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Unique Two-Photoreceptor Scanning Eye of the Nematode *Mermis nigrescens*

Abir Khalil Mohamed, Carolyn Burr, A.H. Jay Burr

Department of Biological Sciences, Simon Fraser University, Burnaby, BC Canada

Abstract. A single eye is present in females of the nematode *Mermis nigrescens.* A pigment cup occupies the entire cross section near the anterior tip of the worm, and the curved cuticle at the tip becomes a cornea. The shading pigment is hemoglobin instead of melanin. The eye has been shown to provide a positive phototaxis utilizing a scanning mechanism; however, the eye's structure has not been sufficiently described. Here, we provide a reconstruction of the eye on the basis of light and electron microscopy of serial sections. Hemoglobin crystals are densely packed in the cytoplasm of expanded hypodermal cells, forming the cylindrical shadowing structure. The two putative photoreceptors are found laterally within the transparent conical center of this structure where they would be exposed to light from different anterior fields of view. Each consists of a multilamellar sensory process formed by one of the dendrites in each of the two amphidial sensory nerve bundles that pass through the center. Multilamellar processes are also found in the same location in immature adult females and fourth stage juvenile females, which lack the shadowing pigment and exhibit a weak negative phototaxis. The unique structure of the pigment cup eye is discussed in terms of optical function, phototaxis mechanism, eye nomenclature, and evolution.

Introduction

In animals with nervous systems, photoreceptors occur at or near the distal end of a nerve dendrite and consist of lamellae or microvilli that expand the surface area of the light-sensitive process. In retina or in compound eyes, many photoreceptors may be arrayed for higher level vision. Simpler eyes of small invertebrates may contain only a few photoreceptors; nevertheless, in combination with accessory structures that block light from certain directions (shadowing, shading, or screening pigment) or focus light (lens or cornea), they can provide useful information about the distribution of light in the environment (Burr, 1984a, b).

In the nematode species in which eyes have been examined by transmission electron microscopy, one photoreceptor is located within each lateral or dorsolateral eye (Burr, 1984a) and is associated with a granular melanin-like shadowing pigment (Bollerup and Burr, 1979). The function of the pigment in these eyes is to cast a shadow on the adjacent photoreceptor for certain orientations of the head, and thus to provide directional sensitivity for the worm's negative phototaxis (Burr, 1979, 1984b). The photoreceptors occur as either a stack of lamellae without ciliary vestiges (Siddiqui and Viglierchio, 1970a, b; Croll *et al.,* 1972, 1975; Van de Velde and Coomans, 1988), or in one case, as a cluster of modified cilia without lamellae (Burr and Burr, 1975).

Mature females of *Mermis nigrescens* are exceptionally large for a nematode (100 mm long) and unusual in having a single eye. The shadowing structure, which provides directional sensitivity for the worm's positive phototaxis, consists of a single hollow cylinder that occupies the entire cross section of the body near the anterior tip of the worm, and the shadowing pigment is oxyhemoglobin (Ellenby and Smith, 1966; Burr and Harosi, 1985; Burr *et al.,* 2000a). The fourth stage juveniles (J4s) and immature females of *M. nigrescens,* which lack shadowing pigment, have a weak negative phototaxis. Whereas the positive phototaxis guides the mature female toward the sky to egg-laying sites in upper vegetation, the negative phototaxis could lead the J4s toward the soil, which they enter soon after leaving their grasshopper host. In 1–2 weeks the J4s molt to adults, and

the females, after mating, remain dormant in the soil for at least a year before emerging to lay eggs (Gans and Burr, 1994; Burr *et al.,* 2000b).

The positive phototaxis of the mature female responds to wavelengths from 350 to 560 nm, and the fluence rate at 420 nm that elicits a half-maximal response is equivalent to predawn twilight (Burr *et al.,* 1989). During phototaxis over a horizontal surface, the anterior 20% of the 100-mm-long body is held above the substrate and becomes oriented towards a light source, while the posterior 80% propels the worm in that direction along its ventral surface. The anterior 2 mm, or "head," is bent both sideways and vertically in a continuous scanning motion, and thereby the eye samples the distribution of light in the anterior field of view. Several cycles of scanning occur before the "neck" begins to reorient to a new source. Orientation of the base of the head toward the light source, due to bending in the neck region, occurs whether the source is horizontal or elevated (Burr *et al.,* 1990). Burr and Babinszki (1990) found that experimentally shifting the phase relationship of photoreceptor illumination and head-bending biased orientation to one side of the source direction. The results support a mechanism based on a pooled photoreceptor signal and rule out a comparison of two photoreceptor signals. In addition, the results strongly indicate the location of the photoreceptor or photoreceptors to be inside the pigment cup. However, a photoreceptor has yet to be identified or described.

We here provide a reconstruction of the eye of mature adult females that is based on serial sections for light microscopy and transmission electron microscopy. We describe the two probable photoreceptors of mature females as well as similar structures in J4s and immature females. The optical geometry raises interesting questions relevant to the unique scanning mechanism of phototaxis. Since this is the first eye known to have only two photoreceptors, the structure and scanning mechanism also bear on the definition of "eye."

Materials and Methods

Specimens of the mature adult female of *Mermis nigrescens* Cobb 1926 were collected as they were ovipositing on vegetation near Vancouver, Canada. Fourth stage juveniles (J4s) were raised by feeding *M. nigrescens* eggs to *Schistocerca gregaria,* the desert locust, and were fixed on the day of emergence from this host. Immature females were obtained by allowing J4s to molt to the adult stage (1 to 2 weeks after emergence) and develop in moist autoclaved soil. They were fixed about 5 months after molting, at which time their eyes have a faint color.

Worms were washed in a detergent (Tween 20) and anesthetized in 0.1 mol l^{-1} sodium azide. Anterior pieces were cut posterior to the ganglionic region in a buffered glutaraldehyde fixative (described below). To facilitate penetration of reagents, the cuticle was incised in the ganglionic region after about 15 min in glutaraldehyde and allowed to fix in the same solution for 1.5 h. Observation under the dissection microscope ensured that morphology was not changed by the cutting or fixative solution. After a wash in buffer, all specimens were postfixed in 2% OsO_4 for 2 h at 22 °C. Specimens were otherwise processed by two somewhat different procedures: (A) The glutaraldehyde fixative was 3% glutaraldehyde in 0.05 mol l^{-1} phosphate buffer. After osmium fixation, specimens were dehydrated in an acetone series to 100% acetone and then in a propylene oxide series to Spurr's resin and were polymerized overnight at 60 °C. (B) The glutaraldehyde fixative was 2.5% glutaraldehyde in 0.05 mol l^{-1} Na cacodylate, 1 mmol l^{-1} $CaCl_2$ buffer; microwave radiation was used to speed penetration (200 W for 4 min in a Pelco model 3450 polymerizing processor). After osmium fixation, specimens were dehydrated in an ethanol series to 100% and then in an acetone series to 1:1 Epon 812:Spurr's resin with microwave radiation (200 W for 6 min each step). Polymerization was overnight at 60 °C or under microwave radiation (500 W) at 90 °C for 1 h. Sections were examined of 4 mature females prepared by procedure A (Figs. 5 and 7–9) and of 2 mature females, 1 immature female, and 2 J4s prepared by procedure B (Figs. 3–4, 6, and 10).

A diamond knife and ultramicrotome (Leica Ultracut T) were used to cut serial semithin sections (0.5 μm) or ultrathin sections (60 nm) from the anterior tip to the end of the pigmented region. Approximate distances from the tip were calculated by multiplying the numbers of ultrathin and semithin sections by their respective thickness. These dimensions were confirmed by comparison with dimensions in longitudinal semithin sections and whole mounts. Ultrathin sections were mounted on Formvar- and carbon-coated grids, stained in 2% aqueous uranyl acetate and lead citrate, and examined with a Hitachi H-7000 electron microscope at an acceleration voltage of 80 kV and an aperture of 50 μm. Semithin sections were stained with 1% toluidine blue for 5 s on glass slides and photographed by phase contrast or differential interference contrast microscopy. Contrast in these photographs was enhanced using Adobe Photoshop ver. 7.0.

Results

Females collected as they are laying eggs in grass have a bright red pigmented region near the anterior tip (Fig. 1). This is seen to be cylindrical in transverse sections (Fig 2, upper left). The diagrams of Figure 2, constructed from serial sections, illustrate the anterior morphology of the mature adult female. The non-muscular esophagus, vestigial as only a cuticular tube in fourth stage larvae (J4s) and adults, passes through the pigmented region and the ganglionic region (associated with the nerve ring) and into the

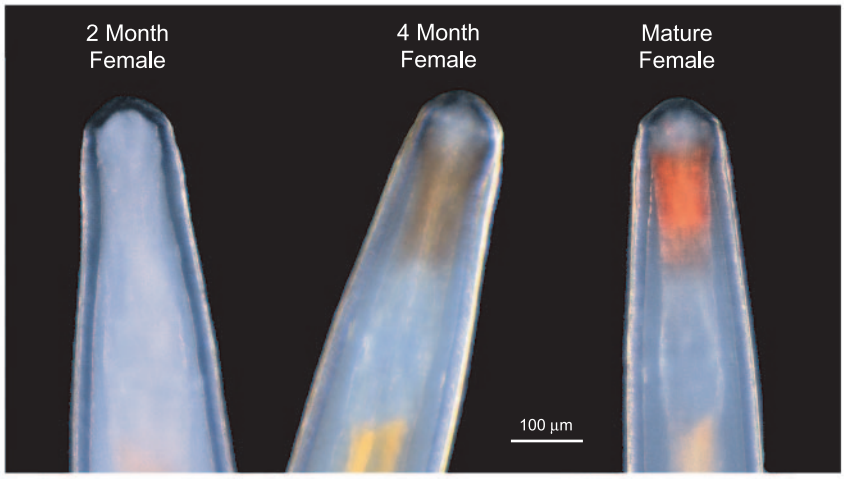

Figure 1. Pigmentation in *Mermis nigrescens* females at different ages after the adult molt (12 months for the mature female). Dark-field views of whole mounts illuminated from the sides. Bluish white is due to light scattering by the tissue; red is oxyhemoglobin; yellow is food stored in the trophosome. Adapted from Burr *et al.* (2000b).

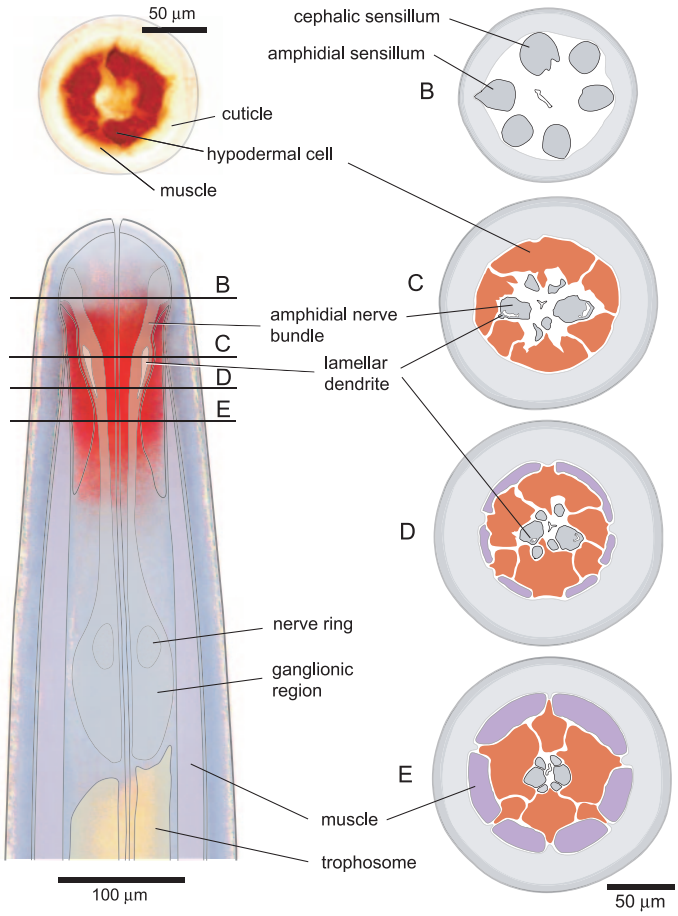

Figure 2. Reconstruction of eye and anterior of the mature adult female *Mermis nigrescens* based on serial sections observed in the light and transmission electron microscopes. Lower left: Dorsal view. Outlines of structures are superimposed on the image of mature female in Fig. 1. Upper left: Unstained thick section through conical shadowing structure at Level C. Modified from Burr *et al.* (2000a). Column at right: Transverse views at Levels B, C, D, E.

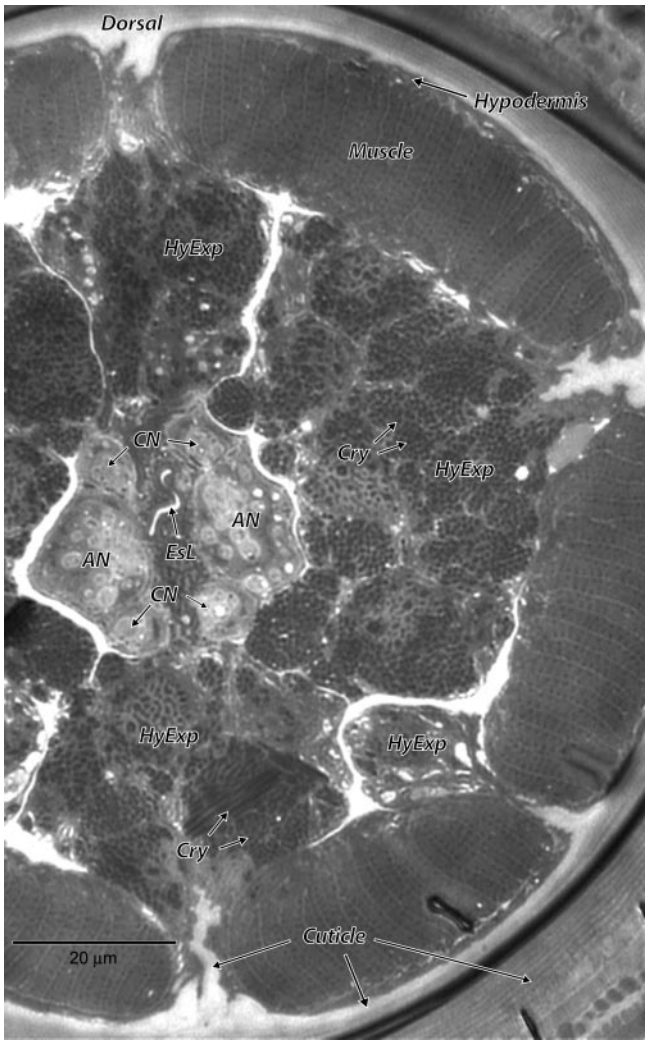

Figure 3. Transverse 0.5-μm section at Level E viewed by differential interference contrast microscopy. The cuticle, hypodermis, and a muscle band are labeled, showing their relationships. Cells of the hypodermis form six hypodermal cords, which at this level are expanded (*HyExp*) and filled with densely colored hemoglobin crystals (*Cry*) to form the pigment cup. The lateral cord expansions are largest and the subventral ones are smallest. *AN,* amphidial nerve bundle; *CN,* cephalic nerve bundle; *EsL,* esophageal lumen.

trophosome, a food-storage body (Figs. 2–4). Six nerve bundles pass through the region surrounded by the pigmented structure (Figs. 2–4). These nerve bundles carry dendrites from cell bodies in the ganglionic region to their sensory processes in the anterior sensilla. The four cephalic and two amphidial sensilla of *Mermis nigrescens* are attached to the anterior cuticle about 46 μm and 64 μm, respectively, from the tip (Fig. 2). The morphology of the cephalic sensillum of *M. nigrescens* has been described previously in detail (Lee, 1974). Labial sensilla are located within the cuticle at the anterior tip (not illustrated). The amphidial sensillum, or amphid, is an important sensory organ found in all nematodes. It includes chemosensory cilia that connect to the exterior *via* a channel through the cuticle, mediating taste and olfaction. Depending on species, amphids may include other chemosensory processes or a thermosensory process (Jones, 2002).

Shadowing structure and pigment

The shadowing structure is a tube, 150–300 μm long and 80–100 μm wide, of dense pigment that begins 100 μm behind the anterior tip of the nematode (Fig. 2). Serial transverse sections indicate that the nonpigmented space inside the cylinder is conical, 60 μm in diameter anteriorly and narrowing to 30 μm posteriorly (Fig. 2). Two putative photoreceptors (lamellar dendrites) are located within the nonpigmented space (Fig. 2; levels C and D). Thus the single eye of *M. nigrescens* has the overall form of a pigment cup, but it is unusual in containing two photoreceptors and occupying the entire cross section of the body inside the cuticle.

The shadowing structure is formed by the expansion of six anterior hypodermal cord cells into the pseudocoelom. These contact each other to form the cylindrical pigment structure that encircles the sensory nerve bundles (Figs. 2 and 3). In the pigmented region, the hypodermal expansions contain densely packed inclusions that are red under the light microscope and dark in grayscale differential interference micrographs (Fig. 3). These correspond to the 0.3–1.0-μm diameter electron-dense inclusions observed in transmission electron micrographs (Fig. 4). At higher magnifications, in favorable transverse sections, the inclusions are seen to have an internal hexagonal pattern of electron density (Fig. 5A). Pointed streaks are observed in longitudinal sections where crystals pass obliquely through the section (Fig. 5B); occasionally they are also observed in transverse sections (Fig. 3, bottom). This general pattern would be expected for needlelike crystals that are constrained in the narrow cells to be oriented mostly longitudinally.

The cytoplasm is densely packed with hemoglobin crystals in mature females (Figs. 3–5). The crystals appear to have a profound mechanical effect on the cell. They often penetrate into a nucleus (Fig. 5B), and the nuclei appear to be displaced from their location in nonpigmented hypodermal cells of J4s and immature females. Rough endoplasmic reticulum is squeezed into small spaces between crystals (Fig. 5A).

Nerve bundles and multilamellar dendrite

The cephalic nerve bundle has a smaller diameter and carries fewer dendrites (probably 6) than the amphidial nerve bundle (probably 19) (Fig. 6). Branching may explain the variable number of cross sections seen. The dendrites normally pass through the region to sensory processes in the four cephalic sensilla and two amphids.

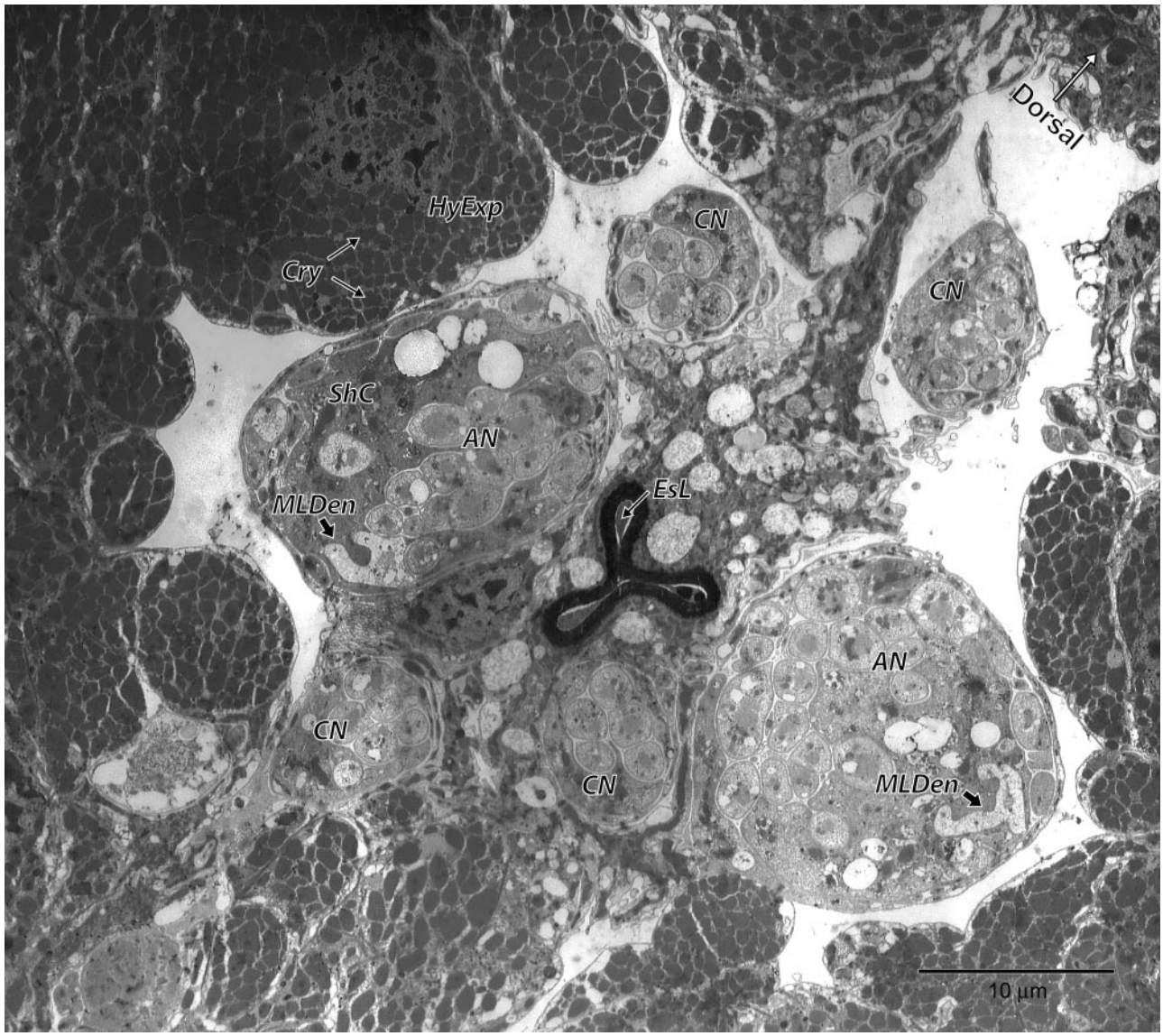

Figure 4. Montage of transmission electron micrographs of transverse section at Level D. Hypodermal expan-sions (*HyExp*) are densely packed with electron-dense hemoglobin crystals (*Cry*). Lateral amphidial nerve bundles (*AN*) contain nerve dendrites and a sheath cell (*ShC*) with a darkly stained cytoplasm. The multilamellar dendrites (*MLDen*) have a few lamellar extensions at this level. *CN,* cephalic nerve bundle; *EsL,* esophageal lumen enclosed by cuticle.

In the *M. nigrescens* female, one of the dendrites in each amphidial nerve bundle forms a multilamellar structure within the pigmented cup, beginning about 100 μm posterior to the amphid (Figs. 2, 6–8). Serial sections reveal a complex, more-or-less-longitudinal folding that reduces to an irregular profile posteriorly (Fig. 8B). Where membrane proliferation is most extensive, the lamellar folds extend more than 10 μm (Fig. 7A). The lamellae project into a sheath cell and are invaginated by the sheath-cell membrane (Figs. 7–9). The cytoplasm enclosed by the lamellae includes transversely sectioned microtubules and neurofilaments; this cytoskeleton appears identical to that of other dendrites (Figs. 8–9). However, the plasma membranes of the lamellae and sheath cell are closely apposed, while other amphidial dendrites passing through these sections are coated by a thick basal lamina, whether or not they are enveloped by the sheath cell (Figs. 8–9). The more posterior sections have one or two processes, in addition to the multilamellar process, that have membranes closely apposed to the sheath-cell membrane. These additional processes could be posterior branches of the multilamellar dendritic process (arrows, Fig. 8A, B), as has been observed for the lamellar sensory dendritic process of another nematode (Ashton *et al.,* 1995). Farther posterior, the dendrite that forms the multilamellar process is indistinguishable from other amphidial dendrites. Anteriorly, it was difficult

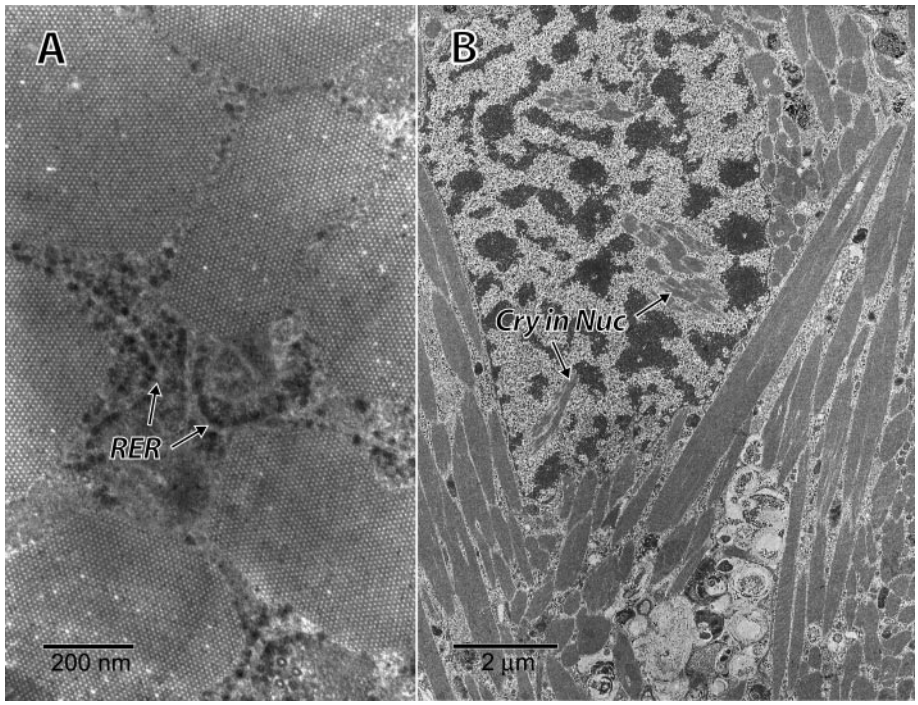

Figure 5. Crystals of oxyhemoglobin in sections perpendicular (A) and parallel (B) to the body axis. The hexagonal arrangement of globin molecules is apparent in (A). The mostly longitudinally oriented needlelike crystals sometimes penetrate the nucleus (*Cry in Nuc*). RER, rough endoplasmic reticulum.

to follow the dendrite past the multilamellar structure, and it is uncertain whether the dendrite ends anteriorly with the multilamellar structure or continues into the amphid.

Fourth stage juveniles and immature females

Immature females (5 months after the molt to adult stage) and J4 females (1–2 weeks before the molt) were also investigated. The immature stages had a faint red color in the eye region and an electron-dense hypodermal cytoplasm without large crystals. The J4s had no visible color, and their hypodermal cells contained granular material (Fig. 10A, B). The latter may be material accumulated for synthesis of the cuticle during the molt that occurs shortly after the J4 emerges from the host. The hypodermal cell nuclei of J4s are larger and more centrally located in the cell. In both J4s (Fig. 10) and immature females, a multilamellar dendrite is located in regions of the amphidial nerve bundle that correspond both laterally and longitudinally to the position of the multilamellar dendrite in mature females. The lamellae are less extensive in the J4s.

Discussion

When an adult female of *Mermis nigrescens* crawls on a horizontal surface, it glides on its ventral surface (unlike other nematodes), its anterior is elevated above the surface, and there is a continual side-to-side (lateral) and vertical (dorsoventral) bending of the "head" (Burr *et al.,* 1990). Similar motion occurs in three-dimensional media such as grass. Whether the light source is horizontally located or elevated, the region behind this scanning motion ("base of the head") becomes oriented preferentially toward the light by bending of the neck (Burr *et al.,* 1990), and locomotive forces applied to grass or fibers by the remainder of the body propel the body in that direction (Gans and Burr, 1994; Burr and Robinson, 2004). When the base of the head is directed toward the light source, the continuous scanning motion of the head would cause the interior of the pigment cup to be alternately shadowed by the pigment and illuminated by light entering through the transparent anterior. By experimentally interrupting the light so that illumination of the cup interior was out of phase with the scanning motion, median orientation of the base of the head and direction of crawling were caused to be biased to one side of the light direction in a way that could occur only if the photoreceptors were located inside the pigment cup (Burr and Babinszki, 1990). We have here described the pigment structure and a pair of sensory processes within the transparent center that are likely to be the predicted photoreceptors.

Putative photoreceptor of the mature female

The multilamellar process in many respects has the appearance of a sensory process. It is a specialized membra-

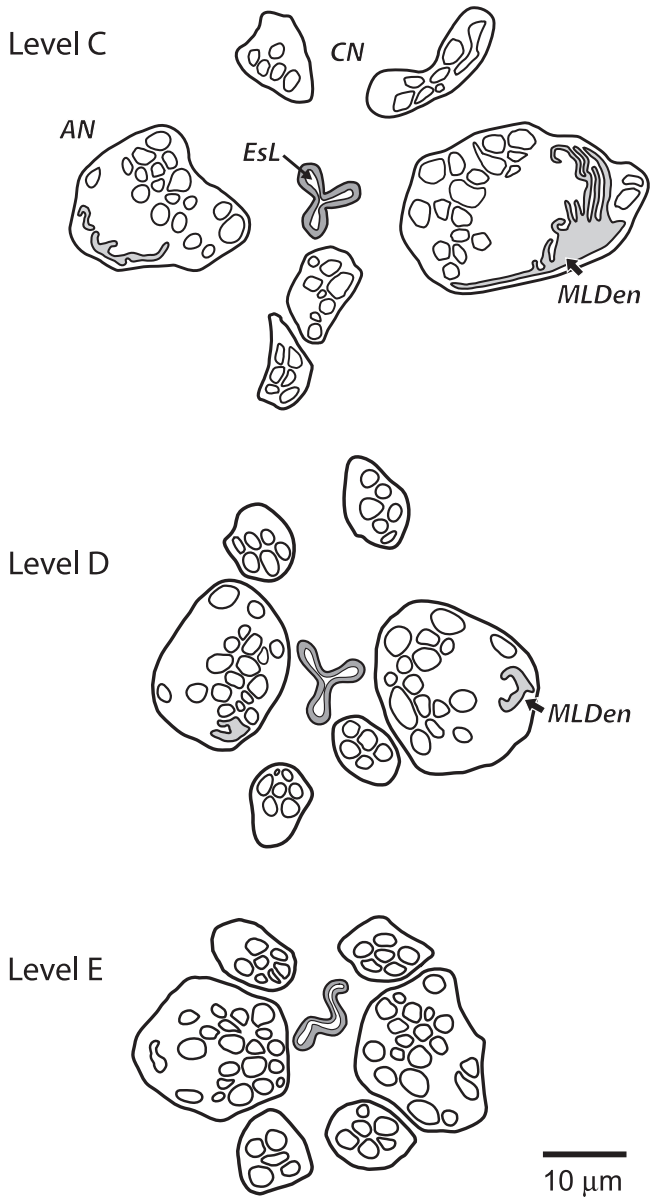

Figure 6. Arrangement of nerve bundles, dendrites, and lamellar extensions of the multilamellar dendrites (*MLDen*). Tracings from transmission electron micrographs of sections at Levels C, D, and E. *AN,* amphidial nerve bundle; *CN,* cephalic nerve bundle; *EsL,* esophageal lumen.

nous structure formed by a nerve dendrite, like the sensory processes in many anterior sensilla. However, it is not located in a sensillum, but in the amphidial nerve bundle with other sensory dendrites that continue anteriorly to their sensory processes in the amphid. Like most sensory dendritic processes of nematodes and other animals, it has an intimate association with a sheath cell. Cells equivalent to the sheath cell in eyes of other animals are thought to have a supportive function (Insausti and Lazzari, 2002).

The multilamellar process, like the photoreceptors of eyes of other nematodes and most animals, consists of an expanded plasma membrane at the distal end of the dendrite. A multilamellar or microvillar structure is not, however, a sufficient indication of a photoreceptive function. Moreover, in nematodes it is sometimes difficult to interpret the function of a sensory process from its morphology. Although the modified cilia that project from amphidial dendrites into a channel that connects to the exterior are predictably chemosensors (Bargmann and Horvitz, 1991; Bargmann *et al.,* 1993), and while other modified cilia have auxiliary structures that indicate a mechanical function (Jones, 2002), there are several sensory processes whose structure is misleading. Examples include the amphidial processes of the three "wing cell" dendrites of *Caenorhabditis elegans* and related nematodes (Ward *et al.,* 1975; Li *et al.,* 2000a). Although enclosed by the amphid sheath cell such that there is no aqueous connection to the exterior, they are chemosensory to lipid-soluble, volatile odorants (Bargmann and Horvitz, 1991; Bargmann *et al.,* 1993; Troemel *et al.,* 1997). Another example, found in *C. elegans* and relatives but not all nematodes, is a sensory process composed of numerous microvilli that arise from the amphidial "finger cell" dendrite and are invaginated by the amphid sheath cell (Baldwin and Hirschmann, 1973, 1975; Ward *et al.,* 1975; Wergin and Endo, 1976; Endo, 1980, 1998; Jones, 2002). This structure is thermosensory and is involved in thermotaxis on temperature gradients (Mori and Ohshima, 1995; Mori, 1999; Li *et al.,* 2000b; Bhopale *et al.,* 2001; Kimura *et al.,* 2004).

In nematodes and other simple invertebrates, because better evidence is usually lacking, the identifying morphological characteristic of photoreceptors, in addition to a ciliary, lamellar, or microvillar fine structure, has been their location adjacent to a pigment spot or within a pigment cup where they can provide directional sensitivity to light (Burr, 1984a, b). Location within a pigment cup is also the best evidence that the multilamellar processes described here in *M. nigrescens* may be photoreceptors. We could find no other obvious sensory process within the shadowing structure.

Roles as mechanoreceptor or chemoreceptor seem less likely. Both structure and location make the multilamellar processes unsuitable as mechanoreceptors: in nematodes these have specialized auxiliary structures and are close to and usually attached to the cuticle (Jones, 2002). Because the multilamellar processes are located far from the amphid and deep within the head (Fig. 2), they would also be poor candidates for olfactory or taste sensors. Even though the chemoreceptors for volatile odorants in *C. elegans* amphids are not connected to the exterior *via* an aqueous channel, they are nevertheless located less than 3 μm from the cuticle at the anterior tip of the head, where they would be readily accessible to the lipid-soluble odorants.

The multilamellar process of *M. nigrescens* appears very similar to a thermosensory lamellar process found in the

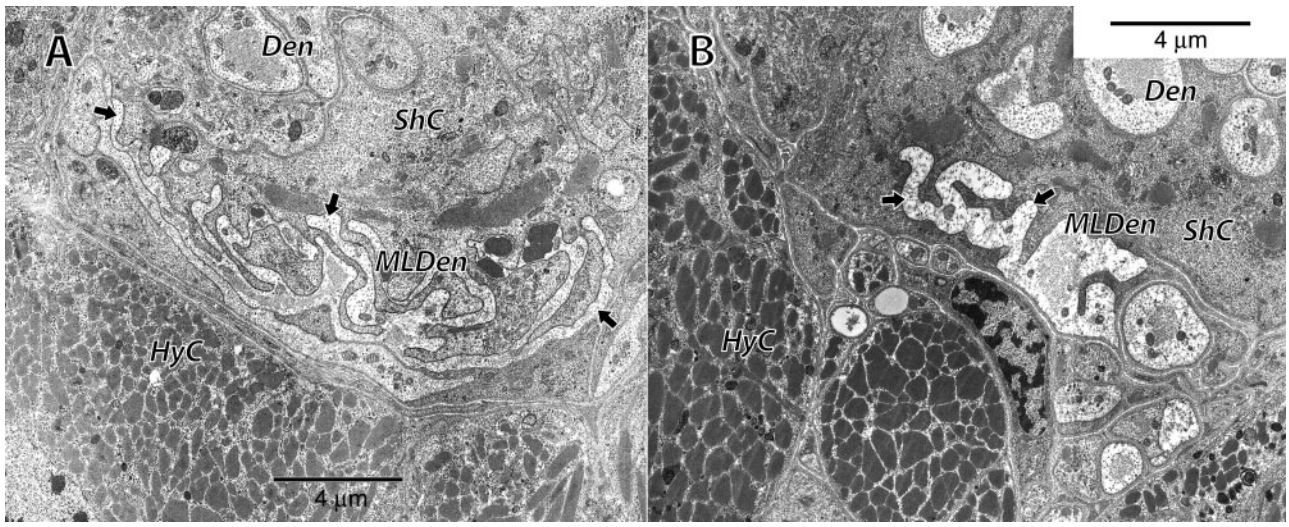

Figure 7. Multilamellar dendrite (*MLDen*) and other dendrites (*Den*): (A) at Level C, and (B) at Level D. *HyC,* hypodermal cell; *ShC,* sheath cell; *thick arrows,* lamellar extensions and possible branches of the multilamellar dendrite.

amphidial sensillae of *Strongyloides stercoralis* (Ashton *et al.,* 1995; Ashton and Schad, 1999; Lopez *et al.,* 2000). A complex of longitudinal lamellar sheets arises from the ALD dendrite and is invaginated by the amphidial sheath cell. In transsection it resembles the multilamellar array in Figure 7A except for being more compressed, in keeping

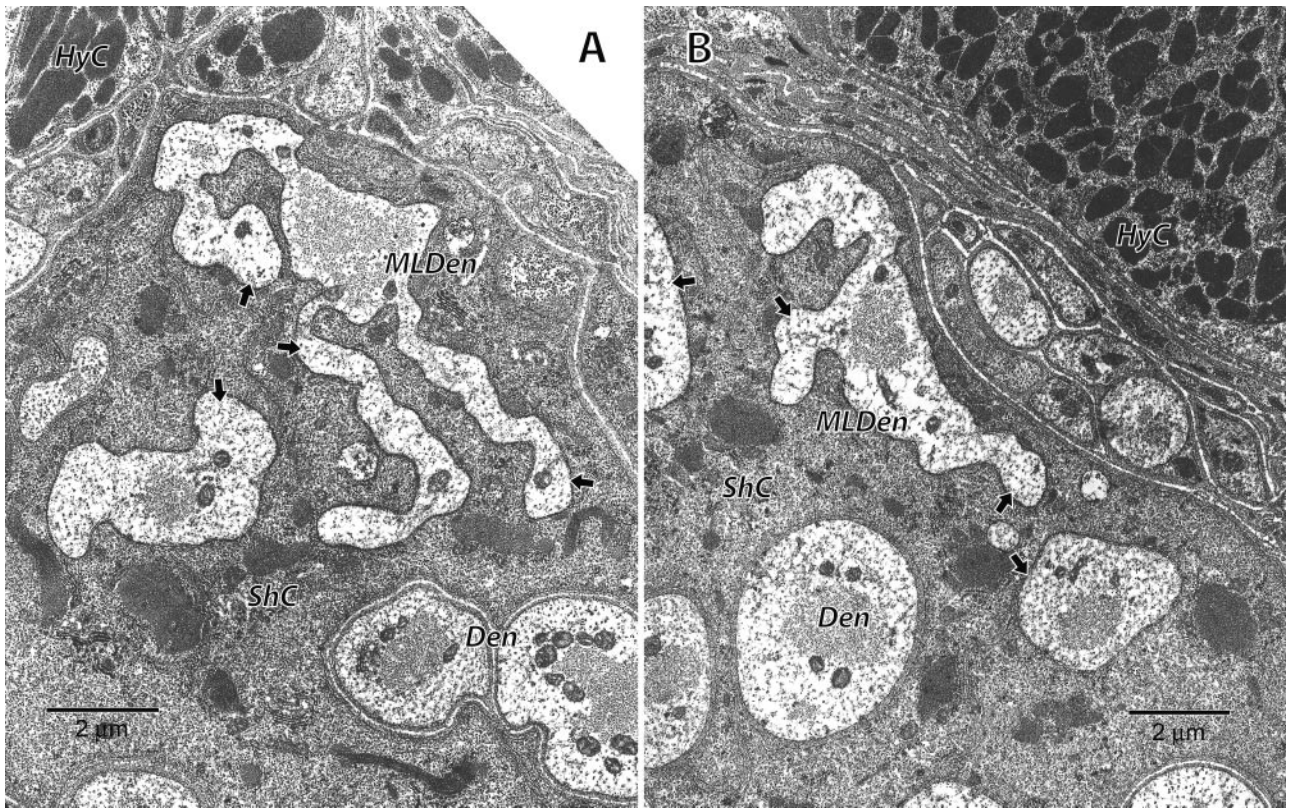

Figure 8. Multilamellar dendrite (*MLDen*) and other dendrites (*Den*): (A), at Level D, and (B) at Level E. *HyC,* hypodermal cell; *ShC,* sheath cell. *Thick arrows,* lamellar extensions and possible branches of the multilamellar dendrite.

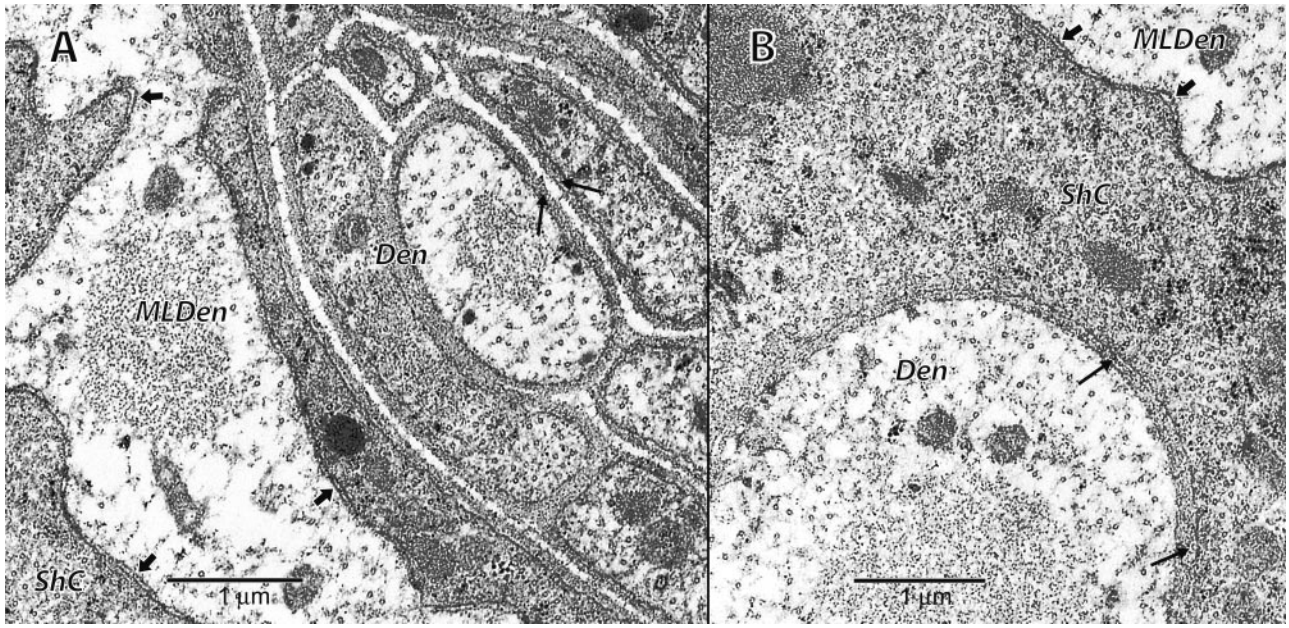

Figure 9. Comparisons of multilamellar dendrite (*MLDen*) with other dendrites (*Den*). The membranes of the sheath cell (*ShC*) and multilamellar dendrite are closely apposed (*thick arrow*), whereas other dendrites are surrounded by a thick basal lamina (*thin arrow*). The other dendrites may pass through the sheath cell (B), or may not (A).

with the smaller space within the *S. stercoralis* amphid. Unlike the similar structure in *M. nigrescens,* the thermoreceptor of *S. stercoralis* is located in the amphid at the anterior tip of the animal and only 2 μm from the cuticle (Ashton *et al.,* 1995; Ashton and Schad, 1996)—a more suitable location for sensing changes in local temperature as the worm crawls over temperature gradients.

It is difficult to understand why a thermoreceptor would be moved 100 μm posteriorly in *M. nigrescens* unless it functions together with the hemoglobin shadowing structure as a directional detector of infrared (IR) radiation, something like the organ found in pit vipers (Hartline, 1974; Desalvo and Hartline, 1978). For this to work, the hemoglobin must effectively block near-IR from directions other than the anterior. Oxyhemoglobin has a weak near-IR absorption band at 800–1100 nm. However, from known extinction coefficients and the visible-band absorbance of oxyhemoglobin in the eye region (Burr and Harosi, 1985), it can be calculated that less than 1% of this near-IR would be blocked by hemoglobin in the walls of the shadowing structure. Thus, the multilamellar process with the hemoglobin shadowing structure is unable to fulfill this role. By comparison, 35% to 99% of light at the wavelengths effective in phototaxis, 350–560 nm, is absorbed by the walls of the hemoglobin shadowing structure (Burr and Harosi, 1985; Burr *et al.,* 1989). Moreover, a beam of 630–980 nm near-IR radiation did not elicit a taxis (Burr *et al.,* 1989).

All things considered, we conclude that the most likely function for the multilamellar dendritic process in *M. nigrescens* is photoreception. The structure is a specialized membrane process originating from a dendrite in a bundle of other sensory dendrites, and like most sensory processes of nematodes and other invertebrates, it is enclosed by a supporting cell. It consists of extensive folded membranes like those in most photoreceptors. Although this morphology does not eliminate the possibility of other sensory functions, others appear to be less likely for a number of reasons discussed above. In particular, the process is displaced about 100 μm posteriorly from the amphid sensillum to a location within the shadowing structure, where it can be effective in phototaxis and where the results of the shuttering experiment predict that it must be located (Burr and Babinszki, 1990).

In retina or compound eyes, the photoreceptor lamellae or microvilli are arranged in tightly regular arrays in narrow columns parallel to the direction of incoming light. This arrangement maximizes membrane surface area, spatial resolution, and the number of photoreceptors that can be crowded into the eye (Land, 1981; Land and Nilsson, 2002). In the eye of *M. nigrescens,* on the other hand, the lamellae can be loosely arrayed because there is plenty of room in the pigment cup with only the two receptors. All other eye-bearing nematodes are 50 times smaller than *M. nigrescens,* have two eyes with only one photoreceptor apiece, and have lamellae that are tightly arrayed to maximize membrane surface area within the tiny shadowing structure.

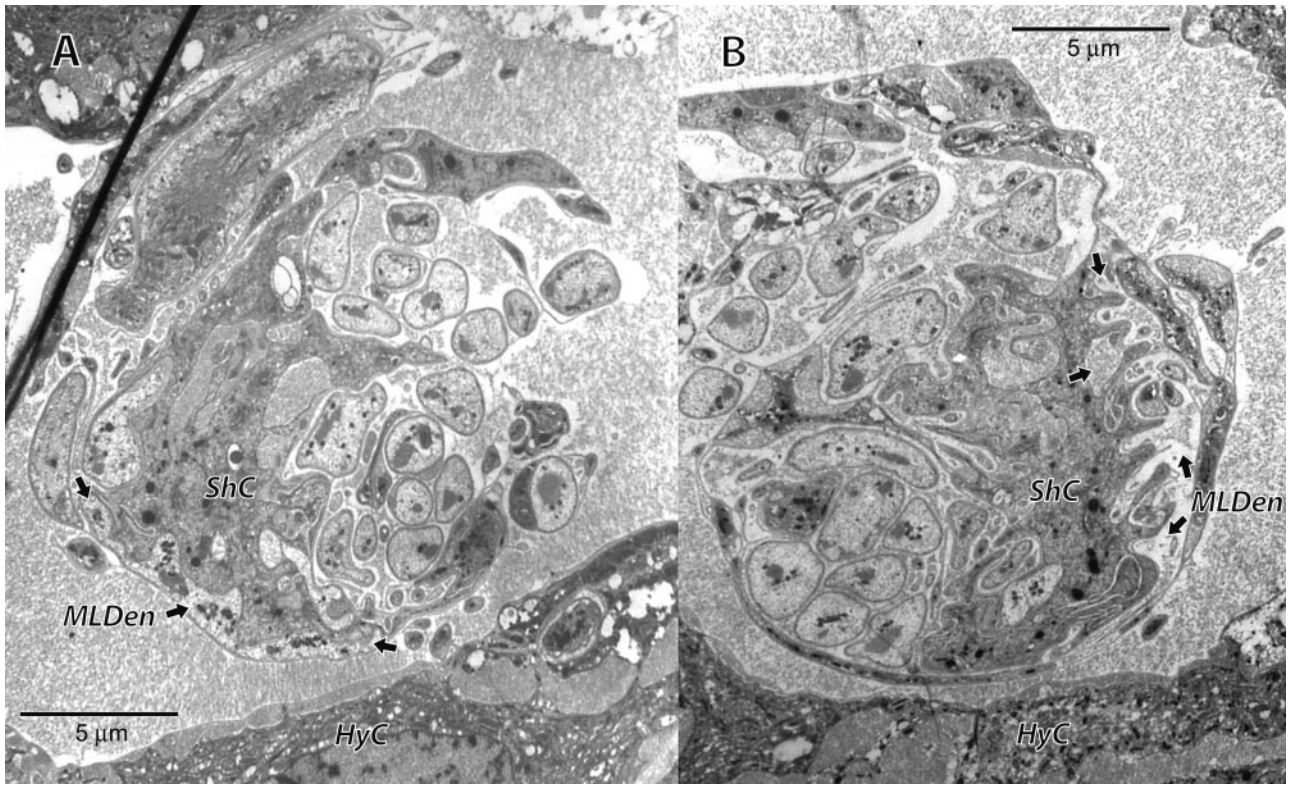

Figure 10. Multilamellar dendritic processes (*MLDen*) in amphidial nerve bundle of a fourth stage juvenile (J4) at Level C: (A) in left amphidial nerve bundle 110 μm from tip, and (B) in right amphidial nerve bundle 117 μm from tip. *Thick arrows* point to lamellar projections. Note the absence of hemoglobin crystals in hypodermal cells (*HyC,*) of fourth stage juveniles, which lack eye pigmentation (compare with adult eye, Fig. 4). *ShC,* sheath cell.

Optical properties of the eye

Because of the large change in refractive index at an air-cuticle interface, light will be refracted as it enters an individual of *M. nigrescens.* When the female points her head toward a light source, refraction by the curved anterior cuticle focuses the light into the pigment cup (Fig. 11). Thus the anterior tip is equivalent to the cornea in eyes of other terrestrial animals, where most of the focusing occurs and any lens plays a minor role. This phenomenon cannot occur for other known eye-bearing nematodes, because they all live in aquatic or marine environments where the refractive index of cuticle and tissues is close to that of water.

Interestingly, the putative photoreceptors are located near the lateral walls of the shadowing structure, where they would have different but overlapping fields of view in the lateral (horizontal) dimension (Fig. 11). In the dorsoventral (vertical) dimension, however, the laterally located photoreceptors would have the same field of view, with extent approximately that of the axial photoreceptor illustrated in Figure 11. The acceptance angle, the angle subtended by the photoreceptor in space, is about 50° in either the lateral or vertical dimensions.

The source used in previous studies of phototaxis in *M. nigrescens* was masked to subtend 1° of the field of view, and thus is essentially a point source (Burr *et al.,* 1989, 1990; Burr and Babinszki, 1990). Figure 11 predicts that whenever the eye is oriented 20° to the left of such a source, the illumination would pass through the left-hand receptor and miss the right-hand one. The focal point, located $r + f$ from the cornea surface, is deeper than the photoreceptor, which would intercept an out-of-focus representation of the point source. However, this would not affect the resolution of a point source by these widely separated receptors (see below). An advantage of a defocused representation is that a small source is more likely to be intercepted during scanning than if sharply focused.

A measure of the resolution would be the reciprocal of the smallest angular separation between bright stripes in an alternating bright/dark pattern that can be resolved (Land and Nilsson, 2002). With the eye pointed at a boundary between stripes in a vertical grating, one photoreceptor will be centered on a dark stripe while the other is centered on the neighboring bright stripe. The inter-receptor angle can be calculated from photoreceptor separation s and the prin-

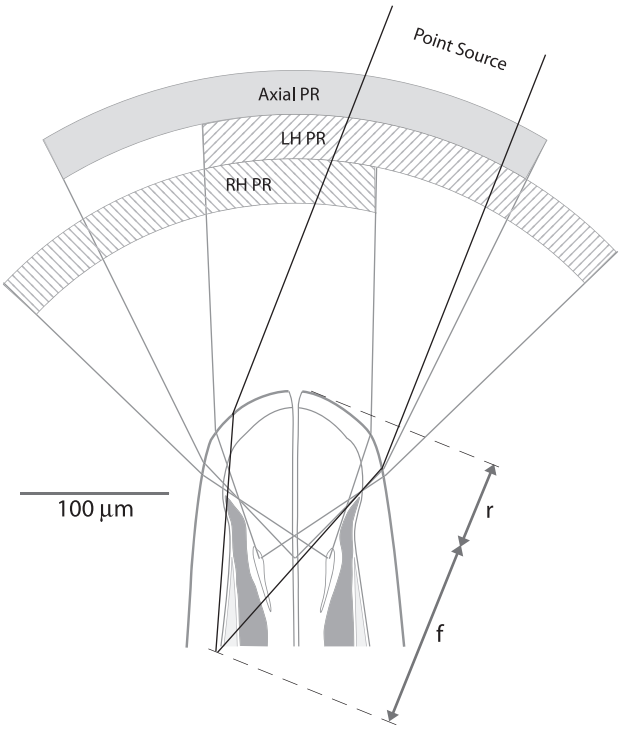

Figure 11. Optical geometry of the eye of *Mermis nigrescens.* Fields of view of the putative photoreceptors were estimated by projecting rays past the edges of the shadowing structure and through the cuticle/air boundary with radius of curvature *r.* Snell's Law was used with refractive indices of 1.4 and 1.0 in the two media. Acceptance angle is defined by the rays leaving the cornea. *RH PR* and *LH PR,* fields of view of the right and left photoreceptors in the lateral dimension. *Axial PR,* field of view of a photoreceptor located axially, which approximates the field of view of the left and right photoreceptors in the dorsoventral dimension. Also diagrammed is the path of light from a point source oriented 20° to the right of the body axis and passing through the left photoreceptor. *f,* principal focal length.

cipal focal length *f* (which we obtained from the graphical construction with Snell's law given in Fig. 11). For *M. nigrescens,* $\Delta\varphi = 53°$ (Table 1), and 2 $\Delta\varphi$ (the period of the grating) = 106°. Calculation of $\Delta\varphi$ does not take into account any screening effect of the shadowing structure. Figure 11 suggests that with screening by the anterior extension of the pigment, two point sources separated by only 80° should be resolved. However, the figure oversimplifies the shadowing structure. The pigment at the anterior margin of the cup is considerably less dense and gaps are discernible. Thus the points should more likely be separated by at least 100° to be resolved.

Resolution of phototaxis was tested with two horizontally directed point sources oriented 90° or 120° with respect to each other (Burr *et al.,* 1990). As the worm crawled, the position of the worm's anterior was maintained at the junction of the two beams by sliding the arena (Burr *et al.,* 1990). With the 120° sources, the neck sometimes oriented the base of the head toward one source, sometimes toward the other, and occasionally in between. With the 90° sources, the base of the head was oriented primarily in between the sources, with only short periods directed toward either source. It appears that two point sources are resolved more reliably when separated by 120° than when separated by 90°.

This result is in agreement with the prediction from the optical geometry and thus supports the possibility of a phototaxis mechanism based on a simultaneous comparison of two receptor signals during sideways scanning but not vertical scanning. However, the optical geometry and behavior also agree with a mechanism involving a pooled-receptor signal during both sideways and vertical scanning. From inspection of the optical geometry with a point source (Fig. 11), one can predict that a pooled signal would peak when the eye is directed within ± 10° of the source, and

Table 1

Optical geometry

Quantitity (definitions from Land and Nilsson, 2002)	Symbol	Units	*Mermis nigrescens*	*Planaria*[a,b]	*Perga* sp. larva[a,c]
PR acceptance angle		deg	50		
Radius of curvature		µm	60		
Aperture diameter	A	µm	120	30	200
Principal focal length or posterior nodal distance	f	µm	53	25	200
Photoreceptor separation	s	µm	49	10	20
Photoreceptor diameter		µm	8	10	10
Photoreceptor length		µm	40	6	120
Inter-receptor angle	$(180/\pi)s/f = \Delta\varphi$	deg	53	35	5.7
Minimum separation of two resolvable bright stripes	$2\,\Delta\varphi$	deg	106	69	11
Relative aperture[b]	A/f or $A'/$"f" $= a$		2.3	1.2	1.0

[a] From table 5 in Land (1981).

[b] For a pigment cup eye without lens or cornea, "*f*" = "focal length" = distance from cup rim to PR.

[c] *Perga* sp. larvae have a corneal lens like *M. nigrescens,* but with a fully developed retina.

would fall to low values at 40° to the left or right and 40° upward or downward, approximately as postulated previously (fig. 4A in Burr and Babinszki, 1990). During horizontal phototaxis, the observed range of left-right swings of the tip was 70° ± 46° (Mean ± Std Dev) for one worm (Burr *et al.,* 1990) or 82° ± 40° for another (Burr and Babinszki, 1990). The range of vertical scanning was 96° during orientation toward a source elevated 45° (Burr *et al.,* 1990). Thus it would appear that, to be consistently resolved by a pooled-receptor signal, the minimum separation of two point sources would have to be at least 80°, and probably considerably more if the variability of swings were accounted for.

Mechanism of phototaxis

The optic geometry described in the present work and discussed above could enable a mechanism involving a simultaneous comparison of two photoreceptor signals for orientation in the left-right dimension. However, results of experiments on the phototaxis support a mechanism based on a pooled signal collected during scanning cycles and rule out one based on a two-receptor comparison signal. The head-bending motion occurs continuously, in both the dorsoventral and sideways dimensions, whether the base of the head is oriented or not. When the light direction was suddenly changed by 90°, several scanning cycles that intercepted the new source direction were required before reorientation of the base of the head to the new source direction began (Burr *et al.,* 1990). This process is to be distinguished from the sweeping motion of the head used by fly larvae to discover the direction away from the light. In that phototaxis, any sweep that decreases the light entering the forward-directed eyes usually initiates a turn in that direction, and the sweeping motion stops once the body is oriented (Bolwig, 1946). Scanning behavior with more complex eyes is reviewed by Land and Nilsson (2002).

In another experiment (Burr and Babinszki, 1990), turning off (shuttering) the light source during swings to one side of the source direction shifted the phase relationship between the scanning motion and the cyclical illumination of the pigment cup interior. This resulted in the median "base of the head" orientation to be biased about 28° to the side of the source direction *opposite* to the shuttered side. With a two-receptor comparison signal, the bias should be to the *same* side. This is explained as follows. Whenever the base of the head is correctly oriented toward the source, the average illumination over a scanning cycle would be equal for the two photoreceptors, the time-averaged comparison signal over a scanning cycle of the head should be zero, and the signal to the neck that corrects the orientation of the base of the head should be zero. If, while the base of the head is correctly oriented towards the source, the source is shuttered whenever the head is bent to the left but not to the right, then the illumination of the left photoreceptor averaged over the scanning cycle would be decreased relative to the right one. This inequality of averaged photoreceptor illumination (left $<$ right) is what would occur normally when the base of the head is actually oriented to the right of the source, and is what would normally produce an averaged comparison signal that would direct the neck to bend to the left until averaged illumination is equalized. Equal average photoreceptor illumination in the presence of left-shuttering would not occur until the base of the head is directed somewhat to the left of the source. Thus, with a two-receptor comparison signal, the left-shuttering should bias orientation to the left of the source direction—the opposite of what is observed. In the case of a pooled-receptor signal, on the other hand, left shuttering would shift the relationship of pooled signal and bending toward the condition that normally causes a correction to the right; therefore, orientation during left-shuttering should be biased to the right, as observed (Burr and Babinszki 1990).

Thus the available evidence indicates that the orientation of the base of the head, under control of the motion of the neck, is corrected after analysis of information collected during several cycles of the scanning motion of the head. The signals for orientation appear to be based on the phase relationship of a pooled photoreceptor signal and signals that indicate the bending of the head. In *C. elegans,* it has been shown, by ablating one of the pair of amphidial chemoreceptors or thermoreceptors, that signals from lateral receptors may be pooled for nematode chemotaxis or thermotaxis (Bargmann and Horvitz, 1991; Bargmann *et al.,* 1993; Mori and Ohshima, 1995).

Definitions of eye and photoreceptor

The use of the term "photoreceptor" to refer to the organ used by small invertebrates to detect light direction probably arose among early biologists who surmised that the pigment spots observed in the light microscope were sensitive to light. Electron microscope and other studies have since clearly shown that this pigment forms a shadowing structure and that an adjacent specialized dendritic process detects the light. Usage of "photoreceptor" to refer to these light-direction-sensitive organs is unfortunate because that term has long been applied to the part of the nerve cell that absorbs light and initiates phototransduction (Eakin, 1968, 1982; Coomans, 1981; Land, 1981; Burr, 1984a).

Coming up with a definition for an "eye" on the basis of morphology has been difficult, as there is a continuous grade of complexity from lens-less pigment-spot eyes to the high-acuity camera eyes of molluscs or the compound eyes of arthropods (Burr, 1984a; Land and Nilsson, 2002). Land and Nilsson defined "eye" as an organ of spatial vision; that is, one that has the ability to compare intensities in different directions. On this basis they chose to limit the term to

organs with at least two receptors and an associated nervous system that can compare intensity in different directions *simultaneously.* An organ involved in comparisons sequentially in time would then be called a photoreceptor.

This distinction becomes problematic with the eye of *M. nigrescens,* as well as with other simple eyes. The optical structure clearly indicates that the two photoreceptors, with shadowing structure and cornea, are capable of making simultaneous comparisons of light from two sideways directions; however, the same organ is used for orientation in the dorsoventral dimension, for which simultaneous comparisons of the photoreceptor signals would be useless. Thus, for sideways orientation it would be called an eye and for vertical orientation it would not. If the two-dimensional orientation capability of *M. nigrescens* were not known, its two-photoreceptor organ would be assumed to use simultaneous comparison and be classified as an eye by this definition.

The behavioral observations and the shuttering experiment suggest that the eye achieves a true spatial vision by a unique scanning mechanism, in which light from different directions and concurrent information about head bending are sampled sequentially and integrated over several scanning cycles before a signal for reorientation of the base of the head is provided (Burr *et al.,* 1990; Burr and Babinszki, 1990). If a scanning mechanism with integration is acceptable as spatial vision, then the number of photoreceptors is unimportant for the definition of an eye: a single photoreceptor with screening pigment would serve the purpose. Such an eye would be morphologically indistinguishable from one in an organism that reflexively reacts to shadowing or unshadowing by the pigment.

These considerations highlight problems with a definition of "eye" that depends on knowledge of behavior and physiology when it is applied to poorly known animals in which only structural information is available. A less problematic definition that depends only on simple morphological criteria was proposed by Burr (1984a): an eye is an *organ* that can extract *directional* information from the photic environment. The alternative would be an unshielded light sensor—a photoreceptor. The minimum criterion would be at least one photoreceptor associated with a shadowing, reflecting, or focusing device that could select light from certain directions. This would include, for example, the single-photoreceptor optical organs found in nematodes other than *M. nigrescens,* as well as those found in rotifers and other animals in the lower phyla (Clement *et al.,* 1983; Burr, 1984a). The definition would exclude photoreceptors not located in an organ having such direction-discriminating accessories and would avoid the need for using "photoreceptor" ambiguously as an organ as well as a light sensor.

Putative photoreceptor of fourth stage juveniles and immature females

The fourth stage juvenile of *M. nigrescens* has a weak negative phototaxis that may guide it through matted vegetation toward the soil into which it burrows shortly after emerging from its host (Gans and Burr, 1994). This sensitivity persists after the adult molt in the immature female, which also lacks pigmentation (Burr *et al.,* 2000b). Thus photoreceptors are expected in these stages, and we find in both stages a multilamellar dendritic process with a structure and location identical to that of the mature female, although the dendritic process of J4 has less extensive lamellae. It appears that the photoreceptors develop in the transparent *M. nigrescens* pre-female juvenile during or before the J4 stage and persist into the adult. With the later addition of the pigment cup and the change to a preference for an illuminated rather than a shadowed photoreceptor, the phototaxis is changed from weakly negative to strongly positive.

The weakly negative phototaxis of J4s and immature females would be optically possible if the body posterior to the photoreceptor shades the photoreceptor sufficiently when the anterior is turned away from a light source. When the J4 and immature female heads are viewed with perpendicular light (Fig. 1), the bluish cast is evidence of a significant amount of light scatter by small refractile structures in the body, such as mitochondria or muscle filaments. This would remove the shorter wavelengths, to which *M. nigrescens* females are more sensitive (Burr *et al.,* 1989), most effectively from a light beam passing through the body, and thus provide shadowing suitable for negative phototaxis. Although orientation with respect to light direction occurs, the morphology in this case is not that of an "eye"—there is no identifiable organ that includes an accessory structure selective for light direction.

Hemoglobin pigmentation

The longitudinally aligned crystals we observe in the *M. nigrescens* eye are densely packed, corresponding to the extremely high concentration of oxyhemoglobin in the pigmented region (Burr *et al.,* 1975). Only anterior hypodermal cells in the eye accumulate hemoglobin to the high concentration necessary to cast a shadow with only a 20–25-μm optical path. Surprisingly, eye globin mRNA is expressed at low levels along the rest of the body, even though hypodermal cord cells posterior to the eye do not have visible amounts of hemoglobin (Burr *et al.,* 2000a). Thus *M. nigrescens* eye hemoglobin may be another example of a protein that has a dual function. These include a number of different proteins, of both vertebrates and invertebrates, that at high concentration provide the high refractive index and transparency of lenses and corneas, while at low concentrations in other tissues preserve their biochemical function as

metabolic enzymes or stress proteins (Frank *et al.,* 2004; Piatigorsky, 2007). Like them, *M. nigrescens* eye hemoglobin may have been recruited during evolution for a shadowing function simply by changing gene regulation, without need for change in protein sequence, so that it would be expressed at high concentration in anterior hypodermal cells.

Evolution of the eye and photoreceptor

The eye in *M. nigrescens* is unique in location and morphology. Unlike any other nematode eye (1) it is unpaired, with the pigmented cup occupying the entire body cross section; (2) the curved anterior tip, used in air like a cornea, concentrates light into the pigment cup; (3) the shadowing pigment is oxyhemoglobin rather than a melanin-like pigment; (4) the pigment develops in hypodermal cells, unlike other eye pigments; and (5) the pigment occurs only in mature females, whereas eyes in other nematode examples develop in the late embryo, usually in both sexes (Bollerup and Burr, 1979). Thus the eye in *M. nigrescens* appears to have evolved independently within Nematoda.

M. nigrescens is the only species of nematode Clade I (Blaxter *et al.,* 1998; De Ley and Blaxter, 2002) discovered so far that has a pigmented shadowing structure. However, the infective juveniles in two other mermithid species, *Agamermis* sp. and *Hexamermis* sp., have an interesting transverse phototaxis (orientation perpendicular to a light beam) (Robinson *et al.,* 1990). They have a terrestrial habitat, therefore it is possible for lateral illumination to be focused, as in a cylindrical lens, at the air-cuticle interface onto suitably located photoreceptors. It would be interesting to investigate these species for evidence of a similar multilamellar structure in the amphidial nerve bundle.

The *M. nigrescens* photoreceptor is very similar to the thermosensory lamellar process found in the amphid of *Strongyloides stercoralis.* Thus, it may have evolved from a similar ancestral process, acquiring photosensitivity and moving 100 μm posteriorly from the amphid to a location suitable for phototaxis, within the pigment cup. This is the second nematode example in which one of the amphidial dendrites has apparently been relocated and modified so as to provide a light-sensitive process within an eye. In *Oncholaimus vesicarius,* a clump of 10 modified cilia projects from the terminal bulb of an amphidial dendrite medially to a position adjacent to a pigment spot, while groups of similar cilia project from the three other amphidial dendrites anterolaterally into a channel through the cuticle, for a chemoreceptive function (Burr and Burr, 1975). Thus, in *O. vesicarius,* the photoreceptor appears likely to have evolved by modifying an amphidial chemoreceptor (Burr, 1984a). Such modality interconversions are reasonable because chemoreceptors and thermoreceptors in nematodes utilize the same classes of intracellular signaling molecules and transcription factors as animal photoreceptors (Svendsen and McGhee, 1995; Coburn and Bargmann, 1996; Mori, 1999; Troemel, 1999; Komatsu *et al.,* 1999; Satterlee *et al.,* 2001; Arendt, 2003; Kimura *et al.,* 2004; Inada *et al.,* 2006). All that is required, initially, is to express an existing opsin gene in the new cell. More difficult, but still evolutionarily reasonable, would be to convert into an opsin one of the hundreds of chemosensory proteins that are expressed in nematodes and belong to the same family of seven transmembrane-domain, cyclic nucleotide-coupled proteins (Troemel *et al.,* 1995). *M. nigrescens* provides an exquisite example of how a photoreceptor and eye might evolve: changing an ancestral chemoreceptor or thermoreceptor into a photoreceptor and translocating it to a position suitable for shadowing, while altering the regulatory region of a gene so that a shadowing pigment is over-expressed in adjacent cells.

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