

A six-rhabdomere, open rhabdom arrangement in the eye of the chrysanthemum beetle *Phytoecia rufiventris*: some ecophysiological predictions based on eye anatomy

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ABSTRACT: We are describing a rhabdom organization of the eye of the chrysanthemum beetle *Phytoecia rufiventris* that to date has not been described from any other insect. In cerambycid beetles free rhabdomeres, forming a circular, open rhabdom, surround a central rhabdom made up of the rhabdomeres of one or two cells. In *Phytoecia rufiventris* the central rhabdomeres are missing throughout the eye and the microvilli of the outer 6 rhabdomeres are regularly oriented in three directions. Following the classification of rhabdom types suggested by Wachmann (1979), we suggest to name the rhabdom arrangement seen in the retina of *Phytoecia rufiventris* "Grundmuster 3". This pattern ought to facilitate polarization sensitivity and movement perception, features that agree with the behavioural repertoire of *Phytoecia rufiventris*.

Introduction

Photoreceptor organization can provide valuable clues on a species' phylogenetic position and its ecophysiological needs and adaptations. For instance, it has long been known (Eakin, 1963) that there are two lines of photoreceptor evolution: the ciliary line, exemplified by the rods and cones of the vertebrate eye and the rhabdomeric line, evident in insects and crustaceans. In addition to the morphological differences between the two types, the transduction mechanisms also differ significantly from each other. This has led to the discovery

that in the non image-forming pathways of the vertebrate eye, that involve melanopsin-containing retinal ganglion cells and not rods or cones (Erren *et al.*, 2008), phototransductive mechanism are based on the rhabdomeric system (Isoldi *et al.*, 2005). On the other hand, a few examples are known in which eyes from the rhabdomeric line possess photoreceptor cells with ciliary rootlets or ciliary characteristics (Home, 1972; Juberthie and Munoz-Cuevas, 1973; Arendt *et al.*, 2004). It has, thus, been argued that the archaic photoreceptor originally combined both types of photoreceptive mechanism (Plachetzki *et al.*, 2005).

In the insect and crustacean compound eye, rhabdoms are formations of numerous, narrow fingerlike projections, known as microvilli. Hundreds or thousands of such microvilli, collectively forming a rhabdomere, house the photopigment in their membranes and are confined to a modified part of a photoreceptive cell in the retina. In most species there are 8 such

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cells, usually termed retinula cells, present in each ommatidium of the compound eye. Studies on the precise arrangement and organization of retinula cells as well as their microvillar rhabdomeres can provide valuable clues on an eye's visual capability and limitations. Therefore, not surprisingly, examinations of compound eye ultrastructures have been used with considerable success to predict aspects of a species' behaviour and general biology, be that a crustacean (Meyer-Rochow and Reid, 1994; Mishra *et al.*, 2006) or an insect (Meyer-Rochow and Gokan, 1988; Gokan and Meyer-Rochow, 2000).

In so-called fused rhabdoms, rhabdomeres of all photoreceptive cells form a central column, in which each rhabdomere can be likened to the segment of an orange, with their narrow ends pointing centrally. Fused rhabdoms can be short or long, wide or narrow and may be separated from the distal dioptric structures of cornea and cones by a clear zone devoid of screening pigment granules (Horridge, 1975). Long and thin fused rhabdoms are more common in diurnal species, while shorter and wider rhabdoms, especially when in eyes with a clear-zone, tend to be indicative of higher sensitivity (Warrant, 1999). Obviously, the latter eye type is particularly useful for nocturnal species.

Microvillus orientations that are either orthogonal to each other in one plane of sectioning or are staggered with layers of orthogonally oriented microvilli, visible in longitudinal sections and arranged in tandem positions, indicate an ability of the insect (or crustacean) to detect the plane of polarized light (Eguchi, 1999; Horvath and Varju, 2003). Such specialized, polarization-sensitive ommatidia are often located along the dorsal rim of a compound eye, which are pointing predominantly skyward (Labhart and Meyer, 1999).

In open rhabdoms, neighbouring rhabdomeres are either totally separated from each other or are not fully centrally-fused, forming a rhabdom that is annular (i.e., ringlike) in transverse section, surrounding two long and thin rhabdomeres in its midst. That a special gene, termed spacemaker, controls the development of such non-fused rhabdoms, has recently been shown by Zelhof *et al.* (2006) and possible consequences of the open or annular rhabdom structure have been discussed by Osorio (2007). The latter author distinguishes a lower acuity scotopic system, made up of the outer 6 rhabdomeres, and a high spatial acuity system, dependent on the two thinner and centrally-placed rhabdomeres. Polarization sensitivity in the eyes of Diptera (which possess open rhabdoms) has so far been reported to be either small or non-existent and recent

behavioural observations have shown that the mosquito *Aedes aegypti* (unlike other aquatic insects) does not use polarotaxis to detect water surfaces (Bernath *et al.*, 2008).

On the basis of ultrastructural examinations of the eyes of 55 species of long-horned beetles (Cerambycidae), Wachmann (1979) concluded that an open rhabdom (with very few exceptions) was the rule in this family of Coleoptera and that two types could be distinguished: Grundmuster 1, in which two central rhabdomeres, labelled 7 and 8, were completely separated from the six peripheral ones and Grundmuster 2, in which the two central rhabdomeres 7 and 8 were structurally attached to the peripheral rhabdomeres. We now report the occurrence of a rhabdom in a cerambycid beetle, in which (a) there are only six totally separate rhabdomeres per ommatidium, (b) a microvillar orientation of the six rhabdomeres occurs that is indicative of polarization sensitivity throughout the eye, and (c) in which central rhabdomeres, surrounded by peripheral ones, are completely absent.

Materials and Methods

The eyes of two adult *Phytoecia rufiventris* beetles from Japan, procured by Professor N. Gokan, were available for study. However, both specimens exhibited identical retinal organizations. The eyes were fixed during the day in the light-adapted state. The head, split in half, was first fixed in a mixture of cold 2% paraformaldehyde and 2.5% glutaraldehyde, buffered in 0.1 mol/L cacodylate buffer (pH 7.4) for 1 d, and then postfixed for 1 h in 2% OsO₄. The samples were then rinsed three times in the same buffer and passed through a graded series of ethanol before being kept in acetone/Epon mixture (50/50) for 1 d. The specimens were finally embedded in pure Epon-812 resin and hardened at a temperature of 60°C for 3 d.

Semithin sections for light microscopy (from cornea to basement membrane) were cut on an ultramicrotome (RMC, Tucson, AZ) with a glass knife and stained with 0.5% aqueous solution of toluidine blue on a hot plate for a few seconds. Ultrathin sections were cut with a glass or diamond knife and picked up on uncoated 300-mesh copper grids. The ultrathin sections were then stained with Reynolds' lead citrate and 2% aqueous uranyl acetate for a few minutes each, washed in distilled water, dried, and finally observed under a Zeiss EM 10 transmission electron microscope (Zeiss, Oberkochen, Germany), operated at 60 kV.

Results

The eye of *Phytoecia rufiventris* conforms in its gross anatomical organization to that of other cerambycids (Wachmann, 1979). Underneath the cornea four cone cells, abbreviated CC in figure 1, surrounded by two massively developed primary pigment cells with large, black screening pigment granules (P), are present in each ommatidium. Eight retinula cells can be counted per ommatidium, but only six of them, corresponding to the “peripheral” ones (numbered clockwise 1 to 6 according to the terminology used by Wachmann, 1979), possess rhabdomeres. The central two retinula cells, known as cells 7 and 8, turn into axons without forming rhabdomeres.

More distally, closer to the cornea, the six rhabdomeres come closer together, but not to the extent that they might form a closed ring. In the contrary,

their rectangular outline (Fig. 1) and the orientation of the rectangle, which measures approximately 7.0-8.0 μm by 3.0-3.5 μm , are maintained across the entire eye. The rhabdomeres are distributed within these rectangles like the dots on a domino and measure roughly 1.75 to 2.00 μm in diameter each. Secondary screening pigment cells surround each ommatidial group of retinula cells. In the micrographs they appear to be ‘grey’ and, pushing projections inward into spaces between the retinula cells, contain a dense, dark ground substance in addition to some screening pigment granules (Figs. 1-3).

The microvilli of the six rhabdomeres are almost identical in length (approximately 1.50 - 1.75 μm) and diameter (55 nm), but their most important characteristic is that the orientation of the microvilli in the two corner rhabdomeres on either side of the rectangle is that of an X (Fig. 2). Thus, the microvilli of these four

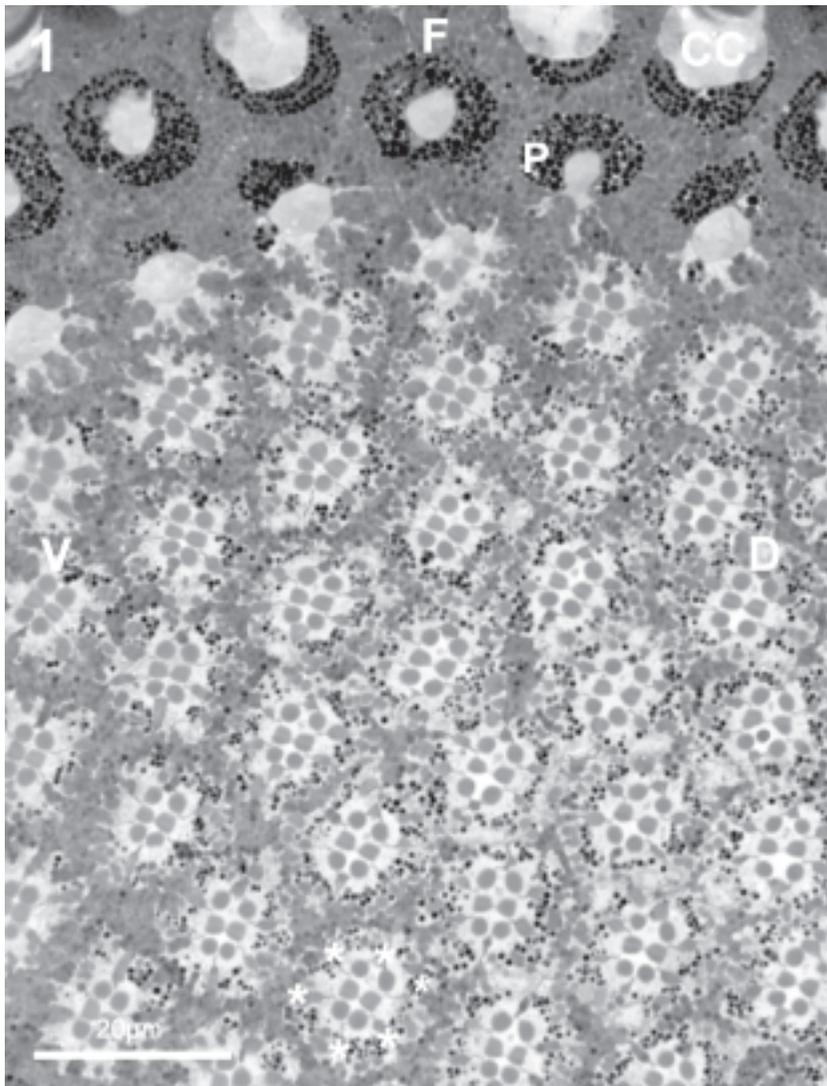


FIGURE 1. Light micrograph of transverse section through the retina of *Phytoecia rufiventris*, showing open regularly oriented rhabdoms with 6 rhabdomeres in characteristic rectangular arrangement throughout eye. Abbreviations: D dorsal, F frontal, V ventral, CC crystalline cone, P screening pigment. Asterisks show the locations of the secondary pigment cells.

cells run in two orthogonal directions. The microvilli of the two cells occupying positions between the four corner cells, in other words those forming the sides of the rectangle, have their microvilli arranged in parallel, but offset to those of the corner rhabdomeres by 45 degrees.

Although this pattern is illustrated by Wachmann (1979) in his review on cerambycid eyes as "Abb. 14A (E1 top row)" for the beetle *Molorchus minor*, the same author also illustrates numerous other rhabdom arrangements seen in that species' eye. Yet, in the eye of *P. rufiventris* we could consistently throughout the retina only find the pattern of six outer cells with their rhabdomeres, but no inner (or central) rhabdomeres despite the presence of two central cells (Fig. 2) and their axons (Fig. 3) per ommatidium. The nuclei are of the retinula cells, which are mottled and of irregular outlines, are present in the proximal region of the retina just above

the basement membrane. The highly regular pattern of rhabdomere and microvillar arrangements in the eye of *P. rufiventris*, appear to be no coincidence, but should represent the basis for highly specialized visual functionality to be discussed below.

Discussion

Wachmann (1979) distinguished two basic types of open rhabdom eyes in cerambycid beetles, but central rhabdomeres were present in both of these types. In the cerambycid eye that we described, the central rhabdomeres are missing, although 8 retinula cells per ommatidium are developed. On that basis we would suggest that it is appropriate to expand the known rhabdom types of cerambycid beetles by one (the one described

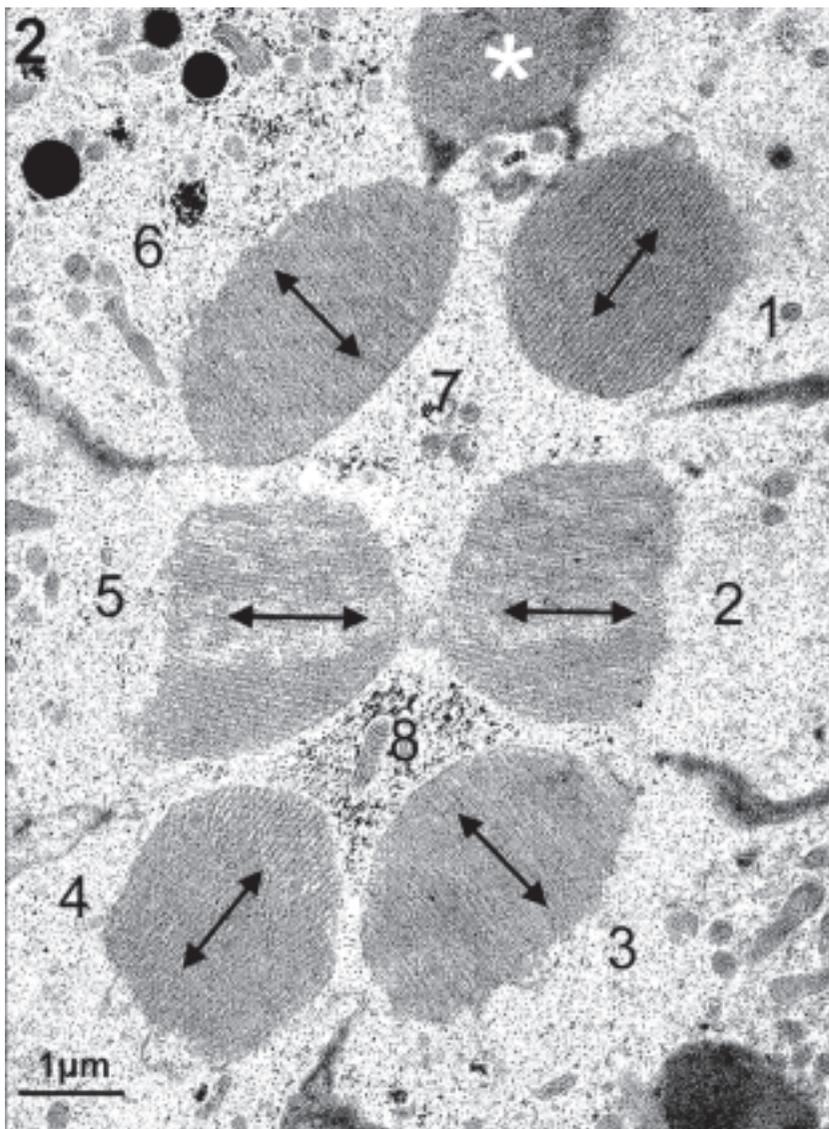


FIGURE 2. Transmission electron micrograph of transverse section through a single rhabdom, showing orientation of microvilli in three directions (arrows) and absence of central rhabdomeres despite the presence of retinula cells 7 and 8. The peripheral rhabdomere-possessing retinula cells are numbered 1 to 6. The asterisk points to a secondary pigment cell.

in this paper) and, following the terminology introduced by Wachmann (1979), call it ‘Grundmuster 3’.

The rather narrow and isolated rhabdomeres seen in the eye of *P. rufiventris* cannot be an adaptation for scotopic vision as suggested for the outer, but thicker rhabdomeres in, for example, higher flies, which possess central and narrower rhabdomeres for photopic vision (Osorio, 2007). A “division of labour” between narrow central and wider outer rhabdomeres can, therefore, not take place in the eye of *P. rufiventris* and the narrow diameter of its six rhabdomeres, thus, indicates an adaptation to see in bright and sunny conditions. Moreover, assuming the outer rhabdomeres R1 – R6 in *P. rufiventris* (as in the open rhabdoms of the eyes of Diptera: Kirschfeld and Lutz, 1974) are the ones responsible for movement perception and central rhabdomeres would exert an inhibiting effect (Srinivasan and Guy, 1990), then not to have the latter must help to improve the movement perception.

Most importantly, the extremely regular orientation of the entire rhabdoms, as well as their rhabdomeres and their constituent microvilli, collectively suggest high polarization sensitivity. In order to simply detect whether the sunlight is polarized or not, two orthogonal microvillus orientations will suffice (Eguchi, 1999; Horvath and Varju, 2003), but if an immediate and unambiguous E-vector detection is required, three regular microvillus orientations are needed (Kirschfeld, 1972). There can

be no doubt that the eye of *P. rufiventris* meets both requirements and we, therefore, predict that *P. rufiventris* individuals use this ability, be it to maintain a straight flight path, to spot polarizing surfaces, or for some other unknown purpose.

How do our findings on the retinal organization of this beetle tally with what is known of its biology? It has been reported that a sex pheromone is *not* involved in this species and that “a male runs to a moving individual (male or female) 1.5 – 5.5 cm away” (Wang *et al.*, 1966). Moreover, the adults are diurnally active and inactive between 21.00 and 07.00 (Wang *et al.*, 1966). For individuals of the closely related and similarly-sized cerambycid species *Agapanthia violacea* Paulus (1974) found that they only fly in bright sunshine and that under an overcast sky disturbed individuals drop rather than fly away.

Although we were not able to find any published behavioural evidence in support of our conclusion that *P. rufiventris* possesses and uses its polarization sensitivity, our prediction that individuals of *P. rufiventris* ought to be active in bright light and possess a high capacity to detect movement are certainly vindicated by the published reports. We are, thus, confident that our prediction of this beetle’s polarization sensitivity will also be correct, even if not yet noticed in either field or laboratory observations.

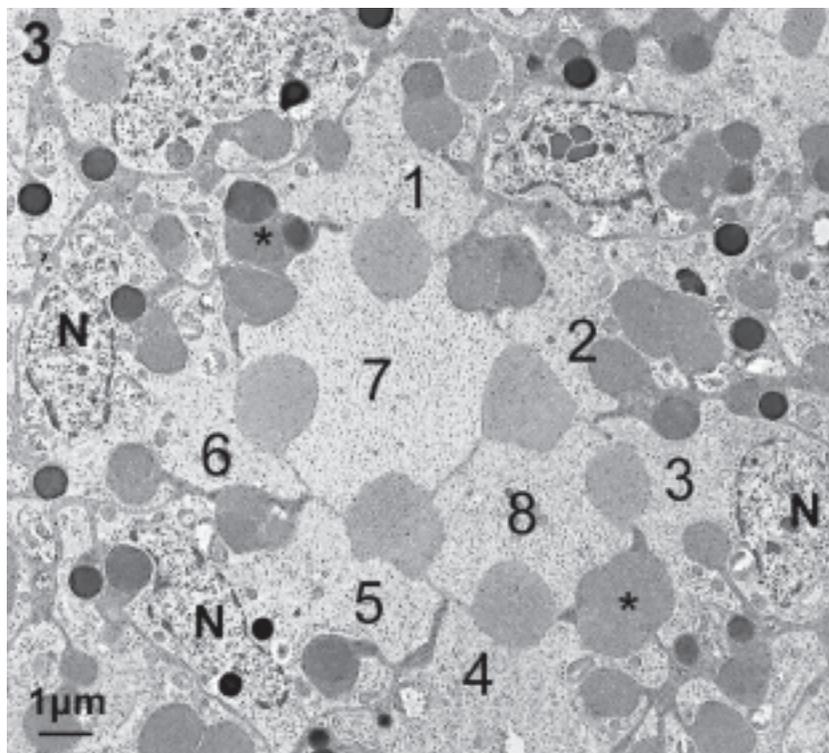


FIGURE 3. Transmission electron micrograph just above the basement membrane, showing that the central cells 7 and 8 have already turned into axons (note presence of neurotubules in them) at a level where the proximal retinula cells still contain nuclei (N). The labelling of the retinula cells is the same as in figure 2. The grey structures, indicated by asterisk, are the proximal cytoplasmic ends of secondary screening pigment cells that occupy spaces between the retinula cells.

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