An Eye for the Extreme: Photoreceptor
Fine-Structure in the Antarctic Midge
Belgica antarctica (Diptera:
Chironomidae)¹

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With regard to temperature and light intensities, the terrestrial environment of Antarctica is undoubtedly the most extreme in the world and only one species of insect (not counting apterygote entognaths) has managed to gain a foothold there in some coastal areas: the ant-like, approximately 2.5 mm long, wingless, black chironomid *Belgica antarctica*.

The species was described by JACOBS (1900) from the Antarctic peninsula, but the first detailed study of its external eye morphology did not appear until quite recently in this journal (MEYER-ROCHOW and REID, 1994). One of the referees had expressed that some information on the fine-structural organization of the eye would have been desirable. We now possess such information and wish to supplement the earlier scanning electron microscopic observations with results obtained from transmission electron microscopy.

MATERIALS AND METHODS

We managed to examine 2 male and 1 female specimen of *B. antarctica* from the Chilean Antarctic "Yelcho Station" (64°38'S; 64°02'W). The habitat was described in the previous paper by MEYER-ROCHOW and REID (1994). Although 4% unbuffered formaldehyde is hardly an ideal pre-fixative for material destined to be examined by transmission electron microscopy (MEYER-ROCHOW and REID, 1995), we had no alternative. We felt that in view of the elusive-

ness of the insect and the difficulty with which its localities in Antarctica can be reached, relatively poorly fixed material was better than none at all and proceeded with the inquiry.

Following 4 weeks of prefixation in 4% unbuffered formaldehyde, the specimens were thoroughly rinsed in 0.4 M Na-cacodylate buffer (pH 7.4) and fixed for 2 h in 1% Na-cacodylate-buffered OsO₄ solution at room temperature. Dehydration of the specimens was carried out in a graded series of acetone and followed by embedding in standard araldite (polymerized at 65°C for 3 days in an oven). Ultrathin sections were cut on a Reichert "Super-Nova" ultramicrotome with glass and diamond knives, picked up on carbon-coated 300 mesh copper grids, double-stained for a few minutes each with uranylacetate and lead citrate, and observed under a JEOL JEM 100CX-II transmission electron microscope, operated at an accelerating voltage of 80 kV.

RESULTS AND DISCUSSION

The eyes of the female individual were found to yield little useful ultrastructural details. We believe that, in addition to the poor conservation of the tissue, this was mainly due to the greater lipid stores in them when compared with those of the male specimens. The following results are, therefore, mostly derived from the eyes of the two male individuals. Despite the lack of standard EM-fixatives at the Chilean Antarctic Base, a surprising amount of anatomical information was, nonetheless, available.

The cornea measures approximately 10 µm in diameter and possesses a maximum thickness of 4.5–5.0 µm at its centre (Fig. 1a). It is strongly bulging outward (of the plano-convex lens type) and has an external radius of curvature of approximately 4.4 µm (Fig. 1a, c). Interommatidial cuticle thickness was found to be 0.8–1.0 µm. Up to 38 horizontal, in the bulging part of the cornea more widely spaced cuticle layers were counted. The layers were also present, but much narrower, in the interommatidial regions, from which numerous bristles had been described earlier (MEYER-ROCHOW and REID, 1994). However, with one obvious exception of a larger than usual hair, none of the interommatidial bristles seemed to be endowed with axons or dendrites and it appears as if the interommatidial bristles are nothing more than cuticular surface specializations with purely physical rather than sensory properties.

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Fig. 1. Transmission electron micrographs of ultrathin sections through the eye of *B. antarctica*. (a) Cone cells (CC) occupy the narrow space between the laminated cornea (CO) and the rhabdom (R). Principal pigment cells (PC) surround the cone cells and contain large pigment grains as well as lipid stores. The scale is 1 μm . (b) The rhabdom (R) is distally made up of 6 pigment-grain (PI) containing retinula cells (RET); these are surrounded by two principal pigment cells, which possess lipid stores (L). Only occasionally the distal tips of retinula cell nuclei (NU) can be spotted at this level. The scale is 1 μm . (c) This longitudinal section through one ommatidium shows the thick, convex cornea (CO) capping cone cells (CC) and rhabdom (R). Distally large screening pigment grains of the principal pigment cells (PC) surround the rhabdom, but at the level where it permits the central rhabdomeres to dominate (O) only retinula cell pigment grains (PI) are present. Retinula cell nuclei (NU) are mostly placed proximally, just above a substantial layer of mitochondria (M) and the basement membrane (BM). The scale is 1 μm . The inset shows regularly-arranged rhabdom microvilli in cross section at higher magnification. The scale is 300 nm.

Fig. 2. Semi-schematic representation of serial sections through one ommatidium of the eye of *B. antarctica*, giving cross-sectional profiles and longitudinal aspects (framed portions) of ommatidial organization at different distances from the cornea (top). The labels 1a, 1b, 1c on the right margin of the drawing refer to approximately corresponding levels of sectioning shown in the electron micrographs of Fig. 1. Numbering of retinula cells (i.e. 1–8) is after DIETRICH (1909). Abbreviations used (from top to bottom of figure) are: Co, cornea; cc, cone cells; PC, principal pigment cells; l, lipid stores; R, rhabdom; O, open rhabdom; b, multivesicular bodies; pi, pigment granules; nu, nucleus; v, vesicles; m, mitochondria; ax, axons.

Although a crystalline cone is not present below the cornea, 4 cone cells with meandering cell boundaries between them, occupy the $5.5\ \mu\text{m}$ wide space between inner corneal surface and distal end of rhabdom. The cone cells are surrounded by two well developed principal pigment cells, which contain black, spherical pigment grains measuring up to $0.7\ \mu\text{m}$ in

diameter and at least equally large lipid droplets (Fig. 1a, c).

The eye is of the apposition type, i.e. the rhabdom is in direct contact with the cone cells and the total length of one ommatidium, measured from inner corneal surface to basement membrane, is approximately $13\ \mu\text{m}$. At its distal end the rhabdom possesses a diameter of $4.8\ \mu\text{m}$, is made up of 6 rhabdomeres, and conforms to the “fused type” of organization (Fig. 1b). Its constituent microvilli (Fig. 1c, inset) are regularly aligned and measure approximately $55\ \text{nm}$ in diameter (based on counts of 40 microvilli). No light-induced membrane- or retinula cell distortions whatsoever (known from other insects eyes when exposed to ultraviolet radiation: EGUCHI and MEYER-ROCHOW, 1983) were apparent. Pigment granules, only half the size of those present in the principal pigment cells, are relatively abundant in the distal third of the retinula cells.

Halfway down the $4.5\ \mu\text{m}$ long rhabdom, the latter increasingly opens up centrally and turns into a sleeve- or ring-like structure, leaving the “central cavity” for retinula cells 7 and 8. The boundaries between rhabdomeres are not clear and the cytoplasm of retinula cells 7 and 8 contains large, loosely arranged vacuoles (Fig. 1b, c). Multivesicular and other intracellular bodies are observed in the more proximal regions of the peripheral retinula cells and up to $1.0\ \mu\text{m}$ long and $0.6\ \mu\text{m}$ thick mitochondria of the crista type become more and more abundant just above the basement membrane before the retinula cells turn into axons.

On the basis of external eye morphology it was already established that the eye of *B. antarctica* curiously combined features of photoreceptors designed to perform optimally under bright conditions with adaptations normally associated with a dim environment (MEYER-ROCHOW and REID, 1994). Our new fine-structural data (graphically summarized in Fig. 2) confirm that this trend is not restricted to the outer appearance of the eye, but involves internal structural design as well.

Being a dipteran insect, *B. antarctica* is provided with an apposition eye and a photoreceptor organization which is extremely useful for movement perception in bright light. But *B. antarctica* rarely ventures out into the open and the bright Antarctic summer light, but prefers instead to hide away under stones and boulders; yet, signs of eye-regression as in grylloblattids (NAGASHIMA, 1990) are absent. To improve its absolute sensitivity to light, a dipteran insect

has two major strategies: (a) enlargement of eye and facets, and (b) fusion of the peripheral rhabdomeres 1–6, at least at their distal ends, if not over their entire lengths. Clearly, *B. antarctica* has not chosen the first option (its facet-size is one of the smallest reported for any insect), but fusion of peripheral rhabdomeres occurs throughout the rhabdom. In fact, the central “invasion” of retinula cells 7 and 8 does not reach the distalmost end of the rhabdom, so that the cross sectional area available to intercept photons just below the dioptric elements is at least $18 \mu\text{m}^2$ and, thus, actually larger than that of rhabdomeres of *Drosophila* reared in the dark for many generations (IMAIZUMI, 1979).

The relatively large amounts of screening pigment granules can only be explained on the basis that the eye requires protection from excessive and/or damaging radiation and a high density of mitochondria indicates high metabolic activity. Given the environment which *B. antarctica* has to contend with (almost constantly cold, windy, and bright), the retinal adaptations appear to allow the insect to take residence in the darker regions between rocks and boulders, while at the same time offering a reasonable amount of protection against light-induced photoreceptor damage should the animal find itself outside the shelter. Mosquito (SATO, 1957) and chironomid eyes (TUURALA, 1963) show somewhat similar retinal adaptations without, however, the strong reduction in facet-size and -number characteristic of the *B. antarctica* eye.

What intrigued us most, was that despite the small corneal lenses and overall eye reduction, rhabdom microvilli possessed diameters that were not in the least different from those of other insects (diptera and non-diptera alike). This finding suggests to us that certain, optical and structural constraints operate which make it impossible for the light-perceiving elements to become infinitesimally reduced. We are

forced to conclude that there is a lower limit of functional miniaturization not only for the insect eye as a whole (BARLOW, 1952), but for the rhabdom microvilli as well. What precisely determines this limit is still unknown, but we believe that “constructional” factors (i.e. membrane properties and -chemistry) must be involved to safeguard that the phototransduction mechanism (a) can continue to function in “small diameter microvilli” and (b) will provide some gain in vitality to the insect.

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