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Diurnal Changes in Angular Sensitivity of Crab Photoreceptors

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Summary. The electrophysiological and anatomical consequences of diurnal changes in screening pigment position were investigated in the apposition eye of the portunid crab *Scylla serrata*. Intracellular recordings revealed that the acceptance angles of dark-adapted photoreceptors enlarged up to four-fold at night compared with photoreceptors dark-adapted in the day. Furthermore, while light adaptation at night caused acceptance angles to narrow, dark adaptation in the day caused no significant broadening of angles. These electrophysiological changes correlated with pigment movements in the eye observed both histologically and in the deep pseudopupil. It is found that the distal pigment cells change diurnally so that the field-stop which these cells form in front of the photoreceptors is opened in the night and closed in the day time.

One feature of the diurnal rhythm is that it prevents photoreceptor fields of view enlarging when eyes are dark adapted in the day. In *Scylla*, photoreceptor fields of view take tens of minutes to narrow upon exposure of crabs to light at night. By preventing a similar broadening in the day, the diurnal rhythm may enable animals suddenly leaving dark refuges to be pre-adapted to daylight. To a range of species which utilise refuges such a mechanism would be of significant advantage, especially after disturbance by predators.

Introduction

In the course of studies of the spectral sensitivity of the eye of the portunid crab *Scylla serrata* (Leggett 1979), the gross repositioning of a number of screening pigment layers was considered. It was thought likely that such pigment movements should also affect the acceptance angle of photoreceptors. It was known (Kleinholz 1937) that the pigment movements were under both light and diurnal rhythm influence, and the aim, therefore, was to investigate in single photoreceptors the effects of both light level and circadian rhythm on absolute sensitivity and acceptance angle. Ophthalmological, histological and electrophysiological methods were used.

Adjustment of the acceptance angle of the photoreceptor is one of the possible strategies by which animals with compound eyes optimise the resolving power of their eyes (Laughlin 1975; Snyder 1979). The ratio of dark versus light-adapted acceptance angles found so far in apposition eyes has an extreme value of three for the cockroach (Butler and Horridge 1973). This paper presents evidence for the largest change of acceptance angle in an apposition eye so far reported: a factor of four in the best result. The way in which light intensity and the circadian rhythm interact to influence acceptance angle is demonstrated and the possible significance of the mechanism is discussed.

An abstract of these results has appeared elsewhere (Leggett and Stavenga 1977).

Materials and Methods

All experiments were on *Scylla serrata*. Crabs were used within a week of being caught, and were maintained until use in a circulating seawater aquarium under a light-dark cycle matched to the natural light-dark cycle. In the context of the diurnal rhythm experiments, it is worth mentioning that both capture site and experimental laboratory were at similar longitude.

In each preparation crabs were legless but otherwise intact. The right eye was cemented to prevent movement, and the crab was mounted in a clamp.

Photoreceptors were stimulated at various known intervals by flashes of narrow band green (505 nm) light which approximates

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the wavelength where spectral sensitivity is maximal for *Scylla*, when dark-adapted (Leggett 1979). Flashes were emitted from a light guide tip which subtended 1.0° at the cornea of the eye, and was mounted in a perimeter device which enabled it to be positioned normal to and equidistant from any of a large number of the facets of the eye. Input to the light guide was from a 300 W tungsten lamp, the beam of which could be attenuated by neutral density and spectral filters, and a shutter.

Calibration of the absolute intensity of the stimulus and of the attenuation values of the neutral density and spectral filters was carried out with a Hewlett Packard 8334A radiant flux meter. A number of calibrations during the course of the experiments showed no significant variation in stimulus conditions.

ERG Experiments

The active electrode was a stainless steel pin which just penetrated the cornea of the eye. The indifferent electrode was a silver wire placed in contact with the blood by means of a hole in a lateral spine on the crab's anterolateral margin. In long term diurnal rhythm experiments, recordings were made from the eyes of animals held entirely in the dark throughout each experiment, except for one dim 40 ms test flash every 10 s.

The ERG method can be used to demonstrate changes in photoreceptor sensitivity. However, despite the attempts of earlier workers (e.g. Jahn and Wulff 1943; Höglund 1966), unless the stimulus illuminates the whole eye, the ERG cannot be further used to quantify, or trace the cause of, photoreceptor sensitivity change. The ERG increase is an overestimate of the sensitivity change both because the photoreceptor response to intensity change is non-linear and because differing numbers of cells contribute to the ERG in differing screening pigment configurations. This latter point also means the ERG cannot be used to trace the cause of photoreceptor sensitivity change because a distinction cannot be made between a greater response per receptor and the response of a greater number of receptors.

Intracellular Experiments

For intracellular recording a small hole was made in the cornea and a glass micropipette (3 M KCl; 80–100 MΩ) inserted. Further recording details are standard (e.g., Leggett 1979). The eye was dark-adapted for at least 20 min, after which the electrode was advanced into the eye. Once a unit with a peak response to light flashes of more than 30 mV was located, the light guide tip was carefully positioned on the physiological axis of the cell. The position of the light guide on axis was then rechecked, and a suitable neutral density filter inserted to bring the axis response to about 50% of saturation. The light guide was then moved in 1° steps through the visual field of the cell.

Early experiments showed that horizontal and vertical runs both gave similar acceptance angle values – i.e. that the fields of view of the retinula cells were approximately circular. Therefore, in the experiments presented here, acceptance angle is derived only from vertical runs as these do not require correction for the horizontal inclination of the perimeter device (Burkhardt and Streck 1965).

In those cells for which both dark and light adapted responses were to be determined, a second series of light adapted measurements was made. Light from a second, 60 W, tungsten lamp was focused upon a highly reflecting white card surrounding the light guide tip. The disc of light so formed subtended an angle of 16° at the cornea. This white adapting light was attenuated (by an iris diaphragm) to give a peak photoreceptor response of approximately 50% of saturation. Movement of the reflecting card during acceptance angle measurement produced no change in the output of the cell. For up to 30 min after light adaptation began, and then for up to 30 min of renewed dark-adaptation acceptance angle runs and intensity series were made at intervals.

Determination of Sensitivity

The following methods were used to determine absolute, relative and angular sensitivities of photoreceptors.

Absolute Sensitivity. There is difficulty in precisely defining absolute threshold – fundamentally on account of photon noise. However, given that cells have similar intensity/response functions, their sensitivities can be defined in terms of some other point on the intensity/response curve, freer of noise problems. The flash intensity at which transient voltage output is 50% of maximum is one such point (Laughlin 1976). In this study dark-adapted absolute sensitivity was determined by the following standard method (Laughlin 1976).

Intensity response functions of cells were determined using a point source subtending 1° at the cornea. Monochromatic light of known quantal content and at the wavelength of maximum sensitivity (the peak) was directed to the cell from the direction of greatest effectiveness (the axis). The number of such peak axial quanta ($cm^{-2}s^{-1}$ at the eye surface) required to evoke a photoreceptor response of 50%, is termed the PAQ_{50}, and was found from the intensity response curve of the cell. The reciprocal of the PAQ_{50} value is the absolute sensitivity of the 50% response. The smaller the PAQ_{50}, the more sensitive the cell.

Sensitivity Change on Adaptation. Changes in sensitivity during adaptation were measured by determining the relative log attenuation (at 505 nm) required in the various adaptation states to cause a criterion depolarisation of 50% from dark-adapted resting level. The difference between the relative log intensities required in the two states for criterion depolarisation gave the log sensitivity difference between the two states.

Angular Sensitivity. Responses for each stimulus angle were converted to log sensitivity using the results of an appropriate intensity series. These were, in the dark-adapted state, a dark-adapted intensity series, and in the light-adapted state, an intensity series made in the presence of the adapting light. The photoreceptor acceptance angle is standardly defined as the angular width of the visual field at 50% of maximum response (Washizu et al. 1964).

Microscopy

Eyes were first either light-adapted in the day or dark-adapted at night. Adaptation was for at least 2 h. After adaptation eyes were fixed directly in cacodylate buffered 4% glutaraldehyde in seawater. Eyes were routinely processed for embedding in Epon. One μm sections were taken from glass knives, stained with toluidene blue, and photographed on a Zeiss Photoscope. Silver to pale gold sections were mounted on 300 mesh grids, stained in uranyl acetate and Reynold's lead citrate, and photographed on a Hitachi H300 electron microscope.

Ophthalmoscopy

Living but legless crabs had one eye fixed to the carapace with dental cement. By means of a half-silvered mirror, an illuminating beam was aligned with the optical axis of the microscope used to view the eye. By appropriate focusing, the images of a number of rhabdom tips could be observed superimposed in the deep pseudopupil (Franceschini 1975; Stavenga 1979). Photographs of the deep pseudopupil were taken at various states of adaptation at both day and night.

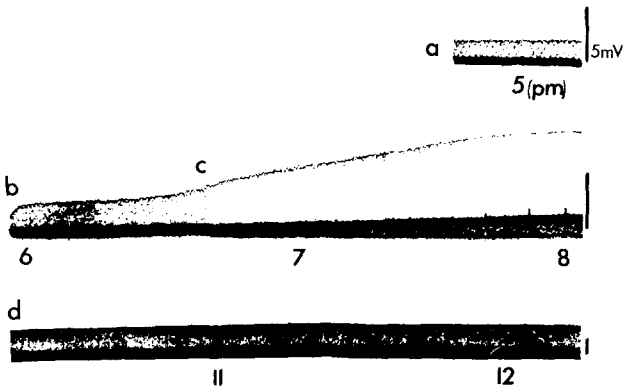

Fig. 1a–d. Details from long term dark adapted ERG records. **a** ERG output to a single test flash every 10 s in the day. **b** Decreasing brightness of test flash leads to rapidly completed increase in output to new steady state. **c** As evening falls, however, the output to the unchanged test flash begins to increase. **d** At night, output is again at steady state (note smaller 5 mt gain bar on record trace)

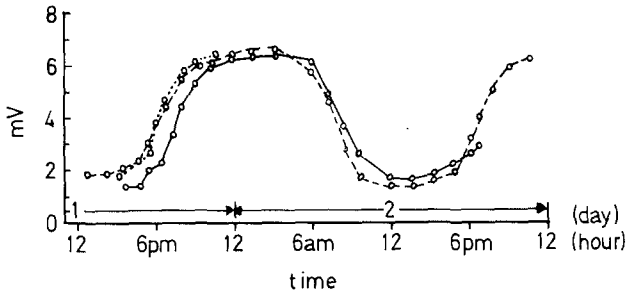

Fig. 2. Diurnal changes in ERG amplitude to a constant test flash in three crabs. ERG amplitude increases each evening and decreases each morning suggesting that the variation is indeed diurnal

Results

To confirm that physiological changes under diurnal rhythm influence indeed took place in *Scylla* the most suitable long term recording technique was that of the ERG. In the experiments, animals were kept in constant darkness, except for a brief test flash every 10 s. Under these conditions, in 12 out of 15 animals ERG output was clearly under massive diurnal rhythm influence, increasing each evening and decreasing again each morning (Figs. 1 and 2).

As explained in the methods section, however, the ERG method is not selective enough to further quantify or trace the cause of photoreceptor sensitivity change. Therefore the rest of the electrophysiological study was carried out on single photoreceptors.

Effect of Light and Dark Adaptation on Acceptance Angle

Day State. Dark-adapted photoreceptor cells (Fig. 3) had acceptance angles which were approximately circular in area and bell-shaped in sensitivity profile, similar to those of other arthropod eyes (e.g. Wilson 1975). Acceptance angles of dark-adapted cells ranged from 2.7° to 5°, with the mean being 4° ($n=30$, Table 1). In this study no attempt was made to correlate acceptance angle properties with eye region.

Initially, when photoreceptors were light-adapted, little or no acceptance angle change from dark-adapted values was found (Table 1 and Fig. 3). This was surprising as a number of insect eyes have shown some narrowing of photoreceptor acceptance angle on light adaptation (e.g., the locust: Wilson 1975) and crab photoreceptors were adapted to back-

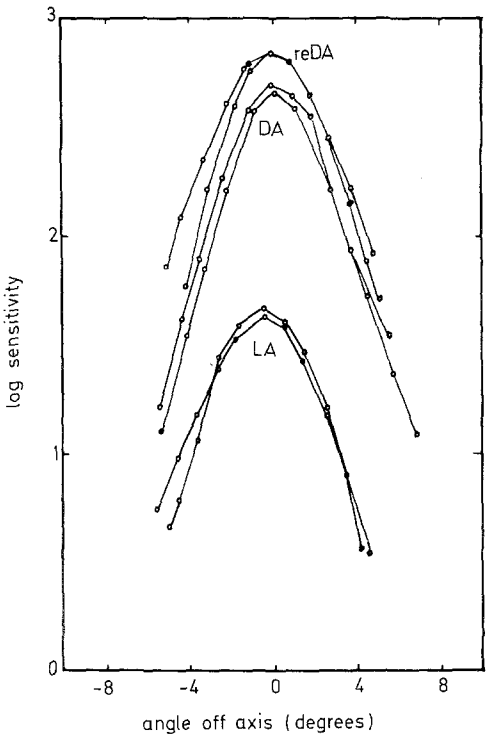

Fig. 3. Angular sensitivity of a single photoreceptor in the day phase of the rhythm, showing little or no difference in angular sensitivity between the dark adapted (*DA*), light adapted (*LA*) and renewed dark adapted (*reDA*) states. To show scatter, two runs are given for each determination

Table 1. Acceptance angles: *a* from dark adapted cells in the day (data from a number of crabs); *b* from single cells, both light and dark adapted, each cell recorded from a single crab in either the day or the night state; *c* from dark adapted cells, all from a single eye, four cells recorded in the day state, a further five recorded at night

	a		*b*			*c*	
	DA	*n*	LA	DA	*n*	DA	*n*
Day	4.0 ±0.6°	30	4.25 ±0.8°	3.9 ±1.1°	10	4.25 ±1.6°	4
Night			5.5 ±1.5°	10.5 ±4.8°	4	11.6 ±3°	5

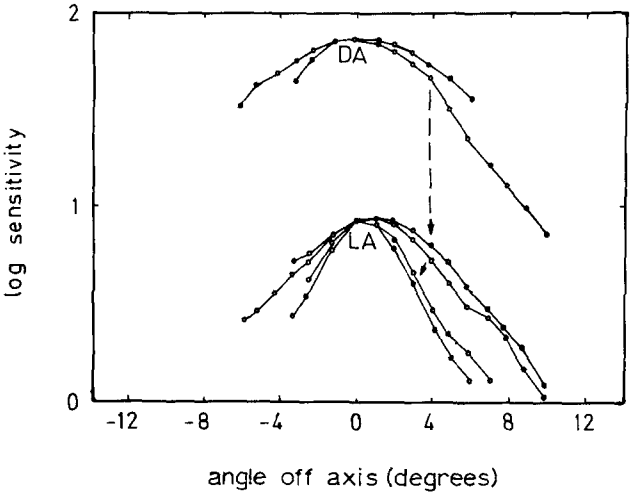

Fig. 4. Angular sensitivity in both light adapted (*LA*) and dark adapted (*DA*) states in a single photoreceptor at night. In this cell the dark adapted acceptance angle was 10°. Upon light adaptation there was a rapid decrease in absolute sensitivity during which acceptance angle was unchanged. After 30 min of light adaptation however, the acceptance angle had halved to 5°. To show scatter, two runs are shown for each determination

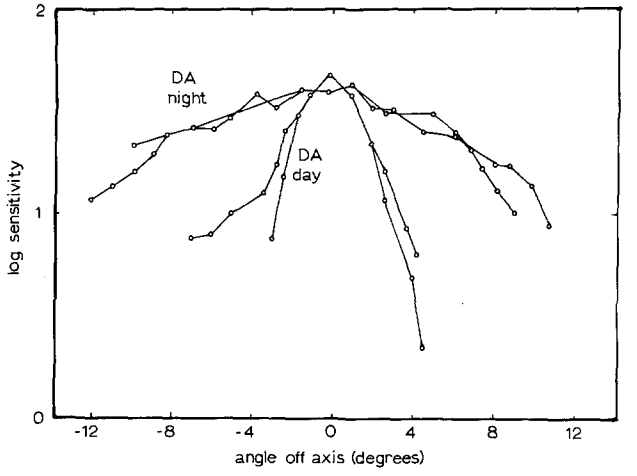

Fig. 5. Angular sensitivities to two photoreceptors from the same region of the same eye. The acceptance angle of the cell dark-adapted in the day is 4°, while that of the cell dark-adapted at night is 16°. In comparison, the absolute sensitivity of the night cell on axis is not significantly different from that of the day cell, suggesting that the diurnal rhythm does not affect dark adaptation mechanisms other than acceptance angle change. To show scatter, two runs are shown for each acceptance angle determination

grounds (maximum of two log units above dark-adapted threshold) which gave significant changes in the insect eyes.

It was known, however, as mentioned above, that crabs, in common with other Crustacea, show movements of screening pigment which are influenced both by ambient light levels and a diurnal rhythm. Kleinholz (1937) showed in *Portunus* that what had been termed 'iris' pigments (Exner 1891) – the distal screening pigments – would not move upon dark adaptation in the day, but would do so at night. It was thought likely that such pigment movement might mediate receptive field size in crabs, and therefore photoreceptor acceptance angles were measured at night as well as in the day.

Night State. At night, the acceptance angles of single cells were measured in both dark and light-adapted states. The results are shown in Table 1 and Fig. 4. Photoreceptor acceptance angle did indeed narrow upon light adaptation: after 30 min of adaptation (Fig. 4) acceptance angle had halved from 10° to 5°. Here, then, was an acceptance angle change analogous to the response to direct change in illumination in other arthropods.

There is also a loss of absolute sensitivity *on axis* upon light adaptation at night. This is attributable to the same rapid mechanisms of light adaptation as seen in the day. At both day and night, on-axis sensitivity dropped by one log unit within 30 s of the commencement of adaptation and was within 80% of that value at the first test flash 4 s after adaptation commenced. This process is presumed to involve the more rapid intraphotoreceptor feedback mechanisms common to many arthropod eyes.

The results posed a further question: did the diurnal rhythm lead to other sensitivity changes independent of acceptance angle change? To observe sensitivity and acceptance angle changes due only to the diurnal rhythm, and not to changes in ambient light intensity, measurements were made, in a single crab, of both the relative sensitivities on axis and the acceptance angles of dark-adapted retinula cells. The experiments began in the afternoon at various times before the diurnal change, and extended through the change into the night phase. Under constant darkness except for occasional test flashes, any systematic acceptance changes or on-axis sensitivity changes that correlated with the time course of the diurnal rhythm as measured by ERG would clearly be an effect of the diurnal rhythm. Single penetrations in *Scylla* could not be maintained throughout the minimum of six hours necessary to conduct the experiments; therefore records were made sequentially from different cells. The records were taken from the same region of the eye as some precaution against variation.

Results are assembled in Table 1. Firstly, as expected from the results already given, there was a clear increase in acceptance angle: in the extreme case, from day values of 4° to night values of 16° (Fig. 5). Secondly, comparing day cells with night cells from the same eye region of the same eye, there was no significant diurnal change in dark-adapted sensitivity to a small spot on axis (Fig. 5). This result was documented more thoroughly by measuring the

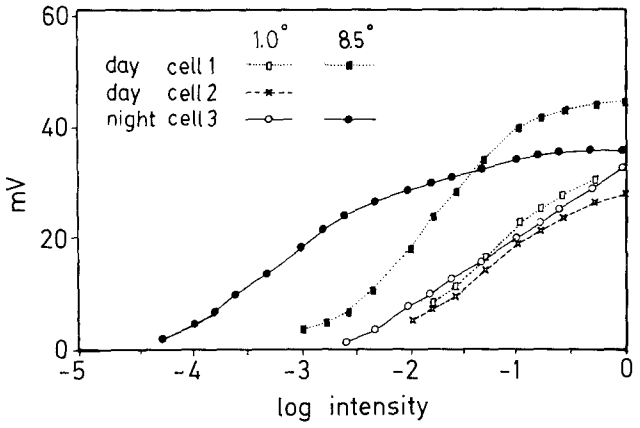

Fig. 6. Intensity response curves from three photoreceptor cells from the same eye of a single crab, two recorded in the day, and one at night. The responses are to spots of equal brightness per unit area, but of different apertures, one subtending 1° at the eye surface, the other 8.5°. It can be seen that while the response to the 8.5° spot is up to ten times greater at night, the response to the 1° spot is very similar at both day and night, suggesting that the diurnal rhythm does not markedly affect the absolute response to a small spot on axis

intensity/response curves of thoroughly dark adapted cells to two spots of different angular subtenses (1° and 5°) at the eye (Fig. 6). The results show that the intensity response curves of both day and night cells to a *small axial* spot show little or no displacement from each other – clear evidence that little or no increase in sensitivity to a small source has occurred. In contrast, however, the response to an *extended source* varies markedly with time of day. While the day response to the 8.5° spot is greater than that to the 1° spot (expected because the acceptance angle even of a day unit is greater than 1°) the night response to the 8.5° spot is greater still, showing that the acceptance angle has widened as a result of the diurnal rhythm. The important control is that the sensitivity to the small spot has shown no significant change at all with time of day while sensitivity to the 8.5° extended source has increased at night, demonstrating a diurnal rhythm effect. These experiments, taken with the earlier experiments measuring acceptance angle and sensitivity to a point source only, offer evidence that acceptance angle change is the main diurnally controlled change in receptor performance and that changes in absolute sensitivity are not involved to the same degree.

The results so far correlate closely in a *qualitative* sense with known anatomical changes in the portunid crab eye (Kleinholz 1937). The next section describes a reinvestigation of movements of screening pigments in the portunid eye which permits the conclusion that the movements of extraphotoreceptor screening pigments are the major if not the only cause of the acceptance angle change. The evidence for this is ophthalmoscopic data which suggests that *quantitatively* the change in screening pigment position is more than sufficient to account for the change in photoreceptor capture area estimated electrophysiologically.

Diurnal Rhythm of Screening Pigment Movement

Four kinds of non-visual pigment occur between ommatidia in the eyes of crabs. These are the primary and secondary distal screening pigments, the proximal screening pigment and the (proximal) tapetum (Kleinholz 1937). Inside each photoreceptor cell, but outside the photoreceptive rhabdom, a fifth non-visual pigment occurs – the intraphotoreceptor screening pigment (Eguchi and Waterman 1967; Ludolph et al. 1973). A red basal pigment lies below the basement membrane, but was never observed in the retina of portunids.

As mentioned above, Kleinholz (1937) studied the anatomy of crab eyes adapted under all four possible combinations of light, dark, day and night. His finding, in *Portunus*, the congeneric of *Scylla*, was that only under one of the four sets of conditions – at night, in darkness – would the extraphotoreceptor pigments of the eye – the screening and tapetal pigments – adopt their 'dark adapted' configurations. These configurations, confirmed for *Scylla* by light microscope, are as follows (Fig. 9).

In the day, light-adapted, state the distal pigments extend from the cones about a third of the way to the basement membrane, so wrapping the distal third of each ommatidium in screening pigment. At the same time the proximal screening pigment covers the proximal third of each ommatidium, and obscures the white tapetal pigment which, in contrast to the case for grapsids (Stowe 1980), exists only at the level of the basement membrane and below.

At night, in the dark-adapted state, the distal screening pigment is contracted distally, and is withdrawn completely from the retina, forming tight knots slightly distal to the level of the cone tips. Proximally, as in the prawn *Palaemonetes* (Kleinholz 1961), the proximal screening pigment has withdrawn well below the basement membrane while the tapetum has moved further into the retina.

Because Eguchi and Waterman (1967) had found no diurnal rhythm effect on the *intraphotoreceptor* screening pigment granules of the decapod crab *Libinia*, it became of interest to repeat aspects of Kleinholz's study of the extraphotoreceptor pigments. The obvious interpretation of Kleinholz's results was (a) that the distal screening pigments acted as an 'iris' for each ommatidium, and that the iris opened at night, in darkness, and (b) that the highly reflective tapetum was occluded by proximal screening pig-

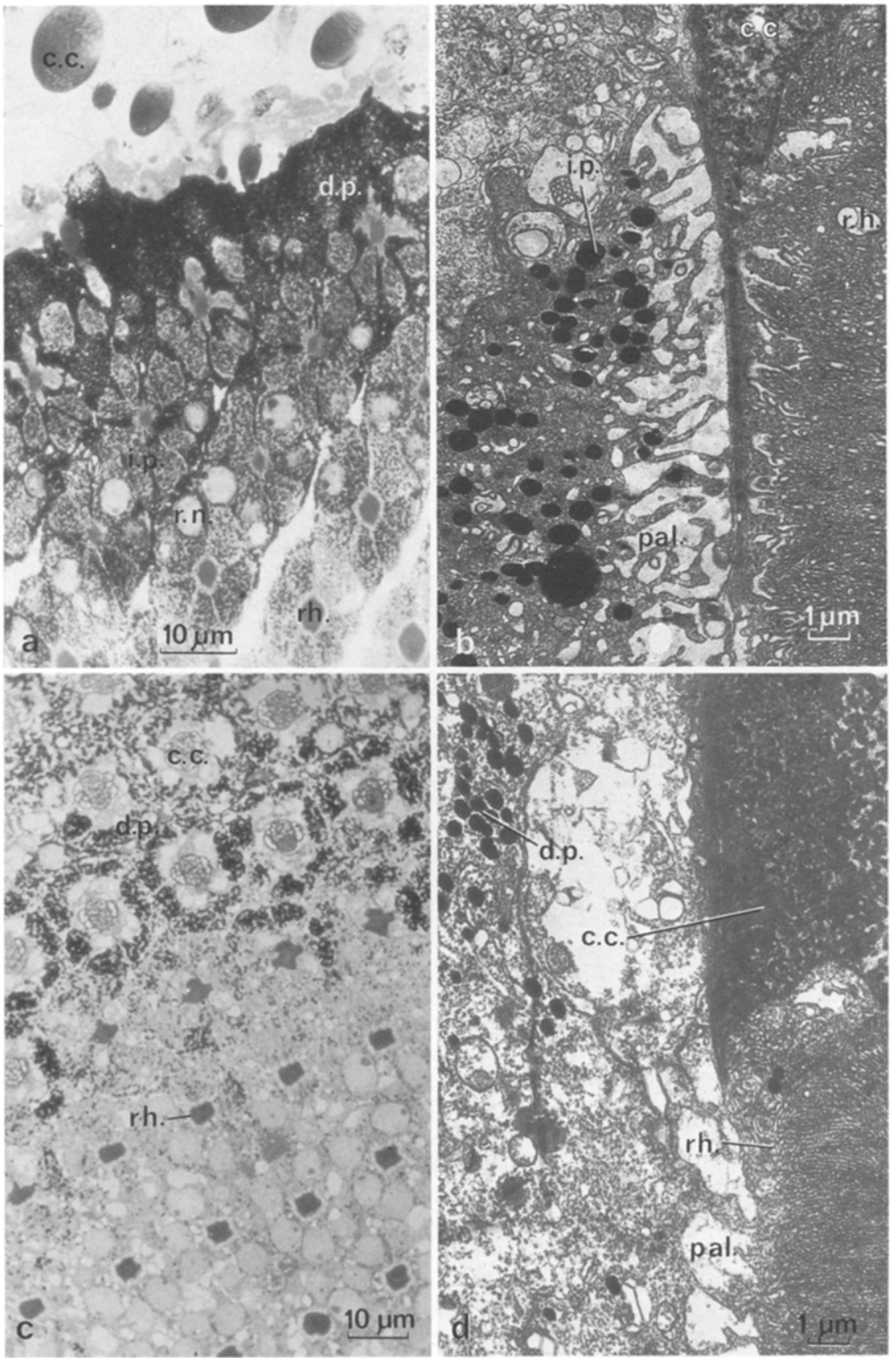

Fig. 7. **a** Light micrograph of the day, light-adapted distal retina of *Scylla* cut in cross section. Moving in from the periphery we have the crystalline cones (*c.c.*), distal pigment (*d.p.*), rhabdoms (*rh.*), intraphotoreceptor screening pigment (*i.p.*), and retinula cell nuclei (*r.n.*). **b** Electron micrograph of the day, light-adapted distal retina cut in longitudinal section at the level of the cone/rhabdom interface. **c** Light micrograph of the night, dark-adapted distal retina cut in cross section. **d** Electron micrograph of the night dark adapted distal retina cut in longitudinal section at the level of the cone/rhabdom interface. Overall, dark adaptation at night causes the distal pigment to migrate radially outward along the cones, opening the pupil. The intraphotoreceptor pigment is dispersed, leaving the rhabdoms the only strongly absorbing structures in the photoreceptor layer. Note the narrower distal rhabdoms (*rh.* in **a**) and the greater peripheral taper of rhabdoms in the light-adapted state (cf. **a**, **c**)

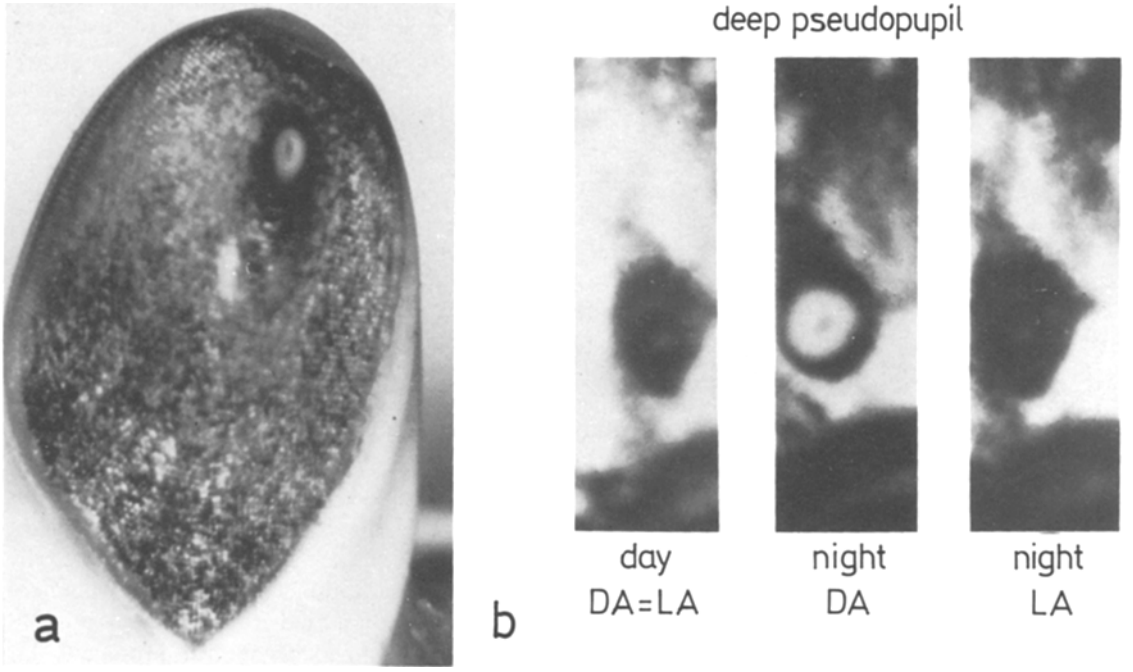

Fig. 8a, b. In vivo photographs of the deep pseudopupil made with orthodromic white light. The deep pseudopupil represents the superimposition of images from a number of facets. Each image is of the plane at which the rhabdom meets the cone. By this means, one can directly observe anatomical changes in this plane. Eye-shine is observed only at night and in the dark-adapted crab eye (see Smith 1948); the photographs show the eye of *Cancer borealis* (**a**) and *Scylla serrata* (**b**, middle). In the day the deep pseudopupil of dark adapted eye and light adapted eye are identical (**b**, left). Light adaptation in the night turns the pseudopupil into the day state (**b**, right); see Stavenga (1979). The iris created by the distal pigment is opened at night so that the exposed tapetum can be seen by way of the eyeshine. The absorbing rhabdom is visible then as the dark centre in the pseudopupil

ments in the day but at night was exposed to the retina. Kleinholz's sections however, had not been sufficiently thin to enable the demonstration that the aperture formed by the distal pigment did indeed enlarge in darkness at night. It was decided, therefore, to reinvestigate the behaviour of the distal pigments at two levels: (a) using EM and LM, to re-examine the configurations of the distal pigments in the night-dark adapted state and in the day-light adapted state; and, (b) to measure by ophthalmoscopy in living eyes the change in iris diameter brought about by the diurnal movements of distal pigment in the dark-adapted state. This would enable the iris change to be compared with the acceptance angle change under identical conditions.

Histology. The histological results are shown in Fig. 7. They show that while the distal pigments indeed withdraw from the retina as expected they also move radially outward just as expected for an iris.

Furthermore, similar to that found in *Grapsus* and *Ocypode* (Nässel and Waterman, 1979) there is some increase at night in rhabdom diameter. In *Scylla* the distal rhabdom diameter increased most, so that rhabdoms were less tapered at night (Fig. 7, cf. a and c). In crabs with eyes of the same facet diameter ($=36$ μm), the rhabdom diameter at the crystalline cone interface in the day (light-adapted) was 4.0 ± 0.5 μm, while the equivalent measurement for eyes dark-adapted at night was 7.0 ± 0.6 μm ($n=8$).

In the context of this larger rhabdomeric capture area at night it is interesting that the sensitivity to a point source measured electrophysiologically did not show a noticeable increase at night. It is possible that any such sensitivity increase might not have been made manifest under the experimental conditions used. The 1° subtense point source is considerably within even the narrowest *Scylla* acceptance angle, and if the point is focused on the rhabdom tip as is likely (Leggett unpublished results), rhabdom widening would not be expected to cause any extra response.

Ophthalmoscopy. The movements of distal pigment observed histologically are confirmed by observations from living crabs. Ophthalmoscopy, in the dark-adapted state throughout, shows that screening pigment forms a tight collar round the ommatidium in the day, and moves radially outward at night (Fig. 8). Although further investigation of the pseudopupil was beyond the scope of the project, the increased area free of screening pigment at night was found in the

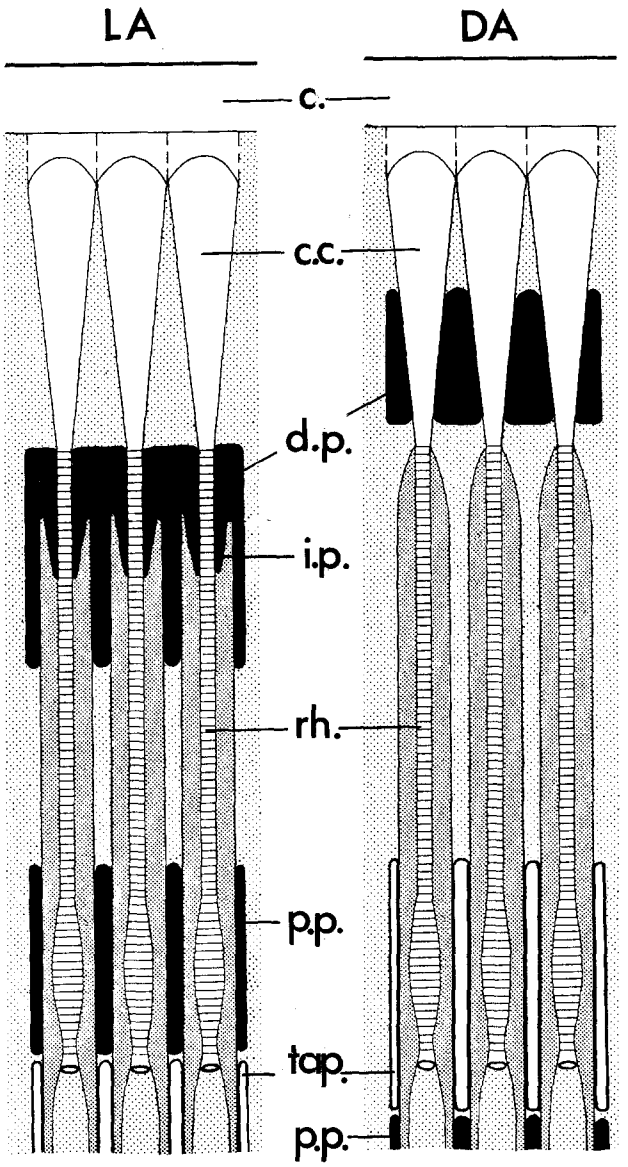

Fig. 9. Reconstruction of ommatidia of the crab *Scylla* as they would appear in the light-adapted state by day, and in the dark-adapted state by night. The reconstructions are diagrammatic: no attempt has been made to delineate all cell membranes, or cell bodies or the palisade. The changes which occur on dark adaptation at night are that both distal and proximal screening pigments are withdrawn from the retina, the intraphotoreceptor screening pigment disperses, the tapetum advances into the retina, and the collar around the rhabdom formed by the distal pigment expands radially outwards. These changes allow more light into the eye and reduce the chance of its being absorbed by screening pigment once it is there. *c* cornea; *c.c.* crystalline cone; *d.p.* distal (screening) pigment; *i.p.* intraphotoreceptor screening pigment; *rh.* rhabdom; *p.p.* proximal screening pigment; *tap.* tapetum

three animals tested to be over twenty times that of the day state. Under conditions such as found in the eye of *Scylla*, where the sensitivity to a point source on axis remains the same, the quantum capture of a photoreceptor is proportional to the square of the acceptance angle (Tunstall and Horridge 1967; Snyder et al. 1977). The acceptance angle of crab photoreceptors increases by up to fourfold from day to night: therefore potential quantum capture from extended sources, in the absence of other changes, should increase by up to sixteenfold.

Thus, the diurnal increase in photoreceptor aperture ($>20\times$) is greater than that of photoreceptor quantum catch ($\simeq 16\times$). As outlined below, the aperture increase caused by the diurnal movement of extraphotoreceptor screening pigments is in principle sufficient to explain the diurnal increase in photoreceptor quantum catch at night. Further, the result corroborates the electrophysiological evidence that the acceptance angle change is the only major electrophysiological change under diurnal influence in the photoreceptors of *Scylla*.

It can be concluded that two patterns of light flux occur in the crab eye, a day pattern and a night pattern. Each light pattern is in turn controlled by a particular configuration of screening pigment (Fig. 9). In the light-adapted day eye, rays not absorbed by the photopigment do not propagate far before they are absorbed by screening pigment. In the dark adapted night eye, however, a wide cone of off-axis rays is now no longer impeded by any screening pigment. These off-axis rays now encounter a retina in which the only highly absorbing objects in the ray path are the rhabdoms. The other structures are either relatively transparent (the ommatidial cytoplasm) or reflecting (the tapetum). Within the ommatidial cytoplasm, the intracellular screening pigment granules are dispersed upon dark adaptation at any time of day (Eguchi and Waterman 1967). Thus, their absorptive effect is reduced in the dispersed state. Between ommatidia the extracellular screening pigments, both distal and proximal, are withdrawn from the photoreceptor layer. Withdrawal of the distal pigment allows a wide cone of rays, otherwise absorbed, to propagate towards other rhabdoms. Withdrawal of the proximal pigment exposes the tapetal layer, which reflects light not absorbed by rhabdoms back into the receptor layer a second time. All these mechanisms act to increase photopigment absorption by decreasing competitive absorption by screening pigment.

Discussion

The present results demonstrate a diurnal change in the acceptance angle of crab photoreceptors of up to a factor of 4. This acceptance angle change is the largest so far recorded in an arthropod apposition eye when compared to the existing data from light-dark adaptation studies; cf. *Locusta:* a factor of 1.6 (Tunstall and Horridge 1967; Wilson 1975), blowfly

Calliphora: 1.2 (Hardie 1979; Beersma 1979), giant water bug: 2.5 (Walcott 1971), cockroach: 3.0 (Butler and Horridge 1973), mantis: 2.6 (Rossel 1979), shore crab: 2.3 (Stowe 1980).

Superposition eyes are commonly regarded as having a much greater capacity to enlarge visual fields, but the *Scylla* result falls neatly halfway between the apposition eye data and the sixfold factor found for the superposition eye of crayfish by Walcott (1974). Although the details of the optics still remain to be elucidated, the data of *Scylla* underscore the point that apposition eyes also can show significant changes in acceptance angle and that the difference between the two eye classes, at least in performance, is more one of degree than of kind.

Significance of Acceptance Angle Change

Per unit time, photoreceptor sensitivity can be increased by increasing either photoreceptor gain or the number of quanta caught. Ultraviolet photoreceptors in the dragonfly (Laughlin 1976) and fly (Hardie 1977) have a larger gain than green photoreceptors. But while this mechanism is advantageous in that it increases the neuronal signal relative to intrinsic neuron noise, its disadvantage is that it increases extrinsic or photon noise along with the signal.

The only strategy which increases the signal relative to both photon and neuron noise is to increase the number of quanta caught. One mechanism is to increase the amount of photopigment in the eye. For example, the spider *Dinopis* greatly enlarges its volume of photopigment upon darkness, and decreases it upon illumination (Blest 1978). A similar system seems to be working in *Limulus* eyes (e.g. Chamberlain and Barlow 1977) and to a less dramatic extent in crabs (Nässel and Waterman 1979; this report).

The mechanism for increasing quantum catch most widely used by eyes, however, is to increase the size of the capture area. Most commonly, many eyes enlarge their pupils in dim light. This is not a true enlargement of part of the eye but a restructuring. The amount of light caught by the eye remains much the same, but there is a decrease in the amount of light caught by screening pigment, and an increase in the amount of light caught by the visual pigment. This is true for pupil change in both "camera" and compound eyes. However, while pupil enlargement in both camera and compound eyes lets more light into the eye, the way in which this occurs is effectively opposite in each case.

In a camera eye, for example, the human eye, an increase in pupil diameter increases the intensity of the retinal image of a point source but only slightly changes its size (Cornsweet 1970). This point correlates with the observation that normal pupil enlargement only slightly changes acuity in humans (Riggs 1965).

The results presented in this paper suggest that pupil opening in crabs has a somewhat different effect. This can be inferred first from the electrophysiological results which show that the on-axis sensitivity of photoreceptors to a point source does not appreciably change, but that the acceptance angle distinctly broadens and, secondly, from the anatomical results which show that after pupil opening light from a point source must reach more receptors.

Such acuity loss may seem to be the unavoidable result of a trade-off which increases sensitivity at the expense of narrow acceptance angles. At the low light levels at which this form of increased sensitivity actually develops, however, it is likely that the increased sensitivity may lead to an *increase* in the maximum acuity possible to the system. As light levels drop, the maximum acuity per unit time possible to any detector is reduced, assuming fixed quantum efficiency, simply because the information content reduces as the square root of the quantum flux (Rose 1973). As has been discussed for the general compound eye case by Laughlin (1975) and Snyder (1979), the increased quantum catch resulting from an enlarged acceptance angle may therefore actually increase the acuity possible to the crab night eye over that possible to the day eye, were it to operate at the same low light level.

Significance of Diurnal Rhythm Influence on Acceptance Angle Change

The overall conclusion from the photoreceptor performance of *Scylla* is that *intraphotoreceptor* mechanisms appear independent of diurnal rhythm influence, and that the main diurnal effect is the change in acceptance angle.

These findings differ from those of Barlow et al. (1977) in *Limulus* which suggested an increase in intraphotoreceptor gain. However, the recordings suggesting this were not directly from photoreceptors but were from the eccentric cell, a second order neuron with input from all 10–12 receptors of an ommatidium. Secondly, the published day intensity-response curve of the eccentric cell was not a simple sigmoid curve and one part of it suggested an inhibition of sensitivity. The implication then is that part of the night effect is the release of a day inhibition. These factors complicate comparisons and suggest that different mechanisms may be at work in these two only distantly related arthropods.

Yet structural changes occur in the lateral eyes of *Limulus* (Barlow et al. 1980), which are functionally similar to those of crab eyes: the position of the distal pigment changes so that an iris opens at night and the acceptance angles increase (in *Limulus* the ommatidial acceptance angle increases twofold).

Diurnal rhythms of change in one or another eye property have been found in a wide range of animals including (vertebrates) rat (LaVail 1976) and catfish (Welsh and Osborn 1936); (arthropods) crabs (Kleinholz 1937; Nässel and Waterman 1979; Stowe 1980), crayfish (Aréchiga and Wiersma 1969), ant (Ventura et al. 1976), beetle (Jahn and Wulff 1943; Meyer-Rochow and Horridge 1975), mantis (Rossel 1979), horseshoe crab (*Limulus*) (Barlow et al. 1977), scorpion (Fleissner 1972) and (molluscs) sea hare (*Aplysia*) (Jacklet 1969). In those visual systems with diurnal rhythms which have so far been studied anatomically, there have almost always been diurnal changes in the receptor layers of the retinas (the exception is in the rat). The changes have been movements or enlargements either of photoreceptors or of areas of screening pigment. In many of the animals exhibiting a diurnal rhythm of screening pigment movement, the response, just as in *Scylla*, while inhibited by light at night, is not, or only slightly, initiated by darkness during the day. In the photoreceptors of both mantid (Rossel 1979) and grapsid crab (Stowe 1980) acceptance angle broadening upon dark adaptation at night is greater than that induced in the day.

Darkness during the day also initiates little or no change in certain diurnally influenced body pigments – again in a phyletically wide range of animals. The range is from vertebrates – salamander larvae, frogs, the horned toad *Phrynosoma*, the chameleon, the minnow *Phoxinus*, and the lamprey *Lampetra* – to the brachyuran crab *Uca*, the phasmid *Carausius* and the echinoderm *Diaderma* (review Brown 1973).

Taken together, the foregoing is evidence that members of a wide range of phyla have an extra diurnal rhythm input to their pigmentary mechanisms of adaptation to light and dark. The overall generalization from all these cases is that certain pigment movements which would otherwise happen upon darkness are prevented from happening, during darkness in the day, by a diurnal rhythm.

The reasons that such diverse animals have this extra control system while many other animals apparently do not, has, to our knowledge and somewhat surprisingly, never been speculated upon.

It is possible that there may be no particular advantage conferred by control by both diurnal rhythm and light level as opposed to control by light level only. As Wright (1968) has pointed out, identical selective forces can lead different populations to quite different phenotypic compositions, each nonetheless sufficient to allow survival: feathers and fur are both adequate insulators. But one possible explanatory hypothesis stems from the following observations.

First, the pigment movements which are under diurnal rhythm influence tend to be slow; in the survey by Brown (1973) none were complete in under an hour. Secondly, a survey of the animals with diurnally controlled light adaptation mechanisms shows that most of them are nocturnal, while some are active either at day or night, and none are active only in the day. Thirdly, many of the animals, nocturnal or otherwise, tend to spend much of the day in dark refuges.

Given these observations, a possible function for the diurnal rhythm of pigment movement can be outlined as follows: in the finite and predictable world of the refuge, where vision could even be dispensed with altogether, a mechanism preventing slow dark adaptation would not be a great advantage. However, there would occur times when after perhaps hours in the refuge an animal would enter the daylight environment 'on short notice', either 'voluntarily' or after disturbance. In either case, there would be great selective advantage in a mechanism which obviated the need for slow light adaptation and thus enabled an animal to emerge into the daylight already light adapted.

In the case of the night, a long, slowly developing, slowly retreating period of darkness, the slow pigment changes would be of advantage without any of the drawbacks of the day situation. The night occurs with a predictable periodicity: therefore the control of the slow adaptation mechanism by a clock is an appropriate way of ensuring that the changes occur only when they are appropriate.

A final prerequisite for adaptation to occur requires not only that it is the right time, but also that it is dark. This possibly enables fine adjustment for the exact timing of nightfall and daybreak, which, of course, varies with the seasons. Such adjustment would further ensure that animals were not dark-adapted while it was light.

Such a diurnal rhythm of light adaptation, of course, would serve no purpose: (i) in animals which appear not to deep dark adapt by slow mechanisms (e.g. the bee: Autrum 1958), or (ii) in those animals which do deep dark adapt by slow mechanisms, but which can rapidly bring a high acuity system into play because it exists as an anatomically separate system (e.g., animals with duplex retinae, such as man).

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