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[SHORT COMMUNICATION]

Not Just an Empty Cavity: the Inter-Rhabdomeral Space in the Jamaican Cavefly *Neoditomyia farri* (Diptera, Mycetophilidae)

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ABSTRACT—Contrary to most other Diptera, the inter-rhabdomeral spaces of the retina of the Jamaican cavefly *Neoditomya farri* are filled neither by extracellular matrix nor dense cytoplasmic material. Instead, a foamy organization of loose vacuoles, measuring approximately 0.7 μm in diameter, appears to keep the rhabdomeres of retinula cells 7 and 8 in place. The vacuoles are bounded by membranes and traces of actin, determined immunocytochemically, are present. The origin of the vacuoles is unclear, but evidence in support of a retinula cell rather than cone cell origin is advanced.

Key words: compound eye, retina, vision, development, insect

INTRODUCTION

No other insect compound eye has been more thoroughly studied than that of Diptera. Over the years developmental research on the eyes of predominantly *Calliphora, Musca,* and *Drosophila* species has led to a detailed description of the cellular pattern formation that produces the ommatidial unit (Ready 2002). Yet, some questions remain and one of them is the origin and nature of the inter-rhabdomeral space (IRS), so typical of the dipteran eye.

While most other insects have centrally-fused rhabdom columns in their ommatidia, Diptera are characterized by the presence of open rhabdoms (Fig. 1). In an open rhabdom individual rhabdomeres do not come together centrally to form a solid, columnar rhabdom, but remain largely separate entities that project into a central cavity, known as the IRS. According to Ready (2002), in *Drosophila* embryologically even the open rhabdom starts as a system of connected rhabdomeres, first visible in cross sections of the developing eye at 37% pd (= pupal development). At 55% pd, when the rhabdomeres have become elongated entities and possess distinct microvilli, they are bracketed by *zonulae adherentes*, the latter inpocketing them into a trapped cavity, the future IRS.

No cellular membrane material or organelles have been recorded from the IRS and because of its homogeneous content it has generally been assumed to be part of the extracellular matrix (ECM). The creation of the IRS has been linked to the stretching of the epithelium through increases in hydrostatic pressure during pupal "inflation" (Ready, 2002). However, how exactly the rhabdomeres separate and how, once they have separated, they maintain their precise alignment in the IRS (so crucial for vision in Diptera: Horridge and Meinertzhagen, 1970), are questions that remain to be answered.

On the basis of immunocytochemical observations, it was claimed by Järvilehto and Harjula (1992) that extracellularly-located actin filaments in the IRS of the fly were involved "in adaptational adjustments". However, since ultramicroscopically no such filaments could be seen in the IRS, these findings have met with considerable skepticism and remained controversial and largely unaccepted to this date. In this note, based on the eye of the Jamaican cave fly *Neoditomya farri*, we wish to show that the IRS of the dipteran eye need not always consist purely of ECM material alone, but can contain cytoplasmic remnants. The presence of the latter might be able to explain some of the earlier controversial findings of "extracellular" actin in the fly eye.

MATERIAL AND METHODS

Larvae of the Jamaican cavefly *Neoditomyia farri* were caught by hand in Dromilly Cave, Trelawny, Jamaica (Jamaica Survey Department map 2, 1:50,000; E738 N920) during the months of

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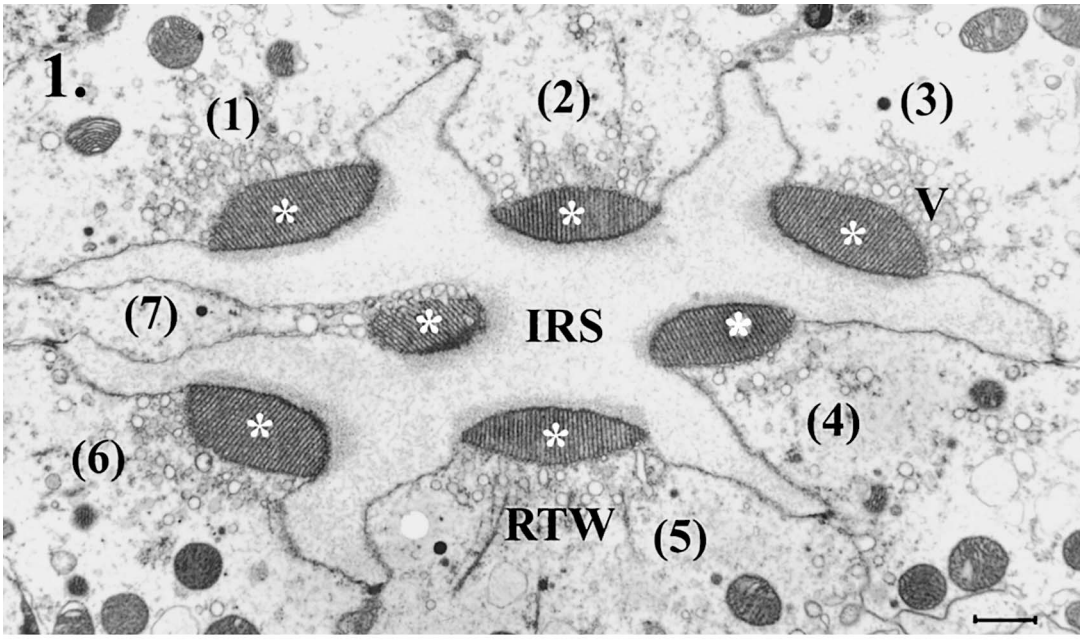
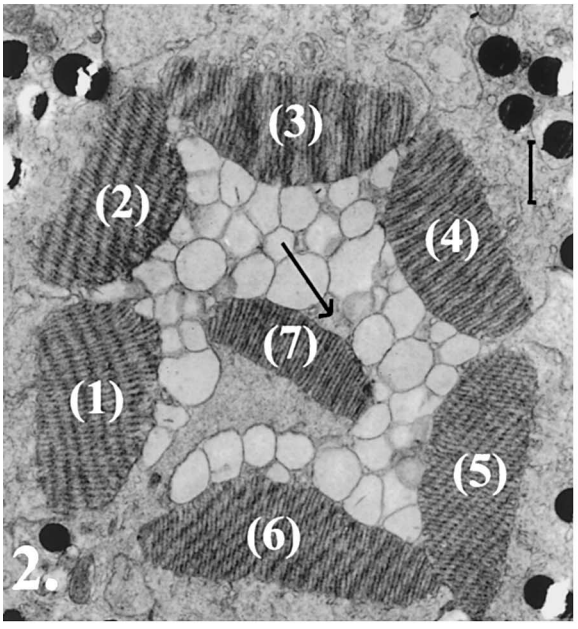
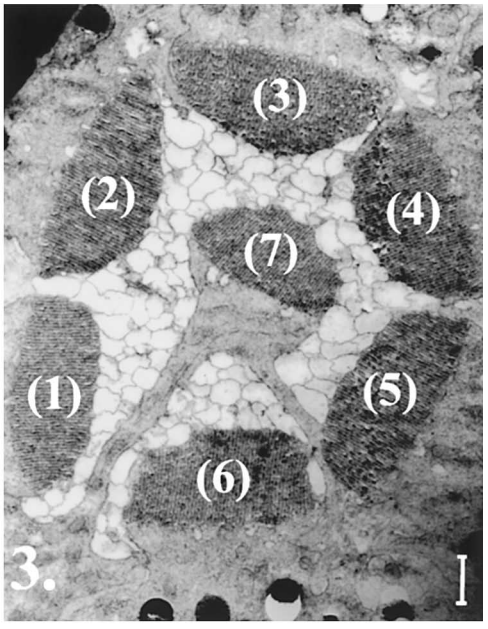
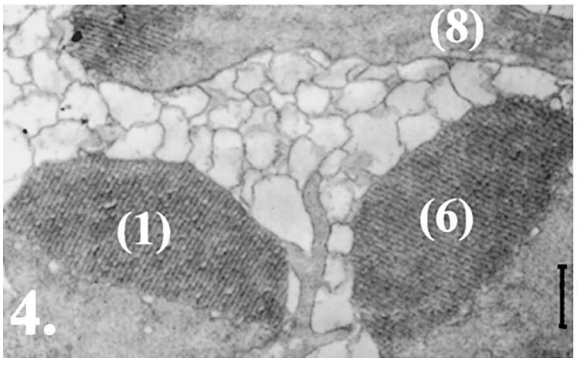
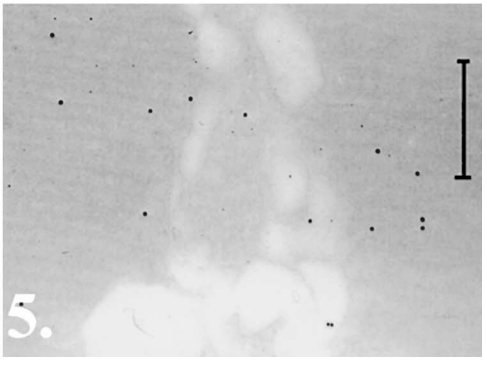
August through December and taken back to the EM-Unit of the University of the West Indies in Kingston. *N. farri* is a tropical, web-constructing mycetophilid fly, but unlike its New Zealand relative with similar habits *N. farri* is non-luminescent (Stringer and Meyer-Rochow, 1996). Due to the relative scarcity and short-lived nature of the males of the species, we used only females for this study,











but following eclosion even they lived only 2–3 days.

Immediately after eclosion some females had their eyes severed from the head and prefixed in Millonig's phosphate-buffered 2% glutaraldehyde 2% paraformaldehyde. Following brief rinsing in buffer the specimens were postfixed 1.5% phosphate-buffered osmiumtetroxide for 2 h, dehydrated in a graded series of acetone, and embedded in araldite. Semi- and ultrathin sections of the eyes of several specimens were cut on a Reichert ultramicrotome. Immunocytochemical methods and reagents were the same as reported earlier (Meyer-Rochow and Royuela, 2002) and unstained sections, collected on Ni-grids, were used for immunocytochemical localizations with 15 nm gold-labelled immunoglobulin M goat anti-mouse actin immunoglobulin. Monoclonal antibodies were used (cf., Meyer-Rochow *et al*. 2003).

RESULTS AND DISCUSSION

The typical arrangement of the rhabdomeres of one ommatidium in the eye of *Calliphora erythrocephala* can be seen in Fig. 1. In the centre of the IRS one finds distally the rhabdomere of R7 and further below in a more proximal position the rhabdomere of R8. The respective retinula cell bodies of these two cells are located between retinula cells R1 and R6 and R1 and R2. The rhabdomeres of retinula cells R1 – R6 do not touch each other, but are arranged in a roughly trapezoidal manner around the central cavity and the central rhabdomeres. Specializations near the rhabdom base/cytoplasm interface in the form of membrane vesicles and the so-called rhabdom terminal web (RTW) are clearly visible in Fig. 1.

Retinula cells and their rhabdomeres in the eye of *N. farri* are in a similar position to those seen in *Calliphora* (Fig. 2). However, the outer rhabdomeres R1 – R6 are less isolated than those of the *Calliphora* eye and, most of all, the IRS is not filled with the homogenous ground substance, thought to represent ECM in *Calliphora*. Rhabdomere shapes and microvillar dimensions (e.g., diameters of ca. 60–70 nm), are approximately the same in the two species.

The IRS in *N. farri* throughout its entire extent, from its most distal end to its proximal termination, consists of more or less circular vacuoles with an average diameter of 0.7 μm, bounded by membranes and resembling foamy material. Embedded in and kept in place by this foam are the cytoplasmic projections of retinula cells R7 and R8 with their rhabdomeres (Fig. 3). In some places rhabdomere-vacuole interactions (Fig. 2) or connections with the retinula cell cytoplasm appear to exist (Fig. 4).

Since actin has been shown to be a component of virtually all cell types in the compound eye of crustaceans and insects, we tested for its presence in the IRS-foam and adjacent rhabdomeres. The microvilli of the rhabdomeres clearly gave positive responses to actin, but some gold particles also appeared to be associated with the IRS foam (Fig. 5). These conjunctions were specific for actin. Yet, where does that foamy material come from? There are two candidates for the origin of the IRS-vacuoles: retinula cells and cone cells.

Cone cells appear to play a pivotal role in the embryogenesis of the rhabdom in flies. According to Ready (2002) they are "zipping shut" the photoreceptor apices at both ends and thus initiate the future positions of the rhabdomeres. It is conceivable that cone cell material invaded the region of the IRS and then, upon "pupal inflation", acquired a loose, foamy, and vacuolar consistency that might have degenerated further, had the eclosed animal a longer lifespan. With only two to three days to live following eclosion, cellular remnants may not have an opportunity to be absorbed or replaced by ECM and, thus, remain there. Under this scenario the presence of the IRS vacuoles would represent a neotenic trait.

The other possibility is that upon separation of the developing rhabdomeres some cytoplasmic strands, linking the ommatidial cluster of retinula cells, remained after the gap between the rhabdomeres became filled largely by ECM material. Evidence in support of a retinula cell origin of the IRS vacuoles comes from observation by Yasuyama and Meinertzhagen (1999), who showed extensive vacuoles in extraretinal photoreceptive cells of the fly brain and secondly, from studies on dipteran species in which no ECM exists (cf., Fig. 15: Stavenga and Wunderer 1999).

Can the presence of actin help decide which of the two views is correct? In the compound eyes of insects and crustaceans it has been found in all cell types, including cone cells of the fly *Calliphora erythrocephala*, where it can have a contractile function or act as a stabilizing cytoskeletal element (Wolfrum 1991). In the adult eye of *Calliphora* actin is predominantly located in a band facing the interommatidial space. In the retinula cells of arthropods actin has been found in palisade-like cisternae of the smooth endoplasmic reticulum backing the photoreceptive microvilli (Baumann 1998). Matsushita and Arikawa (1996), moreover, found that actin was required for the synthesis of rhabdom material.

Fig. 1. Cross section through an ommatidium of an approximately 1 week old *Calliphora erythrocephala*, killed with KCN vapour, showing arrangement of rhabdomeres (asterisks) and corresponding retinula cells, labeled 1 to 7 as well as rhabdom terminal web (RTW) and vesicles (V). The interrhabdomeral space (IRS) corresponds to extracellular matrix (ECM). The scale is 1 μm.

Fig. 2. Cross section through *Neoditomya farri* ommatidium, showing similar arrangement of rhabdomeres (labeled 1 – 7) to that of *C. erythrocephala*, but an IRS filled with vacuolar material rather than ECM. The arrow points to a close vacuole-rhabdomere link. The scale is 1 μm.

Fig. 3. Cross section through *N. farri* ommatidium where the central rhabdomere 7 gets replaced by the more proximal rhabdomere 8. The vesicular IRS remains in place. The scale is 1 μm.

Fig. 4. Close-up of the edge of the IRS, showing cytoplasmic extensions surrounding IRS-vacuoles and the base of rhabdomere 8. The scale is 1 μm.

Fig. 5. Part of an unstained section of the rhabdom edge in *N. farri*, tested immunocytochemically for the presence of actin. Some gold particles indicate interrhabdomeral (but not intercellular) actin. These conjunctions were specific for actin. The scale is 0.5 μm.

Where does that leave us? Small amounts of actin appear to be associated with the surroundings of the vesicles in the IRS of *N. farri* and some actin may be detectable by immunocytochemical means well after the membranous strands surrounding the vacuoles have become so thin that they are no longer easily observable under the electron microscope.

In conclusion it appears that much of what we see in the eye of *N. farri* is a consequence of an arrested morphogenesis of the rhabdom. The development of the rhabdom has seemingly not reached the stage in which a truly open rhabdom with rhabdomeres embedded in an extracellular matrix is developed. The reason for the abridged morphogenesis could be the short lifespan of 2–3 days of the adult insect and/or the lightless cave environment, in which *N. farri* thrives. That the open rhabdom in *N. farri* represents an evolutionarily more primitive type of rhabdom is yet another possibility, but since no fossil record or genetic comparison can back up this notion, it has to remain speculation for the time being.

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