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The Ultrastructure of Ocelli of a Pea Aphid *Acanthosiphon pisum* (Harris).

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Abstract

The morphology and fine structure of the ocelli of *Acanthosiphon pisum* have been analyzed by means of light and electron microscopy. The three ocelli of this species are located near the compound eyes. Externally, the ocelli are marked by the corneal lenses virtually spherical in form. A single layer of corneagen cells lies below the cuticular lens. The corneagen and retinal cells are arranged in a cup-like manner below the cuticular lens. Each retinal cell contribute their microvilli to form rhabdom. Below the rhabdom the oviform nuclei of the retinal cells appear. Screening pigment granules are present within the retinal cell. Spherical mitochondria are homogeneously distributed in the cytoplasm of the cell body. At the proximal region retinal cells form axon and exit the basal lamina.

Keywords: Vision, Ocelli, Ultrastructure, insect, Pea aphid

1. Introduction

Invertebrate photoreceptors exemplify a wide variety of structures accommodating a broad range of functions. Most insects have evolved two photoreceptors i.e. compound eyes and ocelli. The compound eye is used for image formation, where as the ocelli help to sense the light intensity [1, 2]. The function of ocelli in adult insects is reviewed by Goodman, 1981. The functions include (a) flight control [3]; (b)reading e-vector information from the sky [4]; (c) phototactic behaviour [5].

The ocellar visual systems have been studied both at the subcellular and molecular level [6-8]. Although the structure of ocelli varies from compound eye, most of the molecules involved in the functioning of compound eyes is also functional in ocelli. Conservation of various molecules offers ocelli as an ideal model to study the function of various genes two different model system. The morphology of an insect photoreceptor is very closely associated with it's function. Only hand full of literature is available on the morphological studies of ocelli. The more insight into the structure will help us to understand the function of ocelli. To gain further insight into the structural organization of this photoreceptor system the current study investigated the ocelli of a Pea Aphid *Acanthosiphon. pisum* not reported in earlier studies.

2. Materials and Methods

Fixation of adult heads of *A. pisum* for transmission electron microscopic analysis was performed as previously described [9]. Whole heads of *A. pisum* were dissected from adult aphids (4mm in length) under light microscope and immediately fixed with 2.5% glutaraldehyde and 2% paraformaldehyde overnight at 4°C. Heads were washed with PBS, followed by a secondary fixation with 2% osmium for 1h at room temperature in the dark. Heads were dehydrated in 50%, 70%, 90%, 2x100% ethanol and 2xpropylene oxide. Dehydration step is followed by infiltration with a mixture of resin and polypropylene oxide overnight at room temperature. Finally samples were embedded in pure resin. Samples were allowed to polymerise at 60°C for overnight. Semithin sections of 500 nm were cut (Leica Ultracut Microsystems) and stained with toluidine blue. Ultrathin sections of 70 nm were cut from the appropriate areas with a diamond knife and stained with 2% uranyl acetate and lead citrate for contrast enhancement. Digital images (Morada digital camera SiS) were taken using a FEI Tecnai 12 Bio Twin electron microscope, and mounted using Adobe Photoshop.

3. Results

3.1 General morphology

Gross morphology of *A. pisum* typically possess three ocelli close to the dorsal margin of the compound eyes (Fig. 1) which, are hardly visible in necked eyes. The ocelli is formed by dioptric apparatus and a photoreceptor cell. Photoreceptor cells displayed closed rhabdomeres, i.e., rhabdomeres of adjacent photoreceptor cells were fused with each other on their distal lateral surfaces. A biconvex corneal lens (diameter: 20µm) is located at the apex of a cone-shaped cuticular elevation of the head.

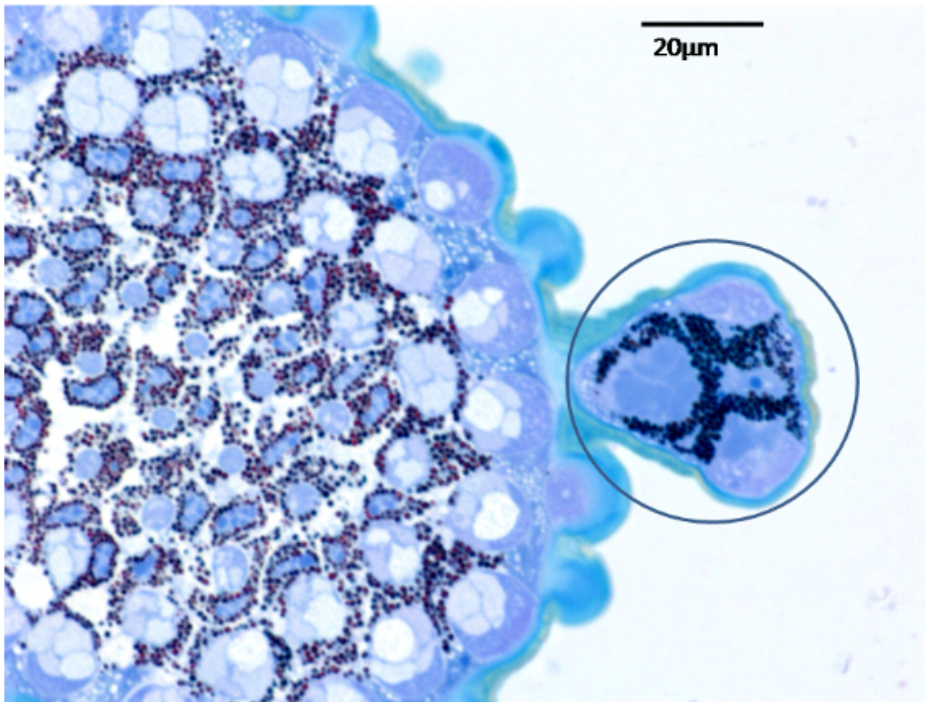

Fig 1: Light micrograph the eye of the *A. pisum* depicting the compound and ocelli. The ocelli is marked in a circle.

3.2 Dioptric apparatus

The corneal lens is thick and, biconvex in structure (Fig. 2, 3). The cornea is layered and formed by chitin and protein. There are approximately 40 layering found in the cornea. At the distal level the layering is more tight and become more wider at the proximal level (Fig. 2, 3).

Proximal to the cornea, single-layered epithelium of corneagenous cells (about 4.4µm thick) are found. The corneagenous cells are arranged in a cup-like fashion beneath the cuticular lens (Fig. 3). The neighboring cells interdigitate extensively at their apical zone with septate desmosomes providing the intercellular contact. The large oviform nuclei of 2.6 µm lie beneath the cuticular lens. The cytoplasm of the corneagenous cells, and sometimes also the nucleus, can extend processes between two retinal cells, making a broad contact with them (Fig. 2). The corneal epithelium is followed proximally by the retinal cells, which comprise the main mass of the ocellus.

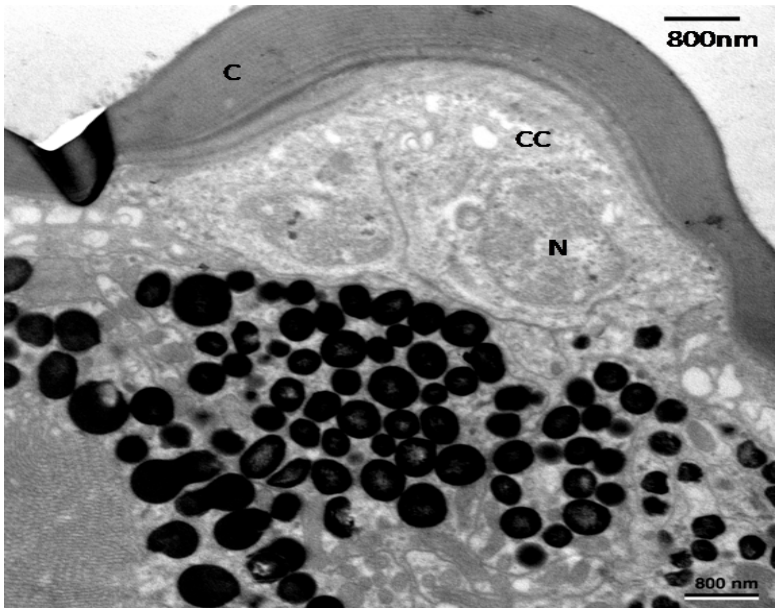

Fig 2: Transmission electron micrograph taken thought the ocllei of *A. pisum.* Distal level is the cornea. Numerous layering is observed in the cornea and proximally the lining is wider. Below the cornea (C) is the crystalline cone (CC) with their nuclei (N). Pigment granules are surrounded by the crystalline cone.

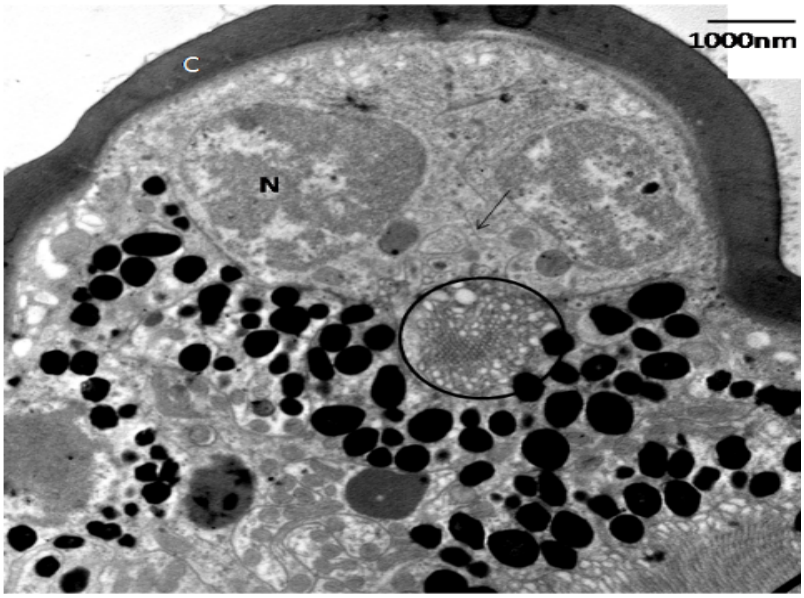

Fig 3: Transmission electron micrograph taken thought the ocllei of *A. pisum.* The section contains cornea (C), followed by crystalline cone with their nuclei (N). The crystalline cone attached with the rhabdom by adherences junction. At the distal level the microvilli of the rhabdomere are irregular in shape. Pigment granules are surrounded by the crystalline cone.

All the ocellus is invested by a layer of thin flat epithelium with pigment granules into the cells (Fig.1). In mature animals, the latter encircle the lens like a ring. The pigment ring appears as a kind of broad 'iris' that give rise to a narrow elongated false 'pupil'. The iris is formed by pigmentary cells that lie beneath the corneagenous cells. The pigment granules are circular and oval in shape. Their diameter varies from 100 to 600nm.

3.3 Ocellar Retina

Retina consists of receptor cells, which are ensheathed by glial cells. The receptor cells are not arranged in an ordered array as observed in compound eye. At the distal level the receptor appear in an irregular manner. However, the retinal cells are arranged perpendicularly to the internal surface of the corneal lens (Fig. 3). Three zones can be recognized in a retinal cell: (i) a distal rhabdomeric zone (about 56m diameter), (ii) a middle zone free of rhabdom, where the nuclei are

located, and (iii) a proximal zone, comprising from the origin of the axon up to its synaptic contacts into the neuropile with secondary neurons, which cell bodies are located in the brain. In light-adapted conditions the pigment granules move upward to the distal region where the rhabdomeric meshwork is located.

In the distal zone, the retinal cells are modified to form microvilli, which are connected with those of neighboring photoreceptors, forming a meshwork of rhabdomeres (about 1.1µm thick) (Fig.3). At the distal most level the microvilli are large and irregular in shape of 260nm in diameter. Rhabdom below the distal level reveals dense packing of microvilli of about 50nm diameter at the centre (Fig. 4). Some irregular microvilli are observed at the peripheral region. At more proximal level the rhabdom appears at it's maximum size of 7µm in diameter (Fig. 5). The size of the microvilli remains in an ordered array at the centre and some irregular microvilli of 80nm are observed at the marginal region. The neighboring retinal cells are connected to each other by desmosomes located at the portion adjacent to the rhabdomeric structure. The cytoplasm of the receptor cell contains, a large number of mitochondria exhibiting diverse shapes, i.e. spherical, elongated, irregular, generally concentrated near the microvillar border. Smooth and rough endoplasmic reticulum is also found in the cytoplasm.

In the middle zone, the retinal cells nuclei are homogeneously distributed over the area of the retina. The nuclei are large of 3µm in diameter (Fig.6). The perinuclear cytoplasm are filled with smooth and rough endoplasmic reticulum, several Golgi complexes as well as multivesicular bodies (few vesicles of about 0.16 µm in diameter). Frequently, just before the beginning of the axon, the smooth endoplasmic reticulum runs in several concentric layers, of about 0.6m in diameter. The glial processes surround the retinal cell (except the desmosomes) filling most of the inter-cellular space. In the proximal zone, the retinal cell tapers off into the axon (with about of 1.9m in diameter).

Each photoreceptor cell contained pigment granules in the cell body between the rhabdomere and the proximally located nucleus (Figs. 3, 4). They were larger in size (~400 nm in diameter) than those in the photoreceptor cells in the compound eye (~150 nm in diameter) (Fig. 4). The contents of pigment granules appeared electron dense under electron microscope, The photoreceptor cells were interconnected by belt desmosomes located close to the proximal ends of rhabdomeres (Figs. 3–5). Proximal to the nuclei were observed axons surrounded by glial cells. The axonal cytoplasm contained microtubule bundles and septum-like membrane-bounded structures (Fig. 3).

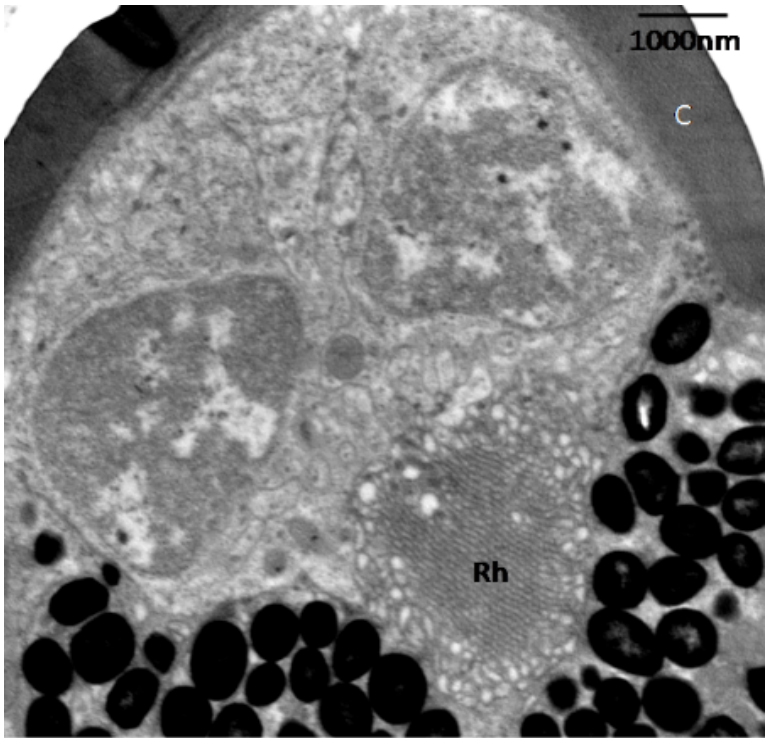

Fig 4: Transmission electron micrograph taken thought the ocllei of *A. pisum.* The section contains cornea (C), followed by crystalline cone with their nuclei. The crystalline cone attached with the rhabdom by adherences junction. The microvilli of the rhabdomere (Rh) appear in a regular manner at the central part of the rhabdom are irregular microvilli appear at the peripheral region.

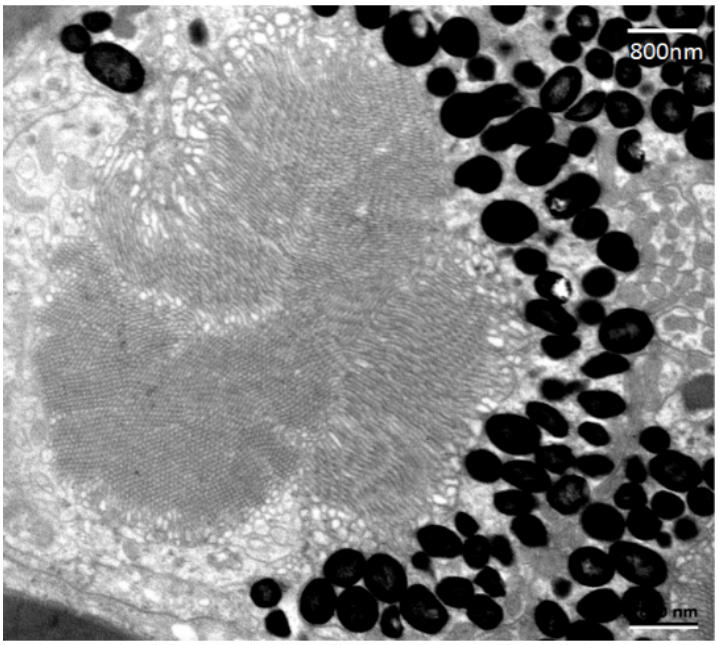

Fig 5: Transmission electron micrograph taken thought the ocllei of *A. pisum.* The rhabdom occupies maximum size at this level. Surrounding the rhabdom is the pigment granule.

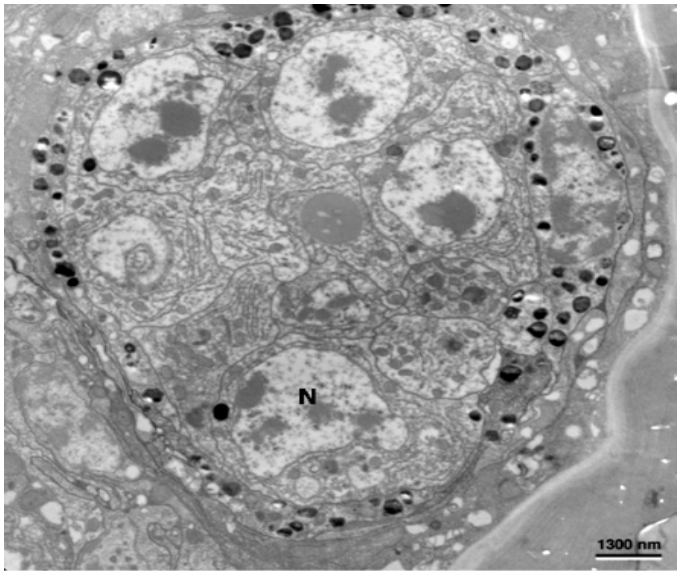

Fig 6: Transmission electron micrograph taken though the ocllei of *A. pisum.* The proximal part of photoreceptor cell filled with nuclei and some other cell organelle like mitochondria.

4. Discussion

The ocelli of *A. pisum* was observed under light and electron microscope. The diameters of the individual facets are larger than what one might expect to find in an insect of small size [10]. The larger facet helps to capture more light thus help in forming a brighter and clearer image [11, 12]. According to calculations by Barlow, 1952 [13] the minimum functional size of lenses in arthropod eyes should be around 8–10µm. Average facet width of ocelli in *A. pisum* is 20µm and, thus, lies significantly above the value given by Barow. The cornea is laminated and has more similarity with the structural arrangement of the facets of compound eye of *A. pisum* (personal observation).

In *A. pisum* most part of the retinal cell is involved in the formation of rhabdom. In insects like bees, dragonflies and cockroaches only a portion of the retinal cells is involved in the rhabdom formation [14-16]. In insects like *T. infestans*, and *R. prolixus* [17], the lateral walls of the photoreceptive cells are involved in the formation of rhabdom. Furthermore, only the distal zone of each retinal cell is associated with rhabdom

formation. A similar arrangement of rhabdom is reported from Dipteran ocelli [18, 19]. The large and massive ocellar rhabdom of *A. pisum* could be related with a specific functional characteristic, such as an increased sensitivity to light or the perception of polarized light. Future work will reveal the relation between structure of the ocelli with the behavior of the animal. Many irregular microvilli is observed in the distal rhabdom. The large irregular microvilli are symbol of photic stress [8, 20, 21]. The irregular microvilli of the distal level of rhabdom suggest probably the light passes through the ocelli are to strong and causes damage to the rhabdom.

Pigment granules are observed inside photoreceptor cells. The pigments are observed close to the rhabdomere. In other reported species pigments are capable of moving around the rhabdom during light adaptation and migrate down, towards the proximal region of the cell, in the dark-adapted ocellus [22, 23]. The pigment size is larger then reported from compound eye. The electron dense pigment granule suggests the pigment can give better protection to the rhabdom.

In the photoreceptor cells of the *A. pisum* the nucleus of the photoreceptor cell appears at the proximal level. Inside the ocellar photoreceptor cells numerous multivesicular organelles, composed by 4–5 vesicles surrounded by electron dense material are observed. These organelles, typically present in compound eyes, use to be associated with the rhabdom. The presence of acetyl- choline/acetylcholinesterase inside these vesicles suggests that they could participate in photic transduction [24].

The ultrastructural arrangement of ocelli suggests this minute photoreceptor organ is functional in *A. pisum*. An electrophysiology study of the ocelli will further answer more regarding the functionality of the ocelli.

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