lateral eye	scz	subcorneal zone
ncc	neck of the cone cells	t	trachea
np	nodal point	tc	tracheal cell
oc	ocellus		