The Journal of Experimental Biology 204, 239–248 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
JEB2795

DIURNAL CHANGES IN RETINULA CELL SENSITIVITIES AND RECEPTIVE FIELDS (TWO-DIMENSIONAL ANGULAR SENSITIVITY FUNCTIONS) IN THE APPOSITION EYES OF *LIGIA EXOTICA* (CRUSTACEA, ISOPODA)

TAKAHIKO HARIYAMA[1,*], V. BENNO MEYER-ROCHOW[2], TSUTOMU KAWAUCHI[1], YASUHARU TAKAKU[1] AND YASUO TSUKAHARA[1]

[1]*Graduate School of Information Sciences, SKK Building, Tohoku University, Katahira 2, Sendai 980-8577, Japan* and [2]*Department of Biology (Section of Animal Physiology), University of Oulu, PL 8000, SF-90014 Oulu, Finland*

*e-mail: hariyama@biology.is.tohoku.ac.jp

Accepted 27 October 2000; published on WWW 3 January 2001

Summary

The structural organization of the retinula cells in the eye of *Ligia exotica* changes diurnally. At night, the microvilli elongate, losing the regular and parallel alignment characteristic of the day condition. Crystalline cones and distal rhabdom tips are not pushed into each other during the day, but at night the rhabdoms protrude into the crystalline cones by up to 5 μm. Screening pigment granules in the retinula cells disperse during the night, but migrate radially towards the vicinity of the rhabdom during the day. No such displacements of the pigment granules of either distal or proximal screening pigment cells were observed.

The sensitivity of the eye, monitored by electroretinogram (ERG) recordings, changes diurnally: values at midnight are, on average, 10 times those occurring during the day. However, intracellular recordings from single retinula cells (50 duing the day and 50 at night) indicate that the difference between night and day sensitivities is only 2.5-fold.

Two-dimensional angular sensitivity curves, indicative of a single unit's spatial sensitivity, had considerably less regular outlines at night than during the day. If based on the 50 % sensitivity level, day and night eyes possessed receptive fields of almost identical width (approximately 2 °), but if sensitivities below the 50 % limit were included, then receptive fields at night were significantly more extensive.

We suggest that the morphological adaptations and diurnal changes in chromophore content seen in the apposition eye of *L. exotica* allow this animal to improve its photon capture at night while preserving at least some of the spatial resolving power characteristic of the light-adapted state. This would explain why this animal is capable of performing complex escape behaviours in the presence of predators both in bright and in very dim light.

Key words: circadian sensitivity rhythm, receptive field, vision, photoreceptor, apposition eye, Crustacea, Isopoda, *Ligia exotica.*

Introduction

Ever since the monumental study of Exner (Exner, 1891), compound eyes have broadly been classified as 'superposition' (those with a clear space between the crystalline cones and the retinula cells) or 'apposition' (those in which no gap or clear zone exists between the retinula cells and the crystalline cones). Superposition eyes are frequently encountered in crepuscular and nocturnal arthropods, while apposition eyes appear to be advantageous, and therefore more common, in diurnal species (Warrant and McIntyre, 1993).

To cope with sudden light intensity changes and to function over a large dynamic range of intensities, compound eyes have available to them a variety of adaptations that differ from those that prepare the eyes for the more predictable and regular diurnal intensity changes (Meyer-Rochow, 1999). In the superposition eye, an extension of the cones that connects the latter with the retinula cells across the clear zone, and which is known as the crystalline thread, elongates during light adaptation; concomitantly, screening pigment granules may migrate into the clear zone from distal stores of specialized cells between the cones and/or from below the basement membrane. One consequence of such changes is that in the light-adapted state only light close to the ommatidial axis can reach the underlying retinula cell, so that the acceptance angle of the retinula cell narrows and its sensitivity to light decreases (DeBruin and Crisp, 1957; Bernhard and Ottoson, 1960; Bryceson and McIntyre, 1983). Additional processes within the photoreceptor cells themselves, affecting the transduction cascade and membrane properties, are also frequently involved and help to optimize the structure and function of compound eyes under challenging photic situations (Terakita et al., 1996; Dorlöchter and Stieve, 1997; Kashiwagi et al., 2000). Finally, parallel with the anatomical and physiological modifications

accompanying dark/light-adaptation, the total amount of the visual pigment chromophore may change rhythmically as in the compound eyes of the amphipod *Orchomene plebs* (Hariyama et al., 1993a) and the crab *Hemigrapsus sanguineus* (1.4 times greater at night than during the day; Arikawa et al., 1987).

Although the apposition type of compound eye is more common in arthropods, and some important research on the visual consequences of dark/light-adaptational changes has indeed been carried out on this type of eye (e.g. Meyer-Rochow, 1974; Horridge et al., 1981; Leggett and Stavenga, 1981; Williams, 1983), there has been more interest in the superposition compound eye (e.g. DeBruin and Crisp, 1957; Bernhard and Ottoson, 1960; Bryceson and McIntyre, 1983; Meyer-Rochow and Tiang, 1984; Warrant and McIntyre, 1990).

To help restore the balance, we have chosen to examine the apposition eye of *Ligia exotica*, a semi-terrestrial coastal isopod, which is active both in broad daylight and at night. This species is known to have an eye in which the volume of the rhabdom changes diurnally, even when the animal is kept in continuous darkness (Hariyama et al., 1986). It has also been shown that the amount of chromophore in the retinula cells of this animal oscillates endogenously (Hariyama and Tsukahara, 1992) and that the pigment granules in the retinula cells migrate diurnally even in the absence of light (Hariyama et al., 1986). However, no apparent pigment granule displacements in the primary pigment cells around the crystalline cones and the secondary pigment cells peripheral to the retinula cells were observed.

Since the precise functional consequences of these rather complex dark/light-adaptational changes are still only poorly understood, we felt that the eye of *L. exotica* offered us a double opportunity: first, it would permit us to correlate altered morphological states with some important functional parameters such as retinula cell receptive fields and sensitivity thresholds and, second, it would provide us with an opportunity to expand our knowledge of the workings of the apposition type of eye.

Materials and methods

Animals

Male and female *Ligia exotica* (Roux) (approximately 4–5 cm total body length) were collected from rocky platforms on the local seashore (Shichigahama, Miyagi Prefecture) during the summer months. The animals were maintained in the laboratory for at least 1 week under a 12 h:12 h light/dark photoperiod (06:00 h lights on; 18:00 h lights off) prior to experimentation.

Morphology

For light microscopy and transmission electron microscopy, the compound eyes were dissected out in cold prefixative, consisting of a solution of 2 % paraformaldehyde and 2 % glutaraldehyde in 0.1 mol l^{-1} sodium cacodylate adjusted to pH 7.2. To study night-adapted eyes, they were removed from specimens in total darkness, using night-viewing equipment, and were prefixed in darkness for 2 h in the refrigerator. The tissues were then rinsed in the buffer and postfixed for 2 h in 1 % OsO_4 in the same buffer at room temperature (23 °C). Fixed tissues were dehydrated in a graded series of ethanol, transferred to propylene oxide and embedded in Epon-Araldite. Sections were cut with an LKB Ultratom Nova microtome. For scanning electron microscopy, specimens were prefixed for 2 h in 2.5 % glutaraldehyde in the same buffer. The specimens were then frozen in liquid nitrogen and cut horizontally with a razor blade to expose the desired tissue layer. Following dehydration in a graded series of ethanol, drying with a critical point apparatus (Hitachi HCP-1) and coating with gold vapour (Eico ion coater IB-3), the specimens, glued onto aluminium stubs, were ready for observation using a Hitachi S-310 scanning electron microscope. Schematic drawings (see Fig. 3) were reconstructed on the basis of data generated by light, scanning electron and transmission electron microscopy of both cross and longitudinal sections.

Electrophysiology

An experimental animal was forced, by mechanical (e.g. tweaking with tweezers) or thermal stimulation, to autotomise its legs and antennae. The animal was then glued firmly down at its mouth onto a holder with a wax/resin mixture. The rest of the body could move freely, and the uropods were kept damp with wet cotton throughout the course of the experiment. Although regional morphological differences were not obvious in the eye of *L. exotica*, penetrations of retinula cells for intracellular measurements were performed mainly on cells located in a horizontal direction relative to the animal's body axis.

Schematic drawings of the electrophysiological measurement techniques are shown in Fig. 1. For electroretinogram (ERG) recordings, a sharpened tungsten electrode was introduced just below the cornea. The reference electrode penetrated the head carapace and was positioned near the basement membrane of the experimental eye. The end of the stimulating light guide (diameter 5 mm) was masked by a small plate and a lampshade-like hemisphere behind the plate. Both the small plate and the lampshade-like hemisphere were coated with aluminium oxide and placed near the compound eye such that the beam of light reached the compound eye from several directions (Fig. 1 upper diagram). For intracellular recordings, glass electrodes with resistances of approximately 120 MΩ, filled with 1.5 mol l^{-1} potassium citrate, were used. To enable insertion of the electrode, a small triangular hole (equivalent to 3–5 facets) was made in the dorsal region of the cornea with a sharp sliver of razor blade (Hariyama et al., 1990). The smaller the hole, the better the chance of long-term recordings. The electrode was pushed into a retinula cell with the aid of a manipulator driven by a stepping motor (Narishige SM-21).

L. exotica has three spectral types of retinula cells (Hariyama et al., 1993b). In this study, only one spectral type (with maximum spectral sensitivity at 520 nm) was used

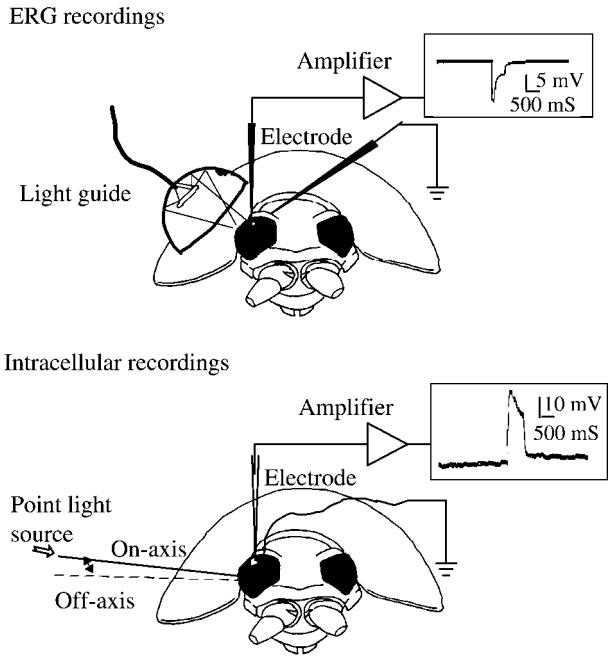

Fig. 1. Experimental arrangement for recording from the compound eye of *Ligia exotica*. For electroretinogram (ERG) recordings (top), the end of the light guide was placed near the compound eye. The light from the light guide was scattered by a small plate fixed inside a hollow light-reflecting hemisphere so that the light reaching the eye would do so from various directions. The inset shows an example of a typical ERG (a cornea-negative mass response recorded extracellularly). For intracellular recordings (bottom), a point light source (subtending 0.4° of the visual angle) was moved across the eye in 0.5° steps using a perimeter device. The inset shows an example of a typical depolarization response to a flash of light.

because of the greater ease with which intracellular recordings could be maintained for longer periods (for at least half a day) in this cell type compared with the other two spectral cell types. The reference electrode, a silver chloride wire, was placed in an unstimulated dorsal part of the same eye. The animal was mounted in the centre of a perimeter device. The end of the light guide was fixed to the perimeter device so that the stimulating light was always the same distance (15 cm) from the surface of the eye irrespective of direction. Optical stimulation came from a small monochromatic source (subtending 0.4° in visual angle terms) functioning as a point source for angular sensitivity measurements. In all recordings, we attempted to keep the optical axis of the ommatidia under investigation accurately in the horizontal plane, but since cells were penetrated by chance, the recorded axis may have deviated from the horizontal plane by a maximum of 3°. Two-dimensional angular sensitivity functions of a retinula cell could be obtained and monitored with the electrode in the retinula cell all day including transitions from day to night and *vice versa*. To confirm that the electrode stayed in the same retinula cell throughout the experiment, the membrane potential was monitored continuously. During the day, the eye (with electrode inserted, stationed on the experimental stage) was kept illuminated, except during electrophysiological measurements. At night, and, in the ensuing continuous darkness, during subjective day and subjective night, the animals were kept in the dark.

Responses were amplified with a high-impedance pre-amplifier (Nihon Koden MZ 8201) and a high-gain amplifier (Nihon Koden AVH-10). The magnitude of the responses (peak amplitude) was measured directly on an oscilloscope (Nihon Koden VC10) and with a chart recorder (Graphtec Linerecorder FWR 3701).

Apparatus for delivering stimuli

An Ushio power supply (XB-15101 AF) was used to run a 150 W xenon arc lamp (Ushio Inc., Type UXL-151D-O). Quartz lenses produced a parallel beam of light, which passed through a heat-absorbing filter (Toshiba IRA-25S) and one of a set of 16 narrow-band interference colour filters (Vacuum Optics Corp., Japan). The light intensities of the stimulating lights were measured with a silicon photodiode (S876-1010BQ, Hamamatsu Photonics K.K.), and the light intensity was altered with quartz neutral density filters (Vacuum Optics Corp., Japan). With the aid of neutral density filters, the monochromatic lights were adjusted so that they contained an equal number of photons. At all wavelengths, the maximum intensity available at the surface of the eye was 6.0×10^{13} quanta $cm^{-2}\,s^{-1}$, a value corresponding approximately to the brightness of a dull day. The light-emitting end of the light-guide was attached to the perimeter device as described above.

Results

Morphology

The two eyes of *L. exotica* are compound and sessile, occupying the lateral edges of the head (Fig. 1). Compound eyes such as those used in these experiments typically possessed approximately 1400 ommatidia, the latter representing the anatomical units of the eye. A single ommatidium consisted of two distinct subunits: the dioptric apparatus and the retina. Scanning and transmission electron micrographs demonstrated that the outer surface of the eye was covered by a multilayered cuticle (see Fig. 3). The outer surfaces of the ommatidia formed a hexagonal lattice of facets with inner-circle diameters of approximately 45 μm. Regional differences in morphology were not obvious. The crystalline cones, located directly below the corneas, consisted of four cells. When the eyes were crudely cut in the physiological solution, numerous crystal-like spheres approximately 30 μm in diameter were released from the dioptric region. Longitudinal (Fig. 2A,B) and transverse (Fig. 2C,D) sections confirmed their circular outlines; their strong affinity to Toluidine Blue suggested an optical role as lens elements within the crystalline cones. An accumulation of screening pigment granules in the distal pigment cells between adjacent groups of crystalline cone cells (Fig. 2A,C,D) probably served to prevent light rays from passing between the ommatidia. The

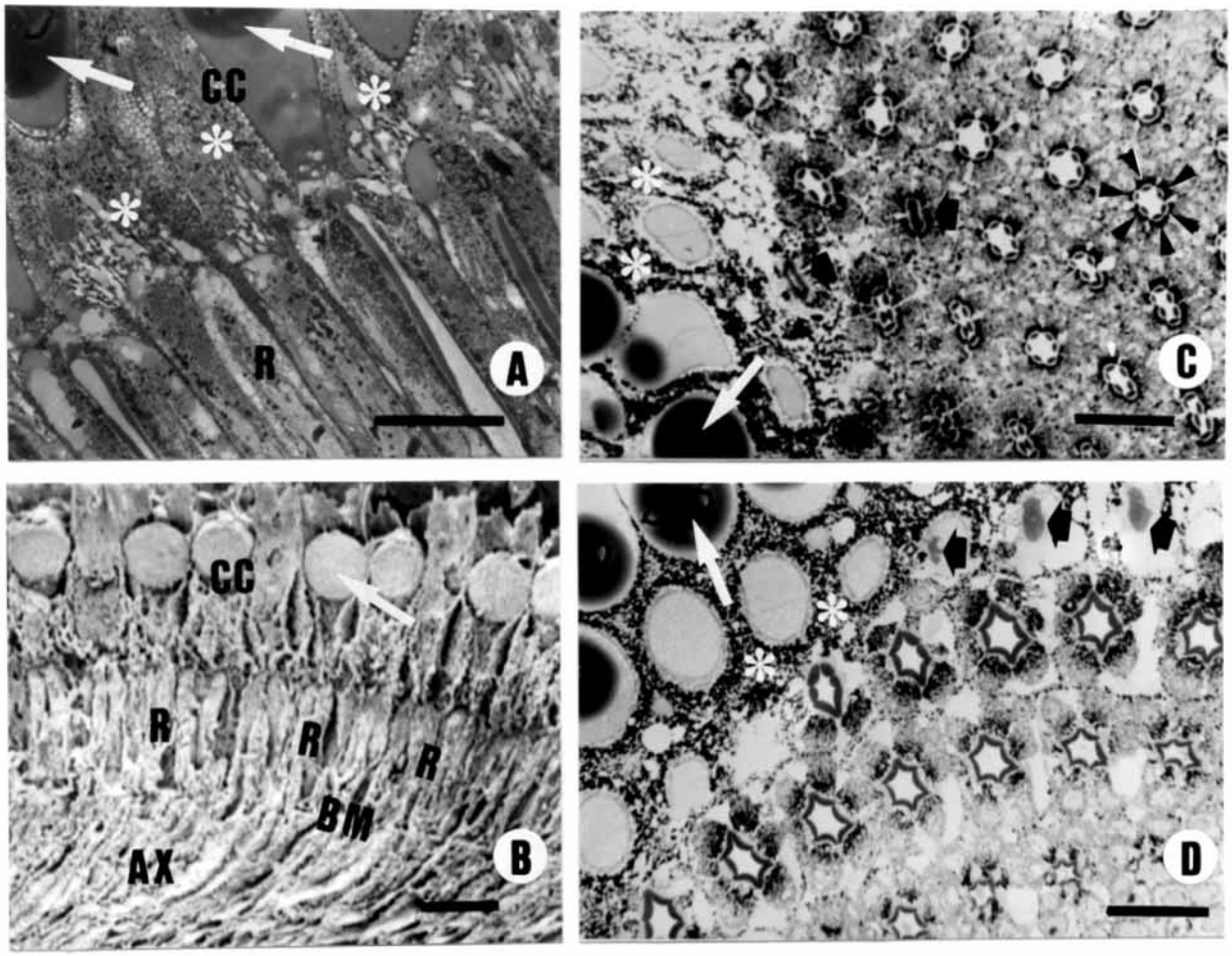

Fig. 2. Longitudinal (A,B) and transverse (C,D) sections through the retina of the eye of *Ligia exotica*. (A) Light micrograph of a longitudinal section through the proximal ends of the crystalline cones (CC) and the distal tips of the rhabdom and retinula cells (R). (B) Scanning electron micrograph of a longitudinally cleaved compound eye showing cone cells (CC) with spherical components (white arrow), retinula cells and rhabdomeres (R), basement membrane (BM) and axons (AX). (C) During the day, the crystalline cones only just contact the distal tips of the rhabdoms (black arrows), which consist of rather small and separate rhabdomeres. Retinula cell screening pigment granules are positioned near the cytoplasmic edges of the rhabdomeres (arrowheads). (D) At night, the rhabdom tips protrude slightly into the proximal ends of the cones (black arrows) and the rhabdomeres are considerably enlarged and laterally in contact with each other. Retinula cell screening pigment has become dispersed. White asterisks show the accumulation of screening pigment granules in the distal pigment cells and white arrows, spherical components. Scale bars, 50 μm.

total length of the dioptric apparatus was approximately 75 μm. There were seven retinula cells and one eccentric cell present in each ommatidium, tapering proximally to form thin axons (Fig. 2B). The retinula cells were approximately 100 μm long and 25 μm wide and surrounded by proximal (secondary) pigment cells. The retinula cells bore centrally facing rhabdomeres which consisted of numerous microvilli, each approximately 0.07 μm in diameter.

Morphological changes affecting the ommatidia were observed to depend on the time of day. Microvilli were elongated at night, so that the area occupied by the ommatidial rhabdom during the night was greater than during the day (Fig. 2C,D and Fig. 3, upper drawings). On the basis of observations made on 20 randomly chosen rhabdoms, we measured the total lengths of the rhabdom in day, night, subjective day and subjective night conditions using light and scanning electron microscopy. The respective lengths of each rhabdom were 101±3.0, 102±2.2, 100±2.3 and 102±1.7 μm (means ± S.D., N=20). With the exception of the distal rhabdom tips, which extended short distal projections, not present during the day (Figs 4A,C), of no more than 3 μm into the cones at night and during subjective night (Figs 4B,D), no significant diurnal changes affecting rhabdom lengths were observed. Rhabdom widths, however, increased very significantly at night and during subjective night over their daytime values, and the basically 'open' rhabdoms with adjacent rhabdomeres not touching each other during the day (Figs 2C, 4A) changed at night into the 'fused' type, in which neighbouring rhabdomeres conjoined to form characteristic ringlike profiles when cut in cross section (Fig. 2D). The screening pigment granules of the retinula cells (having diameters of approximately 0.3 μm) dispersed during the night and the

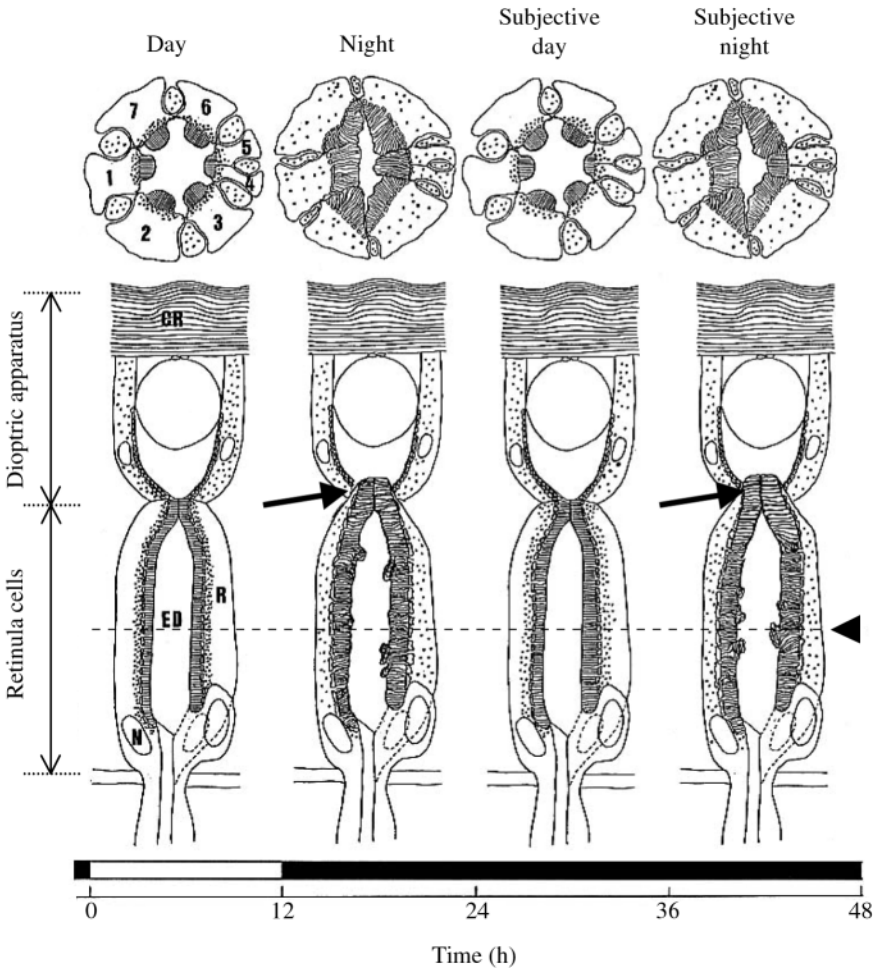

Fig. 3. Semi-schematic drawings depicting cross (upper) and longitudinal (lower) sections through an ommatidium of *Ligia exotica*. Cross sections were taken around the center of the longitudinal sections (arrowhead). There are seven retinula cells numbered 1–7. Retinula cells R1–R3 and R6–R7 have their maximum spectral sensitivity at 520 nm, but the other two types (R4 and R5) have their spectral sensitivity maxima at shorter wavelengths (Hariyama et al., 1993b). At night and during subjective night, the screening pigment granules of the retinula cells are dispersed throughout the cell soma; during the day and subjective day, they migrate into the vicinity of the rhabdom. The arrows point to the distal tip of the rhabdom, which protrudes into the proximal cone-end only at night and during subjective night. CR, cornea; ED, dendrite of eccentric cell; R, retinula cell; N, retinula cell nucleus. White and black bars indicate the lighting regime.

subjective night (Fig. 3), but no obvious displacement of pigment granules (measuring approximately 0.45 µm in diameter) was observed in the distal pigment cells surrounding the crystalline cones and the proximal pigment cells enveloping the retinula cells of an ommatidium.

Physiology

For the ERG recordings, the beam of the light guide was arranged to cover the entire compound eye; thus, the dioptric apparatus of an ommatidium received stimulation not just from a single direction but from several directions simultaneously. The eye was stimulated by a 500 ms pulse of light of 520 nm wavelength and 6.0×10^{10} quanta cm^{-2} s^{-1} once every hour. With this stimulating light intensity, the retinula cells of *L. exotica* do not saturate in intracellular recordings and continue to show a cornea-negative monophasic response in the ERGs (Fig. 1 inset). For analyses, only the highest amplitude in each series of responses was measured. Since the amplitudes of the ERG responses were mainly dependent on the position of the electrode, all responses in an experiment were normalised to the highest response. In each experimental series with one test animal, the highest responses were always obtained at midnight (00:00 h). Twenty-five animals were used in this experiment. The relative amplitudes of the ERGs under continuous darkness are shown in Fig. 5. The lowest ERG amplitudes were observed during the subjective day, while the highest were recorded during the subjective night. Amplitude maxima and minima occurred at midnight and at 10:00 h, respectively. The amplitude difference between midnight and 10:00 h was significant (Student's *t*-test, $P<0.001$, $N=25$). After midnight, the responses gradually decreased, displaying the expected sigmoidal changes. The intensity/response functions (V/log$I$ curve) for night (00:00 h) and day (10:00 h) ERGs are shown in Fig. 6. The average sensitivity difference to light between night and day eyes was approximately 1 log unit, i.e. sensitivity was approximately 10 times greater at night than during the day.

Using the point light source (0.4° in visual angle terms), intensity/response functions (V/log$I$ curves) were recorded intracellularly at the on-axis (see Fig. 1) position from at least 100 retinula cells (Fig. 7A). There was no apparent difference in the slopes of these curves. The average difference between day and night was approximately 0.4 log units. This value corresponded to an approximately 2.5-fold difference in sensitivity. When the curves were normalised to their maximum responses, no apparent differences in the shapes of these two sets of curves could be detected between day and night (Fig. 7B).

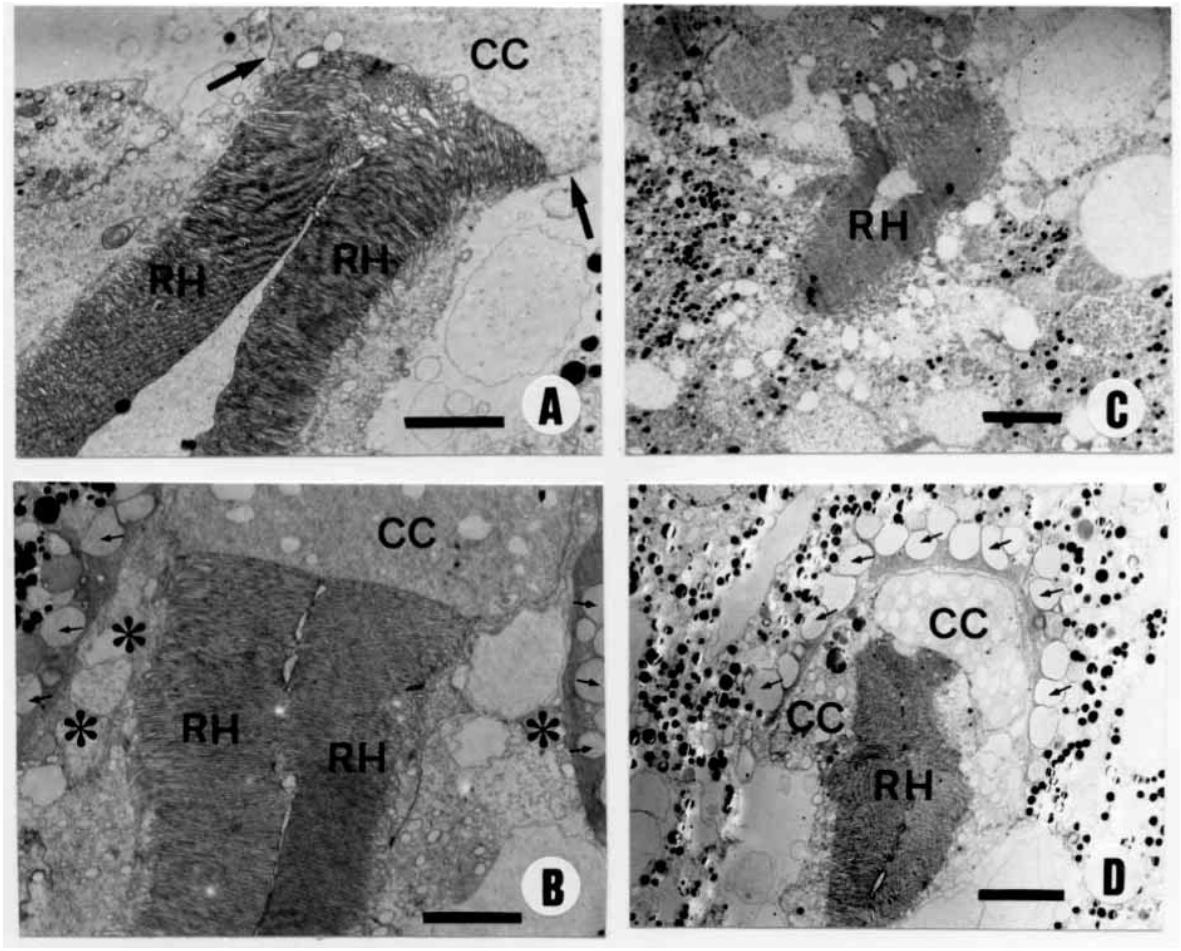

Fig. 4. Longitudinal (A,B) and transverse (C,D) sections through the region of the rhabdom tip of an ommatidium of *Ligia exotica*. (A) Electron micrograph of a longitudinally sectioned junction between the proximal end of the crystalline cone (CC) and the distal end of the rhabdom (RH) during the day, showing the position of cone cell membranes (arrows) and that the tip of the 'open rhabdom' is not surrounded by any cone cells. (B) Electron micrograph of a longitudinally sectioned junction between the proximal end of a crystalline cone (CC) and the distal end of the rhabdom at night, showing how the now 'fused rhabdom' (RH) has pushed into the cone and is enveloped by cone cell material (asterisks) and pigment cells with their vacuoles (small arrows). (C) Transverse sections of a daytime eye at the level of the most distal rhabdom tip (RH) reveal no trace of cone cells. (D) Transverse sections obtained from an eye at night, however, clearly show that the distal rhabdom tip (RH) is surrounded by cone cell material (CC) and pigment cells with their vacuoles (small arrows). Scale bars, 5 μm.

Representative examples of the two-dimensional angular sensitivity functions from a retinula cell during the day, at night, during the subjective day and during the subjective night are shown in Fig. 8. The day and night responses were obtained from one retinula cell, while those obtained under continuous darkness (subjective day and subjective night) came from another retinula cell. The contour lines of the measured receptive fields during the day were less irregular than those at night. A similar difference in the contour lines of the acceptance functions was observed in the recordings from subjective day and subjective night. The contour lines of day and subjective day angular sensitivity functions both show oval rather than circular outlines. The contour lines of the acceptance functions representing the night phases were more complicated, but the long axes of the oval receptive fields were oriented approximately in the same direction as those during the day. There was no apparent difference in the outlines of the

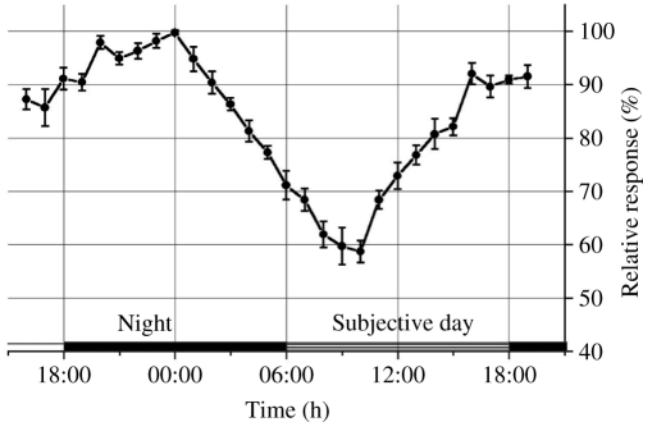

Fig. 5. Relative responses (as a percentage of the midnight response of each animal) of the electroretinogram (ERG) to light of 520 nm wavelength under continuous darkness (N=25). Values are means ± S.D.

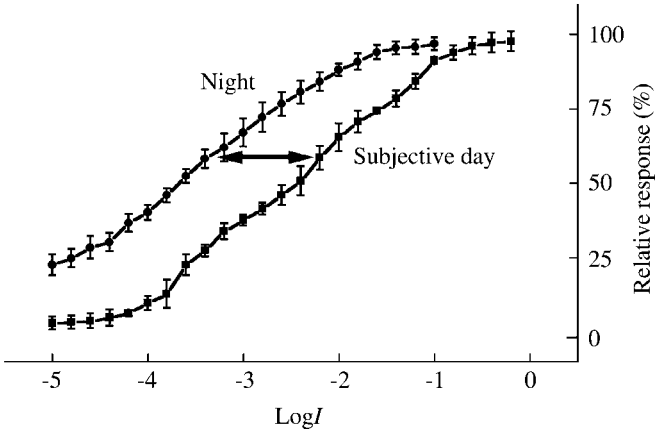

Fig. 6. Intensity/response (*V*/log*I*) curves obtained at night (00:00 h) and during subjective day (10.00 h). The curves are of rather similar shape, but are shifted horizontally by approximately 1 log unit (*N*=50). Values are means ± S.D.

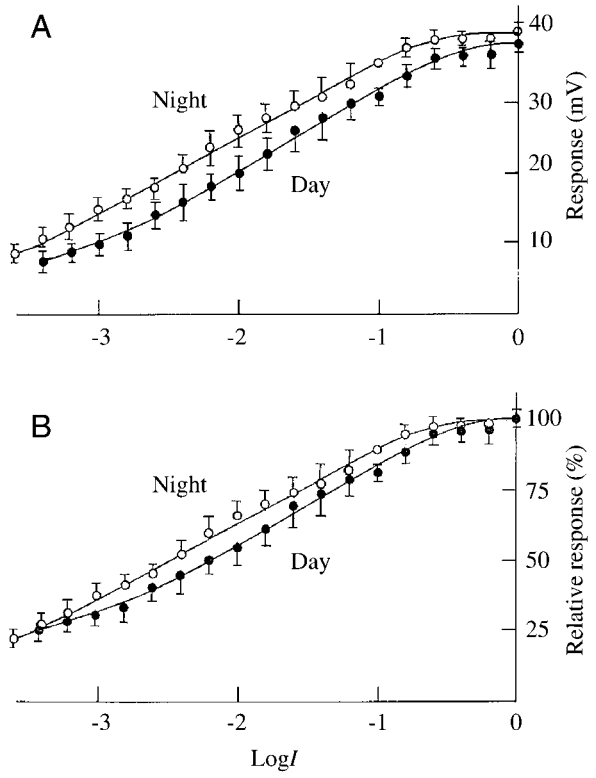

Fig. 7. Intensity/response (*V*/log*I*) curves obtained intracellularly at night and during the day, showing a difference in sensitivity of approximately 0.4 log units when the response is not normalised (A) and considerable similarity in shape when the response is normalised to the highest value (B). Values are means ± S.D. (*N*=50).

acceptance functions when the comparison between day and night recordings was restricted to responses above the 50 % sensitivity level. However, below the 50 % level, the area from which light was able to contribute to the responsiveness of a receptor was considerably greater at night than during the day on account of the increased amplitude of the lateral flanks (Figs 8, 9).

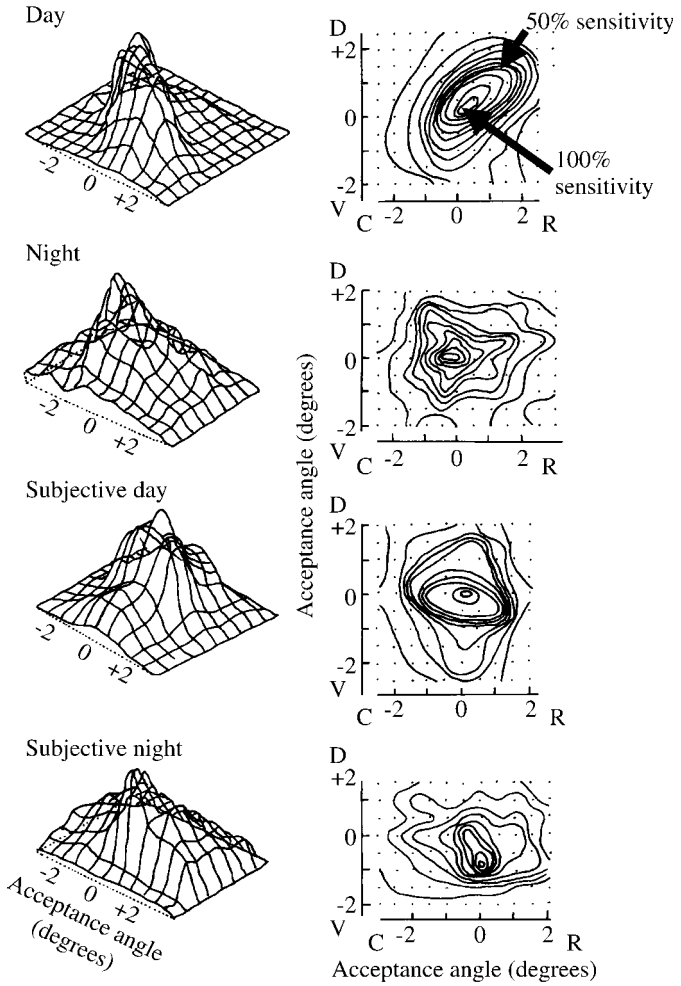

Fig. 8. Examples of the two-dimensional angular sensitivity functions recorded from a retinula cell under a light:dark photoperiod (day and night) and under continuous darkness (subjective day and subjective night). Illustrations on the left are three-dimensional representations of the sensitivity fields, while on the right the corresponding contour diagrams are plotted. The small dots in the contour diagrams correspond to the light stimulation positions, separated from each other by 0.5 °. D, dorsal side; V, ventral side; C, caudal side; R, rostral side.

Because the measured receptive fields obtained from day and subjective day animals exhibited the same tendencies, they were combined. Using the same reasoning, data from recordings at night and subjective night were also combined. Fig. 9 shows the two resulting receptive fields, one for the day/light (Fig. 9A) and the other for the night/dark phase (Fig. 9B) in three-dimensional representations. The three-dimensional images were based on the normalised responses of 50 retinula cells representing the day/light phase and 50 cells representing the night/dark phase. During the day (Fig. 9A), the shape of the receptive field envelope was considerably less irregular than that at night (Fig. 9B). The black disc drawn in Fig. 9 at the 50 % sensitivity level highlights both the similarities between the two three-dimensional representations of the receptive field above the 50 % sensitivity level and the

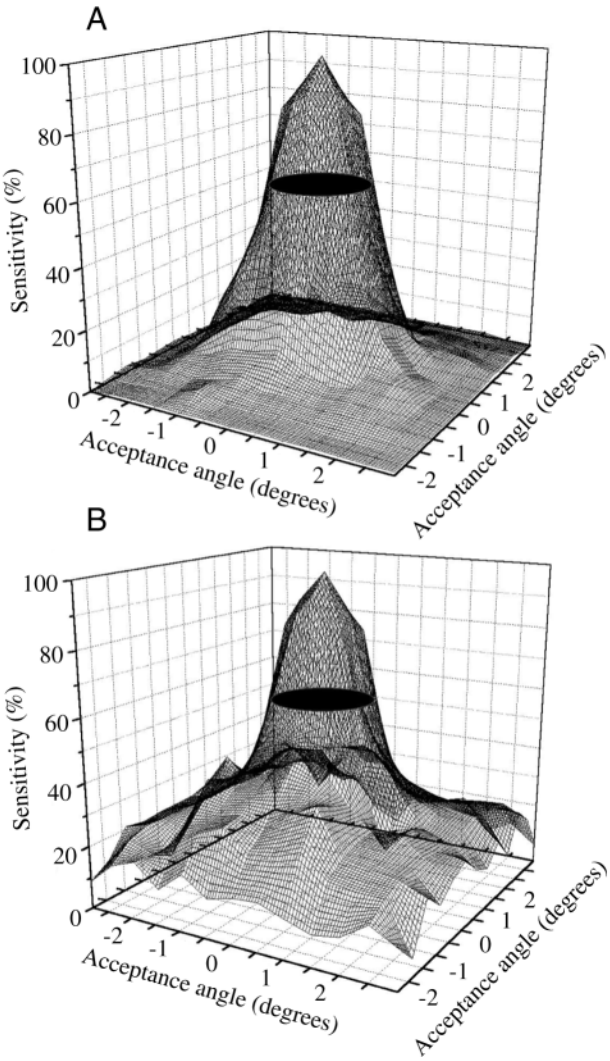

Fig. 9. Acceptance fields, represented as three-dimensional illustrations, based on the means of intracellular recordings from 50 retinula cells during the day and subjective day (A) and from 50 cells at night and during subjective night (B). The responses of each retinula cell were normalised to their maximal response. The black discs, drawn at a relative sensitivity of 50 %, indicate that, at that level and above, almost no difference exists between the two states. Differences between day and night eyes do, however, occur below the 50 % sensitivity level.

differences below this level. The angular sensitivity function below the 50 % level is considerably more complicated and extensive at night than during the day. Secondary sensitivity 'peaks', with 10–15 % peak sensitivities, surround the on-axis peak in all directions as far as at least ±3 ° at night, but not during the day or subjective day (Fig. 10), confirming that night-time sensitivity improvements are likely to depend on events below the 50 % angular sensitivity line.

Discussion

ERG recordings showed the eye's sensitivity to be approximately 10 times greater at night than during the day

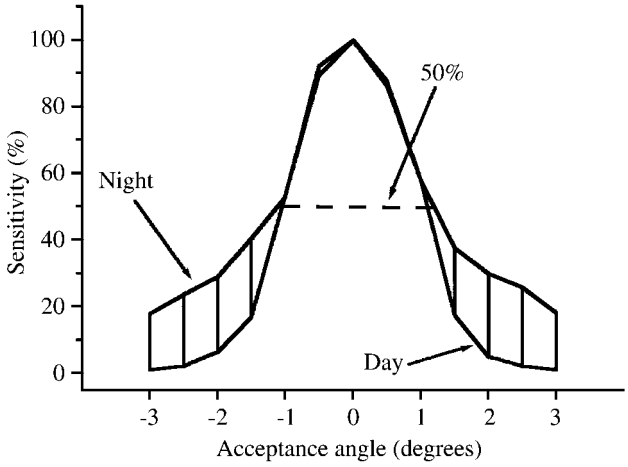

Fig. 10. A diagram showing the acceptance angles of day- and night-adapted retinula cells. No difference exists in the angular sensitivity functions for sensitivities greater than 50 %. Differences of up to 20 %, however, occur in the retinula cells at night below the 50 % angular sensitivity line. Day phase data are for both day and subjective day, and night phase data are for both night and subjective night.

(Fig. 6). On the basis of this result, one could speculate that the sensitivity of a single retinula cell might also change by the same amount between night and day. However, intracellular recordings showed that the average sensitivity difference of retinula cells between night and day was only 2.5-fold (Fig. 7) and, thus, much smaller than the result obtained from the mass response (the ERG).

The ommatidial rhabdom occupation area was three times larger at night than during the day (Hariyama et al., 1986). The small projection of the distal tip of the rhabdom into the proximal end of the cone at night was, however, so short compared with the rest of the rhabdom that it can probably be discounted as a major contributor to the observed sensitivity changes between night and day eyes. However, the approximately threefold increase in the volume of the rhabdom at night when compared with that of the day-adapted eye must have had a bearing on the overall sensitivity of the eye. The rhabdom possesses only one type of chromophore, retinal, and at night the amount of 11-*cis* retinal (26.2±3.5 pmol per eye) is approximately 2.5 times larger than during the day (10.9±2.6 pmol per eye; Hariyama and Tsukahara, 1992). The morphological and biochemical rhythmicities persist in continuous darkness. The concentration of 11-*cis* retinal within the rhabdom, therefore, does not change much between night and day.

If the width of the stimulating incident light is constant, then the sensitivity (according to Hamdorf, 1971), is thought to be principally related to the concentration of visual pigment. In our experiments, the light source subtended 0.4 ° in visual angle terms and was placed 15 cm away from the compound eye. On-axis light rays, incident on the cornea, could cover the whole area of the tip of the rhabdom. Compared with the light-exposed width of the rhabdom during the day, that of the

rhabdom at night should have been approximately three times wider. Day and night intracellular intensity/response curves differed by approximately 0.4 log units. This is equivalent to an approximately 2.5-fold difference in sensitivity, which agrees well with the 2.5 to threefold increase in sensitivity at night predicted on the basis of the morphological and biochemical changes.

The crystalline cone cells are completely surrounded by the distal pigment cells both at night and during the day. The retinula cells are surrounded by proximal (=secondary) pigment cells. Pigment migrations depending on the degree of light adaptation or the diurnal rhythm were observed in the retinula cells only, not in the two kinds of screening pigment cells with their somewhat larger pigment granules. In the crustacean superposition eye, the main causes of sensitivity changes, monitored by ERG recordings at different states of adaptation, have been shown to be related to the positions of the screening pigments within the eye (Arechiga et al., 1974; Bryceson and McIntyre, 1983; Meyer-Rochow and Tiang, 1984), a conclusion also reached for the apposition eye of the portunid crab *Scylla serrata* (Leggett and Stavenga, 1981). However, it seems that the large sensitivity changes seen in the apposition eye of *L. exotica* in ERG recordings were not caused by migrations of the screening pigment granules in the pigment cells (as in a superposition eye), but primarily by radially migrating retinula cell pigment granules (see Warrant and McIntyre, 1992) and diurnal changes in the volume of the rhabdom.

Two-dimensional angular sensitivity functions, recorded from retinula cells during day and night and during subjective day and night, showed that, at night, the acceptance angle contour lines were considerably more complex than during the day. The complexity of the contour lines during the night phase seems to correspond to the more complex structural organization of the rhabdomeres at night (Hariyama et al., 1986). The examples given in Fig. 8 show, however, that the contour lines have similar oval shapes and orientational tendencies irrespective of time of day. Incidentally, oval receptive fields, which are thought to aid pattern discrimination, have also been reported from the apposition eyes of flies (Mimura, 1981). Therefore, rather than being an artefact in *L. exotica*, caused by incorrect alignment of the optical axes of the ommatidia under observation on the horizontal plane, the contours may mirror the anatomical placements of the individual rhabdomeres near the rhabdom tip and, in particular, the group of five retinula cells R1, R2, R3, R6 and R7 (Hariyama et al., 1986). However, to prove that this is, indeed, correct, it would be necessary to stain individual retinula cells.

From the three-dimensional images (see Fig. 9), it became apparent that, below the 50 % level, sensitivities at night were greater and involved considerably more extensive receptive lateral areas than during the day. If we assume that the light rays come from several directions, then the expanded sensitivity field below the 50 % level should contribute to the total sensitivity of the retina. In our study, the ERG measurements represented the summed responses to light rays reaching the receptors from many different directions. To establish the relative sensitivity difference between day and night phases, dependent on the acceptance functions, we integrated the three-dimensional data sets. The resulting volume corresponding to the day phase was 2090, whereas the equivalent value for the night phase was 4760, suggesting an approximately 2.3-fold difference in total sensitivity between night and day phases when a retinula cell is stimulated by scattered light rays. Intracellular recordings based on on-axis stimulation with a point light source revealed a sensitivity difference between day and night of 2.5. When an eye is stimulated by scattered light, the total sensitivity difference should be at least the product of these two values, i.e. 5.75. This suggests that full-field illumination, as used in our ERG measurements, could lead to a spatial recruitment of off-axis units and a further boost in sensitivity. Warrant (Warrant, 1999) offers some receptor cell circuitry that would achieve this, but whether any one of his models apply to *L. exotica* cannot yet be determined.

To perform a particular visual task, an eye must be capable collecting sufficient light and it must be able to determine with a reasonable degree of accuracy from which direction the light was incident. In other words, the eye must have adequate sensitivity to light and adequate spatial resolving power. However, an eye is often forced to function in dim light and, consequently, possesses designs biased towards capturing photons; this compromises the ability of the eye to resolve fine spatial detail. Unless, however, sufficient photons are captured, no spatial resolution is possible (Warrant and McIntyre, 1992). In *L. exotica*, because of the phenomena described above, the total amount of light absorbed by each rhabdom increases at night, but the structure of the two-dimensional angular sensitivity curves above the 50 % level showed no apparent difference between day and night (Fig. 10).

As pointed out in great detail previously (Warrant and McIntyre, 1990) on the basis of observations on the superposition eyes of dung beetles *Onitis alexis*, spatial resolution depends on the form of the entire acceptance function, because, *via* Fourier transformation, the angular sensitivity function is equivalent to the eye's modulation transfer function. The increased flanks of this function (also observed by Warrant and McIntyre, 1990) for dark-adapted eyes could have a profound influence on the transfer of higher spatial frequencies and, thus, on the spatial resolution of the eye. Yet, even in the model applied by Warrant and McIntyre (Warrant and McIntyre, 1990) to the eye of the beetle *Onitis alexis*, there is no change in acceptance angle with the state of adaptation. In apposition eyes, which show changes in rhabdom widths between day and night conditions and/or migration of screening pigments, the widths of the angular acceptance functions (the acceptance angles) are usually greater at night (Leggett and Stavenga, 1981; Horridge et al., 1981; Williams, 1983), but in *L. exotica*, despite considerable changes in rhabdom size and in the shape and position of the

retinula cell pigment granules, the acceptance angles do not change.

Although we cannot categorically say that the most critical area of the angular sensitivity function concerned with spatial resolution lies above the 50 % level, we can state that *L. exotica* appears to have gone further than most other arthropods with apposition eyes to resolve the tricky problem of increasing the rate of photon capture (through increased spatial summation below the 50 % sensitivity level) while at the same time retaining daytime acceptance angle widths. The increase in low-intensity off-axis glare in the blur circle at night, predicted by Warrant and McIntyre (Warrant and McIntyre, 1990) to contribute to the flanks of the angular sensitivity functions, may be unavoidable but, given the appropriate circuitry, not necessarily detrimental to the quality of the image perceived by *L. exotica*. *L. exotica* is active mainly during the day, but it also moves around when it is dark. If, in the eye of *L. exotica*, spatial and temporal resolving powers are to be preserved at night, then this ability should be an extremely beneficial asset for an animal that moves around in dim as well as bright light (see discussion in Warrant, 1999). It ought to allow *L. exotica* to take evasive action in response to small predators, such as crabs, even at low ambient light intensities. Indeed, *L. exotica* showed typical flight responses at night (half moon) when approached by a crab-sized, dark target at the end of a 5 m long fishing rod in the field, testifying to this animal's visual prowess (T. Hariyama, unpublished observations).

This work was supported in part by a grant-in-aid for Scientific Research (10640659 and 11168206) to T.H. from the Japanese Ministry of Education, Science, Sports, and Culture. We also wish to thank the two anonymous reviewers for thoughtful considerations and useful hints.

References

Arechiga, H., Fuentes-Pardo, B. and Barrera-Mera, B. (1974). Influence of retinal shielding pigments on light sensitivity in the crayfish. *Acta Physiol. Lat. Am.* **24**, 601–611.

Arikawa, K., Kawamata, K., Suzuki, T. and Eguchi, E. (1987). Daily changes of structure, function and rhodopsin content in the compound eye of the crab *Hemigrapsus sanguineus*. *J. Comp. Physiol.* A **161**, 161–174.

Bernhard, C. G. and Ottoson, D. (1960). Studies on the relationship between the pigment migration and the sensitivity changes during dark adaptation in diurnal and nocturnal Lepidoptera. *J. Gen. Physiol.* **44**, 205–215.

Bryceson, K. P. and McIntyre, P. (1983). Image quality and acceptance angle in a reflecting superposition eye. *J. Comp. Physiol.* A **151**, 367–380.

DeBruin, G. H. P. and Crisp, D. J. (1957). The influence of pigment migration on vision of higher Crustacea. *J. Exp. Biol.* **34**, 447–462.

Dorlöchter, M. and Stieve, H. (1997). The *Limulus* ventral photoreceptor: light response and the role of calcium in a classic preparation. *Progr. Neurobiol.* **53**, 451–515.

Exner, S. (1891). *Die Physiologie der facettirten Augen von Krebsen und Insecten*. Leipzig: Deuticke Verlag.

Hamdorf, H. (1971). Korrelation zwischen Sehfarbstoffgehalt und Empfindlichkeit bei Photorezeptoren. *Verh. Dt. Zool. Ges.* **64**, 148–157.

Hariyama, T., Gleadell, I. G. and Tsukahara, Y. (1990). Improved fine scalpels for micro-surgery. *Annl. Appl. Inform. Sci.* **16**, 87–89.

Hariyama, T., Meyer-Rochow, V. B. and Eguchi, E. (1986). Diurnal changes in structure and function of the compound eye of *Ligia exotica* (Crustacea, Isopoda). *J. Exp. Biol.* **123**, 1–26.

Hariyama, T., Terakita, A. and Meyer-Rochow, V. B. (1993a). Rhythmicity of chromophore turnover of visual pigment in the Antarctic amphipod *Orchomene plebs* (Crustacea; Amphipoda). *J. Comp. Physiol.* A **173**, 615–619.

Hariyama, T. and Tsukahara, Y. (1992). Endogenous rhythms of the amounts of 11-*cis* retinal in the compound eye of *Ligia exotica* (Crustacea, Isopoda). *J. Exp. Biol.* **167**, 39–46.

Hariyama, T., Tsukahara, Y. and Meyer-Rochow, V. B. (1993b). Spectral responses including a UV-sensitive cell type, in the eye of the isopod *Ligia exotica*. *Naturwissenschaften* **80**, 233–235.

Horridge, G. A., Duniec, J. and Marcelja, L. (1981). A 24-h cycle in single locust and mantis photoreceptors. *J. Exp. Biol.* **91**, 307–322.

Kashiwagi, T., Meyer-Rochow, V. B., Nishimura, K. and Eguchi, E. (2000). Light activation of phospholipase A_2 in the photoreceptor of the crayfish (*Procambarus clarkii*). *Acta Neurobiol. Exp.* **60**, 9–16.

Leggett, L. M. W. and Stavenga, D. G. (1981). Diurnal changes in angular sensitivity of crab photoreceptors. *J. Comp. Physiol.* **144**, 99–109.

Meyer-Rochow, V. B. (1974). Fine structural changes in dark/light adaptation in relation to unit studies of an insect compound eye with a crustacean-like rhabdom. *J. Insect Physiol.* **20**, 573–589.

Meyer-Rochow, V. B. (1999). Compound eye: circadian rhythmicity, illumination and obscurity. In *Atlas of Arthropod Sensory Receptors* (ed. E. Eguchi and Y. Tominaga), pp. 97–124. Tokyo, Berlin, New York: Springer Verlag.

Meyer-Rochow, V. B. and Tiang, K. M. (1984). The eye of *Jasus edwardsii* (Crustacea, Decapoda, Palinuridae): electrophysiology, histology and behaviour. *Zoologica* **45**, 1–61.

Mimura, K. (1981). Phototactic behaviour of walking flies in response to some visual patterns: correlation with receptive field patterns of photoreceptors. *J. Comp. Physiol.* **144**, 75–82.

Terakita, A., Takahama, H., Tamotsu, S., Suzuki, T., Hariyama, T. and Tsukahara, Y. (1996). Light-modulated subcellular localization of the alpha-subunit of GTP-binding protein Gq in crayfish photoreceptors. *Vis. Neurosci.* **13**, 539–547.

Warrant, E. J. (1999). Seeing better at night: life style, eye design and the optimum strategy of spatial and temporal summation. *Vision Res.* **39**, 1611–1630.

Warrant, E. J. and McIntyre, P. D. (1990). Limitations to resolution in superposition eyes. *J. Comp. Physiol.* A **167**, 785–803.

Warrant, E. J. and McIntyre, P. D. (1992). The trade-off between resolution and sensitivity in compound eyes. In *Nonlinear Vision* (ed. R. B. Pinter and B. Nabet), pp. 391–421. Boca Raton, FL: CRC Press.

Warrant, E. J. and McIntyre, P. D. (1993). Arthropod eye design and the physical limits to spatial resolving power. *Progr. Neurobiol.* **40**, 413–461.

Williams, D. S. (1983). Changes of photoreceptor performance associated with the daily turnover of photoreceptor membrane in locusts. *J. Comp. Physiol.* **150**, 509–519.