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POSTEMBRYONIC DEVELOPMENT OF DIOPTRIC APPARATUS AND LIGHT INSULATING APPARATUS IN THE COMPOUND EYE OF DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (L.)

(Lepidoptera: Plutellidae)

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Chung-Hsiung Wang, Chu-Fang Lo, Yun-Hsin Duann and Yien-Shing Chow (1987) Postembryonic development of dioptric apparatus and light insulating apparatus in the compound eye of diamondback moth, *Plutella xylostella* (L.). *Bull. Inst. Zool., Academia Sinica* **26**(2): 165-177. The organization of the dioptric and light insulating apparatus in the compound eye of diamondback moth was studied using electron microscopes (SEM and TEM). The central region and interfacets of cornea are formed by two prospective primary pigment cells (corneagenous cells) and six prospective secondary pigment cells respectively. The four cone cells are involved in the formation of crystalline cone by the deposition of glycogen-containing substances from center of cone and along the adjacent membranes of cone cells.

The insulating pigment apparatus consists of two groups of cells: two primary pigment cells and six secondary pigment cells. In both of them, the cytoplasm is rich in rER, glycogen, mitochondria and microtubules at the early stages. The comma-shaped primary pigment cells and the spindle-shaped secondary pigment cells contain many morphologically mature pigments at P4 stage. The structure of secondary pigment cell is obviously different from those of primary pigment cells at the early stage.

The compound eye of diamondback moth, *Plutella xylostella* (L.) is a typical superposition eye; and many characteristics are similar to the compound eye of the nocturnal moths (Wang and Hsu, 1982; 1984; Wang *et al.*, 1983). The progressive organization of the compound eye shows two major stages: cell differentiation stage, a prepupal occurrence, and the organization stage occurring during the P1-P5 stage of pupal development (Wang and Duann, 1984).

The various aspects of postembryonic development of the compound eye of Lepidoptera have been studied by light microscopy (Wolsky, 1949; 1956; Yagi and Koyama,

1963). The present paper discusses certain aspects of ultrastructural studies of the organization of the dioptric apparatus and the light insulating apparatus of the compound eye of *Plutella xylostella* (L.).

MATERIALS AND METHODS

The postembryonic development of the compound eye of *Plutella xylostella* (L.) can be classified into six stages, PP, P1-P5, from prepupal stage to image stage based on the color changes in the compound eye (Wang and Duann, 1984).

The presumptive compound eyes of the moth with the developmental stages ranging from P1 to P5 were dissected out and processed for EM as described earlier (Wang and Hsu, 1982).

OBSERVATION AND DISCUSSION

The compound eye of diamondback moth is composed of 1500-2000 ommatidia. Each ommatidium consists of 20 cells and can be divided into 3 functionally distinct units: dioptric apparatus (containing 4 cone cells), light-insulating apparatus (containing 2 primary and 6 secondary pigment cells), and photosensitive apparatus (containing 8 retinular cells) (Wang and Hsu, 1982).

The developmental patterns are described in sequence with formation of the functionally distinct units in each stage. This report focuses on the developmental aspects of the dioptric and light insulating apparatus, and that of photosensitive apparatus will be reported elsewhere.

I. Cornea

As in the case of many moths, the cornea of diamondback moth has corneal nipples and lamellated structure (Wang and Hsu, 1982). After pupation, the development of preommatidial cell clusters is clearly seen. At the middle phase of P1 stage, the differentiation of preommatidial corneagenous cells are observed first (Wang and Duann, 1984). The surface of primitive corneal area is covered with molting fluid and many budding nodules are arranged regularly on this area (Fig. 1b). It is believed that the nodules are protruded by the differentiating corneagenous cells. The irregularly protrusive microvilli are found on the distal surface of corneagenous cells (Fig. 1a). The tips of microvilli are covered by electron-dense materials, possibly the plasma membrane plaques (Locke, 1966; 1976).

At the later phase of P1 stage as well as the early phase of P2 stage, the round facets of primitive cornea are observed. Each facet measured about 16 μm in diameter and is arranged at a distance of about 1.25 μm from one another (Fig. 2b). The microvilli of corneageous cells of this stage are regular and measure about 600 nm×80 nm (height× width) (Fig. 2a). The first epicuticular layer with bilayer electron-dense structure secreted next is the cuticulin, which aligns in regular semicircular shape along the apical part of microvilli (Fig. 3). Each microvillus on primitive cornea is a mold for a corneal nipple. Therefore the number of nipples correspond to the number of microvilli except in interommatidial area (interfacet area) where the microvilli are arranged irregularly and the smooth epicuticular layer is secreted (Fig. 2a; 3a).

During the late phase of P2 stage and early phase of P3 stage, the semicircular discontinous cuticulin grows along the microvilli and formsa continous layer. The underlying layer of epicuticle is already beginning to secrete beneath the cuticulin. So far the corneal nipples are 260 nm in height and 113 nm in diameter with an internipple space of 133 nm. At this time the microvilli run through the homogenous epicuticle to the tips of the nipples. A thin layer of ecdysial membrane is formed probably from the condensation of the molting fluid (Fig. 4a; b; c).

On P4 stage, the facets are more convex than in earlier stages and are still covered with a thin layer of molting fluid (Fig. 5a;

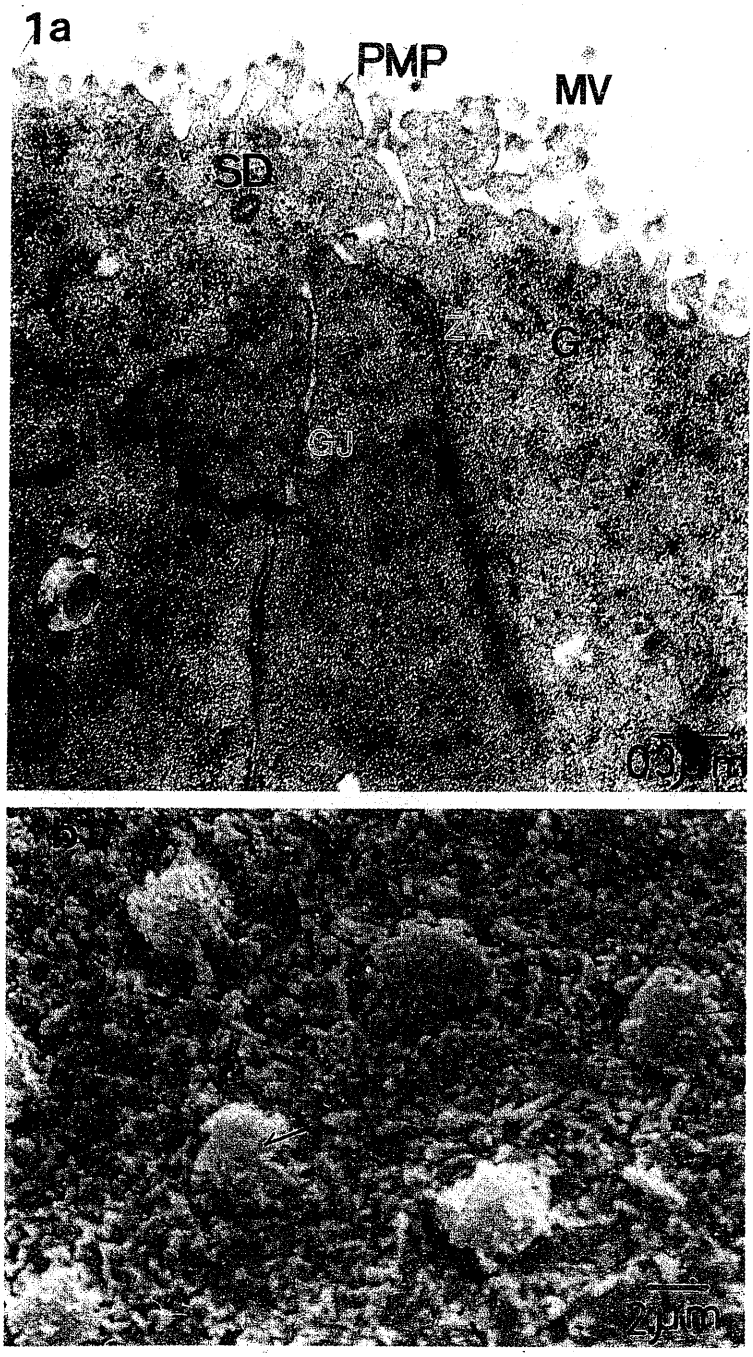

Fig. 1. The middle phase of P1 stage:

a. Section through the distal end of preommatidial cell cluster. The irregularly protrusive microvilli with the plasma membrane plaques are seen on the surface.

b. The surface structure of the developmental cornea, many budding nodules (arrow) are projected from the amorphous surface.

G: glycogen; GJ: gap junction; PMP: plasma membrane plaques MV: microvilli; SD: secretary droplets; ZA: zonula adherens

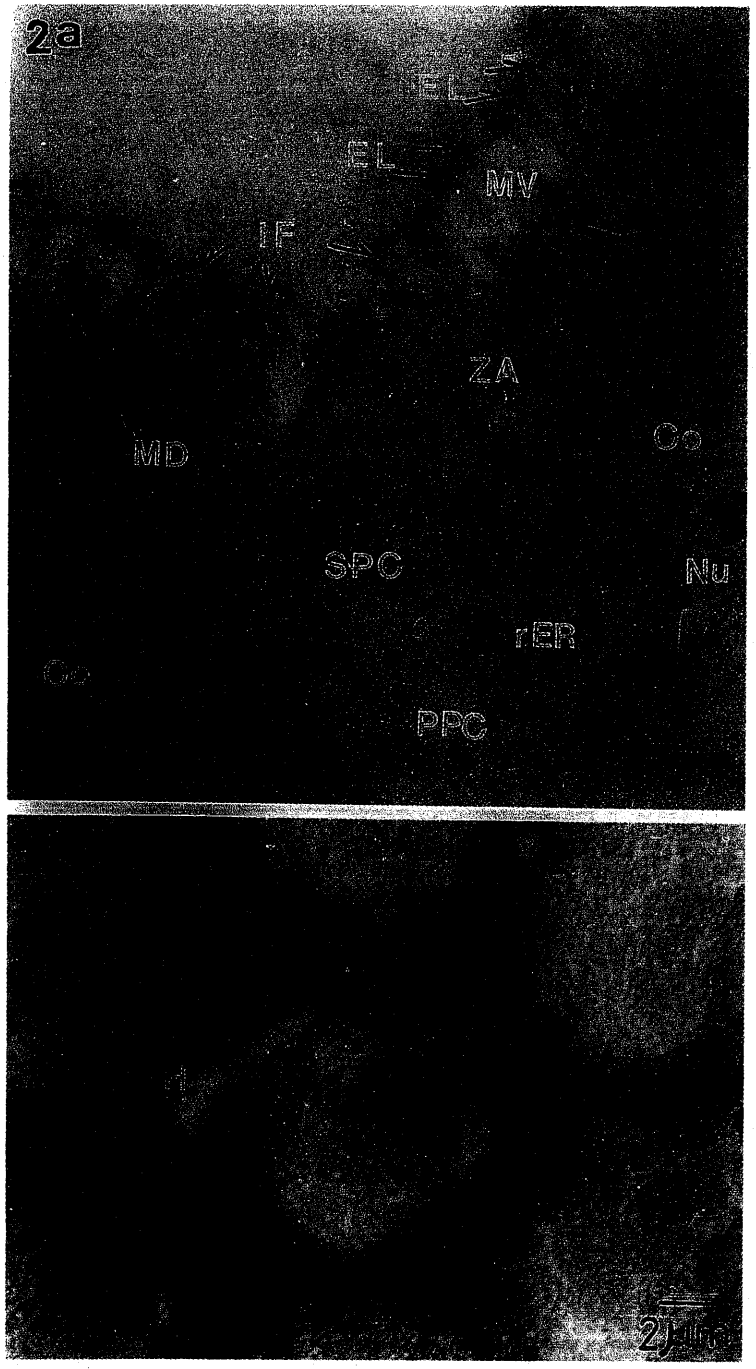

Fig. 2. The late phase of P1 stage as well as the early phase of P2 stage:
a. Section through the interommatidial area. The microvilli of corneagenous cells are more regular and covered with a epicuticular layer. Except in the interfacet area the regular foldings of epicuticular layer (EL) along with the apical microvilli are clearly seen.
b. The round facets of primitive cornea covered with molting fluid are shown.
Co: cone cell; EL: epicuticular layer; IF: interfacet area; H: interfacet hair; MD: molting droplet; MV: microvilli; Nu: nucleus; PPC: primary pigment cell; rER: rough endoplasmic reticulum; SPC: secondary pigment cell; ZA: zonula adherens.

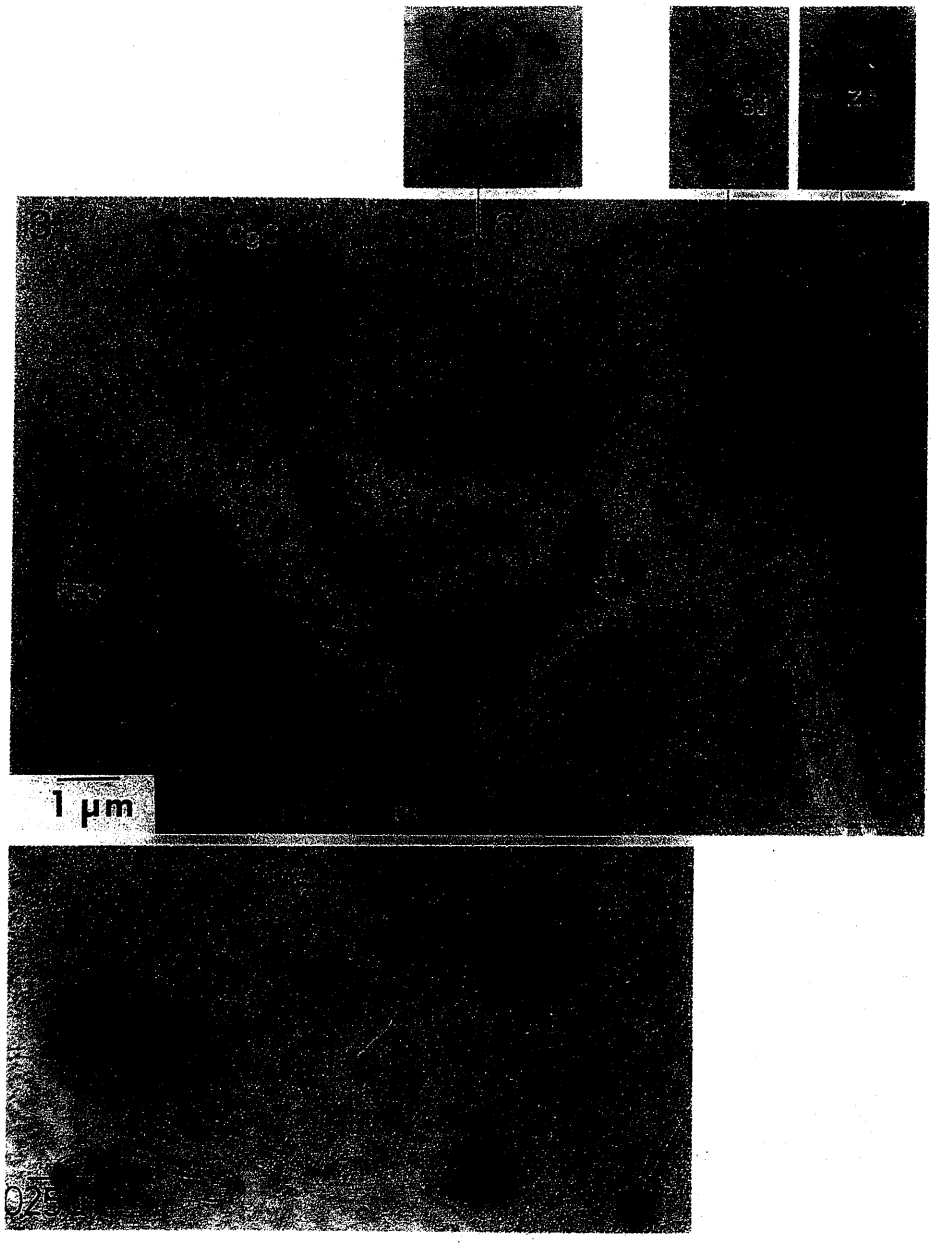

Fig. 3. The late phase of P1 stage as well as the early phase of P2 stage, an oblique section through the dioptric apparatus showing the fine structure of developing dioptric apparatus. The same higher magnifications, a to d, showing.

a. the cuticuline with bilayer electron-dense structure (arrows).
b and c. the cellular junctions between primary pigment cell (corneagenous cell) and secondary pigment cell.
d. rER-rich and immature pigments in primary pigment cells.

CC: crystalline cone; CgC: corneagenous cell; Co: cone cell (1-4);
P: pigment droplet; PPC: primary pigment cell; M: mitochondria;
RC: retinular cell; SJ: septate junction; ZA: zonula adherens.

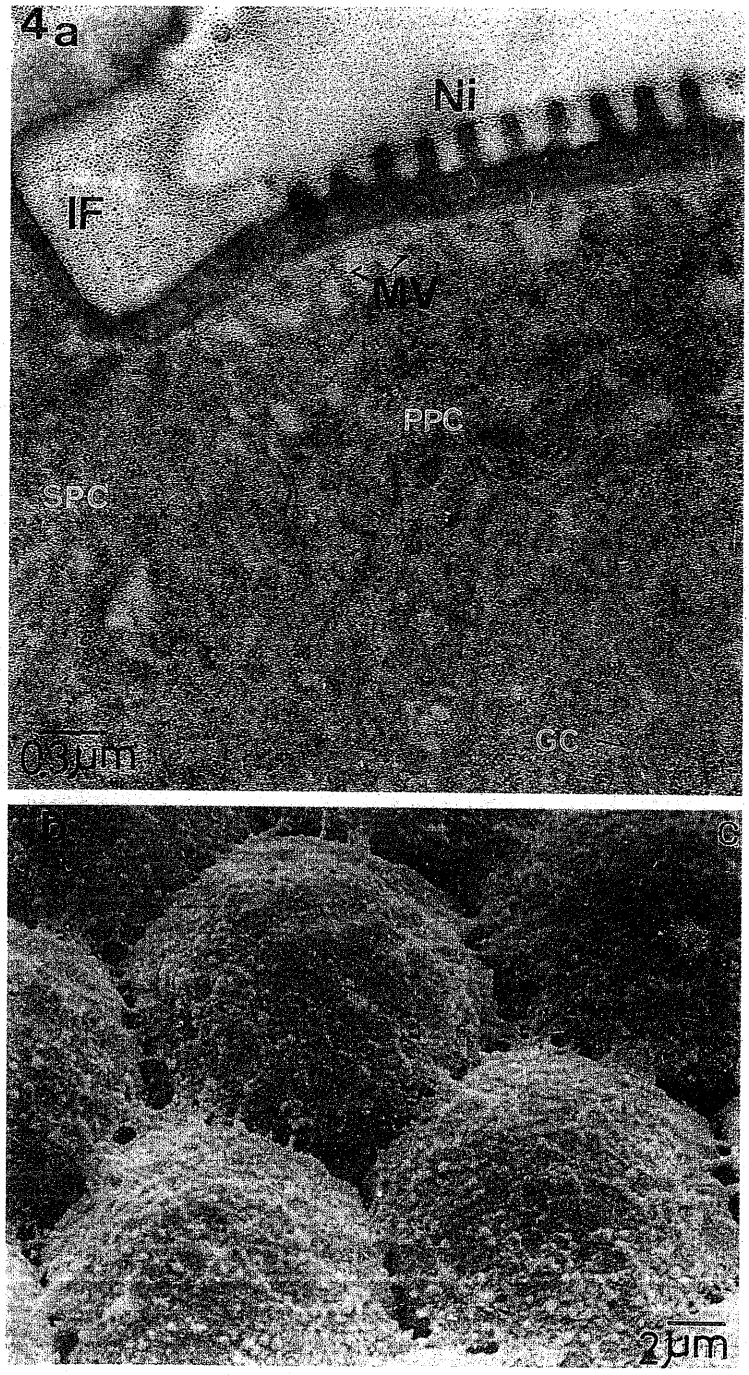

Fig. 4. The late phase of P2 stage as well as the early phase of P3 stage:
a: The corneal nipples on the primitive cornea are seen.
b: A thin layer of ecdysial membrane covers the well-developed facets.
GC: Golgi complex; IF: interfacet area; Ni: nipples;
PPC: primary pigment cell; SPC: secondary pigment cell,

Fig. 4. The late phase of P2 stage as well as the early phase of P3 stage:
c: A longitudinal section of developing compound eye shows the whole view of eye.
CC: crystalline cone; MV: microvilli; PC: pigment cell;
RC: retinular cells;

b). The hexagonal arrangement of corneal nipples is clearly seen in scanning electron micrographs and the nipples appear elongate and cone-shaped with 250 nm in height, 166 nm in diameter and with an internipple space of 200 nm (Fig. 5a; b). The fibrous cuticle is being deposited at this stage and the lamellae of procuticle are formed one after another. The upper lamellae tend to become more compact than the underlying area. Fifteen layers of lamellae with a thickness of 4.3 μm in the central area of cornea are observed during this stage (Fig. 5a).

At the last stage, the preimago stage, most characteristics are similar to that of the adult as described earlier (Wang and Hsu 1982). The molting fluid on the cornea is completely absorbed. The hexagonally arranged corneal nipples are 240 nm in height and 200 nm in diameter. The cornea is a lamella structure of 19 layers with a periodicity about 0.2 μm. The curvature of the lamellar structure corresponds with the shape of cornea which is convex on surface and flattens inside. There are no microvilli at the space between cornea and corneagenous cells.

Although the structure and the developmental pattern of insect cuticle have been described extensively (Caveney, 1976; Locke, 1976; Weis-Fogh, 1970; Zacharuk, 1976), yet the development of cornea has obviously not been included in them. Nevertheless, much of the developmental pattern of cornea is not different from that of cuticle except the formation of corneal nipples. The nipples

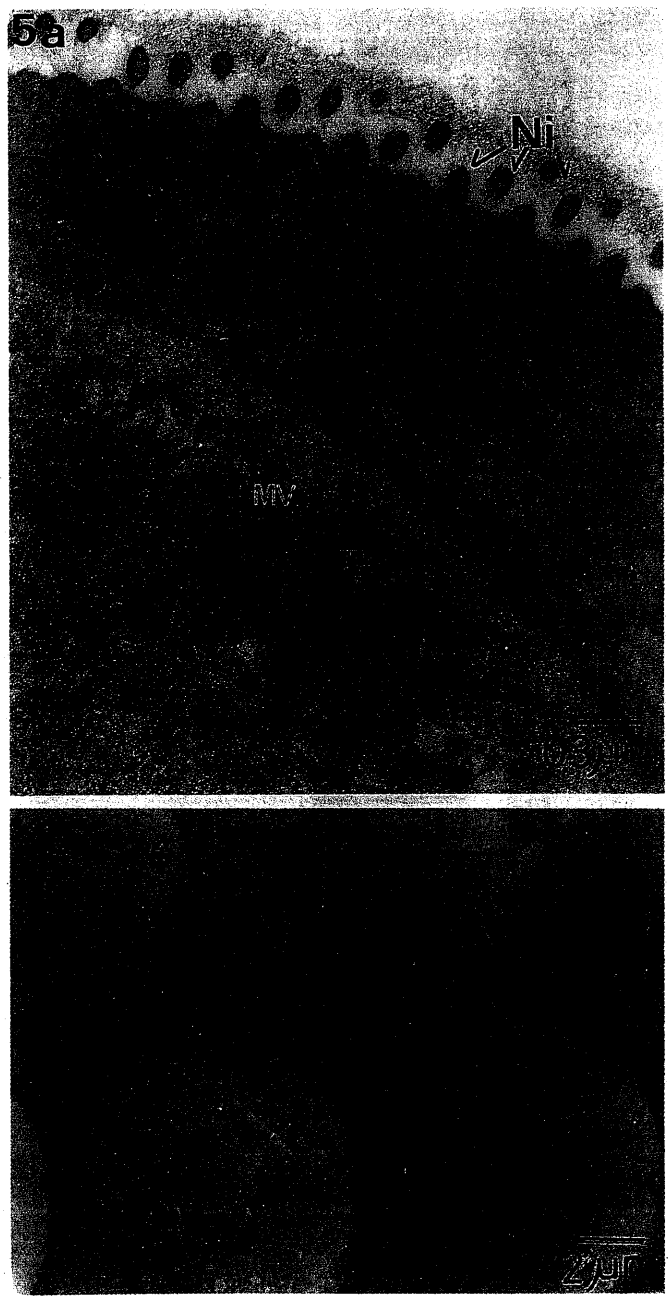

Fig. 5. The P4 stage:

a. The lamellar structure of cornea are formed.

b. The hexagonal arrangement of corneal nipples are covered with a thin layer of ecdysial membrane.

C: cornea; Ni: nipple; MV: microvilli

probably act as an antireflection coating, thus reducing reflection from the aircornea interface, and also act as an impedance transformer (Bernhard, 1967; Miller *et al.*, 1968). They may possibly be a special be a special characteristics of the nocturnal insects. The ontogenesis of these nipples has been examined with the EM in the developing moth pupa (Gemne, 1966). It has been reported that in *Deilephila elpenor*, the immature nipples are of the same height as in the adult but with a smaller periodicity (Bernhard, 1967). In *Plutella xylostella* there is a strong evidence to suggest that the cuticle of interfacet is formed by prospective secondary pigment cells (Fig. 2a; 3; 6) and the primary pigment cells play a role in the formation of major part of cornea. However, this observation needs further confirmation. It is interesting to note that in diamondback moth, two different types of cells, the corneagenous cells (or primary pigment cells) and secondary pigment cells are involved in the secretion of cornea and neither the corneagenous cells alone nor the corneagenous cells with cone cells are responsible for corneal formation. Therefore, both of the primary and secondary pigment cells are called corneageneous cells in the corneal development of diamondback moth.

II. Crystalline cone

The distal parts of four prospective cone cells are surrounded by the corneagenous cells

Fig. 6. The late phase of P1 stage as well as the early phase of P2 stage, the cytoplasm of cone cells is rich in glycogen and mitochondria.
CgC: corneagenous cell; Co: cone cell; M: mitochondria; MV: microvilli; Nu: nucleus; PPC: primary pigment cell; SPC: secondary pigment cell.

with conspicuous nuclei and deep-staining cytoplasm at late P1 as well as early P2 stage (Fig. 2a; 3; 6). The cytoplasm of cone cell is rich in mitochondria and glycogen (Fig. 6). The adjacent plasma membranes between corneagenous cells and cone cells are separated by nonjunction membranes with a distance of 30 nm. At the earlier phase, the homogeneous distrition of glycogen in the cone cells is observed (Fig. 6), then the glycogen accumulate initially from the center of primitive cone and grow along the adjacent borders of cone cells. A compact X-shaped electron-dense structure is formed and surrounded by many aggregating electron-dense particles (Fig. 3).

At the P3 stage the electron-dense structure takes a rhombus shape (Fig. 7). So far the cross section of primitive cone can be divided into 3 distinct regions: (1) central homogenous electron-dense region; (2) middle aggregated electron-dense region; and (3) external electron-lucent region. The crystalline cone tract can be found at P4 stage, and it extends into retinular cells bundle and connects with rhabdome (Fig. 8). The three regions of primitive cone can also be distinguished but the electron-dense structure changes to a compact round shape. The crystalline cone attains its complete development

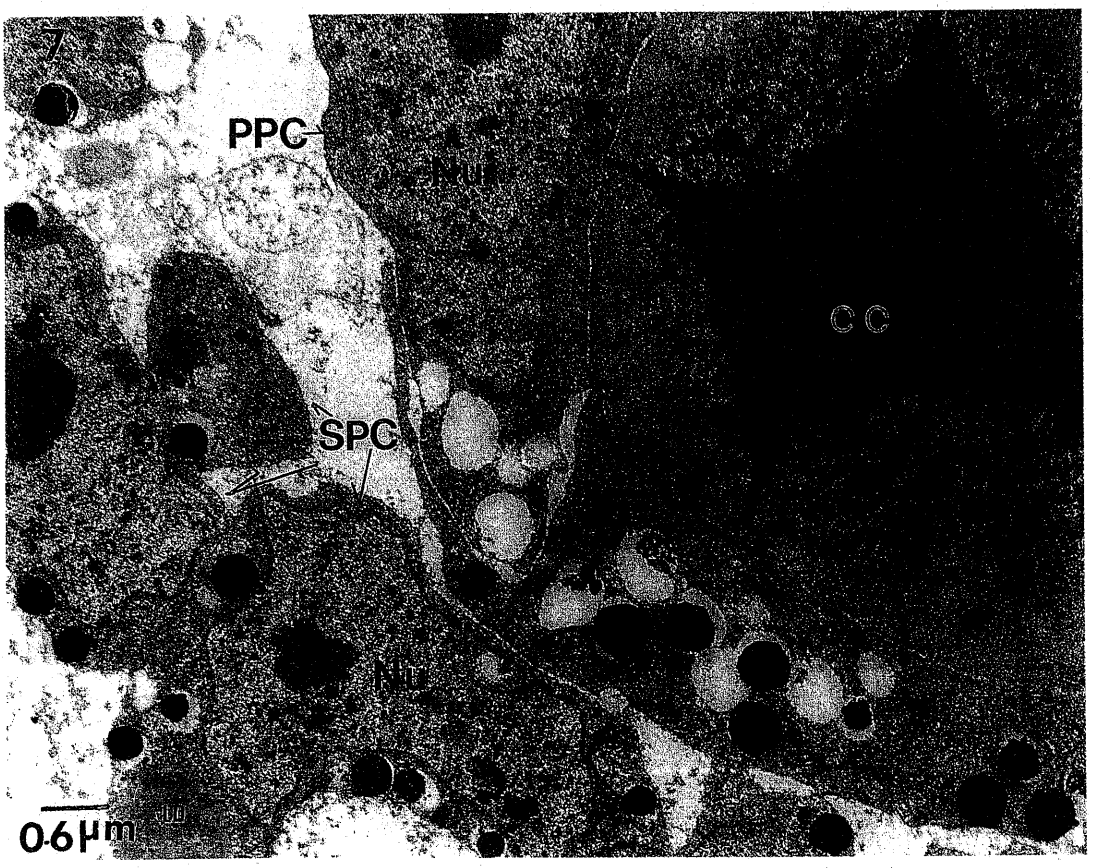

Fig. 7. The P3 stage, a rhombus shape of electron-dense structure in crystalline cone is seen. This primitive cone can be divided into 3 regions from peripheral to central region. The round-shaped pigments in the pigment cells are shown.

CC: crystalline cone; LD: lipid droplet; Nu: nucleus; PPC: primary pigment cell; SPC: secondary pigment cell.

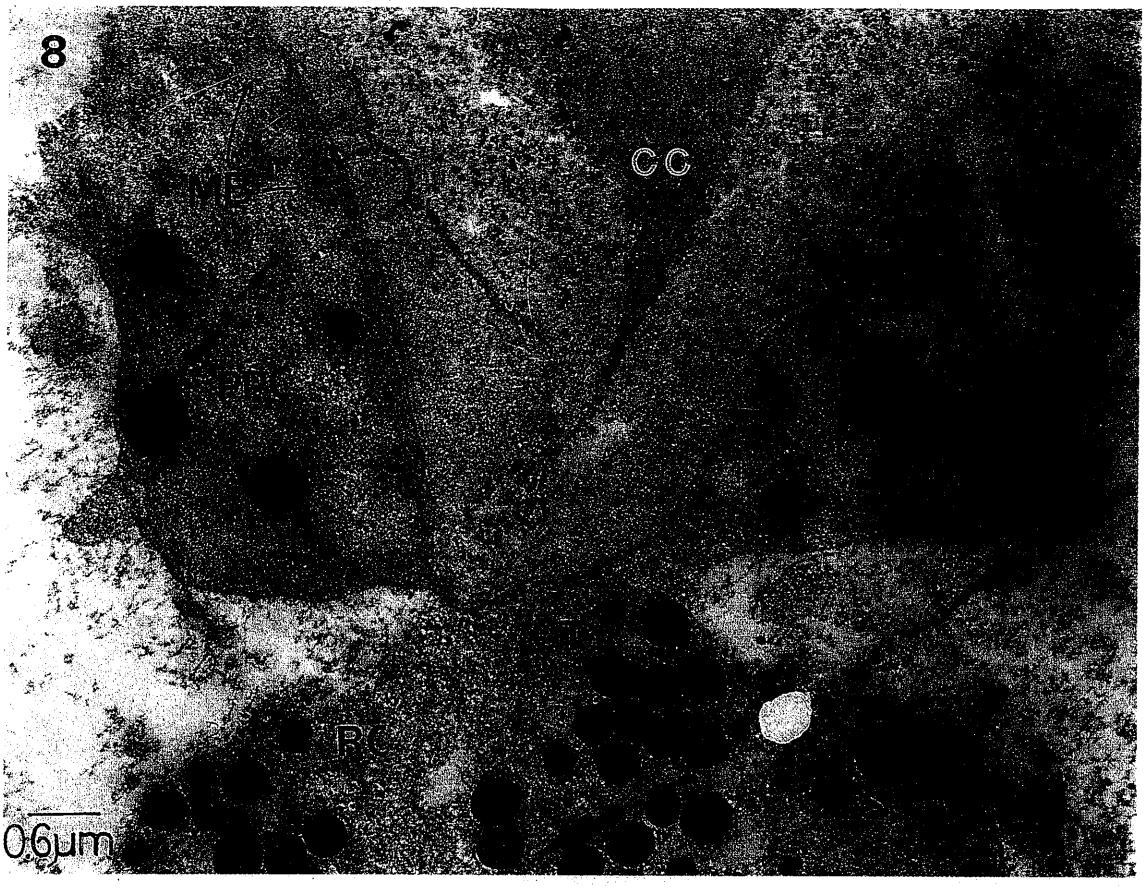

Fig. 8. The P4 stage, section through the proximal end of crystalline cone showing the connection between crystalline cone and retinular cells with crystalline tract and also showing the well-developmental pigments within multivesicular bodies of primary pigment cell.
CC: crystalline cone; CT: crystalline tract; MB: Multivesicle bodies. PPC: primary pigment cell; RC: retinular cell.

at the last stage (P5) and the electron-dense structure spreads gradually from the center to external region.

The main sequence of events in the cone formation during pupation can be summarized as follows: (1) the major components of crystalline cone are particle of glycogen; (2) the glycogen is clumped along the pathway of formation of crystalline cone and could be seen extending from the center to the plasma membrane of the neighboring cone cells. The components in the cone of the eucone type is not fully analysed but the structure of the glycogen-containing cone of the compound eye of diamondback moth corresponds with previous reports (Meyer-Rochow, 1975; Perrelet, 1970).

III. Pigment cells

The pigment cells of diamondback moth consist of primary and secondary pigment cells. As described in section I, both of them take part in the formation of cornea and the former, the corneagenous cells, differentiate out at the middle of P1 stage. These cells are then slowly transformed into pigment cells. The two primary pigment cells extend their major bodies downward beside the

proximal end of crystalline cone. The cytoplasm of primary pigment cells contains abundant rough endoplasmic reticulum, free ribosomes, mitochondria, multivesicle bodies and immature pigment droplets. The immature pigment droplets, amorphous shaped and electron-densed, usually take their place at the side of mitochondria (Fig. 3d) and inside multivesicle bodies even at the early P2 stage. The nuclei of primary pigment cells are large and irregularly shaped. The most pigment droplets are formed at the proximal part of cells while the cornea is secreted by the primary pigment cells. The immature pigments seem to condense after synthesis (Fig. 7) but the intact pigments could be seen in the primary pigment cells of P4 stage (Fig. 8). At the same time the multivesicle bodies containing pigments fill most of the proximal parts of primary pigment cells and exhibit a migratory phenomenon in relation to the light intensity and color.

The secondary pigment cells seem to develop from the basal area of optic disc. While the ommatidia growing, the secondary pigment cells stretch out their bodies from basement membrane to the inner side of cornea. The cell body is spindle-shaped and the size of cell depends on the developmental stage. As noted previously, the distal end of the slender cell body secrets the interfacet cornea. In addition to the cytoplasmic characteristics of primary pigment cells, the secondary pigment cell has many lipid droplets (Fig. 7). The mechanism of pigment synthesis is similar to that of primary pigment cell but the timing is much later. The site of pigment synthesis in these cells is concentrated around the nucleus. As the ommatidia grow, the inter-ommatidial space is gradually filled by the six secondary pigment cells and the retinular column. At the last stage (P5) numerous longitudinally oriented microtubules are also located within the cytoplasm, being especially concentrated around the nucleus and the extent of pigment migration depends on the intensity of the light and the color. Although there are no morphological variation in pigment granules of later P4, P5, and adult stages, in terms of spectroscopic properties, there are pronounced differences (Wang and Duann, 1984).

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小菜蛾複眼的折光器與隔光器之胚胎發育

王重雄　羅竹芳　段鈞心　周延鑫

以電子顯微鏡術 (SEM and TEM) 研究小菜蛾複眼的折光器與隔光器之後胚胎發育，角膜中央部份由 2 個角膜原細胞（即主色素細胞）形成，而四周部份則由 6 個副色素細胞形成。4 個晶體細胞參與晶錐體之形成，晶錐體之形成由中央沿着細胞之相鄰胞膜逐漸由肝醣顆粒沉積而成。

隔光器可分成 2 羣細胞、2 個主色素細胞與 6 個副色素細胞，兩者發育初期之胞質富有粗糙內質網、肝醣、粒腺體和微小管。逗狀的色素細胞和梭狀副色素細胞在 P_4 期已含成熟的色素顆粒。副色素細胞在發育早期時含有主色素細胞所未具有的脂肪顆粒可借此區分。