

## XX. PHYSICAL OPTICS OF INVERTEBRATE EYES\*

### Academic and Research Staff

Prof. G. D. Bernard  
Dr. W. H. Miller†

### Graduate Students

J. L. Allen  
F. Beltran-Barragan

### RESEARCH OBJECTIVES AND SUMMARY OF RESEARCH

The principal research activities of this group concern the structure and function of compound-eye dioptrics. Invertebrate compound eyes contain a wide variety of structures with characteristic dimensions of the order of a wavelength of visible light. Examples of such structures are corneal nipples, corneal layering, crystalline tract, rhabdomeres, tracheoles, and pigment granules. We are particularly interested in the effect of such structures on the light scattered from and transmitted through such eyes, and in understanding their functional role.

During the past year significant progress has been realized in the following problems.

#### 1. Subsurface Corneal Nipple Array

Nonuniform corneal reflection properties of the housefly and the giant silkworm moth have been related to properties of the subsurface nipples. The nipple axes tend to line up with the ommatidial axis. We feel that such nipple arrays function to reduce glare from sources outside of the ommatidial visual field.

We are also studying such surfaces, using microwave models.

#### 2. Interference Filters in Fly Corneas

We have shown that colored reflection patterns from the corneas of many species of Dipteran flies are due to specialized layering at the front corneal surface. Such layer systems filter the light reaching the reticular cells, and probably serve a contrast-filtering function for vision. In this sense, the corneal filters correspond to colored oil droplets found in some vertebrate retinas.

We have developed a technique to preserve such colored eye patterns in dried flies. This is useful because these patterns, which are absent in untreated dried animals, serve as an aid in identification of species.

#### 3. Optics of a Nocturnal Moth Compound Eye

Our research shows that the so-called "superposition" compound eye of the tobacco hornworm moth is not functioning according to the widely held superposition theory of Exner. Studies of unstained sections with the interference microscope have yielded

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quantitative maps of refractive index as a function of position in the compound-eye dioptrics of this nocturnal moth. The crystalline tract is dense relative to the surround, and the index of the crystalline cone is approximately constant throughout its volume. Our numerical and experimental studies have shown that the cornea-cone combination concentrates the incident light into the crystalline tract, which then guides the light into the reticular cells. The excitation and transmission properties of the tract, and consequent detection by the reticular cells, have been analyzed by using dielectric waveguide mode theory.

### 4. Tapetal Glow in the Skipper Compound Eye

Under appropriate conditions of illumination, the eyes of skippers (Family Hesperidae) exhibit an unusual bluish glow that persists even when the eye is fully light-adapted. We have found the cause of this reflection to be diffraction from an unusual system of taenial ridges of the tracheol cells that surround the sensory part of each ommatidium.

In addition to studies related to these projects, we are also working on the following problems:

- (a) Simulation of the nipple-in-air insect corneal nipple array, using the Argon laser and high-resolution photographic plates.
- (b) Microwave studies of excitation and detection systems in multimode cylindrical dielectric waveguides related to structures found in nocturnal moth compound eyes.

G. D. Bernard

## A. CORNEAL INTERFERENCE FILTERS IN THE COMPOUND EYES OF FLIES

Six months ago, we reported that specialized layering just beneath the front corneal surface was causing colored reflection patterns from the compound eyes of Tabanid flies (horseflies and deerflies).<sup>1</sup> The present report includes further observations from Tabanidae, observations from other Dipteran families, and discusses the function of such layering.

### 1. Optical Microscopy

As reported previously,<sup>1</sup> electron micrographs of the specialized layer system show considerable difference in electron density between adjacent layers. We have since observed the specialized layer system in a normal section from the horsefly Hybomitra lasiophthalma, using the Zernike phase-contrast system. This is important because it is firm evidence that there is a significant difference in optical refractive index between adjacent layers in the specialized layer system.

Using the Leitz Mach-Zehnder interference microscope, we were able to measure the average refractive index of the layer system. Since the rare layers entirely collapse upon drying while the dense layers do not, we assume that the rare layers are largely water; therefore, we assume a refractive index of 1.40 for the rare layers. With the measured average value used, the dense layers are then of refractive index 1.74.

## 2. Contribution of Facet Intersections to Reflection Patterns

In the Horseflies and Deerflies we have found that about half of the specialized layers continue across the facet intersections, while the remainder terminate. The reflection properties in the neighborhood of the intersections differ, therefore, from the properties of the remainder of the facet surface. Consequently, the colored reflection patterns that one observes from such eyes are formed from two components, facet reflections and intersection reflections. For instance, consider an "orange" stripe when viewing the eye of H. lasiophthalma at low magnification from the direction of illumination. In the central part of the pattern the orange spots originate from the facet surfaces, while at the periphery of the pattern the green spots originate from the intersections. In the "blue" stripes the blue reflections come from the facet surfaces, and the red spots come from the intersections.

## 3. Corneal Filters in the Deerfly

We had observed that the colored reflections from deerflies of the Genus Chrysops were noticeably brighter than from the horseflies, and decided to find out why. Having ruled out difference in facet curvature, we turned to electron microscopy and discovered that there are more layers in the deerfly filter system than in the horsefly system. The "orange" filters in the deerfly contain 20 layers, while there are only 12 in the horsefly.

## 4. Corneal Filters in Diptera Other than Tabanidae

We have found in observations of other Diptera that corneal interference filters are rather widespread. In particular, filters similar to the "orange" filters of H. lasiophthalma are very popular. If one observes such an eye in the field under a magnifying glass, the eye will probably appear greenish but will become orangish if illuminated from the direction of observation. Steyskal<sup>2</sup> reports observations of color and pattern in Dipteran compound eyes of 72 species from 23 families (excluding Tabanidae) captured in Michigan. Sixty-four of his descriptions involve the word "green," and 13 involve "coppery" or "golden" or "bronzy." He makes no mention of conditions of illumination, but it seems reasonable to assume that these observations were made in the field with the available illumination used. His work supports our impression that these filters are widespread in Dipteran flies.

The pattern shapes reported by Steyskal are almost all of a banded or a striped type, which might suggest that all filtered eyes have facets with identical filters organized in stripes or patches containing many facets. We have observed exceptions to this in long-legged flies of the Family Dolichopodidae. Such eyes contain two types of filters that appear red or green when illuminated at normal incidence. The two types of facets are organized so that each "red" facet has four "green" nearest neighbors, and

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vice versa. Our electron microscopy shows that in one such fly the filter system consists of 4 dense layers and 3 rare layers, layers in adjacent facets have different thicknesses, and the layer system does not continue through the intersections but terminates in a rare region that is a little thicker than the total layer system thickness.

### 5. Theoretical Transmission Filter Characteristics

A mathematical model was used to gain insight into the reflection properties of the layer systems. The model assumes plane uniform layers resting upon a uniform cornea. Reflection and transmission coefficients were computed for a monochromatic polarized plane wave incident on the filter at an arbitrary angle. Effects of layer thickness variation, refractive index variation, and variation in number of layers were studied.

As an example, Figs. XX-1 and XX-2 show theoretical intensity transmission coefficient vs wavelength for a plane wave normally incident upon typical corneal filters.

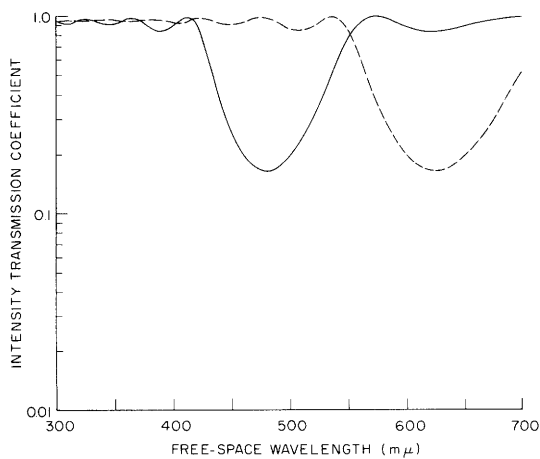


Fig. XX-1. Theoretical transmission filter characteristics for typical twelve-layer horsefly "blue" filters (————) and "orange" filters (-----).

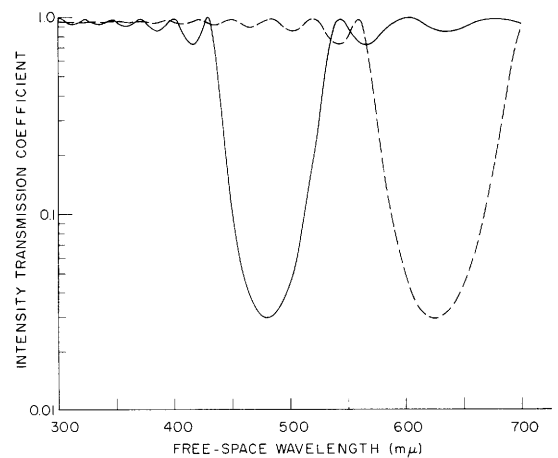


Fig. XX-2. Theoretical transmission filter characteristics for typical twenty-layer deerfly "blue" filters (————) and "orange" filters (-----).

Figure XX-1 is for the model of typical twelve-layer horsefly filters, showing filter characteristics of both the "orange" and "blue" filters. Similarly, Fig. XX-2 is for typical twenty-layer "blue" and "orange" deerfly filters.

Generally, insects are sensitive to wavelengths between roughly 300 mμ and 650 mμ.<sup>3</sup> In this band of wavelengths, reference to Figs. XX-1 and XX-2 shows that the "blue" filters tend to preferentially exclude the center of the band, and to pass the shorter and longer wavelengths. The "orange" filters tend to exclude the longer wavelengths, and pass the shorter wavelengths, as well as those rejected by the "blue" filter.

## 6. Function of the Filter System

Since these filters are so widespread, the question of function weighs heavily. What, if anything, are these interference filters doing for their owners? At this point in our work, we can only speculate and offer a little circumstantial evidence. Although the function or functions may not be related to the owner's vision, the possibility that it is related to vision is attractive.

The visual field half-angle of a given ommatidium is of the order of  $10^\circ$ , or less.<sup>4</sup> Therefore, the transmission properties for any ray of light originating from within this sector and passing through the corneal filter to the rhabdom are essentially identical to those of the on-axis ray. Also, because of the lenticular shape of the corneal surface, parallel rays impinge upon the center of the facet at a different angle from that at the edge of the facet, where the difference in angle is less than  $20^\circ$ . This leads to a slight difference in filter transmission properties for central rays as compared with edge rays, but, for the present, suppose that this difference is negligible. The effect of the specialized layer system, then, is simply to filter the light entering a particular ommatidium according to the transmission filter characteristic for normal incidence. Therefore, this specialized layer system behaves like a type of contrast filter that changes the intensity spectrum of the incident light.

We feel that the interference filters in Dipteran corneas probably correspond to the colored oil droplets or colored lenses (absorption filters) found in some vertebrate eyes.<sup>5,6</sup> When oil droplets occur in a vertebrate retina, they are located in cones at the distal ends of the outer segments; this means that light must first pass through the oil droplets before entering the outer segments, the sites of photodetection. The oil droplets are most striking in birds, in which 8 varieties have been distinguished. In the chicken the droplets are red, golden or greenish. Also, in birds, certain regional distributions of colors may be made out, designated as the red field or the yellow field.<sup>5</sup>

Walls<sup>6</sup> discusses at length vertebrate intraocular color filters such as oil droplets and lenses. He suggests that different colored droplet filters are important for enhancement of color contrasts in particular parts of the visual field. An example is the pigeon, in which the ventronasal droplets are yellow, giving maximum contrasts of objects seen against the sky by eliminating the blue color, while the dorsotemporal quadrant, being especially rich in red droplets, affords maximal visibility to objects seen against green fields and trees over which the bird is flying. Walls treats several other examples in which he correlates filter properties with habits of the animals.

In order to proceed from this point to the important conclusion of how, if at all, corneal interference filters affect the Dipteran visual process, one would like to know the spectral characteristics of the rest of the dioptrics and of the photochemical transduction from optical stimulus to neural response. The life history and natural history of

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such insects is also of great interest. Unfortunately, because this knowledge is quite incomplete, we can only suggest that the specialized layer system functions as a type of contrast filter system.

Another possibility is that the colored eyes play a role in display or mutual recognition of species. The eye of a fly must, however, be closer than 1 cm to a striped pattern 10 facets wide in order to resolve the pattern. It would be impossible for the animal to resolve the red-green pattern of the Dolichopodid flies, mentioned above, since the facets subtend approximately  $0.2^\circ$  at this range. It seems reasonable that the red-green filters of such flies are relatively useless for display or recognition, but serve an optical filtering function for vision.

We have no direct evidence to support this position, but are continuing to work with these animals with this possibility in mind.

### 7. Preservation of Colored Eye Patterns in Dried Flies

Colored corneal reflection patterns disappear in death, because of shrinkage of the rare layers in the corneal filters as the liquid contained therein evaporates. Therefore, such patterns are absent in dried flies, but may be restored by moistening the eye.

Eye pattern details vary considerably among species of the Family Tabanidae and are therefore an aid in identification of species.<sup>7</sup> According to Professor L. L. Pechuman, Curator of the Insect Collection, Department of Entomology, Cornell University, although it would be valuable to be able to preserve the eye patterns in dried specimens, attempts at doing so have proved unsuccessful.<sup>8</sup>

We have recently developed a procedure to preserve the colored eye patterns in dried flies. The principle is to replace the liquid in the layers with a substance that will not evaporate.

The method that we have successfully used is to take either a living specimen, or a dead one that has been in 70% ethanol, and immerse it in polyethylene glycol (average molecular weight 300; liquid at room temperature) for 24 hours, then remove and drain on tissue.

At the writing of this report, the colored pattern in flies so treated has shown no signs of deterioration several weeks after treatment. To all indication, this method affords permanent preservation of eye patterns. We must simply wait to see if this is true.

At low magnification, say 10X, the colored pattern is easily recognizable as that of the living animal. One disadvantage of this method is that the treatment changes the color and contrast of body and wing markings used in species classification.

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B. OPTICAL FUNCTIONING OF THE TOBACCO HORNWORM  
MOTH EYE

Our research into the functioning of the superposition type of invertebrate eye has been concentrated during this reporting period on two major aspects of the waveguide mode theory, which have been outlined in a previous report<sup>1</sup>: (i) the experimental determination of the refracting properties of the cornea and crystalline cones, and (ii) the implication of these refracting properties for the excitation and transmission of light through the crystalline tracts.

The refracting properties of the cornea-cone combination have been determined by a dual series of experiments.

The first series was directed at determining the shape of the component lenses and the index of refraction ( $n$ ) of the lenses and the surrounding media. The  $n$  of the liquid surrounding the optics was determined by direct measurement with a microrefractometer to be  $1.371 \pm .001$ , measured at  $23^\circ\text{C}$  at a wavelength of  $5461 \text{ \AA}$  (the green line of mercury). The index of refraction of the edge of the cones was measured by the Becke line technique as  $1.523 \pm .001$  under the same conditions. Thin unstained sections of the moth eye were then examined with a Leitz Mach-Zehnder interference microscope to determine the relative index of refraction of the optics. Since the exact thickness of the specimen was unknown, calibration was accomplished by observing the boundary between the mounting medium ("Permout" by Fisher Scientific) and the embedding medium (epon). The  $n$  of samples of these calibrating media were measured by the

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microrefractometer and the Becke line technique, respectively. The resulting values were  $1.526 \pm .002$  and  $1.546 \pm .002$  for Permunt and epon, respectively, at  $22^\circ\text{C}$ . The former was measured at  $\lambda = 5461 \text{ \AA}$ , the latter in white light. The results indicate that the crystalline cone consists essentially of a core of constant (within  $\pm .01$ ) index of refraction, surrounded by a thin cortex of slightly lower index. The cornea appears tangentially layered, as reported previously<sup>2</sup> from phase microscope observation. Under the assumption that the embedding process replaces the surrounding cytoplasm with epon, the microscopy indicates that cornea, cone, and crystalline tract all have indices greater than that of epon. Using our measured  $\underline{n}$  of the epon sample, we arrive at a value for  $\underline{n}$  of the cone from the interference microscopy that is slightly higher ( $\approx .04$ ) than that obtained from the direct Becke line measurement of the cone. In view of the following experiment, the discrepancy does not appear to warrant great concern and its resolution has not been pursued.

The second series of experiments consisted in observing a distantly illuminated pin-hole through an eye scalp from which some of the cones had been removed. The focussing action of the cornea alone and the cornea-cone combination could be observed, as well as the dependence of the focal spot location on incident angle and the focal spot diameter dependence on wavelength. By substituting oil with  $n = 1.520$  for the normal surrounding cytoplasm, the focal planes of the cornea and the cornea-cone combination were made coincident; thus, it was substantiated that the cone is essentially of constant  $\underline{n}$ , of value  $\sim 1.52$ . The focal distance of the spot from the proximal cornea surface was measured at  $90 \mu$ , with the focal plane at the tract-cone junction. The spot diameter was within 1.5 of the diffraction limit, and was essentially directly proportional to the incident wavelength over the visual spectrum.

We determined the transmission properties of the crystalline tract analytically by conventional dielectric waveguide theory, assuming a tract diameter of  $4 \mu$  and  $\underline{n} = 1.523$ . The surround was assumed initially to be lossless and of  $\underline{n} = 1.371$ . Fifty modes were found to be above cutoff, even at a wavelength of  $5500 \text{ \AA}$ . The extent to which these modes are excited depends upon the distribution of light at the cone-tract junction. Proceeding from our experimental results, the input distribution was modelled as having a diffraction-limited shape  $\left[ \frac{J_1(a\rho_1)}{a\rho_1} \right]$ , where  $J_1(x)$  is a Bessel function of the first kind of order 1, and  $\rho_1$  is the radius from the center of the spot (the location of which varies with the incident angle of the light). The value of  $a$  was chosen to reproduce the measured spot size.

The mode excitation as a function of incident angle was determined for the two visual peaks associated with the Hornworm moth  $\lambda = 5500 \text{ \AA}$  and  $3600 \text{ \AA}$ . For normal incidence, only the  $\text{HE}_{11}$  mode with even symmetry is strongly excited. For displacements up to and slightly exceeding the interommatidial angle, a combination of the nearly degenerate



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$TM_{01}$ ,  $HE_{21}$ , and  $TE_{01}$  modes is also excited, which produces a pattern with odd symmetry. As the angle increases, the  $HE_{11}$  excitation decreases and that of the odd symmetry pattern increases. For angles much greater than the interommatidial angle, the excitation of all modes decreases as the focal spot moves to the edge of the tract.

The known phase velocities of the modes have been used to compute the relative power in each rhabdomere, with the use of a rudimentary rhabdom model that considers the individual cells to be arranged as 6 pie-shaped segments. The results are twofold: (i) the coverage of each ommatidium exceeds the interommatidial angle by approximately 2:1, and (ii) the degree to which the incident light (for pinhole illumination) lies off the axis of the ommatidium can be inferred from the relative excitation of the individual rhabdomeres in a dark-adapted eye.

As the eye light adapts, the different modes undergo different rates of attenuation, with the  $HE_{11}$  mode having only about half the attenuation (expressed in db or log units) of the next higher modes ( $TM_{01}$ ,  $HE_{21}$ ,  $TE_{01}$ ), and much less attenuation than any waves that might propagate in the surround (such as those that contribute to the "eye shine" or "tapetal reflection" in the dark-adapted eye). Thus, in the light-adapted eye, the  $HE_{11}$  mode in the tract is the dominant light-transmission vehicle.

Our analysis predicts that slightly more than 80 per cent of the light normally incident on an ommatidium is conducted by the tract. We have used these results, and a measurement by S. Carlson<sup>3</sup> showing that a dark-adapted Hornworm produces a neural response for an incident flux level of  $3.5 \times 10^{-11}$  watt/cm<sup>2</sup>, to determine whether a single ommatidium functioning by tract propagation alone could provide adequate light-gathering capability to account for such a threshold. If a flicker fusion frequency of 100/sec is assumed,<sup>4</sup> the reported flux corresponds to 10 quanta per flicker interval at the cornea, 8 into the tract, and 1<sup>+</sup> into each rhabdomere. Since incident fluxes at this level have been found adequate to produce vision in other insects,<sup>5</sup> we conclude that the tract propagation could account for the observed sensitivity of the Tobacco Hornworm Moth.

J. L. Allen

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### C. SKIPPER GLOW

The skippers (Family HesperIIDae), named for their erratic flight patterns, have features in common with both moths and butterflies. Their compound eyes, though, are more closely related to those of the moths. The compound eyes of butterflies are apposition eyes; those of the skippers and moths are superposition eyes. The rhabdom is directly juxtaposed to the crystalline cone in the former type of eye. In the latter type, the rhabdom and crystalline cone are separated by the crystalline tract.

#### 1. Ommatidial Structure: Skipper vs Moth

The skipper and moth ommatidia have the same basic superposition construction. But there are differences too. We shall discuss differences in pigment location and differences in the reflecting layer, or tapetum, found in the two kinds of eyes.

The location of pigment is depicted schematically in Fig. XX-3, which shows a moth and a skipper ommatidium as each would appear in the light-adapted condition. In both the moth and skipper, long thin iris cells surround the crystalline tract. The moth iris cell contains numerous granules of screening pigment which are located at the distal parts of the cells when the eye is dark-adapted.

This screening pigment migrates proximally to surround the crystalline tract when the eye is light-adapted (as shown in Fig. XX-3). See Höglund<sup>1</sup> for a recent review of pigment migration in the moth eye.

The crystalline tract is schematically represented in Fig. XX-3 as a tube connecting the crystalline cone to the rhabdom. This representation may be taken literally for the moth eyes that we have studied in the electron microscope because the tract is composed of wedge-shaped extensions of the retinular cells formed into a single cylindrical tract. In the hobomok skipper, though, these

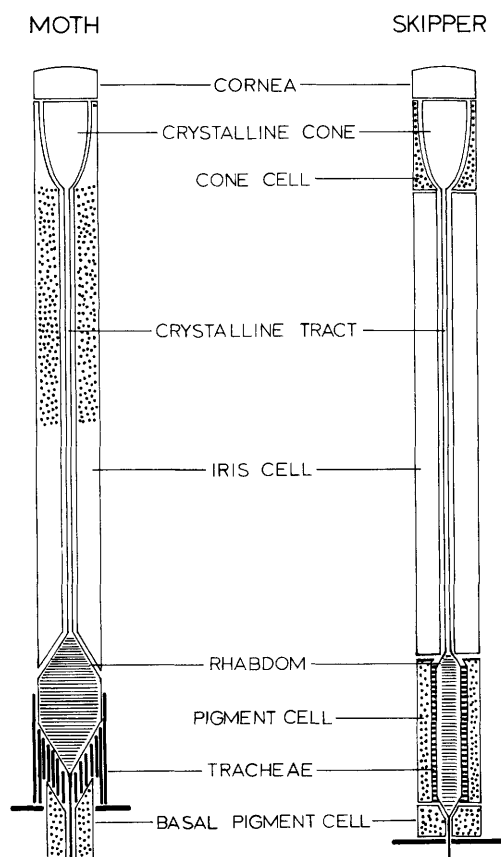


Fig. XX-3.

Schematic drawing showing pigment location in the light-adapted moth and skipper ommatidia. Information for the skipper is based on studies of the hobomok skipper.

cells are separate. There is not one fused crystalline tract, rather there are 7 separate cylindrical tracts, each of which is only approximately 150  $\mu$  in diameter, with what appears to be an equivalent empty space between them. Our experience is too limited to know if this difference between the moth and skipper crystalline tracts can be generalized to be a characteristic difference between most moths and skippers.

The iris cell of the hobomok skipper appears to be uniformly transparent with bright-field microscopy. The schematic drawing in Fig. XX-3 shows the skipper iris cell as seen with bright-field microscopy, and it is intended to illustrate the absence of a screening pigment comparable to that of the moth eye. The moth iris cell has dense screening pigment; the skipper iris cell does not.

Within the sensory part of the ommatidium where the rhabdom is located, the situation is reversed. There is no screening pigment in the moth, but the sensory part of each ommatidium of the skipper eye is surrounded by pigment-containing cells.

At the proximal ends of the ommatidia of both the moth and skipper there are basal pigment cells as depicted schematically in the figure.

## 2. Moth Glow and Skipper Glow

It is well known that many moth compound eyes show a glow in reflected light when the eye is dark-adapted (Höglund<sup>1</sup>). It can easily be shown by observation of freshly sectioned living eyes that the glow has its origin in the dense layer of tracheols which underlies the rhabdoms in this eye. Without exploring in detail the mechanism of tapetal reflection in these eyes, we can state that the reflection appears white to incident white light if the rhabdoms are carefully sectioned off the tapetal layer, but the reflection has a rosy tinge when the rhabdoms remain intact on the tapetum. The light that we see from moth glow originates by reflection from the tracheols and is faintly colored by double passage through the rhabdoms.

The skippers, too, have tapetal reflections. Fernandez-Moran<sup>2</sup> shows beautiful colored pictures of these metallic bluish reflections in tropical skipper compound eyes whose dioptric apparatus had been cut away to expose the underlying retinulae. We have confirmed this observation in skippers native to Massachusetts and Connecticut. Not only do we find a reflection from the scalped eye; we also find a bluish glow in the intact eye under suitable conditions of illumination.

The skipper glow is shown in Fig. XX-4 which is a photograph of the eye taken from the same direction as the illumination. The reflection of the light source is seen as faint specular reflections from the facets over much of the eye surface. The tapetal glow is the bright area approximately 10 facets in diameter at the center of the eye. The original color photograph from which this was reproduced shows the glow as a

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metallic bluish hue. We have found that the skipper glow, unlike the moth glow, is essentially unaffected by light or dark adaptation. It is visible in the fully light-adapted animal, and the size of the glow is independent of the degree of dark adaptation. This is so because there are no migrating screening pigment granules within the skipper's iris cells.

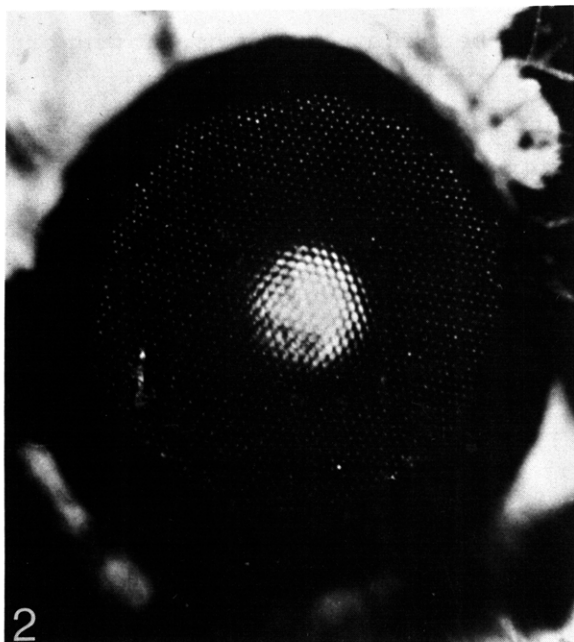


Fig. XX-4.

Photograph of a light-adapted hobomok skipper compound eye taken from the same direction as the illumination, in order to show the glow. Color of the glow is bluish.

### 3. Origin of Skipper Glow

Fernandez-Moran<sup>2</sup> has suggested two possible explanations for the blue color that he observed in the exposed retinulae of the tropical skipper. First, the color could originate as a result of scattering and diffraction by the microvilli of the rhabdomeres. Second, the color could originate by complex diffraction effects produced by an intracellular network of "ultratracheoles" with spacings in the cytoplasm of the order of a free-space wavelength (500 m $\mu$ ).

Neither of these explanations are satisfactory to us. First, the rhabdomeres of virtually all compound eyes are composed of similar microvilli, yet only the skipper exhibits the blue glow. Second, the ultratracheoles reported by Fernandez-Moran are very fine threads, less than 25 m $\mu$  in diameter, which is much smaller than a wavelength of blue light as measured in the ommatidium. The scatter from individual ultratracheoles is therefore extremely small, and their spacing within the ommatidium is not sufficiently regular to cause the observed bluish glow.

We believe that the bluish glow in skippers probably originates from a specialized system of taenidial ridges in the tracheol cells surrounding the sensory part of each ommatidium. These specialized tracheol cells are shown in Fig. XX-5. Figure XX-5a

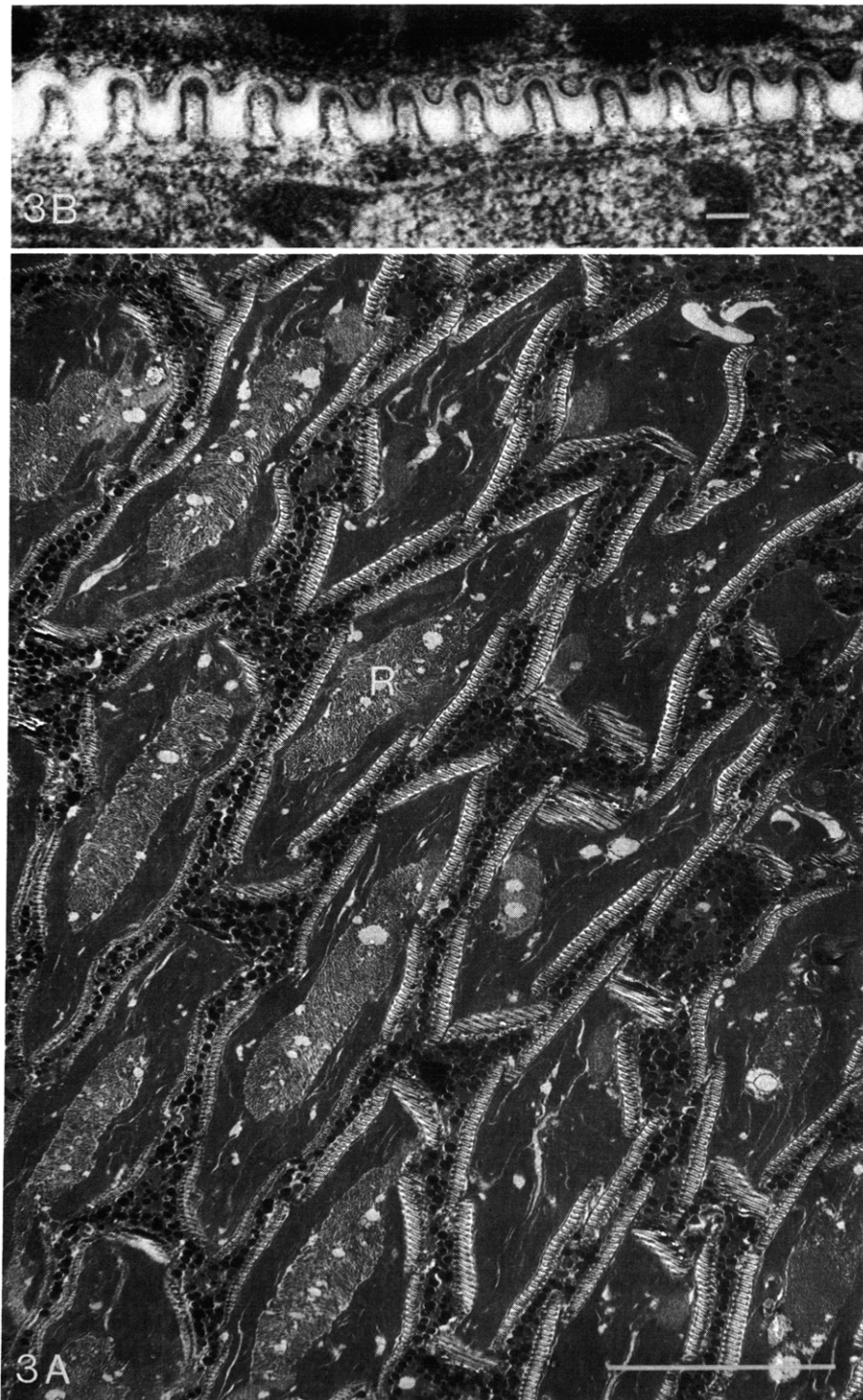


Fig. XX-5. a. Electron micrograph of oblique section through the hobomok skipper ommatidia showing the relationship of pigment cells and modified tracheols to the ommatidia. "R", rhabdom. Magnification marker, 10  $\mu$ .  
b. Detail of tracheolar wall. Magnification marker, 0.1  $\mu$ .

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is an electron micrograph of an oblique stained section through the sensory part of the ommatidia. The sensory part of each ommatidium is isolated from its neighbors by pigment cells bordered by the specialized tracheol system. These tracheols are shown in longitudinal section at higher magnification in Fig. XX-5b, in which it is seen that the outer walls of the tracheol cell exhibit a regular succession of deep taenidial ridges, while the inner wall has shallow ridges. The usual air space between the two walls is almost obliterated in this skipper.

Therefore, incoming light propagating into the reticular cell along the ommatidial axis meets a periodic structure, the taenidial ridges of the tracheol cell. Because the period is approximately  $180 \text{ m}\mu$ , a single diffracted wave exists within the ommatidium for free-space wavelengths shorter than approximately  $520 \text{ m}\mu$ . For free-space wavelengths visible to human observers this diffracted wave propagates back out of the eye, thereby causing the glow. For free-space wavelengths longer than approximately  $520 \text{ m}\mu$  there are no propagating diffracted orders present within the ommatidium.

As shown in Fig. XX-5, the taenidial ridges are composed of cytoplasmic ridges  $\sim 70 \text{ m}\mu$  wide, separated by air spaces  $\sim 110 \text{ m}\mu$  wide. For wavelengths corresponding to bluish hues these dimensions are approximately one-quarter wavelength in each region, which for layered media is a condition for maximal reflection at normal incidence. For the skipper, we feel that this is a mechanism of increasing the energy in the diffracted wave for blue light.

Such considerations lead us to believe that the source of the bluish glow in skippers is the taenidial ridges of the tracheol cells that surround the reticular cells.

The tracheol cells in Fernandez-Moran's tropical skipper are almost identical to those of our northern hobomok skipper. The only difference is that in the tropical skipper there is more air space between the inner and outer walls of the tracheol cells.

### 4. Skipper Glow and Superposition Optics

According to Exner's classical theory of superposition optics, the light in each ommatidium in the dark-adapted eye is not confined to the crystalline tracts. Instead the cornea-crystalline cone combinations act to project an erect image on the rhabdoms. There is strong evidence that this theory does not apply in crustacean superposition eyes where Kuiper<sup>3</sup> has shown that the light is indeed confined to the crystalline tracts.

Direct experimental evidence is difficult to obtain in the moth eye because the crystalline tracts do not retain their natural positions when the eye is scalped. Indirect argument tells us very little more. In the fully dark-adapted moth eye, the glow elicited by a light source of small angular dimensions lights up a substantial portion of the eye, approximately 60 facets in diameter. This suggests that not all of the light is confined to the crystalline tracts, and one could argue that this supports the superposition hypothesis. The argument, however, seems to hinge on an exact numerical solution to

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the problem of how much of the light is confined to the crystalline tracts and how much is scattered inside the eye. John Allen is working on a solution of this problem which is touched on in Section XX-B. He concludes that most of the light incident from within the interommatidial angle is confined to the crystalline tract, so it appears likely that the moth eye does not form a superposition image.

The skipper eye is in some respects easier to analyze. There is no migrating iris pigment; there is excellent optical isolation between the sensory parts of the ommatidia; and each ommatidium is supplied with its private tapetum. We find that the glow is very directional. To observe it, one must be exactly aligned, looking in the same direction as the illumination. The glow is not visible if the illumination is more than  $10^\circ$  off axis. We find that the glow is confined to a spot approximately 10 facets in diameter. This figure is consistent with the known ommatidial beamwidths in other animals, and suggests that the light is largely confined to the crystalline tracts in these skipper eyes. In order to know if this is actually the case, we must determine whether the cornea and cone function to focus incoming light on the crystalline tract and whether the tract, in fact, acts as a waveguide. With regard to the last question, we find that the tract in our Maraglas-embedded specimen has a higher refractive index than that of the iris cell. Thus all of the evidence that we now have suggests that the skipper does not form a superposition image.

W. H. Miller, G. D. Bernard

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