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STRUCTURE OF THE RETINAE OF THE PRINCIPAL EYES OF JUMPING SPIDERS (SALTICIDAE: DENDRYPHANTINAE) IN RELATION TO VISUAL OPTICS

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INTRODUCTION

Jumping spiders (Salticidae) are among the most vision-dependent animals. Much of their behaviour is initiated by visual stimuli, and controlled in its course by visual information. This behaviour includes prey-catching, courtship (which involves mate-recognition and an elaborate display of visual signals), escape from enemies, and the ordinary business of getting around the environment. To guide this repertoire the animals have eight simple eyes (Fig. 1). These are divided on functional and anatomical grounds into two groups: the principal eyes and the side eyes. The former group consists of a single pair of large eyes (antero-median, AM) which have movable retinae and, as we shall see, retinae with a very complicated structure. The side eyes (antero-lateral, AL; postero-lateral, PL) are relatively small, incapable of movement, and have a simple, single-layered retina.

The principal eyes are aptly named as they play by far the largest role in the control of the animals' behaviour. Homann (1928) and Crane (1949) found that the only effect of blinding all the side eyes was to prevent the animal seeing and orienting towards a stimulus (e.g. a moving fly) which lay outside the field of view of the principal eyes. But, blinding the principal eyes prevented the occurrence of all the other stages of prey-catching (stalking, jumping, etc.), as well as the complex series of visually directed activities involved in courtship. None of these activities was affected by blinding the side eyes.

The structure of the principal (AM) eyes of jumping spiders has been described twice, by Scheuring (1913–14) and Homann (1928). Scheuring found that the eyes were long tubular structures with a retina consisting of a narrow strip of receptors. He also described the harness of muscles which move the eyes—or rather, the retinae since the lenses are fixed to the carapace. Homann determined the focal lengths of the eyes, using an ingenious hanging drop technique, and gave a slightly more detailed description of the retinae. Both Scheuring and Homann assumed that the retina contained a single layer of receptors, like other retinae, but the present author found in the course of a general histological examination of the eyes that this was not the case. In frontal (horizontal) sections of the retinae one can see four separate mosaics of receptors, one behind the other along the optic axis (Fig. 3).

This finding raises a number of questions. As the total thickness of the retina is

large compared with the focal length of the eye (about 12 %) it is likely that if an object in space is brought to a focus on one of the layers it will be out of focus on the others. Do the other layers receive better images of objects at different distances, or are they situated in the focal planes for light of different wavelengths? It is clear from the work of earlier authors that jumping spiders possess, and make use of, extremely high visual acuity, so these questions are worth asking; the animal is unlikely to provide itself with three out-of-focus retinae.

This paper contains a new description of the retinae of the AM eyes of two species of jumping spiders, and a re-examination of their optical design. The intention is to discover from a consideration of the nature and quality of the image in the region of the retina, what reasons there may be for the retina having this unique structure.

MATERIALS AND METHODS

Materials

Jumping spiders of the sub-family Dendryphantinae were collected locally. Adult *Phidippus johnsoni* (Peckham) were collected during April and May from grassy hillsides, and *Metaphiddipus aeneolus* (Curtis) were obtained at most times of the year by shaking trees and scrub. They were identified from Peckham & Peckham (1909). The spiders were kept in Petri dishes containing pieces of damp cotton wool until required, and were fed on houseflies (about one a week). Those that were not used survived many months under these conditions. *P. johnsoni* is a large species, 8–11 mm. long, and *M. aeneolus* is half this size, 4·5–5·5 mm. The animals used here were all female.

Methods

Histology

For each species, two complete series of sections of the cephalothorax were made (frontal and transverse). The material was paraffin-embedded, sectioned at 10 μ, and stained with either Mallory's Trichrome, or haematoxylin and eosin. The animals in each species were selected to be as nearly as possible identical in size to one another and to the animals later used in optical experiments. Reconstructions of the retinae and optic glomeruli were made by photographing every section in each series, enlarging and tracing each negative, and superimposing the tracings. This process is tedious, and complete reconstructions were made only of the right AM eye and right optic glomerulus of *M. aeneolus*.

Optics

The methods used here to determine the optical properties of the eyes are similar to those devised by Homann (1928). To measure the focal lengths of the lenses, the part of the carapace containing the eyes was removed from a freshly killed spider, cleaned of all tissue except the lenses, and suspended from a hanging drop of spider Ringer (Parry & Brown, 1959). The lenses thus had their corneal surfaces in air, and their internal surfaces in fluid, as in the intact animal. The lenses had to be judiciously oriented until the optic axes were at right angles to the microscope slide. The focal length of each lens was then found by measuring with a microscope the size of the image of a target of known dimensions at a known large distance in front of the cornea;

the first focal length ($-f_1$ = the posterior nodal distance) was then obtained from the magnification ratio. In addition, the axial distance in Ringer from the back of the lens to the focal plane—i.e. the position of the image of sharpest microscopic appearance—was measured, and from this measurement and the focal length the position of the nodal point in the eye was obtained (Fig. 10). The same measurements were also made with the Ringer on *both* faces of the lens; these are not physiologically significant, but were useful in determining the refractive indices of the lenses. All distances measured through Ringer are 'apparent' distances, and gave real distances when multiplied by 1·335, the refractive index of the Ringer. The radii of curvature of the same lenses were measured by orienting the pieces of carapace in Ringer so that the lenses appeared in profile; then, after removing obstructing cuticle, they were photographed through the microscope. From the radii of curvature, measured from these photographs, and the image-lens distances, the refractive indices of the lenses were obtained by calculation (see Table 3).

The retinae were examined using the eyes' own optics. This was done with the aid of the ophthalmoscope described in the next paper (Land, 1969). This instrument was also used to determine the exact position of the focal plane in the eye, and the principle of this determination will be given in the Optics section of the Results.

RESULTS

Anatomy of the visual system

General description

Figure 1 is a diagram of the cephalothorax of a jumping spider seen from above, showing the positions of the eyes and their fields of view in the horizontal (frontal) plane. Each eye consists of a cornea, which is a transparent, convex region of cuticle, beneath which lies the lens, which is not chitinous, and behind that a cellular 'vitreous'. These structures are common to all eyes; the differences lie mainly in the fine structure of the retinae. In the retinae of the side eyes the receptors are arranged in a single-layered hexagonal lattice, their receptive segments having their long axes all perpendicular to the curve of the retina (see Homann, 1928, figs. 12–15). Each receptive segment is separated from its neighbours throughout its length by pigment contained in processes of cells whose nuclei lie beneath the retina. The structure of the receptors is interesting in that the receptive part of the cell is the central not the terminal portion; the cell bodies in fact lie outside the eye, to the side, and send in processes over the front of the retina which expand to form the rhabdoms and then contract again to form the optic nerve fibres (Fig. 2*b*). This contrasts with the situation in the AM eyes and with the structure of photoreceptors in most other animals (Fig. 2*a*). Behind the retina each optic nerve fibre terminates in a discrete patch of neuropile, where it interdigitates with one, or possibly a small number of second-order fibres. This region is the first optic glomerulus—the 'erste Sehmasse' described by Hanström (1921, 1928). As can be seen from Fig. 1 the side eyes between them cover almost the entire 360° around the animal in the horizontal plane, with a small (25°) region of binocular overlap in front. A complete account of the fields of view of these eyes is given by Homann (1928).

The AM eyes differ from the side eyes in three main ways: (i) They have a much

longer focal length than the side eyes, giving a more magnified image covering a smaller field. (ii) The histological structure of the receptors is different, the receptive part being in this case terminal, separated from the axon by the soma. The receptive segments are not individually surrounded by pigment, as in the side eyes, but the entire array is backed by pigment, more like vertebrate retinae. (iii) The AM eyes

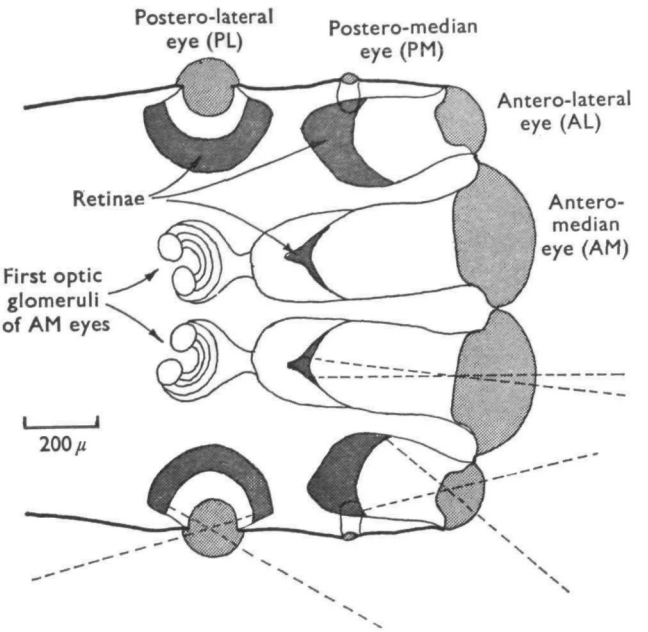

Fig. 1. Diagrammatic frontal section of the cephalothorax of *M. aeneolus*, showing the positions of the eyes and their fields of view in the horizontal plane.

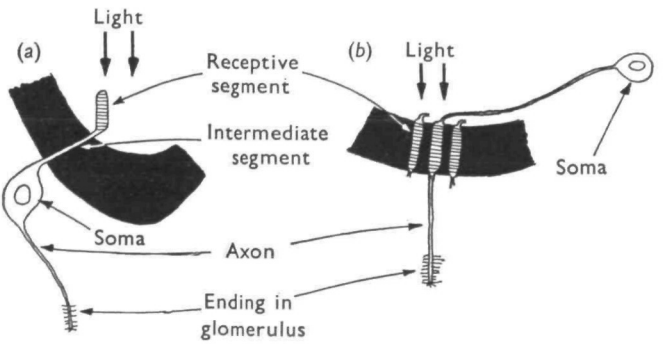

Fig. 2. (*a*) Structure of a receptor in a principal (AM) eye. (*b*) Structure of a receptor in a side (AL or PL) eye.

are movable. The eye tubes pivot about the regions immediately behind the lenses, and a set of six eye muscles permits each retina to move vertically, laterally or obliquely in one plane, and in addition the retinae can be rotated about the visual axes (Scheuring, 1913–14; Dzimirski, 1959; Land, 1969). The side eyes are fixed.

Structure of the retinae of the antero-median eyes

The receptive part of the retina consists of a long, narrow strip of receptor endings lying in a V-shaped depression at the back of the eye, the walls of which are formed by pigment cells. The terminal segments containing the rhabdomeres are embedded in a rather deeply staining matrix which has a somewhat fibrillar appearance by light microscopy. Professor R. M. Eakin, who is engaged on an electron microscopic study of these eyes, tells me that this matrix consists of an intertwining tangle of processes from the pigment cells underlying the receptive part of the retina. The cell bodies of the receptors lie behind the pigment layer, and are connected to the receptive segments by thin intermediate segments. There is a sharp discontinuity in the appearance of the parts of the cell designated 'receptive' and 'intermediate' segments, the former being thicker and more highly refractile, and in all except the most superficial layer of cells only this terminal portion of the cell is oriented towards the lens; according to Professor Eakin, only this part of the cell contains arrays of microvilli. The long dimension of the retinal depression is vertical (dorso-ventral), and in *M. aeneolus* it extends for approximately 200 μm. with a width in most places not greater than 30 μm. Half-way down the retina there is a bend of about 30°, so that looking at a transverse section of the cephalothorax, one sees the retinae as a pair of boomerang-shaped structures with their convexities directed outward.

The arrangement of receptors in the retina is shown in Figs. 3–5. Figure 3 is a drawing of a horizontal section through the right retina in *M. aeneolus*, close to the centre (i.e. half-way up), while Fig. 4*a*, *c* are sections through the outer regions of the retina, 50 μm. above and below the centre respectively. It is immediately clear that the retina contains several layers of receptors, one behind the other with respect to the light path. In the upper and lower regions there are two layers, while the central part contains two further layers overlying the previous pair. To determine the exact arrangement of receptors in these layers, a complete reconstruction of the retina was made from transverse sections, and this is shown in Fig. 5. I propose for convenience to designate these layers 1, 2, 3 and 4, from the deepest layer (1) forwards.

Layers 1 and 2 are very similar. Both occupy the whole of the long dimension of the retina, and both consist of a basically hexagonal lattice of receptors which is nowhere more than ten receptors wide. The two layers differ in that in layer 1 the receptive segments protrude directly from the pigment layer, so that the intermediate segments lie entirely within the pigment. In layer 2 the cell bodies lie to the side of the retina, and send their intermediate segments into the retina at an angle. There is thus a bend of about 45° between the intermediate and receptive segments (which point straight towards the light), and the intermediate segments are exposed to light for some of their length. In layer 2 about two-thirds of the receptors have their cell bodies on the medial side of the retina (open circles in Fig. 5) and the remaining one-third on the lateral side (closed circles). In both layers 1 and 2 there is a marked difference in the packing distance of the receptors as one passes from the centre to the periphery. In layer 1 the central receptor spacing is 1·7 μm., and in layer 2, 2·4 μm. At the periphery of both layers the spacing reaches 6 μm., and the receptors are also shorter and thicker. A further difference between central and peripheral parts of the retina is that the central receptors appear to be contiguous, whereas the outlying receptors are separated

by a considerable amount of matrix. These spacing distances indicate that receptors in the centre of these two layers subtend 11 and 16 min. at the nodal point of eye, and hence in object space (these values are for *M. aeneolus* where the AM eyes have a focal length of 512 μm.). These values agree well with those given by Homann (1928) for the similar sized *Evarcha blanchardi* where, in what was presumably layer 1, he found a central receptor spacing of 1·6 μm., corresponding to a visual angle of 12 min.

Layer 3 contains receptors structurally similar to those in layer 2, but differs from this layer in that it occupies only a small distinct region close to the centre of the

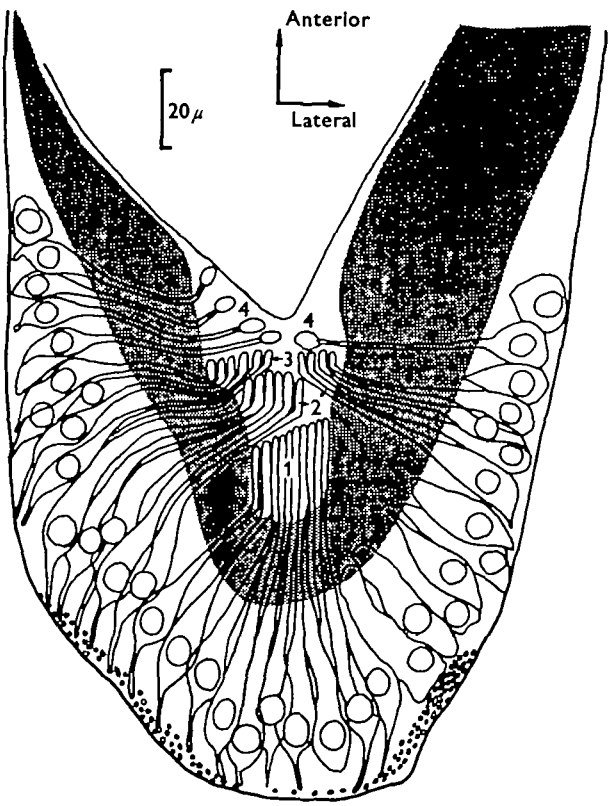

Fig. 3. Frontal (horizontal) section of the retina of the right antero-median eye of *M. aeneolus*. The section is taken close to the centre of the retina (level B in Fig. 6) and shows the four layers of receptor endings (1–4). In layers 1, 2 and 3 the presumed receptive part of each receptor is the straight terminal portion, in layer 4 it is the terminal ovoid swelling. Light would be incident from the top of the page.

retina, medial to most of layer 2, but in places overlying it. Near the centre of the retina those receptor endings of layer 2 whose cell bodies lie on the lateral side of the retina occupy a more superficial (anterior) position than they do elsewhere on the retina, so that in this region they come to lie in the same plane as layer 3 (Fig. 3). Figure 3 also shows that the intermediate segments of layer 3 arise from a distinct 'shelf' in the pigment layer, whereas the pigment walls of the depression containing layers 1 and 2 are both parallel to the visual axis.

Layer 4 differs considerably from the other three. While in layers 1, 2 and 3, the

receptors form hexagonal lattices, whose axes of symmetry change direction systematically throughout the length of the retina, in layer 4 this lattice structure does not appear to exist, or if it does it is very much more loosely defined. The cells in layer 4 also differ from the others in that most of them do not turn up at the end of the intermediate segment to point parallel to the visual axis. The receptive segments are short fat ellipsoids, not rod-like, and the intermediate segments are very long and thin. The other oddity of this layer is that the intermediate segments reflect blue-green light quite well, and it is these structures which make the retina visible opthalmoscopically (Fig. 7*a*, *b*). The reason for this is not yet known.

The preceding description is based on a reconstruction of the eye of *M. aeneolus*, but a less exhaustive study of the retina of *P. johnsoni* revealed exactly the same structures. All four retinal layers are present in the same relative positions, and the only

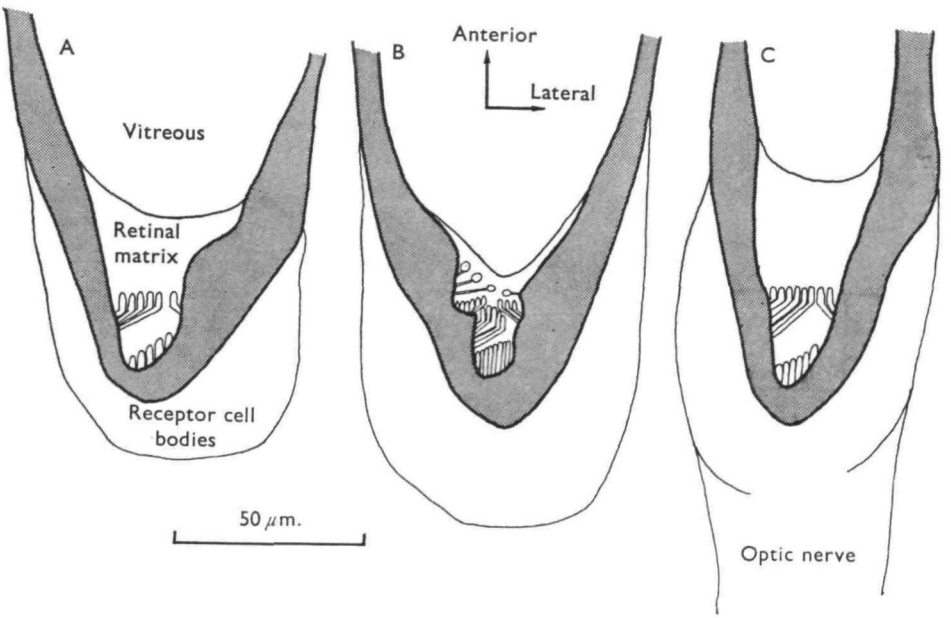

Fig. 4. Frontal sections of the retina at the three levels indicated in Fig. 6, showing the layout of the receptor endings. The retina contains four layers of receptors only in the central region (B); dorsal and ventral to this there are only two layers.

detectable differences between the species were numerical. The retina is larger in *P. johnsoni* by a factor of about 3/2 in linear dimensions, but since this is the same as the ratio of the focal lengths on the two eyes, the angular subtense of the retinae in object space (about 21° × 2° for layers 1 and 2) is the same in both species. The total number of receptors in *P. johnsoni*, based on a count of axons in the optic nerve, is greater than in *M. aeneolus*; the total numbers are 1184 and 794 respectively. The cell spacing in *P. johnsoni* is also somewhat greater, the minimum receptor spacing in layer 1 being 2·0 μm. as opposed to 1·7 μm. Because the focal length is also greater in *P. johnsoni* this actually represents a slight gain in angular resolution over *M. aeneolus*; this receptor spacing corresponds to 9 minutes of arc rather than 11. Finally, the total thickness of the retina, from the top of layer 4 to the bottom of layer 1, is larger in the larger species—90 μm. instead of 60 μm.

Structure of the first optic glomerulus

The first structures encountered by the optic nerves after leaving the eye are the first optic glomeruli. These are paired horseshoe-shaped structures lying flat on top of the brain, immediately behind the AM eyes, and they are regions of neuropile where the primary fibres (the axons of the receptors) terminate, and the second-order fibres

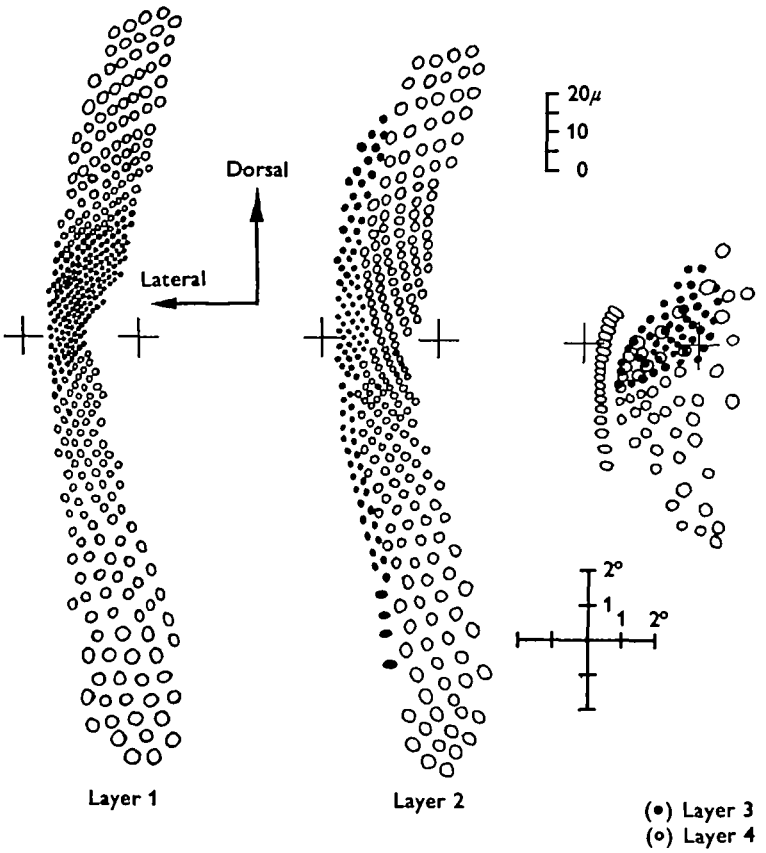

Fig. 5. Complete reconstruction of the right antero-median retina of *M. aeneolus*, from serial transverse sections, showing the disposition of receptor endings in each of the four layers. Every attempt has been made to show the exact numbers and positions of the endings, and to preserve the axes of symmetry of their lattices. In layer 2 the closed figures indicate those elements whose cell bodies lie on the lateral side of the retina, the open figures on the medial side. The small crosses are fiducial marks showing how the three drawings superimpose. The scale in degrees indicates the subtense of retinal structures at the nodal point of the eye, and hence in object space; it assumes a focal length of 512 μm. (see Table 2).

begin. The second-order fibres are T-shaped, with the cell bodies off to one side outside the glomerulus. Hanström (1921, 1928) made Golgi preparations of the glomeruli, which show the endings of first-order and second-order fibres interdigitating in small discrete tufts of neuropile. These tufts of neuropile can be seen in conventionally stained preparations (Mallory) where they stand out as rather deeply staining structures in a lighter matrix. They give the junctional region of the glomerulus a lattice-like or

banded appearance, depending on the orientation of the section (Fig. 8). The size of these tufts varies somewhat with their position in the glomerulus. They are cylindrical, 13–23 μm. long and 3·5–6 μ in diameter.

We are interested here in how the optic nerve fibres map on to the glomerulus. Does the arrangement of the junctional regions in the glomerulus bear a simple relationship to the arrangement of receptors in the retina? It appears that it does. A reconstruction was made of the first optic glomerulus of the right AM eye of *M. aeneolus*, and is shown

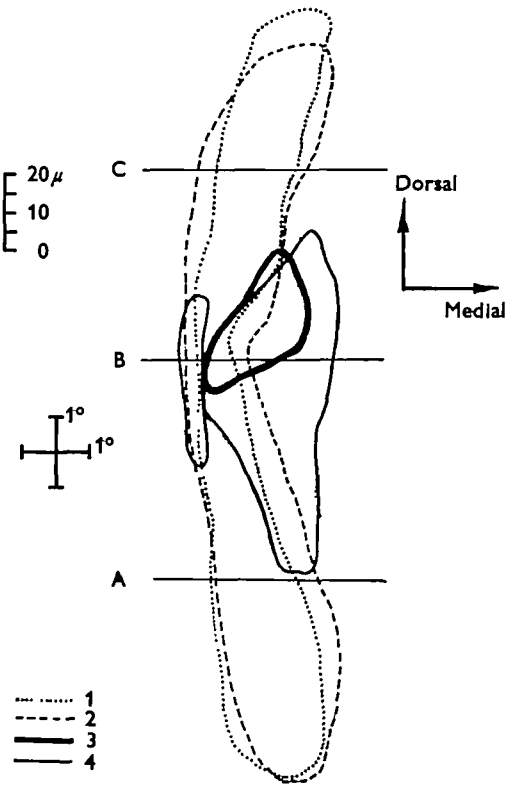

Fig. 6. View of the retina from in front to show how the four layers in Fig. 5 superimpose.

in Fig. 8. The glomerulus contains two semicircular strips of tufted neuropile: a single strip on the outside (*a*), encircling a second strip which is made up of a dorsal (b_1) and a narrower ventral part (b_2), the two parts separated by a narrow gap through which the second-order fibres from the outer strip of neuropile pass. Towards the rear of the structure, immediately above the two ends of the horseshoe, are two additional more or less circular patches of neuropile (*c* and *d*). Thus one can distinguish five separate regions of neuropile in the glomerulus: the outer (anterior) strip, the paired inner strips, and the two postero-dorsal patches of neuropile.

In dissections one can see that the optic nerve has a twist. In *P. johnsoni* which has a relatively long (1 mm.) optic nerve, one can see that the nerve as it leaves the eye is flattened laterally, while by the time it reaches the glomerulus it is flattened dorso-ventrally. The twist is in opposite directions in each eye, so that on both sides the top

of the nerve at the eye becomes the lateral part of the nerve at the glomerulus. The presence of this 90° twist means that the long dimension of the retina does in fact map on to the long dimension of the glomerular strips. If this is so, then the geometry of the nerve also demands that the medial part of the retina maps on to the upper surface of the glomerulus. If the retina and the glomerulus were both single-layered structures, one could think of one of the glomerular strips, uncurled, as being a direct projection of the retina, rotated through 90°. This geometrical argument can be extended to

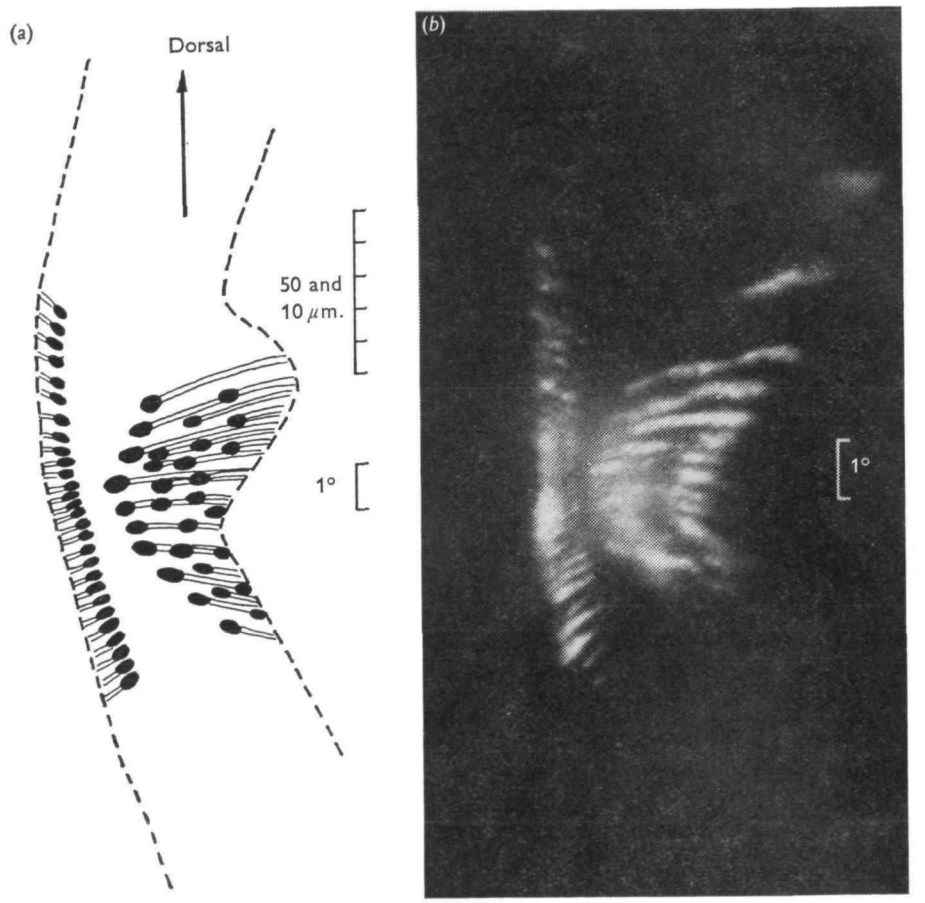

Fig. 7. (*a*) Drawing of layer 4 of the right retina of *P. johnsoni*, seen from in front (i.e. from the direction of the incident light). It is a reconstruction from five serial transverse sections. The scale in degrees was obtained from the μm. scale by assuming a focal length for the eye of 767 μm. (Table 2). (*b*) Ophthalmoscopic photograph of the retina of *P. johnsoni*, taken using the apparatus shown in Fig. 11. It is clear that the structures made visible by ophthalmoscopy are the intermediate segments of the receptors of layer 4. The 1° scale was derived directly from the calibration of the ophthalmoscope; it can be seen that the sizes of the structures shown in (*a*) and (*b*) are, as expected, virtually identical.

cover the three-dimensional structure of the retina and glomerulus, if one assumes that the anterior layers of the retina have their axons in the outer parts of the nerve, with the axons of the receptors of layer 1 in the centre; this is the way they would lie if the geometry of the retina was preserved in the optic nerve

(Fig. 9). Then at the glomerulus the central (layer 1) fibres would encounter the anterior strip of neuropile (*a*), outside these the layer 2 fibres would pass over and under this strip, and encounter the second strip (b_1 or b_2 according to whether the fibres originated from the medial or lateral parts of the retina). The fibres in the outermost parts of the nerve, predominately from the medial cells of layers 3 and 4, would then pass over both strips of neuropile, and terminate in the two dorsal patches (*c* and *d*).

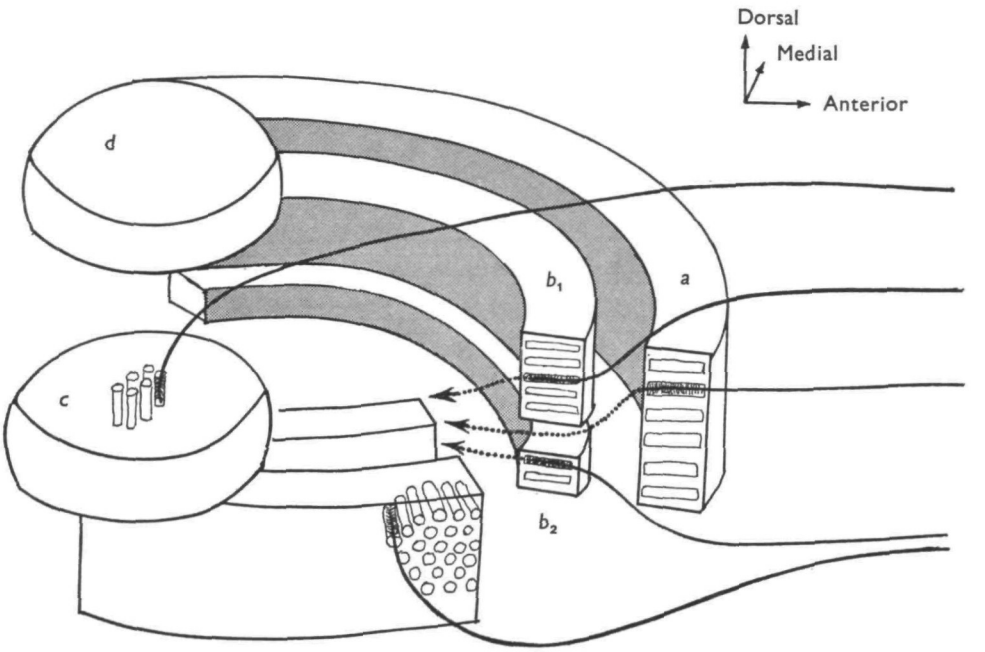

Fig. 8. Diagrammatic reconstruction of the first optic glomerulus of the right antero-median eye of *M. aeneolus*. The glomerulus is seen from the side and slightly from above. The diagram shows the two horseshoe-shaped strips of tufted neuropile (*a* and *b*) and the two dorsal patches of neuropile (*c* and *d*). The paths of the primary fibres (solid lines) and the second-order fibres (broken lines) are indicated in the cut-away part of the figure. The figure should not be taken to imply a one-to-one relationship between primary and second-order fibres. The cylindrical objects are the 'tufts' which are visible in conventionally stained histological sections.

This suggested arrangement relies heavily on the assumption that the essential geometry of the retina is preserved in the organization of the fibres in the optic nerve. Examination of the course of fibres in the nerve is not possible over large distances, but there seem to be no obvious complications in the organization of the nerve (apart from the twist) to suggest that the fibres pursue a more tortuous path than just described, which is the simplest and most direct.

Another line of evidence that could be used to support the scheme suggested in Fig. 9 would be to count the number of tufts of neuropile in each part of the glomerulus. and show that there is a simple numerical relationship between the numbers of receptors in each layer and the number of tufts in its proposed projection. Unfortunately, the tufts are not sufficiently well defined to be counted individually in many parts of the glomerulus, although an approximate estimate can be formed by counting

tufts where the definition is good, and then estimating the areas of the various regions of neuropile. The estimates obtained in this way are given in Table 1.

The anticipated simple ratios were not forthcoming; Hanström's drawings (1928, fig. 421) indicate that each optic nerve fibre ends in a single tuft, and one would thus expect a ratio of 1 receptor to 1 tuft. However, despite the use of several different methods to calculate tuft numbers, this ratio remained in all cases close to 1·5:1, and slightly higher for parts *a* and *b* (assuming these to be the projections of layers 1 and 2). Detailed examination of the tufts showed that while in most cases the optic fibres (1–2 μm. in diameter) terminated within individual tufts, others ended between tufts,

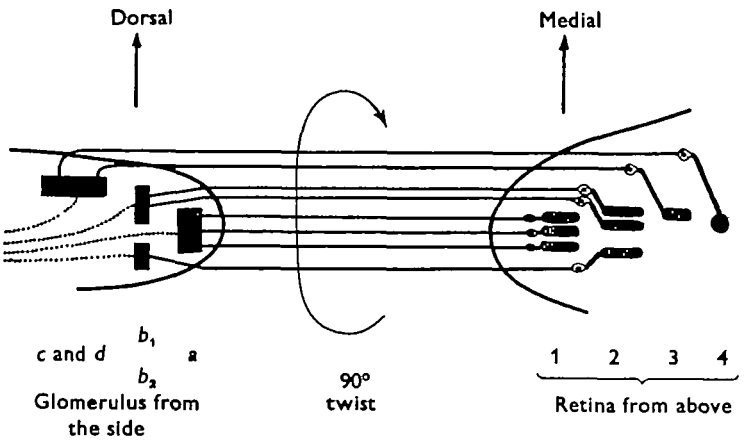

Fig. 9. Hypothesis suggesting the way the retina projects on to the glomerulus. The simplest path the optic nerve fibres could take would bring fibres from the medial part of the retina on to the dorsal part of the glomerulus, and the lateral part of the retina on to the ventral part of the glomerulus. Similarly, the long dimension of the retina (into and out of the page) would map onto the long dimension of the glomerulus, fibres from the dorsal retina reaching the lateral part of the glomerulus. This hypothesis assumes that the optic nerve has a single 90° twist; this twist is readily observable in dissections.

Table 1. *Comparison of the numbers of receptors in each retinal layer with the approximate numbers of tufts of neuropile in each part of the first optic glomerulus, for* Metaphidippus aeneolus

Retina and number of receptors		First optic glomerulus and numbers of tufts of neuropile (approx.)		Ratios, receptors/tuft (approx.)
Layer 1	376	Strip *a*	231	1·63
Layer 2 (medial part)	204	Strip b_1	119	1·72
Layer 2 (lateral part)	98	Strip b_2	51	1·92
Layer 3	48	Lateral dorsal patch *c*	75	0·8
Layer 4 (medial part)	49	Medial dorsal patch *d*	70	
Layer 4 (lateral part)	19			
Totals	794		546	1·45

or in small 'subtufts' distal to, and to the side of, the main tufts. It is also possible that some fibres pass through or past the glomerulus, although no such fibres were found. All that can be said is that the relative numbers of tufts in areas *a*, b_1 and b_2 are similar to the relative numbers of receptors in layer 1 and the two parts of 2, and this is in accord with the geometry of Fig. 9; the situation in areas *c* and *d* is less clear.

Nothing is known about the numbers of second-order fibres. In the homologous glomerulus in wolf-spiders, Trujillo-Cenóz (1965) found that each tuft consisted of a calyx of several second-order fibres making synaptic contacts (reciprocal as well as direct) with each primary fibre. The situation seems to be similar here; the second-order fibres are much smaller, and apparently much more numerous than the primary fibres of the optic nerve.

Optics

Introduction

There are two reasons why, in a simple eye, it might be an advantage to have a many-layered retina. First, such a retina could be used in lieu of a focusing system; if light from distant objects is brought to a focus on one layer, light from near objects will be focused on a deeper layer. The animal could then use whichever retina possessed the sharper image to examine objects at different distances. Secondly, each retinal layer might contain a different photo-pigment, and be situated in the plane containing the best resolved image for light of the wavelength of maximum absorption of the pigment. This would be an excellent way for an animal with colour vision to overcome the problem of chromatic aberration—a defect inevitably inherent in a simple refraction system such as this. To determine whether either of these hypotheses is compatible with the structure of the retina it will be necessary to (i) measure the focal lengths of the eyes; (ii) determine where on the retina light from infinity is normally focused, and then estimate how the position of best focus changes for different planes in object space, and for different wavelengths; (iii) estimate how the quality of the image changes as the receiving plane moves away from the ideal focus.

There will be no point in postulating either hypothesis if the depth of focus—i.e. the distance range in image space over which the quality of the image is substantially the same—is large compared to the separation of the receptor layers.

Focal length

To determine the subtense on the retina of an object in space one needs to know the focal length of the eye. The focal length ($-f_1$) which is defined as the ratio of the size of the image to the angle in radians subtended by an object at infinity, can be obtained by direct measurement of the size of the image of a distant object. Alternatively, it can be found by calculation, once the radii of curvature of the refracting surfaces and the refractive indices of the components of the eye have been determined. The refractive indices present a problem as the eyes are too small to permit direct refractometry; however, they can be obtained indirectly by measuring the distance from the rear surface of the lens to the position of the best image, and then, from the radii of curvature of the lenses (obtained photographically), the refractive indices of the lenses can be calculated by conventional thick-lens optics. Table 3 shows the results obtained this way. The focal lengths are then given by

$$\frac{1}{-f_1} = \frac{n_2 - n_1}{r_1} + \frac{n_3 - n_2}{r_2} - \frac{d(n_2 - n_1)(n_3 - n_2)}{n_2 r_1 r_2},$$

where $n_1 = 1$ (air), n_2 is the refractive index of the lens, and n_3 is the refractive index of the material in the posterior chamber of the eye, which is assumed here to be identical to spider Ringer ($n = 1{\cdot}335$). r_1 and r_2 are the radii of curvature of the front

and rear surfaces of the lens, and d its thickness. If the posterior chamber of the living eye is optically rather denser than spider Ringer, e.g. if it contains much dissolved protein, the actual focal lengths would be somewhat shorter than the measurements given, but these differences would be only a few μm., and they will be ignored.

Table 2. *Optical constants for the front and side eyes of* Phidippus johnsoni (10 mm. ♀) *and* Metaphidippus aeneolus (5 mm. ♀)

(For explanation of symbols see text. All dimensions are in μm. The signs of r_2 and f_1 refer to a Cartesian sign convention with light entering from the left. See text for further explanation of symbols.)

Eye	r_1	$-r_2$	d	n (lens)	$-f_1$ (measured)	$-f_1$ (calculated)
P. johnsoni						
AM	344	∞(179)	435	1·40 (1·37)	767	1160 (830)
AL	201	129	223	1·43	385	383
PL	157	91	192	1·49	254	242
M. aeneolus						
AM	217	525	236	1·41	512	504
AL	112	162	150	1·45	256	219
PL	86	65	152	1·51	174	142

Table 3. *Derivation of the refractive indices used in Table 2 from the lens-image distances*

Eye	Distance from back of lens to image with Ringer on both faces of lens l_1 (μm.)	Distance from back of lens to image with air on front surface of lens and Ringer on rear l_2 (μm.)	Refractive index (n) from l_1	Refractive index (n) from l_2	Mean
P. johnsoni					
AM	10,000	700	1·38 (1·35)	1·41 (1·39)	**1·40 (1·37)**
AL	1,166	330	1·42	1·44	**1·43**
PL	382	211	1·51	1·47	**1·49**
M. aeneolus					
AM	3,140	464	1·40	1·41	**1·41**
AL	741	176	1·44	1·45	**1·45**
PL	204	82	1·52	1·50	**1·51**

n can be obtained from l_1 by thin-lens optics after making a correction for the position of the optical centre of the lens:

$$\frac{1}{l_1'} = \left(\frac{n}{1{\cdot}335} - 1\right)\left(\frac{1}{r_1} - \frac{1}{r_2}\right), \quad \text{where} \quad l' = l_1 - \frac{dr_2}{r_1 - r_2}.$$

When air is on the front face d is not short compared with l_2, and the thick-lens formula must be used to obtain n:

$$l_2 = \frac{1{\cdot}335 r_2\,(\{nr_1/[n-1]\} - d)}{nr_2 - (n - 1{\cdot}335)\,(\{nr_1/[n-1]\} - d)},$$

the signs of all quantities are as given in Table 2.

Complete optical data for all eyes of representative specimens of *M. aeneolus* and *P. johnsoni* are given in Table 2. Partial sets of results were obtained for other specimens of each species, and these were very similar to those given here. In general the measured and calculated values for the focal lengths ($-f_1$) agree well. The only exceptions are the AM eye of *P. johnsoni*, and the PL eye of *M. aeneolus*. In the first

case the discrepancy depends on what value of r_2 is adopted. This lens has a soft' flat-surfaced layer behind a core of much higher curvature, and if one takes the radius of curvature of the core in calculating n_2 and f_1, rather than the flat rear surface, the discrepancy between measured and calculated values for f_1 is greatly reduced (these figures are given in parentheses). The implication is that the soft rear layer of the lens is of lower refractive index than the core, and can be regarded optically as part of the

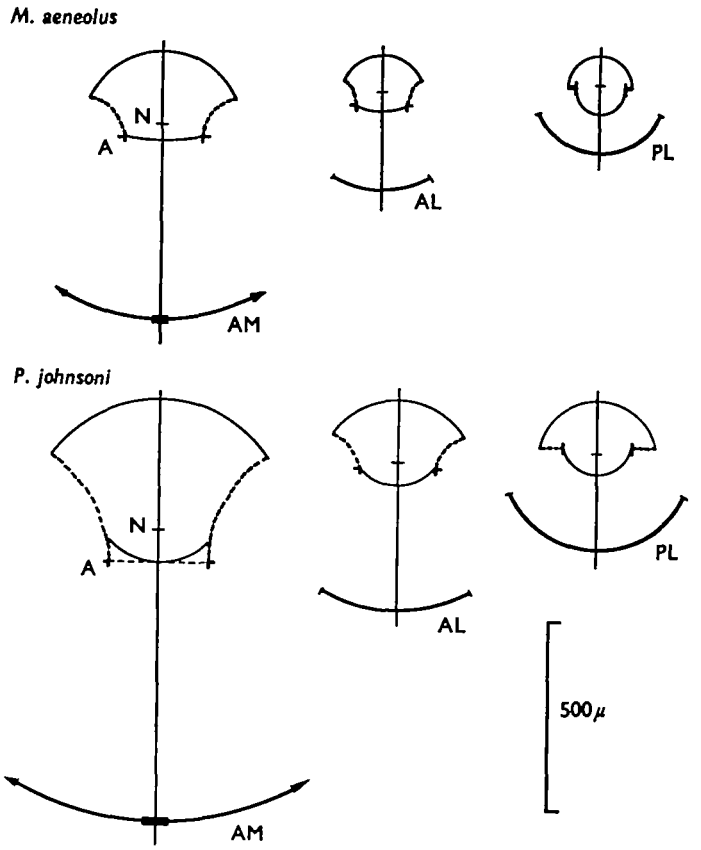

Fig. 10. Scale optical diagrams of the eyes of *M. aeneolus* and *P. johnsoni*. The diagrams were constructed from the data in Table 2. They show the curvatures of the lens faces, the positions of the images, of the nodal points (N) and the aperture stops (A). The extents of the retinae in the horizontal plane were determined histologically. In the case of the AM eyes the thickened part of the line indicating the retina is the actual lateral extent of the structure, the rest of the line shows the 'extended' field of view resulting from the lateral movements of the retina.

rear chamber, even though it stains histologically like the rest of the lens. In the case of the PL eye of *M. aeneolus* the discrepancy can probably be accounted for by the relative increase in measurement errors resulting from the eye's small size.

The values for f_1 used in calibrating Fig. 5, and in constructing the optical diagrams in Fig. 10, are the directly measured ones, and these will also be used in future calculations; the direct measurements are simple to repeat, and values obtained by the direct method involve fewer measurement errors than those obtained by calculation.

All the eyes have a basically similar optical structure. In each case most of the refraction occurs at the cornea, the rear surface of the lens being of secondary importance; in fact in the AM and AL eyes, where the refractive index of the lens differs only slightly from that of water, the ratios of the front and rear surface powers of the lenses are 13:1 and 6:1 respectively (*M. aeneolus*), and for most purposes the systems can be regarded as ones involving refraction at a single spherical surface. If refraction at a single interface is to produce a tolerable image over a wide angle (50°–60° for these eyes, when the eye movements of the AM eyes are taken into account) the position of the aperture stop will be important. Ideally, the stop should be located at the centre of the curvature of the refracting surface, since with the stop in this position oblique pencils of rays from objects lying well away from the optic axis will make the same range of angles of incidence with the refracting surface as do axial pencils, and consequently the defects of the image will be no greater for objects off the axis than they are for axial points. In each case the principal defect of the system will be spherical aberration, the magnitude of which will depend on the diameter of the aperture. In real eyes the aperture stop is a ring of pigment of fixed diameter which surrounds the rear surface of the lens (Fig. 10), and in the AM and AL eyes the rear surface is situated very close to the centre of curvature of the first, so that the above condition is met: all image-forming pencils will be effectively axial. In the PL eyes the situation is similar. Here the pigmented stop occupies the flat region close to the centre of the lens, which, at least in *M. aeneolus*, lies at the centre of curvature of ***both*** faces. This eye covers a very wide angle (130°), and second-surface refraction is of relatively much greater importance than in the other eyes (the second surface has half the power of the front surface), so one may assume that the stop position is chosen so that extra-axial rays do not make excessively large angles with ***either*** face of the lens.

It is clear that in the 'design' of the jumping spider's various eyes different compromises have been struck between resolution, aperture, field coverage and physical size. In the AM eyes the long focal length and fine receptor 'grain' indicate that high resolution is the most important consideration, this being achieved at the expense of large physical size (the eyes occupy one-third the length of the cephalothorax) and possibly aperture. In the side eyes, particularly the PL eyes, wide field coverage is achieved by eyes of very small volume, although the resolution, as judged by the angular subtense of single receptors, is only about one-sixth that of the AM eyes (see Table 4). As Homann pointed out (1928), what the spider has done is to divide the functions normally performed by the foveal and peripheral parts of the human retina between two systems—the AM eyes being responsible for tasks requiring high acuity, like pattern recognition, and the side eyes being concerned principally with movement detection requiring relatively low resolution. The beauty of this arrangement is that it saves an enormous amount of space; if both functions were performed by a single vertebrate-like eye, with the same focal length as the AM eyes, such an eye would occupy most of the front part of the animal's head.

Ophthalmoscopy and the position of the image

There are two ways in which the exact position of the image could be found. One would be to determine with great accuracy the focal length of the eye and the physical distance from the lens to each of the retinal layers, and then construct the image

position from these measurements. The accuracy this method would require is not attainable. Alternatively, the retina can be examined ophthalmoscopically, i.e. through the eye's own lens, and by determining what visible landmarks on the retina are conjugate with what planes in space, one can determine from the histology of the retina at what level the image of *any* plane in object space will be situated. The principle of the method is illustrated in Fig. 11, and the instrument actually used to make the measurements is described in Land (1969). Briefly, light from the retina (A) emerges from the pupil approximately parallel and is focused by a microscope objective (L_4) at B. L_7 collimates this light again, and the parallel beam is observed through a telescope (L_5 and L_6) which forms a final image at C. The eye is illuminated by a beam of light which enters the system via a beamsplitter between L_4 and L_7. If the point on the retina being observed is strictly conjugate with infinity (i.e. it is at the focal point

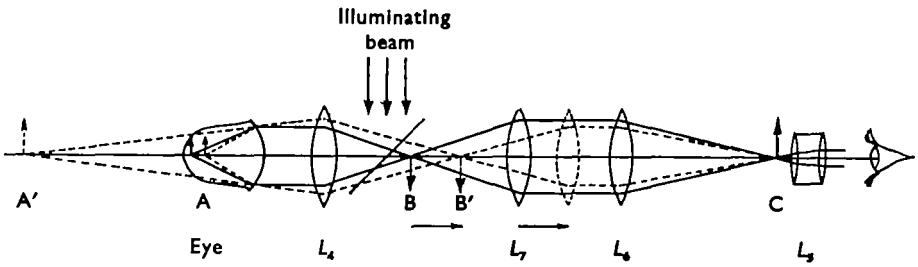

Fig. 11. Ophthalmoscopic arrangement used to determine the position of the focal plane in the eye. For explanation see text. This diagram is not to scale; for a full description of the ophthalmoscope, see Land (1969). The numbering of the lenses refers to this description.

of the eye), then the beam emerging from the lens will be parallel, and will be imaged at B; on the other hand, if it is not conjugate with infinity, light will be seen by the system to come from some other point A', the conjugate image of the retinal point, in front of or behind the eye. Light from A' will be imaged at B', and for this to be imaged at C, L_7 will have to be moved towards or away from L_4 according as light from A' is converging or diverging. The movement of L_7 can be calibrated directly for different positions of A', by removing the eye and focusing the system on real targets to the left of L_4. This method is very sensitive: a shift of only 13 μm. from the focal point in *M. aeneolus* requires a 1 cm. adjustment of L_7. The following observations were made on living animals which were restrained by means of a strip of card waxed to the top of the cephalothorax, and attached to a micromanipulator which permitted the animal to be positioned exactly with respect to the optical system.

When the spider is active the retinae of the AM eyes are in almost continuous motion and it is difficult to make out details of their structure. After a time, however, the animal settles down and the retinae adopt a definite resting position (see Land, 1969) and certain parts of the retinae are seen distinctly by virtue of scattered and/or reflected light. It turns out that the parts of the retina which are most visible are the intermediate segments of the cells of layer 4. This is shown by Fig. 7*b*, which is a photograph of the retina of *P. johnsoni* taken through the ophthalmoscope, and Fig. 7*a* which is a drawing reconstructed from the five serial sections containing layer 4. It can be seen that the linear structures visible in the ophthalmograph can correspond to,

and only to, the cells of layer 4. Nothing of the receptor mosaic of layers 1–3 can be seen, although the outline of the depression containing these layers is just visible. With the eye in the resting position the plane in space conjugate to layer 4 was determined, with the aid of the focusing movement on L_7. In *P. johnsoni* layer 4 is conjugate with a plane 24 mm. *behind* the animal's cornea, and in *M. aeneolus* 15 mm. behind. Three similar-sized animals were used to make each determination, and in no case was the position of the conjugate image more than 3 mm. from the position given; it seems that the axial length of the eye is fixed rather precisely. In practice, layer 4 is 'in focus' over a considerable range of settings of L_7, (see 'Depth of Focus' below) and the conjugate distance has to be found by taking the midpoint between the settings where the retina is noticeably out of focus. The position of focus did not appear to change during eye movements or when targets were presented to the eye through the ophthalmoscope at different apparent distances—i.e. no accommodatory (focusing) movements have been seen, using this system.

Since the plane conjugate to layer 4 is known, we can calculate the distance from the focal point to layer 4. Where the difference in focus is small, this distance is given by

$$\Delta f = \frac{nf_1^2}{u},$$

where n is the refractive index of the rear chamber, taken as 1·335, and u is the distance of the plane conjugate to layer 4 from the cornea. For *P. johnsoni* and *M. aeneolus*, layer 4 is found to lie 33 and 23 μm. in front of the focal point respectively. Referring back to the histology (Figs. 3, 12), we find that in *P. johnsoni* the focal plane lies near the middle of layer 2, and that in *M. aeneolus* it lies at the bottom of this layer. The best images of objects at infinity will therefore be located in these planes.

The ophthalmoscopic measurements were made using white light, but since the visible structures in layer 4 reflect mainly green and blue-green light the position of focus given by these measurements can be taken to refer to wavelengths in the range 500–550 nm. (where they would inevitably be biased by the human spectral sensitivity curves).

Change of focus with object position (*Fig.* 12)

If the best image for mid-spectral light from infinity is formed on layer 2 in both species, we can calculate the positions of the planes in object space that are conjugate with the other retinal layers. Layers 3 and 4 lie closer to the lens than the focal point, and thus are conjugate with planes *behind* the animal's head, which means that they will always receive a worse image than layer 2, no matter where in space the object is situated. However, layer 1 lies behind the focal point, and is thus conjugate with a range of object distances in front of the animal. The object plane conjugate with the centre of the cells in layer 1 is given by

$$u = \frac{nf_1^2}{\Delta f},$$

where Δf is the separation of the centres of the receptors in layers 1 and 2. For *M. aeneolus* this separation (Δf) is 20 μm. and in *P. johnsoni* it is 32 μm., so that the centres of layer 1 receptors are conjugate with planes 17·5 and 24·6 mm. in front of the corneas of the respective animals. These distances are actually rather close (about

3 body lengths) and are likely to be the closest distances at which the animals would require good vision (see Discussion).

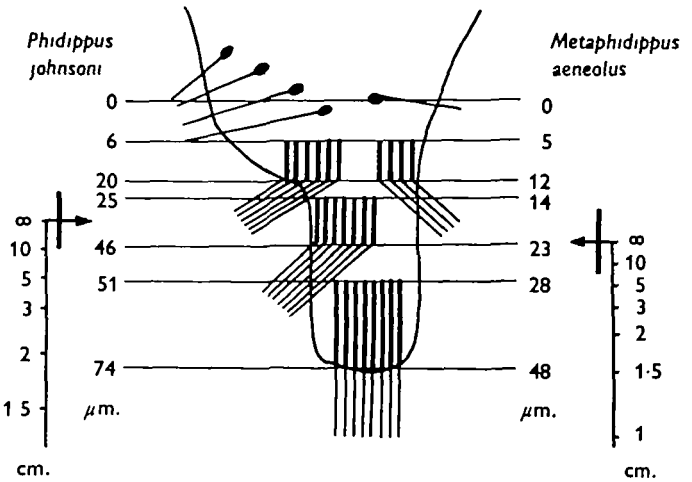

Fig. 12. Image positions for different object distances. The diagram shows the relative positions and dimensions of the four retinal layers for each species. The position of the image of an object at infinity (blue-green light) is shown by an arrow: the outer scale on each side shows the distance from the cornea of the planes in object space conjugate to different depths on the retina. The bar crossing each arrow shows the calculated depth of focus, supposing the eye to possess a perfect aberration-free optical system. With two wavelengths of spherical aberration (see text) the depth of focus would be roughly twice as great as indicated here.

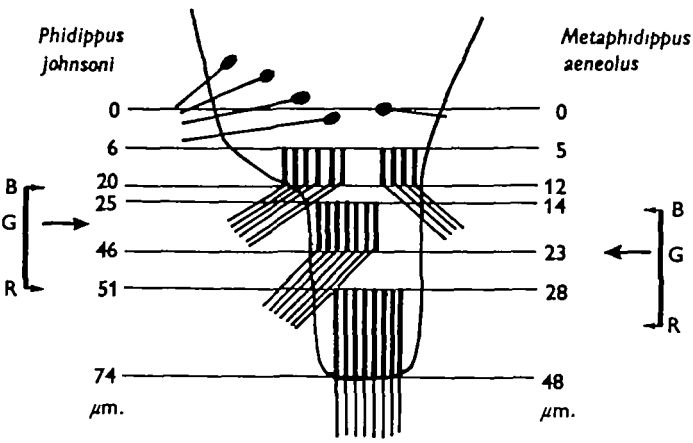

Fig. 13. Image positions for light of different wavelengths. As in Fig. 12, the ophthalmoscopically determined position of the image, for distant objects in blue-green light (G) is shown by the arrow. The effect of chromatic aberration is to decrease the focal length for short wavelengths and increase it for long wavelengths; the calculated positions of the focal planes for blue (B) and red (R) light are shown.

Chromatic aberration: changes of focus with wavelength (Fig. 13)

One can calculate the longitudinal chromatic aberration, i.e. the distance between the focal planes for different colours, if the refractive indices for these colours are known. The optical media of the AM eyes (the lens and the 'vitreous') are aqueous solutions, presumably of protein, and will have optical dispersions close to that of

water. Helmholtz gives the refractive indices of water for the C (red) and G (violet) spectral lines as 1·331705 (λ = 656·3 mm.) and 1·341285 (λ = 434·0 mm.) (Southall, 1924, p. 173).

Regarding the optical system as a single air/water interface, the difference in focal length for these two wavelengths will be given by

$$\Delta f = nf_1\left[\frac{n_g - n_c}{n-1}\right],$$

where the expression in brackets is the *mean dispersion* of the medium (it is more usual to take the blue F line rather than the violet G line when determining the dispersion, but this change makes very little difference). For water the dispersion is 0·0288, and this means that for the AM eyes of *M. aeneolus* Δf = 19·7 μm., and for *P. johnsoni* Δf = 29·5 μm. In other words, the focal planes for violet light will be approximately 20 and 30 μm. closer to the lenses than the focal planes for red light in the two species respectively. These distances are very similar to the distances separating the centres of the receptors in layers 1 and 2 (20 and 32 μm.) so that if wavelengths close to the blue end of the spectrum are focused on layer 2, those close to the red end will be focused on layer 1 (Fig. 13). We have already seen that blue-green light is brought to a focus on layer 2, so that it is entirely reasonable that layers 1 and 2 should contain red-sensitive and blue-sensitive receptors respectively. However, for *no* wavelength in the visible spectrum will a good image be formed on layer 4 (layer 3 is too close to layer 2 for one to assert this—it might make use of the best violet or near-ultraviolet image). This could mean either that the receptors in layer 4 are ultraviolet-sensitive, or else that this layer performs some other task for which a well-resolved image is not required. The different structure of these cells, and their relatively wide spacing, suggests that this may be the case (see Discussion).

Depth of focus and depth of field

In any optical system there is a range of positions in image space over which the image of a plane in object space is nearly equally well resolved, and outside which it deteriorates rapidly (depth of focus). Similarly there is a range of object distances that are 'in focus' on a given plane in image space (depth of field).

If an optical system is reasonably free from aberrations, so that quality of the image is limited mainly by the size of the diffraction pattern due to the pupil, physical optics can be used to determine the depth of focus. This is given by the distance that a receiving plane can stray from the 'ideal' image plane before the wavefront converging on it from the aperture of the system contains differences in optical path-length greater than a quarter of a wavelength (the Rayleigh limit). Another way of looking at the problem is to estimate the range of distances, on either side of the ideal image point, within which the size of the geometrical 'blur-circle' is smaller than the separation of the receptors. Such a criterion will only be relevant if it suggests a value greater than the physical limit.

By physical optics the depth of focus is given by Born & Wolf (1965), p. 490:

$$\Delta f = \pm\frac{2\lambda}{n\pi}\left(\frac{R}{a}\right)^2.$$

λ is taken as 0·5 μm., n as 1·335, a is the semi-aperture of the exit pupil, and R is the radius of the spherical wave converging on the ideal image point. In this case, because the nodal point of the eye and the pigmented aperture stop almost coincide (Fig. 10), R can be replaced by the focal length ($-f_1$) and a by the radius of the pigment ring (see Table 4). For *M. aeneolus* $\Delta f = \pm 6{\cdot}2$ μm., and for *P. johnsoni* $\Delta f = \pm 8{\cdot}3$ μm. (Fig. 12).

Geometrical optics gives the depth of focus as

$$\Delta f = \pm Rd/2a,$$

where d is the arbitrarily chosen permitted diameter of the out-of-focus blur circle—in this case the receptor separation (1·7, 2·0 μm.). Then for *M. aeneolus* $\Delta f = \pm 4{\cdot}4$ μm., and for *P. johnsoni* $\Delta f = \pm 5{\cdot}9$ μm.

The two estimates of depth of focus agree fairly well, although the more legitimate physical limit is slightly greater. In both species the total depth of focus (12 and 17 μm.) is slightly more than half the distance between the centres of the receptors in layers 1 and 2 (20 and 32 μm.). This leads to the conclusion that if a good image of one object plane, for one wavelength, is formed on one receptor layer, the image on another receptor layer will be significantly worse. This in turn means that it *would* be worth the animal's while to employ separate receptor layers, either to receive images from different object planes, or to receive light of different wavelengths from the same object plane.

If we assume for the moment that the second (wavelength) hypothesis is correct, it will be helpful to calculate the depth of field of a single receptor layer. This is simply given by the distance range in object space conjugate to the range of image positions limited by the depth of focus. Assuming the outer limit of the depth of field is infinity, in *M. aeneolus* the near limit will be 2·8 cm., and in *P. johnsoni* 4·7 cm. This means that an animal in which each receptor layer contains a different pigment would only have *perfect* vision down to the distances mentioned—a few body lengths away.

These calculations are necessarily approximate; if the quality of the image is limited by aberrations rather than diffraction the depth of focus (and field) will be somewhat greater than suggested here (although, of course, the best image will be of poorer quality). Conversely, if the receptors are of finite length they will occupy much of the available depth of focus, and this will have the effect of restricting their depth of field. Preliminary calculations suggest that these two effects should roughly cancel out.

Image quality; diffraction and aberrations

The factors that may affect the physiological resolving power of an eye are: (i) the density of receptors, (ii) the size of diffraction pattern due to the aperture, (iii) the aberrations of the optical system.

Of these three, the first will, in a 'well-adapted' eye, be related to the other two; selection is likely to endow an eye with a density of receptors sufficient to exploit fully the spatial information contained in the image, but not an excess. It is thus instructive to compare receptor density with the fundamental limit of resolution set by the size of the aperture (ii). The most useful measure of the diffraction limit is the *spatial cut-off*. If the eye is looking at a grating pattern in which the intensity is sinusoidally

modulated, then as the period of this grating decreases the contrast of the image will decrease until a period is reached where the image has zero contrast. This is the *spatial cut-off* and it is a useful measure of the resolving power of an optical system because information about structures in space whose separation is smaller than this value will not reach the image; further, there would be no point in a retina containing receptors whose separation is less than one-half of the period of such a grating (in image space). The period of such a grating, in the plane of the image, is given by Born & Wolf, p. 485, eqn. (24), which can be written

$$\text{cut-off period} = \lambda R/2na,$$

where λ/n is the wavelength of light in the image space, R is the radius of the converging wavefront, and a is the semi-aperture of the exit pupil. The calculated values of the *spatial cut-off* in the various eyes are given in Table 4. It can be seen that for the AM eyes the receptor separations are approximately twice the spatial cut-off value, i.e. four times what they would be if the optical systems were completely aberration free, and the image was being exploited fully. For the side eyes the receptor separations range from three to nine times the cut-off value. We can conclude that in none of the eyes is resolution limited by diffraction, although this condition is most closely approached in the AM eyes.

Table 4. *Comparison of the theoretical limit of resolution with receptor spacing*

(The theoretical limit given here is the period of a sinusoidal grating, in image space, which would just not be resolved by an aberration-free system. Ideally the receptor separation should be half this value.)

Eye	Focal length ($-f_1 = R$) (μm.)	Semi-aperture of exit pupil (a) (μm.)	Reciprocal of the spatial cut-off frequency [$\lambda R/2a$], where $\lambda = 0{\cdot}5/1{\cdot}335$ μm.		Minimum receptor separation	
			(μm.)	(min.)	(μm.)	(min.)
P. johnsoni						
AM	767	130	1·09	4·9	2·0 (layer 1)	9
AL	385	100	0·71	6·3	3·2	28
PL	254	90	0·52	7·0	4·5	60
M. aeneolus						
AM	512	100	0·95	6·3	1·7 (layer 1)	11
AL	256	65	0·73	9·7	2·0	27
PL	174	65	0·50	9·7	3·0	59

The other possibility is that aberrations limit the performance of the optical system, causing image contrast to be lower than it would be if the system were well corrected. This is very likely to be the case. Apart from chromatic aberration, the principal defect of the image in an eye such as this will be spherical aberration. The magnitude and effect of this can be found by determining (by ray tracing) the point of intersection of the optic axis and the marginal ray, and applying the formula: $(\Delta Z)_{max} = 4\,(R/a)^2\,A$ (Born & Wolf, p. 471), where $(\Delta Z)_{max}$ is the distance between the marginal and paraxial foci, and A is the required wavefront aberration, i.e. the deviation

of the wavefront from the ideal spherical form. In *M. aeneolus* the wavefront aberration (A) is about two wavelengths—assuming the faces of the lens are spherical, as they appear to be, and this value can be used to estimate the deterioration of image quality using the curves given in Born & Wolf, p. 488. These are reproduced in Fig. 14 and show that with two wavelengths of spherical aberration the contrast of a grating with a period of twice the receptor spacing (3·4 μm., giving a normalized spatial frequency—the upper abscissa in these curves—of approximately 0·6) will be about 25 % that of the object grating, compared with 65 % for a well-corrected system. For grating periods smaller than twice the receptor spacing the image contrast decreases gradually to zero, while for gratings with periods larger than this the contrast improves rapidly. Thus, unless the visual system was sensitive to very small differences in intensity between adjacent receptors, there would be little to be gained by having a greater receptor density, but much image detail would be lost if the receptors were farther apart. This would not be true if the optical system was well corrected—the image would have

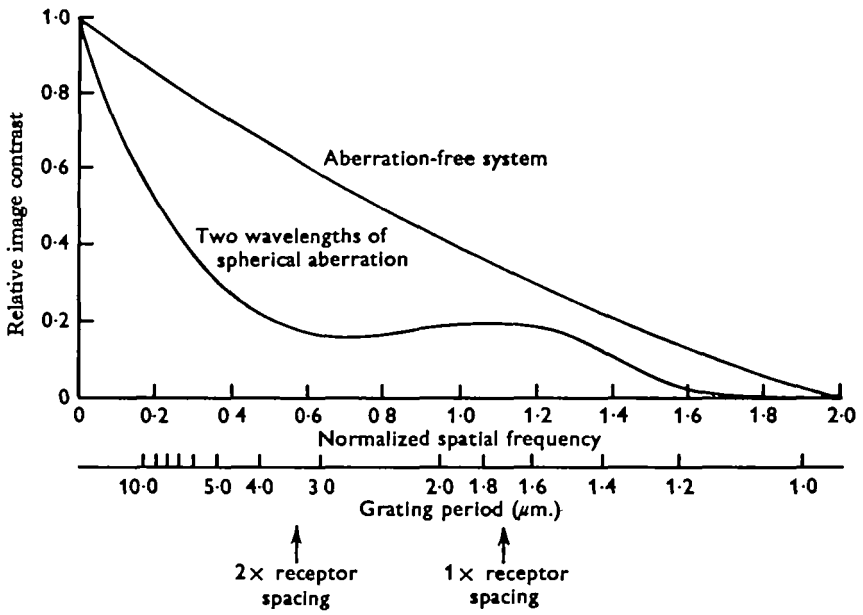

Fig. 14. Effect of spherical aberration on resolution. The ordinate is the ratio of the image contrast of a sinusoidally modulated grating to the contrast of the object producing it. Contrast is defined as

$$\frac{I_{max}-I_{min}}{I_{max}+I_{min}},$$

where I_{max} and I_{min} are the intensities at the peaks and troughs of the grating. The upper abscissa, the 'normalized spatial frequency', is the function: $\lambda/n\ (R/a)\ F$, where λ, n, R and a have their usual meanings (see text) and F is the grating frequency (cycles per unit length); this scale applies to any optical system. The lower abscissa gives the grating period on the retina of *M. aeneolus* ($R = -f_1 = 512$ μm.). In an aberration-free system the image contrast increases steadily with increasing grating period from zero at the spatial cut-off. With two wavelengths of spherical aberration the contrast only begins to improve substantially when the grating period is about three times its value at the spatial cut-off. This grating period is approximately twice the minimum receptor separation for layer 1. The curves are taken from Born & Wolf (1965), p. 488.

tolerable contrast for gratings with periods 2–3 times smaller. It seems likely that in this eye the receptor spacing matches the limit imposed by spherical aberration, rather than diffraction.

DISCUSSION AND CONCLUSIONS

It has been shown that the retinae of the AM eyes of jumping spiders (Dendryphantinae) possess four layers of receptors (Figs. 3, 5). The deepest two layers occupy the entire area of the retina, and the upper two are present only in the central region. As far as I know this arrangement is unique; the only other eyes containing retinae with a remotely similar arrangement are those of certain predatory heteropod molluscs like *Pterotrachea* and *Carinaria* (Hesse, 1900). These too possess long narrow retinae with several layers of receptors, but little is known of their function.

Why several layers?

One suggestion that might be advanced to account for this structure is that by having several layers of receptors the retina will be able to capture more of the light entering the eye, thereby increasing the chance of detecting objects under low levels of illumination. This is extremely unlikely to be the case, first, because jumping spiders are diurnal animals, and would have no need for such an adaptation. Secondly, if the excitation from corresponding cells in each layer were simply pooled at a later stage, this would have the effect of physiologically ruining the image produced by an excellent optical system, by combining information from an in-focus image with one or more out-of-focus images. The structure of the first optic glomerulus (Fig. 8) indicates that each retinal layer has a separate representation, i.e. that information from different layers is *not* pooled, at least at this stage.

Two more realistic hypotheses have already been stated. These are:

(*a*) The several receptor layers act in lieu of a focusing system, the animal using one or another layer to examine objects at different distances from it.

(*b*) Each receptor layer contains a different photopigment and is situated in a plane containing the best image for light of the wavelength of maximum absorption of that pigment.

The results of the optical measurements and calculations do not, on their own, make it possible to choose between these hypotheses. They show:

(i) That the best image for blue-green light from infinity is on the next to deepest layer (2). This means that the deepest layer (1) will receive a good image from planes close to the animal (about three body lengths away), for light of the same wavelength. Alternatively, because of chromatic aberration layer 1 will receive a good image of distant objects for light of longer wavelengths (Fig. 13). As far as layer 1 and 2 are concerned, both hypotheses are compatible with retinal structure.

(ii) Layers 3 and 4 are conjugate with planes behind the spider's head, for blue-green light. This means that they will always receive images that are badly resolved compared with layers 1 and 2. However, if layer 3 contained a pigment sensitive to violet or near-ultraviolet light, it would be able to exploit the image in this region. Similarly, layer 4 could receive the ultraviolet image.

(iii) The depth of focus of the optical system is about one-half of the distance separating layer 1 and 2. This means that an image focused on one layer will be

significantly worse on adjacent layers, so that there *is* a real advantage to be gained from the use of more than one retinal layer. This argument applies equally to both hypotheses.

To sum up, the structure of the retina and visual optics are compatible with both hypotheses for layers 1 and 2, although only the different photopigment hypothesis (*b*) can explain the presence of layers 3 and 4. If the eyes could be shown to have focusing movements, this would abolish hypothesis (*a*). However, ophthalmoscopy, which provides a sensitive method of detecting such movements, has so far failed to reveal any. It should be pointed out that even if each retinal layer is conjugate with a single, distant, plane in space (for different wavelengths) the image of near objects will be by no means unsuable. In *M. aeneolus* it is estimated that the image will be virtually perfect for objects at distances from infinity down to 2·8 cm., and the image will be only slightly out of focus at much shorter distances. Thus it is *not obligatory* for the spider to possess a layer of receptors to cover the near distance range.

Colour vision

If it can be shown that jumping spiders do possess colour vision, the problem would be solved. The retina would then have to contain receptors with different pigments, and there would be only one sensible arrangement for them—that outlined in hypothesis (*b*). Mixing up receptors with different sensitivities in the same layer would have the double disadvantage of a chromatically imperfect image, and reduced resolution for each wavelength because of the increased separation of receptors containing the same pigment. These would clearly outweigh any advantage to be gained from having good images over two distance ranges.

There is considerable evidence to suggest that jumping spiders can discriminate colour. Like other groups that are known to have colour vision, for example birds and teleost fishes, jumping spiders are very colourful animals. *P. johnsoni*, for example, has a bright red abdomen, barred with yellow and white in the female, the falces are irridescent green, and the legs and palps are black banded with orange. Many other species, possibly the majority, contain some specific colourful adornment (see Peckham & Peckham, 1894; Crane, 1949). While a few of these patches of colour could be explained as warning, or possibly cryptic coloration, the majority cannot, as they are present on parts of the body (the face and forelegs) which are seen only by a second spider during courtship, when the male is displaying, and the female is facing him. The suggestion is that these patches of colour are concerned in species recognition. The Peckhams showed that painting various parts of live female jumping spiders in atypical colours greatly reduced their capacity for releasing sexual display from males. Crane found with the aid of models that the yellow patch on the clypeus of male *Corythalia xanthopa* was essential in releasing threat display; crude two-dimensional drawings of a spider containing such a patch were successful in eliciting display but ones in which the yellow was replaced with white were not, and this difference could not be accounted for by intensity differences. Crane's general conclusion was that colour is perceived, but is of secondary importance in courtship, the profile of the animal (a dark object with 'legs' at the appropriate angles) and the kind of movements it makes being relatively more important. (See also Drees, 1952; Land, 1969.)

Kaestner (1950) used the fact that jumping spiders (*Evarcha falcata*) orient to and

climb a surface with a striped pattern in preference to a plain one, to find out whether the animals could distinguish different colours from grey. In a series of well controlled experiments he presented the animals with gratings consisting of orange stripes against backgrounds of twenty-six shades of grey, from black to white, and in another series with blue gratings against the same backgrounds. In all cases the spiders chose the grating surface rather than the alternative plain surface, with the same reliability as they did when the gratings were black and white (80–100% of trials). Kaestner concluded that the animals could indeed distinguish structures on the basis of colour rather than just intensity.

Taken together, the evidence of Crane and Kaestner is compelling and indicates that the AM eyes of jumping spiders must contain at least two types of receptors with different spectral sensitivities. The optical argument, that if such receptors did exist they should be arranged in layers like the receptors in actual eyes, thus appears justified, and it leads to a specific prediction. The receptors of layer 1 should have maximal sensitivity to wavelengths in the red end of the spectrum, and layers 2 and probably 3 to light in the blue region. These differences should be detectable by recording electrophysiologically either from the receptors themselves, or from the neuropile of the first optic glomerulus, where layers 1 and 2 have been shown (provisionally) to have separate representations (Fig. 9). The only electrophysiological work on these eyes so far published is that of DeVoe & Zvargulis (1967) who found that electroretinograms recorded from the cornea showed a peak spectral sensitivity around 535 μm. (*Phidippus* sp.). They were not at that time able to isolate separate pigment systems by selective adaptation.

Layer 4

The remaining anomaly in the structure of this retina is layer 4. It is not conjugate with any plane in front of the animal, nor is it near the focal plane for any wavelength in the visible spectrum. Furthermore, the cells of this layer differ structurally from the other layers in that their terminal segments are short and fat, and are mostly oriented at right angles to the optic axis rather than along it. We should, perhaps, look for a quite different function for these cells. One suggestion is that they are concerned with detecting the plane of polarization of light entering the eye. This is raised as a possibility because Magni *et al.* (1964, 1965) have produced good evidence that wolf spiders (Lycosidae) are able to navigate with respect to the pattern of polarization of skylight, and that this ability depends on the AM eyes (which are homologous with, but simpler than, those of jumping spiders). They also showed that the AM eyes gave different electroretinographic responses for light polarized in different planes, and that the analysing mechanism was in the retina, i.e. it was not due to the optical media being anisotropic, or to differential reflexion at the cornea. Jumping spiders, like wolf spiders, have to get about the environment, and in *Phidippus* at least they have to return after hunting to the same retreat. And while no one has been concerned to show whether or not they use the pattern of polarization of skylight to assist their navigation, this seems a reasonable possibility, particularly in view of the wolf-spider precedent. That layer 4 should be involved in this is suggested by the orientation of the terminal segments. The long axes of these segments are at right angles to the optic axis, and within this plane each terminal segment makes a slightly different angle with the

horizontal (frontal) plane (Fig. 7). Professor R. M. Eakin (personal communication) has found that the microvilli in these receptors are oriented at right-angles to the long axes of the terminal segments and lie mostly in a plane perpendicular to the optic axis. Then, if the conclusion of Waterman & Horch (1966)—that the major dichroic axis of the pigment molecules is parallel to the long axes of the microvilli—applies here as it does in decapod crustacea, each receptor should respond preferentially to light polarized in a plane perpendicular to the long axis of the terminal segment. Then, because each receptor has a slightly different inclination from every other one, each will respond maximally to light polarized in a slightly different plane (inspection of Fig. 7 will show receptor endings making angles of up to 90° with each other). Thus an array of receptors like that present in layer 4 would in theory provide an 'analysing' system capable of supplying the animal with information concerning the pattern of polarization in the light emanating from the part of the environment (sky or otherwise) that the animal is looking at. This suggestion requires behavioural or electrophysiological confirmation, but it has the merit of offering an explanation for the morphological and orientational differences between layer 4 receptors and the others; and, since image quality is relatively unimportant when the animal is looking at the sky, it also helps to explain the absence of a well-resolved image on this layer, for light of visible wavelengths.

SUMMARY

1. The retinae of the principal (AM) eyes of jumping spiders contain four layers of receptors, one behind the other with respect to the incident light. The distribution of receptors in each layer has been determined.

2. The deepest layers (1 and 2) cover the whole area of the retina, and have the greatest density of receptors. The minimum receptor separation is 1·7 μm., or 11 min. visual angle. The more superficial layers (3 and 4) are confined to the central region of the retina.

3. In layers 1, 2 and 3 the rhabdomere-containing segments are rod-shaped, and are parallel to the incident light. In layer 4 they are ovoid, and are oriented approximately at right angles to the light.

4. At the first optic glomerulus the primary fibres from each receptor layer appear to terminate in separate regions of neuropile.

5. The focal lengths, radii of curvature and refractive indices of the lenses of the principal and side eyes have been measured. For the principal eyes, estimates have also been made of the diffraction limit, the depth of focus, and the magnitudes of chromatic and spherical aberration.

6. The normal position of the image in the eye was found by ophthalmoscopy. For blue-green light, the best image of distant objects is formed on the next-to-deepest layer (2).

7. The deepest layer (1) is conjugate with a plane about 2 cm. in front of the animal for blue-green light, or with infinity for red light, because of the eye's chromatic aberration.

8. Two theories are offered to account for the retinal layering. Either the spider uses different layers to examine maximally sharp images of objects at different distances; or each layer exploits the sharpest image of distant objects, but for light of different wavelengths.

9. Optical considerations indicate that either theory is possible, but the second (wavelength) theory is the more probable, because jumping spiders are known to possess colour vision. It predicts that layer 1 receptors contain red-sensitive, layer 2 blue-green sensitive and layer 3 violet-ultraviolet sensitive pigments.

10. The structural peculiarities of the most superficial layer (4), and the fact that it is not conjugate with any plane in front of the animal for any visible wavelength, suggest that it is not resolving an image, but performing some other function. Reasons are given for thinking that this might be the analysis of the pattern of polarization of skylight.

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