Pigment Movement during Light and Chromatic Adaptation in the Retinula Cells of *Formica polyctena* (Hymenoptera, Formicidae)

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Summary. The distribution of pigment granules in the distal part of the retinula cells of *Formica polyctena* is analysed with the electron microscope after light adaptation to natural illumination during sunrise and after selective chromatic adaptation. During the process of light adaptation the pigment granules in the retinula cells move radially towards the rhabdom attenuating the light flux within the light guide structure of the rhabdom. Under natural conditions of light adaptation during sunrise it is found that adaptation by pigment movement is a biphasic process. The pigment granules move closer towards the rhabdom during twilight but suddenly at dawn, they move outwards again and as the sun rises return to the light adapted position. This dramatic break in the pigment adaptation process is explained by postulating that a second adaptation mechanism exists, which regulates the concentration of bleached photopigment as a result of the chromatic change of sky light during sunrise. Chromatic adaptation experiments with wavelengths 337 nm, 447 nm, and 591 nm at different intensities demonstrate 2 UV-receptors (cell Nos. 1 and 6) and 6 green-receptors (Nos. 2, 3, 4, 6, 7, 8) in each ommatidium.


Introduction
The photoreceptive organs of insects adjust to the broad range of naturally occurring light intensities by pigment movement both in
pigment cells and in the retinula cells themselves. In superposition eyes (e.g. in butterflies; Bernhard, 1967) the pigment granules in pigment cells move along the length of the ommatidium thereby attenuating the light reaching the rhabdom and altering the spatial resolution of the ommatidial pattern. In apposition eyes the position of the pigment granules in the pigment cells does not change significantly, but in the retinula cells the pigment moves radially for a few microns thereby altering the amount of light within the rhabdom (Kirschfeld and Franceschini, 1969). The movement of pigment granules towards and away from the rhabdom alters the refractive index of the surrounding medium and thus changes all mode dependent properties (Snyder and Horridge, 1972).

Although the phenomenon of radial pigment movement in retinula cells of apposition eyes has been investigated in a number of insect species (Kirschfeld and Franceschini, 1969; Menzel and Lange, 1971; Kolb and Autrum, 1972; for review see Walcott, 1978) much is still unknown. This study of retinula cell pigment movement in the red wood ant, Formica polyctena, concentrates on two problems: the dependence of radial pigment movement on light intensity under natural conditions and the distribution of spectral cell types within each ommatidium, as resolved by chromatin adaptation. This last experiment depends upon the fact that pigment granules in each retinula cell are seen to move independently and it is assumed that this reflects the dependence of pigment movement upon receptor potential.

**Methods**

The experimental animals were foraging ants taken from a natural nest and dark-adapted over night. Preparation for fixation was made in red light (Schott filter OG 650). This was found not to have any effect upon the pigment movement. Fixation was in a mixture of 2% paraformaldehyde and 5% glutaraldehyde in Sörensen's phosphate buffer (0.12 M, pH 7.1-7.2) for 1.5-3.5 hours at room temperature (Karnovsky, 1961). Postfixation was in 1% OsO4 in phosphate buffer for 6.5-10 hours. After rapid dehydration in acetone the tissue was embedded in Spurr's ERL (Spurr, 1969). Thin transverse sections, orientated with respect to the head axis, were examined under the Elmskop 51 (Siemens) or EM9 (Zeiss). In the first experimental series (light adaptation under natural conditions) the preparation was made in the open at different times during dawn. The eye was adapted by light from sky directly overhead and fixed in this position. The intensity of sky light was measured with a calibrated lux meter which pointed in the same direction as the eye. For the chromatid adaptation experiments, the dark adapted animals were fixed to a holder in red light with the right eye looking downwards at a well in a ground glass disc. The right eye was illuminated for 10 minutes through the disc with the adaptation light (preadaptation). Then after fixative had been dropped into the well the eye was dissected out thus falling into this well. It was adapted here for another 20 minutes. The fixative has low UV transmission but the very thin layer between glass and cornea caused little attenuation of the UV light. The radiant flux of spectral light after passing through the ground glass and the fixative was measured in absolute numbers of quanta with a calibrated UV-sensitive selenium element.

**Results**

Each ommatidium contains 9 retinula cells which form a central fused rhabdom. Eight retinula cells pass through the whole length of the ommatidium (100 μm) while the 9th cell only appears in the proximal third. The following description is restricted to the 8 long retinula cells, as the 9th cell contains no pigment granules. In cross-sections taken just below the crystalline cone six large and two smaller cells are distinguished (Menzel, 1972a, b). The small cell pointing in the dorsal direction is numbered cell 1 and the other cells are counted clockwise (only right eyes are examined) (Fig. 3). At a more proximal level all the retinula cells and their rhabdomeres are about the same size. The shorter microvilli of the 2 smaller retinula cells have the same orientation as one of the neighbouring larger cells, so that the rhabdom consists of 6 groups of microvilli arranged in 3 directions. A detailed description of the ommatidium and its structural changes during light adaptation is given elsewhere (Menzel and Lange, 1971; Menzel, 1972a, b).

All measurements on pigment distribution are made in the 5 μm beneath the crystalline cone, as most of the pigment granules in the retinula cells lie distally, but it is found that there is no major difference in the proximal and distal pigment distribution. After dark adaptation the rhabdom is surrounded by broad intracellular vacuoles and the pigment granules lie further from the rhabdom. During light adaptation the intracellular vacuoles break up into a number of small vacuoles which move into the surrounding cytoplasm and come to be evenly distributed throughout the cell. Meanwhile the pigment granules move radially toward the rhabdom and the greater the light intensity the closer they are to the rhabdom (compare Menzel and Lange, 1971, Fig. 2). It is found that only those pigment granules within 2.5 μm of the rhabdom move in response to illumination, and those in the more peripheral part of the retinula cells appear to remain stationary.

**1. Light Adaptation under Natural Light Conditions**

The distribution of pigment granules in the retinula cells is determined from electron micrographs in two ways. In the first case the number of pigment granules within 1 μm of the edge of the rhabdom in each retinula cell is counted and expressed as a percentage of all the pigment granules found in that cross section of the cell. The percentages for each...
Fig. 1. Comparison of the two methods of determination of pigment distribution expressed with the data from one experimental series (No. II). O, ▲ for the large green cells Nos. 2, 3, 4, 6, 7, 8 (see below). The thin lines (O, ▲) give the average percentage of pigment granules 1 µm or nearer to the rhabdom in relation to all pigment granules on cross-sections (method 1). The thick lines (●, ○) show the average percentage of cells containing one or more pigment granules 0.5 µm or closer to the rhabdom edge (method 2). The difference between the two pairs of curves are greatest for the 6 large cells. This may be caused by the broad contact area between rhabdomere and cytoplasm, which increases the probability for one pigment granule to lie very close to the rhabdom. The values for 5200 lux come from another experiment in which the adaptation was measured only by the second method.

In the dark adapted state only 5% of the retinula cells have pigment granules close to the rhabdom (Fig. 2a). Granule counting shows an average of 4% of pigment granules are nearer than 1 µm at the rhabdom (Fig. 1). Initially, as light intensity rises the number of pigment granules close to the rhabdom increases. At about 1000 lux the percentage of cells with granules near the rhabdom shows a maximum, which is followed by a decrease and a second increase above 10000 lux. There are no differences of the pigment distribution within the two small cells (Nos. 1 and 5), the UV-cell, and the 6 large cells (Nos. 2, 3, 4, 6, 7, 8), the green cells, (see below), therefore they are pooled in two groups and the average of each experimental series (II-IV) and that of all series are given in Fig. 2a. The surprising result is the discontinuity of the curve showing a decrease with increasing intensity between 1000 and 4000 lux. As Fig. 2b shows, the sun rises at about 2000-4000 lux. All 4 experimental series display the same basic curve with a minimum at sunrise. The difference between the minimum and the maximum is highly significant in all series ($\chi^2$-test).

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retinula cell number (1-8) is averaged from many ommatidial cross sections (Fig. 1). In the second case we only distinguished between cells containing one or more pigment granules 0.5 µm or closer to the rhabdom edge and cells without pigment granules in this area. This area around the rhabdom is chosen because the refractive index of the first 0.5 µm is important for the regulation of light flux within the rhabdom (Kirschfeld and Franceschini, 1969; Snyder and Horridge, 1972). The number of cells with pigment granules in this area is expressed as a percentage of all cells of the same cell number examined. In principle both methods describe the dependence of pigment distribution on light intensity (Fig. 1). The second method, however, appears to be more sensitive in reflecting the location of pigment granules in the close surrounding of the rhabdom, because pigment granules in the peripheral part of each cell are not involved in the response to light. In addition this method allows a quicker evaluation of a large number of cross-sections. In the following experiments only the second method is used.

In order to determine the intensity range of the pigment movement response animals are adapted to natural light at various times during daybreak on four different days (experimental series II, III, IV, V). The fixation procedure is slightly changed between the different series but this has no effect on the results. The eyes are always exposed to the sky light from directly overhead and are fixed in this position. On all 4 mornings the sky was hazy, and sometimes a few small clouds appeared. Fig. 2b shows that the brightness changes between 4.30 a.m. and 9.00 a.m. by 6 log units (0.1-100000 lux). As the experiments are performed in shadow to the early morning sun the light intensity increases in two steps. There is a rapid increase at sunrise following a slow increase during dawn and another large increase at the moment when the sun shines over the wall.

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Fig. 2. a) The light intensity dependence of the distribution of pigment granules within the retinula cells during dawn. The percentage of cells containing one or more pigment granules 0.5 μm or closer to the rhabdom is given at the ordinate. As no significant differences are found, between UV cells (Nos. 1, 5) and between green cells (Nos. 2, 3, 4, 6, 7, 8) (see below) these cell types are averaged in two groups (A, C, D, ±, UV cells, 4 different experimental series; A, B, C, D for green cells, 4 different experimental series). The two curves with the large symbols (○ for UV cells, ● for green cells) show the average distribution. As the eyes are fixed at different intensities in the 4 experimental series the values are collected within groups whose ranges are given by lines under the abscissa. b) increase in brightness during dawn at 14.6.72 (II experimental series). This curve corresponds very closely to the average time course of brightness increase of all 4 experimental series (see text).

The curves for the small and the large cells are not significantly different but in all 4 series the minimum for the small cells is lower and occurred later at higher intensities. In 2 series (II and III), the maximum for the smaller cells is much higher. In the 3 series (II, III, IV) which have data for about 650 lux and 2000 lux the difference in adaptation between maximum and minimum is larger for the small cells (28%, 14%, 10%) than for the 6 large cells (13%, 9%, 7% respectively). Therefore the average curve of all series for the small cells shows a much greater decrease than that for the larger cells indicating that the mechanism responsible for the minimum has a greater effect in the 2 small cells.

A possible explanation for the discontinuous intensity function could be a circadian rhythm of pigment movement as exists in Tenebrio (Wada and Scheider, 1968). This was tested by fixing dark adapted control animals between 5.00 a.m. and 12.00 a.m. We found no change in pigment distribution during this time.

Adaptation with monochromatic light (see below) did not reveal such a discontinuous curve (compare Fig. 2a with Fig. 4). This suggests that the discontinuous movement of pigment granules depends on the natural light conditions at dawn, particularly at the moment of sunrise.

2. Chromatic Adaptation

Electrophysiological experiments have shown that the eye of the wood ant most probably contains two colour receptor types; a UV receptor.
Fig. 5. Histogram for pigment distribution in the retinula cells after exposure to UV (337 nm), blue (447 nm) and yellow (591 nm) light with about the same radiation densities given separately for each of the 8 retinula cells (abscissa). Ordinate as in Fig. 4. The inset shows the numbering of retinula cells and the orientation of the cross-section. The small cells Nos. 1 and 5 are marked by hatched bars with maximum at 361 nm and a green receptor with maximum at 500 nm (Roth and Menzel, 1972). The results from behavioural discrimination experiments support the existence of a dichromatic colour vision system. UV and green are seen as two distinct colours while blue and green are not (Kiepenheuer, 1968; Menzel and Müller, unpublished).

The sensitivity maxima of the two cell types lie far apart and the sensitivity of each type is low in the effective range of the other. Thus, if pigment movement in each cell is independent from that of its neighbours then selective chromatic illumination should allow the separation of the UV and green receptor cells. This would enable description of the arrangement of colour receptor types in the ommatidial pattern.

We used spectral lights, wavelengths 337 nm, 447 nm, and 591 nm (Schott, double-band interference filters, half band width 6—9 nm) and
their intensities were varied by 3 log units. The pigmentation distribution was examined in 19 independent experimental series and measured with method 2 as described above. In Fig. 3 six cross sections of UV and yellow adapted ommatidia are superimposed to one schematic drawing. Electron micrographs are given elsewhere (Menzel, 1972b). It is immediately clear that both small cells, Nos. 1 and 5 have more pigment granules close to the rhabdom after UV adaptation. After yellow adaptation only the 6 large cells have granules near the rhabdom. The intensity dependence of the pigmentation distribution for the 3 test wavelengths is shown in Fig. 4. The selective chromatic effect is obvious for the wavelengths 337 nm, and 591 nm. In the small cells pigmentation movement to UV-adaptation begins 1.5–2 logs units earlier than in the large cells. The large cells respond to yellow light more than 2 log units earlier than the small cells. There is no significant difference between the small and the large cell after blue adaptation. In contrast to light adaptation experiments under natural conditions (Figs. 1 and 2) no intermediate minimum was found with chromatic adaptation.

The selective chromatic effect becomes even clearer, if one averages the percentage for each cell in the middle part of the response/log intensities function (at about 10^9 quanta/cm²/sec) and shows this in a histogram (Fig. 5). The small cells are the UV receptors and the large cells are the green receptors. Blue may adapt the UV-receptors a little more than the green receptors but the difference is not significant (t-test). The differences between the 6 large cells after yellow adaptation are not significant but the differences between the small and the large cells after UV or yellow adaptation are highly significant.

**Discussion**

Radial pigment movement in retinula cells of the apposition eye is one of the mechanisms by which the receptor cells adapt to strong and long lasting illumination. Other possible adaptation mechanisms are (1) change of the concentration of unbleached photopigment and (2) change in excitability of the receptor membrane. Kirschfeld and Frangin(1969) found that the attenuation of light caused by pigment movement occurs within 2–5 seconds. These authors also describe pigment movement in the dark in response to CO₂ treatment. As CO₂ depolarises the receptor cell one can assume that the depolarisation of the receptor cell causes the movement of the pigment granules. The selective pigment movement to chromatic illumination described above supports this interpretation. We found a dichromatic colour vision system with UV- and green-receptors in the wood ant using electrophysiological (ERG and selective adaptation) (Roth and Menzel, 1972) and behavioural methods (Menzel and Müller, unpublished; see also Kiepenheuer, 1968). We conclude that the degree of depolarization of each retinula cell regulates the distribution of pigment granules. As receptor potential amplitude depends on the spectral sensitivity of individual receptors the analysis of pigment distribution allows us to describe the colour receptor types and their distribution. Furthermore, because pigment granules move independently in adjacent cells it must be assumed that retinula cells within the ant ommatidium are not electrically coupled. This should be compared with the situation in the bee. Here it is not possible to demonstrate selective pigment movement (Gribakin and Grundler, personal communication), but independent studies demonstrated that the eight long retinula cells are electrically coupled in the bee ommatidium (Shaw 1969; Snyder, Menzel, and Laughlin, in press).

Photoreceptors respond to increasing light intensity with an increase in depolarization. We find the same increase in percentage of pigment granules close to the rhabdom, if spectral light is used. Under natural light conditions, however, the intensity dependence of pigment distribution shows an unexpected minimum between 2000–4000 lux which follows a maximum at 600–1000 lux. Above 4000 lux the number of pigment granules near the rhabdom increases again. This paradoxical result demands an explanation.

The depolarization of a photoreceptor to illumination depends on the light absorbed by photopigment within its rhabdomere. For a given rhabdomere structure the concentration of unbleached photopigment is one of the most important parameters influencing effective absorption.

The concentration of unbleached photopigment is dependent on the equilibrium between the bleaching process and the reisomisation of bleached pigment to the unbleached state. Recent studies on insect UV and green pigments demonstrate that the reisomisation process is partially driven by the absorption of blue light (photoreisomisation) (Hamdorf, Paulsen, Schwemer, Täuber, 1972; Paulsen and Schwemer, 1972; Hamdorf, Högland and Langer, 1972). Thus a change in the spectral composition of incident light, and in particular the relative content of blue (c. 440nm) wavelengths will change the concentration of unbleached photopigment. This may explain the anomalous break in screening pigment distribution.

During dawn and especially at sunrise very complex changes occur in the spectral content of sky light. At night and in the early twilight the sky is much "redder" than a clear sky during the day (Rozenberg, 1966). But the light intensity is so low (less than 1 lux) that the retinula cells show the fully dark adapted state. With increasing brightness the sky becomes more blue because of the predominance of scattered light in the upper atmosphere, which follows the 1/2 rule
long wavelength light and at sunrise a strong increase in long wavelength radiation causes a peak in the relative intensity ratio of green:blue. Condit and Grum (1964) measured an increase in colour temperature from 7500° K to 9700° K with the rising sun between +5° and +20° solar altitude for daylight with a hazy sky, therefore, the curve for the relative intensity ratio UV:blue shows an increase 1/2 hour after sunrise when the ratio green:blue decreases again. It has been estimated that these chromatic changes should be great enough to alter the concentration of unbleached photopigment (Hamdorf, Gogola and Schwemer, 1971).

In all 4 experimental series (II–IV) the minimum of the adaptation curves (Fig. 2) lies at the time of sunrise, and the average curves make clear that the minimum for the two UV receptors is slightly shifted to higher intensities, that means, it occurs later after sunrise. This may reflect the later increase in the UV:blue ratio shown in Fig. 6. Also the UV cells show a more noticeable minimum than the green receptors. The UV-photopigment in insects in contrast to the longer wavelengths photopigments is almost completely reisornerized by blue light and the metabolic pathway plays only a small part although metarhodopsins of UV- and green photopigments have nearly the same spectral absorption with maximum in blue (Hamdorf, Pauleen, Schwemer and Täuber, 1972). This may explain why the UV receptors react more strongly to the chromatic change of the sky light than the green receptors.

The distribution of pigment granules within the retinula cells of the wood ant is found to be a sensitive indicator of the excitation of the photoreceptor cell. The adaptation process under natural light conditions has two phases: an early phase at relative low brightness (1–100 lux) and a second phase at brightness higher than 5000 lux. Our hypothesis is that the change in chromatic light distribution at the time of sunrise causes a decrease in the concentration of unbleached photopigment, thus decreasing the excitability of the receptor cell. The result is a redistribution of pigment granules further from the rhodobium where they, to a smaller degree, attenuate light travelling in the lightguide structure of the rhabdom.

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References


Snydert, A. W., Horridge, G. A.: The optical function of changes in the medium surrounding the cockroach rhabdom. J. comp. Physiol. 81, 1–9 (1972)


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