

The Effects of Light and Dark Adaptation upon the Compound Eye of *Nilaparvata lugens*

벼멸구 겹눈의 명적응과 암적응 효과

Young Nam Youn

尹 泳 南

ABSTRACT The eyes of *Nilaparvata lugens* were examined for ultrastructural changes in the light and dark adapted states. Inspection of light microscope sections taken at similar levels of compound eyes from insects kept in light or darkness for periods up to 72 hours revealed some differences between light and dark adapted eyes. Using the electron microscope, in light adapted eyes the palisade layer was narrower than that in dark adapted eyes. The pigment granules still formed a ring around the palisade layer in the dark adapted eye but, they did not form a tight circle around the rhabdom. No constant difference was found between the diameters of the microvilli in light and dark adapted eyes. The pigment movements at the junction of the cone and the rhabdom took the effect on varying the pigment aperture at the tip of the cone in front of the rhabdom tip.

KEY WORDS Ultrastructure, compound eye, light adaptation, dark adaptation, the rice brown planthopper, *Nilaparvata lugens*.

초 록 명상태와 암상태에서 벼멸구의 겹눈의 미세구조가 변화하는 것을 관찰하였다. 벼멸구의 겹눈을 암조건 혹은 광조건 하에서 최고 72시간 까지 유지하였을 경우에 비슷한 수준에서 겹눈을 절단하여 해부 현미경으로 관찰한 결과, 구조적인 면에서 서로 다른 모양을 관찰하였다. 한편, 전자현미경을 이용하여 암조건과 광조건 하에서의 겹눈의 미세구조를 비교한 결과, 단애층(palisade layer)의 넓이는 광조건 하에서 암조건에서 보다 더 좁은 것을 알 수 있었다. 색소체는 암적응 상태의 눈에서는 단애층 주위에 뿔을 형성하고 있었지만, 잠간체 주위에 밀집되어 있지는 않았다. 암적응과 광적응 상태의 눈에 있는 망막세포 내에서의 미세움도 지름은 차이가 없었으며, 수정체와 잠간체의 접합 부위에 있는 색소체의 이동은 잠간체의 위부분에 있는 수정체의 끝부분에서 변화가 있음을 관찰 할 수 있었다.

검색어 미세구조, 겹눈, 명적응, 암적응, 벼멸구

Ultrastructural studies have shown that insects exhibit a wide range of anatomical adaptations during light-dark adaptation (Walcott 1975). These adaptations included the formation of a palisade around the rhabdom in the dark adapted state formed from sacks of endoplasmic reticulum and the cisternae. The vesicles of the palisade may be quite large, occupying up to 1/3 of the width of the cell. Pigment granules in the cell may be displaced radially (Horridge and Bamard 1965, Tunstall and Horridge 1967). In the light adapted state the palisade was reduced in extent and displaced radially, and

pigment granules in some eyes then moved to lie close against the rhabdom. There was great variation amongst insects displaying this type of adaptation. For example, pigment granule movements were not seen in the locust although there were extensive palisade changes, however, mitochondria migrated instead (Horridge and Bamard 1965). In *Formica*, the palisade in the proximal half of the retina moved only slightly and so a narrower palisade remained in the light adapted state (Menzel and Lange 1971, Menzel 1972). Pigment granules occurred in the distal part of the cell, and most of the pigment granule

movement occurred here (Menzel 1972). The presence or absence of the palisade and the movement of pigment granules or mitochondria altered the efficiency of the rhabdom as a light guide and hence the amount of light that is absorbed by the visual pigment in the rhabdom. The rhabdom has a higher refractive index than the surrounding regions of the retinula cell and so behaves as a light guide. When pigment granules or mitochondria are near the rhabdom in the light state, the refractive index of the region around the rhabdom is increased so that some light escapes and is absorbed by the pigment granules in the cell (Walcott 1975, Nilsson 1989b). In the dark adapted state, a palisade of lower refractive index surrounds the rhabdom and light is better retained within the rhabdom.

Other ultrastructural changes include longitudinal pigment movement in eyes such as lepidopteran superposition eyes where rhabdoms can be screened from each other in the light adapted condition but light from one facet is allowed to pass to more than one rhabdom in the dark adapted condition (Horridge 1971)

Photoreceptor membrane turnover in the rhabdom has been shown to be influenced by the light regime. Light causes a breakdown in membrane and a decrease in rhabdom size. Darkness favours synthesis and leads to an increase in rhabdom size (Schwemmer 1989). In many cases the mechanisms of adaptation in the retina have been shown to be endogenous and controlled by circadian oscillators (Aschoff 1981, Barlow *et al* 1989). This is particularly true of the movements of screening pigments and may also apply to photopigment membrane turnover.

The brown planthopper, *Nilaparvata lugens*, may be attracted to light traps however little is known about the role of vision, if any, in host plant selection. The eye of *Nilaparvata lugens* was examined for ultrastructural changes in the light and dark adapted states in order to provide the basis for further physiological studies of the response characteristics of the eye.

MATERIALS AND METHODS

The fine structure of compound eye retinula cells during the course of light and dark adaptation was examined using 1-2 days old female adults which had been kept in culture in the insect room in a 16:8 (L:D) light regime at $25 \pm 1^\circ\text{C}$. The brown planthoppers were caught at 9 o'clock in the morning and separated into two groups. One group of insects were kept in the dark with rice plants in a double covered box in a dark room for 24 hours. Following the dark adaptation, the insects were exposed continuously to 300 lux light using a tungsten lamp (100 W, Philips). Sample insects were taken from this group for examination at intervals of 1, 2, 4, 12, 24, 48 and 72 hours. This group, in which the course of light adaptation is followed, were referred to "L" group. After removal, they were fixed in 3% glutaraldehyde under the same lighting conditions for 2 hours. This was followed by two hours post fixation in 1% OsO_4 , dehydration in serial ethanol and embedding in TAAB resin as described in Youn (1992 1995)

The second group of brown planthoppers were kept in the light for 24 hours with rice plants at 300 lux (provided by a 100 W Philips tungsten bulb as above). Light measurements were carried out with a light meter (Opto-Meter UDT Model 40X) and were measured at the surface of the leaf-sheath of the rice plant. After the 24 hour period of light, the insects were placed in a double covered dark box in dark room. Sample insects were removed at intervals of 1, 2, 4, 12, 24, 48 and 72 hours. They were caught using the red safety light (30 W with a red filter) since electrophysiological investigations have shown that the compound eye of *N. lugens* is very insensitive to red light (A. Michell and L. J. Goodman, personal communication at QMWC). The whole insect was fixed in darkness and was post fixed and embedded in TAAB resin in the same way as the group L brown planthoppers. These brown planthoppers, in which the course of dark adaptation was followed are referred to group "D". Eyes from all the group L and group D samples were sectioned both for light microscopy and for TEM.

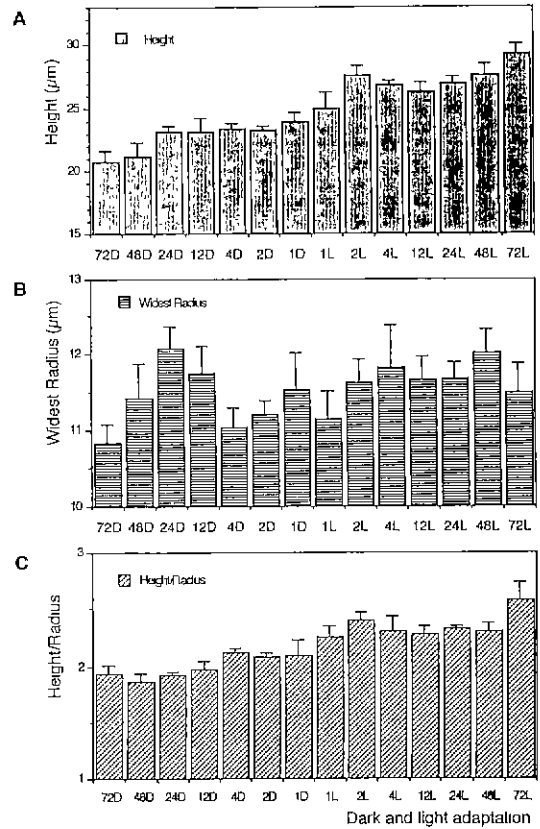
Full details of insect and plant material specimen

preparation were given by Youn (1992, 1995). After curing, thin sections were cut on a Reichert Om U2 ultramicrotome. The colour of the serial sections when observed in reflected light ranged from silver to gold (80-120 nm thick). When areas of interest were reached after TEM inspection of these sections, thinner sections of gray colour (40-50 nm) were taken for high magnification examination. Sections were picked up on 200 and 400 mesh copper grids and stained with uranyl acetate (3% in 70% alcohol) (Stempak and Ward 1964), followed by Reynolds's lead citrate (Reynolds 1963). Light microscope sections were examined with a Zeiss Photomicroscope II, where necessary phase contrast was used. TEM sections were examined using a JEOL JEM-100S electron microscope.

RESULTS

Inspection of light microscope sections taken at similar levels of compound eyes from insects kept in light or darkness for periods up to 72 hours revealed some differences between light and dark adapted eyes. In the two hour light adapted eye, the pigment of the primary and accessory pigment cells appeared to cluster near the base of the cone while in the two hour dark adapted eye, the pigment was aligned at the sides of the cone. The palisade layer seemed larger and the ring of pigment granules within the retinula cells more conspicuous than that in the light adapted eye. In the four hour light adapted eye the pigment of the primary and accessory pigment cells appeared to be even more concentrated around the base of the crystalline cone and that of the retinula cells to have dispersed more peripherally within the cell. In the four hour dark adapted cell, the pigment clustered nearer the center of the cell, especially in the distal region near the crystalline cone.

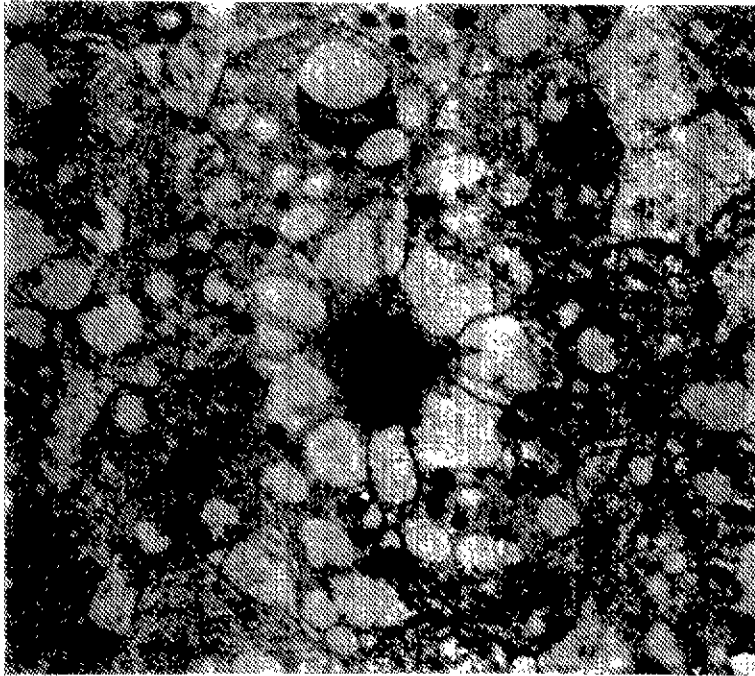
The length of the crystalline cone (i.e., the long axis of the cone between the corneal lens and the rhabdom) increased with the time spent in the light regime (Fig. 1) although the increase was not found to be significant for either length or width (length: $t=0.155$, $p=0.281$; width: $t=0.121$, $p=0.325$; ratio of length and width: $t=0.152$, $p=0.285$, normality



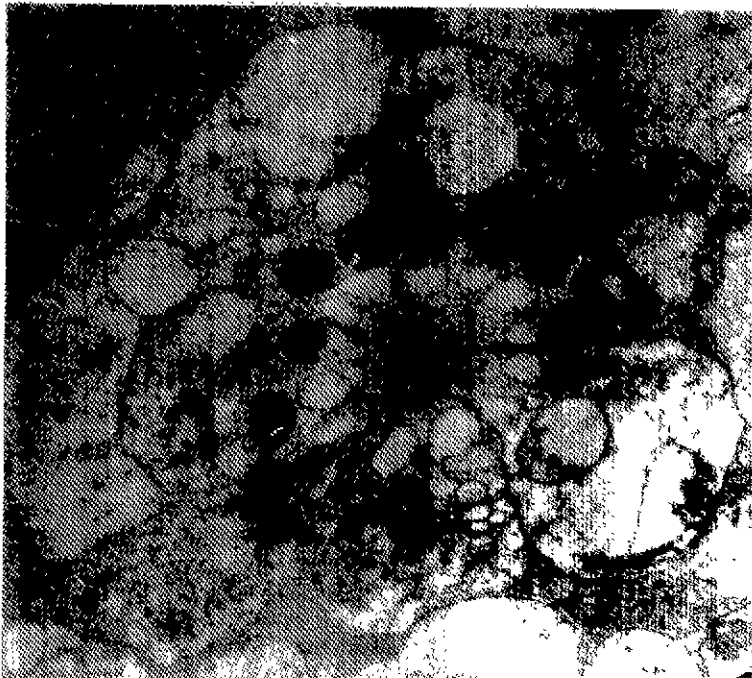
Figs. 1. Crystalline cone changes in dimension with light-dark adaptation of the compound eye of the brown planthopper, *N. lugens*. A: Change of height; B: Change of radius C: Change in the ratio of height and radius. Bar=represents standard error. Data from 5 insects \times 50 crystalline cone cells in each different light-dark category).

test, $df=12$).

Examination of TEM sections showed changes in the fine structure of the retinula cells. In light adapted eyes, the palisade layer was narrower than that in dark adapted eyes (Fig. 2). The pigment granules still formed a ring around the palisade layer in the dark adapted eye, however, they did not form such a tight circle around the rhabdom because of width of the palisade. In light adapted eyes, mitochondria moved closer to the rhabdom as the palisade layer was reduced in size. The rhabdoms of light and dark adapted eyes, were examined for differences in dimension, but it was very difficult to obtain sufficient comparable TEM sections taken at the same

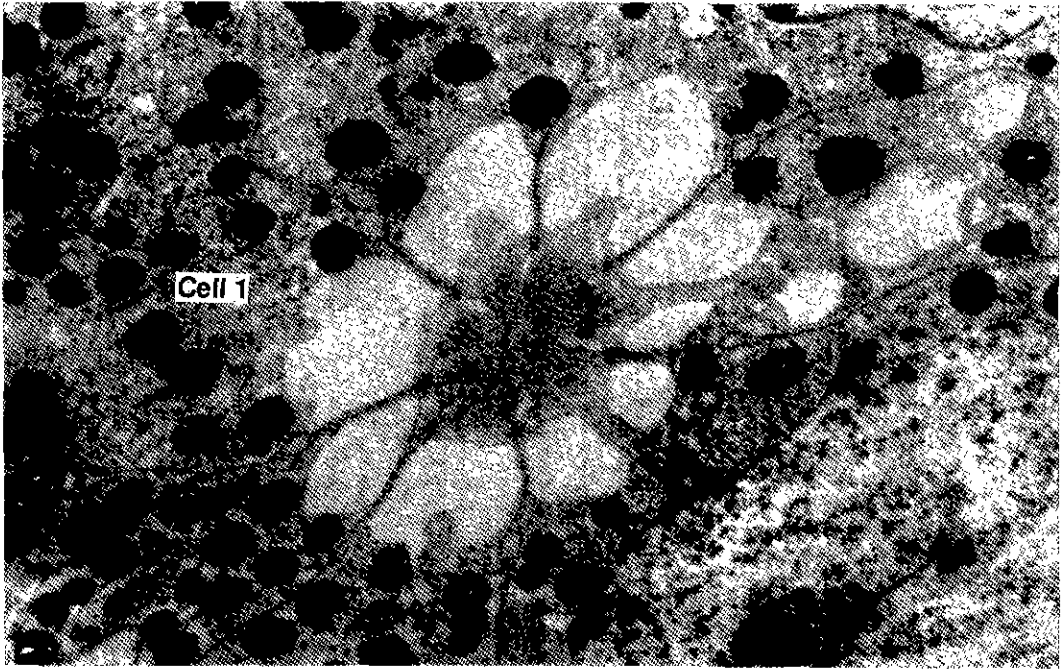


(A) Two hours in the dark

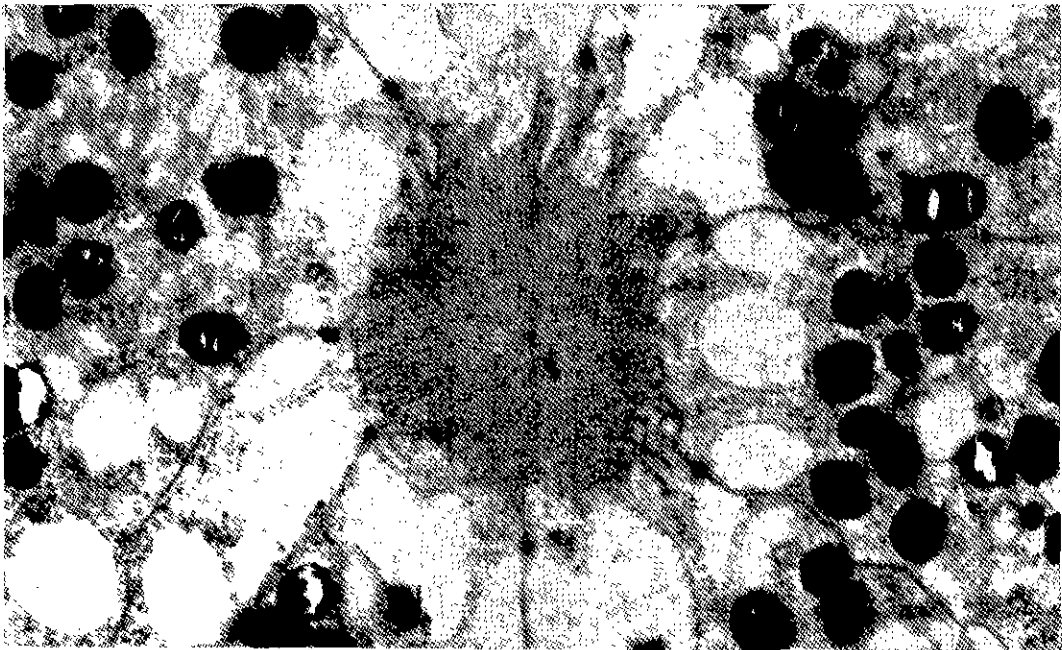


(B) Two hours in the light

Fig. 2. Ultrastructure of retinula cells. (A) kept for 2 hours in the dark and (B) kept for 2 hours in the light. Note the difference in the size of the cisternae and hence in the width of the palisade surrounding the rhabdom. The ring of pigment granules is present in each case, but is further from the rhabdom in the dark adapted eye (A) x5,800; (B) x12,780.



(A) Four hours in the light



(B) Four hours in the dark

Fig. 3. Horizontal sections of the upper region of one ommatidium in (A) four hour light adapted eye (x14,900) and (B) four hour dark adapted eye (x14,800)

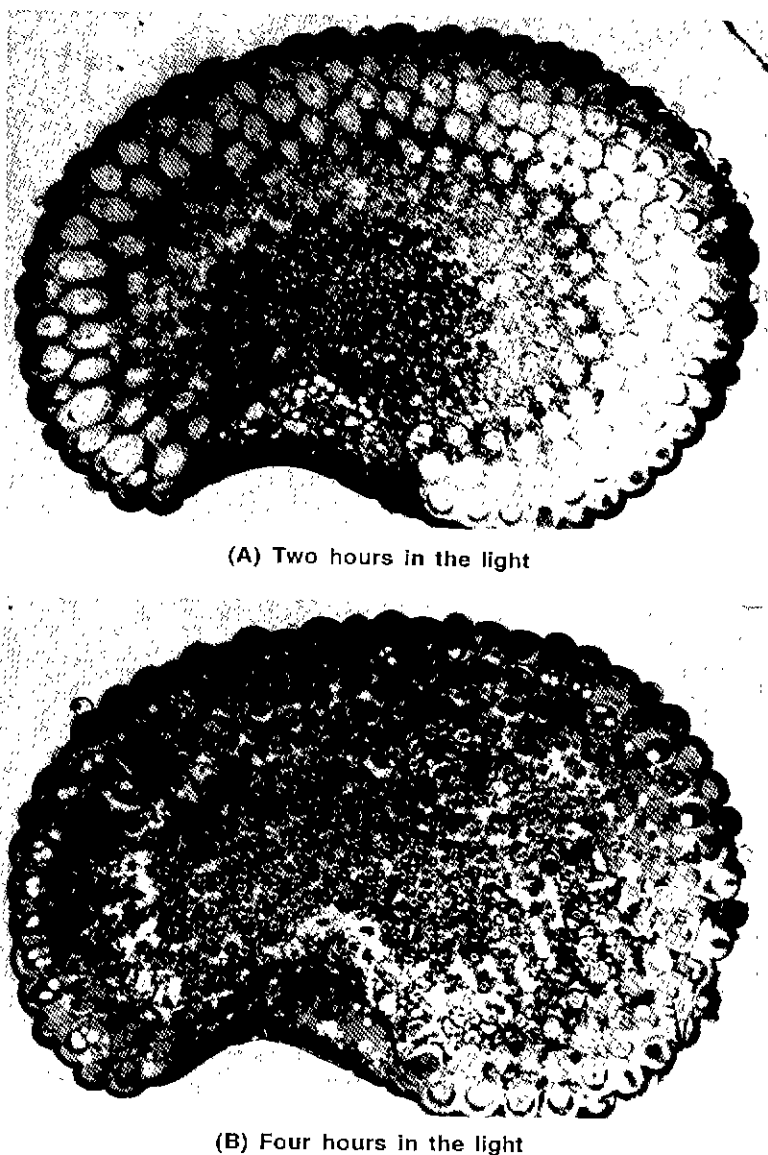


Fig. 4. A and B Light microscope sections through comparable regions of the compound eye of brown planthoppers which had been (a) 2 hrs and (b) 4 hrs in the light. Note that the pigment of the primary and accessory cells is concentrated around the base of the crystalline cone and tips of the retinula cells. x60

level in the retinula cell for measurement. Nevertheless examination of numerous survey pictures suggested that the rhabdom is somewhat larger in the dark adapted eye (Fig. 3). No constant difference was found between the diameter of the microvilli in light and dark adapted eyes. If the insects were maintained in darkness for long periods, then breakdown of the microvilli comprising the rhabdom

could be observed (Fig. 4).

In the outer zone of the retinula cell a variety of organelles were present (e.g. mitochondria rough and smooth endoplasmic reticulum, Golgi complex and pigment granules). Adjacent to the rhabdom, there was an inner zone which contained vesicular components of the cytoplasm and the cisternae. The size of the cisternae varied with the state of adapta-

tion of the eye (Fig. 2). In the light adapted eye, they were smaller, but still always present by forming a ring around the rhabdom. Sections taken parallel to the long axis of the rhabdom showed the extent and regularity of these cisternae. In the distal region of the retinula cells where the nuclei were located immediately beneath the crystalline cone the retinula cells were at their widest. Many pigment granules were found in this region of the retinula cell. The pigment granules, 0.4-0.6 μm in diameter, appeared as vesicles containing material of variable electron density. As the medial region of the retinula cell was approached the number of pigment granules became fewer. In a section perpendicular to the long axis of the cell 2-4 pigment granules per retinula cell were commonly found close to the cisternae. As the cisternae were smaller in the light adapted eye this meant that there was a tight ring of pigment granules outside the palisade layer, quite closed to the rhabdom. Sections parallel to the long axis in the rhabdom showed the orderly alignment of pigment granules adjacent to the cisternae and rhabdom. Near the basement membrane the rhabdom was lost and the retinula cells narrow to form axons descending through the basement membrane into the optic ganglia. Pigment granules were found within the terminals of the retinula cells and the axons themselves.

In the pigment cells, the pigment granules appeared to move centrally in both the primary and accessory cells in such a way that there was a concentration of pigment around the apex of the cone and the beginning of the rhabdom, this took the effect of stopping-down the dioptric apparatus. In the dark adapted eye the pigment granules were mainly concentrated between the corneal cones (Figs. 4 and 5) along the lateral margins of each cone.

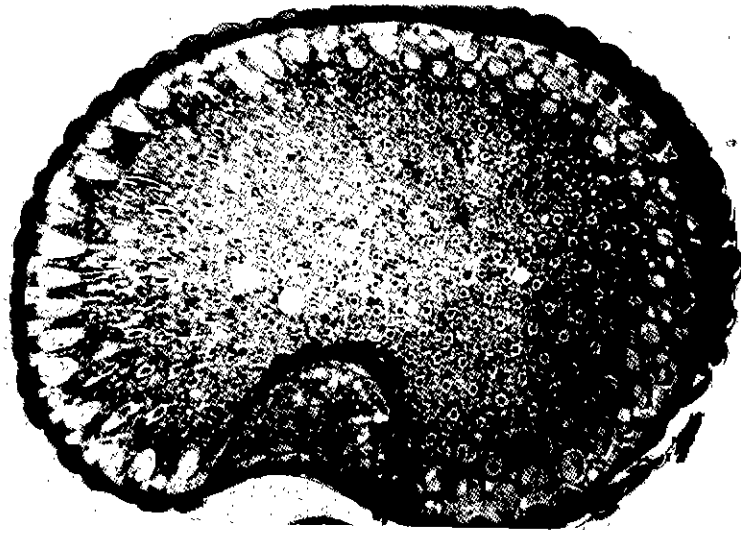
DISCUSSION

The brown planthopper showed differences in its ultrastructure between the light and dark adapted state: changes in the size of the palisade layer, movement of pigment granules in the retinula, primary pigment and accessory pigment cells, a change in length of the cone and a change in the width of

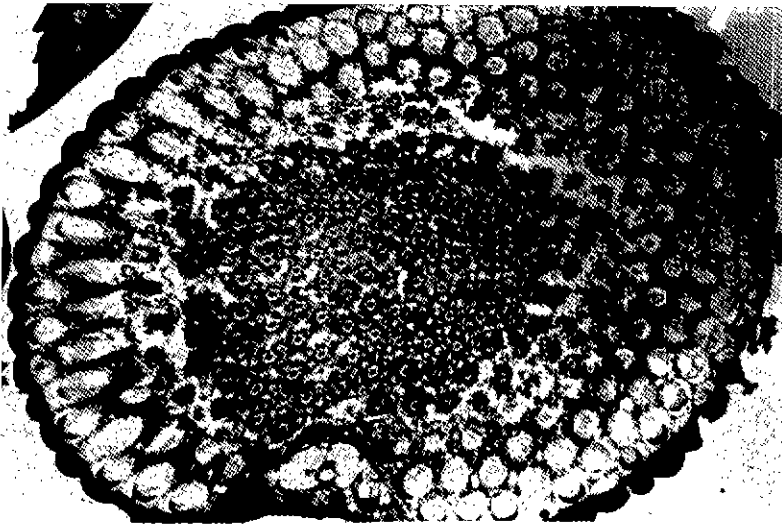
the rhabdom.

In the light adapted eye, the sacs of the palisade layer were small so that the central rhabdom was surrounded by only a thin palisade layer. This was present throughout the eye from the distal to the proximal region of the retinula cell. When the palisade layer was thin, other cell organelles moved nearer to the rhabdom for example mitochondria, but principally the pigment granules. In each cell there were one, two or three granules quite near the palisade layer in each section taken perpendicular to the rhabdom which showed the pigment granules lined up adjacent to the palisade throughout the length of the cell and separated by only 40-50 nm. There was thus a tight ring of pigment granules around the rhabdom. Pigment movement also occurred in the distal end of the retinula cell at the level of the nucleus and above. Here where the retinula cells met the crystalline cone, more pigment was present in the cell and it too moved centrally around the rhabdom. In light microscope sections taken from eyes kept in the light for one, two or four hours it could be seen that the pigment of the primary pigment cells surrounding the cone moved down around the region where the cone and the rhabdom met. The accessory pigment cells ran from the corneal lens to the basement membrane. They were larger and contained pigment at the level of the crystalline cone and the distal-most part of the retinula cells. Their pigment also appeared to migrate to the area of the proximal part of the cone and the distal region of the retinula cell.

When the eye was kept in darkness for 1, 2 or 4 hours the cisternal sacs became larger so that the rhabdom was surrounded by a wider palisade layer. This change took place at all levels of the retinula cell. The pigment granules in the retinula cell were displaced laterally by the thickening of the palisade layer, however, they still remained in a ring around the palisade although it was a looser more dispersed ring. Light microscope sections showed that the pigment of the primary cells and accessory pigment cells had moved away from the base of the cone and was concentrated along the sides of the cone. The pigment in the distal part of the retinula cells appeared to move laterally. Thus in the



(A) Two hours in the dark



(B) Four hours in the dark

Fig. 5. A and B Light microscope sections through comparable regions of the compound eye of brown planthoppers which had been (a) 2 hrs and (b) 4 hrs in the dark. Note that the pigment is located around the side walls of the crystalline cones. The palisade layer is slightly larger in the dark adapted eye. x60.

light adapted eye the pigment granules of the primary and accessory pigment cells appeared to move tightly around the base of the cone and the rhabdom where it met the cone. In the dark adapted eye, the pigment moved away from this region.

In addition to the changes in pigment position, changes in the shape of the crystalline cone were

observed particularly in its length. Although the changes in length between the extreme dark adapted and light adapted states examined were found not to be statistically significant, yet there is steady trend towards an increase in length of the cone as the eye becomes more light adapted with a change of about 10 μm between the cone length after 72

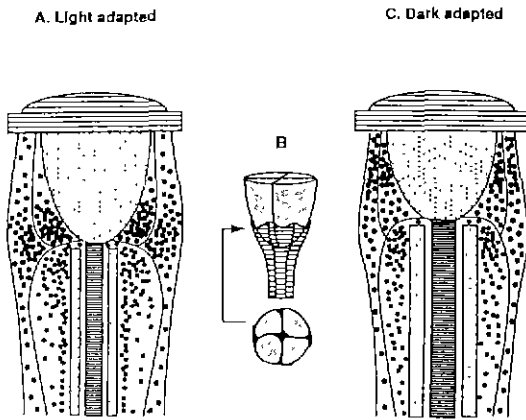


Fig. 6. A diagrammatic illustration of the differences observed between the light and dark adapted states of *N. lugens* (A) Light adapted. (B) and (C) Two possible explanations for the measured shortening of the cone in the dark adapted eye (B) The retinula cells have moved up around the cone and the apex of the cone is buried more deeply within the rhabdom. (C) The cone itself has shortened, the pigment granules of the primary and accessory pigment cells have migrated away from the base of the cone, as have the distal retinula cell granules. The rhabdom is wider, the palisade layer is wider and the granules in the length of the retinula cell are dispersed laterally. It is suggested that the cone shape alters to accommodate the wider rhabdom, the rhabdom moving distally to remain in contact with the base of the cone.

hours in darkness and 72 hours in light

Fig. 6A, B and C shows in diagrammatic and exaggerated from what may be happening at the junction of the cone and the rhabdom in this eye during light and dark adaptation. The pigment movements in this region took the effect of varying the pigment aperture at the tip of the cone in front of the rhabdom tip. This mechanism of stopping-down the pigment iris in the focal plane of the lens in light is found in some insects with open rhabdoms and in some ants and beetles with fused rhabdoms (Nilsson 1989a). It had the effect of reducing the acceptance angle and thus the apparent sensitivity of the eye. In dim light the pigment withdrew, increasing the acceptance angle. As well as this the cone shortens slightly, possible rounding to accommodate the inc-

reased diameter of the rhabdom. Horridge *et al.* (1981) showed that in the locust, widening of the rhabdom at night contributed to the increase of the acceptance angle of the ommatidium in dim light, thus helping to increase the capture of available light. Williams (1983) also examined the increase in sensitivity of the locust retinula cells in response to an increase in the size of the field stop, or aperture at the base of the cone caused by pigment migration, complimented by with an increase in the diameter of the rhabdom. He found that sensitivity to an extended source increased by at least 1 log unit during the first 1-3 hours after dark.

In addition to these mechanisms, the change in the medium surrounding the rhabdom also helped to control the light flux in the receptors. The presence of a well developed palisade, as seen in the dark adapted eye of *N. lugens* improved its light guide properties (Nilsson 1989b). The difference in the reflective index between the palisade and the rhabdom assisted in keeping the light which was caught by the rhabdom tip within the column as it passes down (Horridge *et al.* 1981). In the light adapted eye, with a narrow palisade the ring of pigment granules came close to the narrower rhabdom. Work on the optics of the rhabdom showed that light propagating along a narrow waveguide would have part of its power outside the diameter of the waveguide. This light was absorbed by the screening pigments, helping to attenuate the light flux in the light adapted eye (Snyder 1979, Van Hateren 1989). The changes in palisade dimension and hence radial pigment migration in the retinula cell were normally faster than the changes in pigment migration in the primary and accessory pigment cells and changes in rhabdom size, and were believed to have much less effect on the acceptance angle of the rhabdom (Nilsson 1989b).

Further studies are necessary to examine the timing of events in *N. lugens*. Pigment migration and changes in the size of the palisade layer appear to be largely complete after one hour in light and dark. Physiological experiments are also necessary to measure changes in the acceptance angle of the ommatidium in the light and dark adapted states.

REFERENCES

- Aschoff, J. 1981. A survey on biological rhythms. In Handbook of Behavioral Neurobiology. Vol.4 Biological rhythms. J. Aschoff (ed.), Plenum New York. pp. 3-10.
- Barlow, R. B. Jr., S. C. Chamberlain and H. K. Lehman. 1989. Circadian rhythms in the invertebrate retina. In: Facets of Vision. D. G. Stavenga and R. C. Hardie (eds). Springer-Verlag. Berlin. pp. 30-73
- Horridge, G. A. 1971. Alternatives to superposition images in clear-zone compound eyes. *Proc. R. Soc Lond. B* **179**: 97-124
- Horridge, G. A. and P. B. T. Bamard. 1965. Movement of palisade in locust retinula cells when illuminated. *Quart. J. Microsc. Sci.* **106**: 131-135.
- Horridge, G. A., J. Dumec and L. Marcelja. 1981. A 24-hour cycle in single locust and mantis photoreceptors. *J. Exp. Biol.* **91**: 307-322.
- Menzel, R. 1972. The fine structure of the compound eye of *Formica polyctena*-Functional morphology of a hymenopteran eye. In: Information Processing in the Visual Systems of Arthropods. R. Wehner (ed.). Springer-Verlag New York. pp. 37-49
- Menzel, R. and G. Lange. 1971. Änderungen der Feinstruktur im Komplexauge von *Formica polyctena* bei Helladaptation. *Z. Naturforsch.* **26B**: 357
- Nilsson, D. E. 1989a. Vision optics and evolution-Nature's engineering has produced astonishing diversity in eye design. *Bio. Science* **39**(5): 298-307.
- Nilsson, D. E. 1989b. Optics and evolution of the compound eye. In: Facets of Vision. D. G. Stavenga and R. C. Hardie (eds.). Springer-Verlag. Berlin. pp. 30-73.
- Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* **17**: 208-212.
- Schwemmer, J. 1989. Visual pigments of compound eyes-Structure, photochemistry and regeneration In: Facets of Vision. D. G. Stavenga and R. C. Hardie (eds.). Springer-Verlag. Berlin, New York. London. pp. 112-133.
- Snyder, A. W. 1979. Physics of vision in compound eyes. In: Handbook of sensory Physiology. Vol VII/6. A. H. Autrum (ed.) Springer-Verlag Berlin pp. 225-313
- Stempak, J. C. and R. T. Ward. 1964. An improved staining method for electron microscopy. *J. Cell Biol.* **22**: 697-701
- Tunstall, J. and G. A. Horridge. 1967. Electrophysiological investigation of the optics of the locust retina. *Z. Vergl. Physiol.* **55**: 167-182.
- Van Hateren, J. H. 1989. Photoreceptor optics, theory and practice In: Facets of Vision. D. G. Stavenga and R. C. Hardie (eds.). Springer-Verlag. Berlin pp. 74-89
- Walcott, B. 1975. Anatomical changes during light-adaptation in insect compound eyes In The Compound Eye and Vision of Insects. G. A. Horridge (ed.). Clarendon. Oxford. pp. 20-36.
- Williams, D. S. 1983. Changes of photoreceptor performance associated with the daily turnover of photoreceptor membrane in the locust. *J. Comp. Physiol.* **150**: 509-519.
- Youn, Y. N. 1992. Electrophysiological recordings of olfactory receptors, ultrastructure of compound eyes and electrical monitoring of feeding behaviour in *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae). Ph. D. Thesis. Queen Mary and Westfield College, University of London. England 392pp
- Youn, Y. N. 1995. Ultrastructure of the compound eye of the rice brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Auchenorrhyncha: Delphacidae). *Kor. J. Appl. Entomol.* **34**(3): 266-277.

(Received August 23, 1995)